

The Pennsylvania State University

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**FEEDING SYSTEMS COMBINING PASTURE WITH CONCENTRATE
AND TOTAL MIXED RATIONS FOR HIGH PRODUCING DAIRY COWS**

A Thesis in

Animal Science

by

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ABSTRACT

Studies were conducted to determine: 1) the effect of pasture allowance on substitution rate, pasture and total dry matter intake, milk production and composition, rumen digestion, and grazing behavior of unsupplemented and supplemented high producing dairy cows in early-mid lactation, and 2) the effect of feeding systems combining pasture and total mixed rations on dry matter intake, milk production and composition, body weight and body condition score changes, rumen digestion and grazing behavior of high producing dairy cows in early-mid lactation. Pastures grazed in the studies were based on smooth brome grass (*Bromus inermis* L.) and orchardgrass (*Dactylis glomerata* L.).

In the first study, twenty multiparous Holstein cows (4 ruminally cannulated) in five 4 x 4 Latin squares with 21-d periods were used to study the effect of concentrate supplementation when grazed at two pasture allowances. The four dietary treatments resulted from the combination of two pasture allowance targets (low, 25 vs. high, 40 kg DM/cow per d) and two concentrate supplementation levels (zero vs. 1 kg concentrate/4 kg milk). Concentrate supplementation decreased pasture dry matter intake 2.0 kg/d at the low pasture allowance (17.5 vs. 15.5 kg/d) and 4.4 kg/d at the high pasture allowance (20.5 vs. 16.1 kg/d). Substitution rate was lower at the low pasture allowance (0.26 kg pasture/kg concentrate) than at the high pasture allowance (0.55 kg pasture/kg concentrate). Total DMI of both supplemented treatments averaged 24.4 kg/d. Milk production of both supplemented treatments averaged 29.8 kg/d, but was increased with higher pasture allowance in the unsupplemented treatments (19.1 vs. 22.2 kg/d). Milk response to concentrate supplementation was 1.36 and 0.96 kg milk/kg concentrate for the low and high pasture allowance, respectively. Concentrate supplementation reduced milk

fat percentage but increased milk protein percentage. Rumen pH and $\text{NH}_3\text{-N}$ concentration were decreased by concentrate supplementation. Substitution rate was likely related to both negative associative effects in the rumen (reductions in rumen pH, rate of pasture digestion and NDF digestibility) and reductions in grazing time. This latter was quantitatively more important, explaining between 80 to 100% of the reduction in pasture DMI observed.

In the second study, the effect of pasture allowance on the grazing behavior of unsupplemented or supplemented high producing Holstein cows was studied in two single reversal experiments. Ten multiparous cows (2 ruminally cannulated) were unsupplemented, and six multiparous cows (2 ruminally cannulated) were supplemented with a corn-based concentrate. Within each supplementation group cows grazed a brome/orchardgrass-based pasture offered at two pasture allowances: 1) 25 kg DM/cow/d, and 2) 40 kg DM/cow/d. Recorders were used to measure grazing time and bites per d. Bite mass was calculated dividing the pasture DMI, measured by chromic oxide, by the total number of bites per d. With unsupplemented cows, pasture DMI (18.8 vs. 15.6 kg/d) and milk production (17.6 vs. 15.2 kg/d) were higher at high pasture allowance. The higher pasture DMI was a result of a longer grazing time and a greater number of bites after the p.m. milking. Rumen pH was lower (6.33 vs. 6.60) and total VFA concentration was higher (134.5 vs. 119.5 mmol/L) at high pasture allowance. With supplemented cows, pasture and total DMI (14.7 and 22.8 kg/d) and milk production (27.8 kg/d) were not affected by pasture allowance. Grazing behavior of supplemented cows was not affected by pasture allowance. Neither rumen pH (6.05) nor total VFA concentration (148.4 mmol/L) differed between low and high pasture allowance. Pasture allowance had important effects on grazing behavior, pasture DMI and milk production of unsupplemented cows, however

neither grazing behavior nor performance of supplemented cows were affected by pasture allowance.

In the third study, forty-five Holsteins cows (15 primiparous, 30 multiparous) were used to compare three feeding systems combining pasture and total mixed ration (TMR) on animal performance in a 21-wk repeated measures experiment. The three treatments were: 1) pasture plus concentrate (PC), 2) pasture plus partial TMR (pTMR), and 3) TMR (non pasture). Total dry matter intake (DMI), using chromic oxide as a marker, was 21.6, 25.3, and 26.8 kg/d for PC, pTMR, and TMR, respectively. Milk production was highest for TMR (38.1 kg/d), lowest for PC (28.5 kg/d), and intermediate for pTMR (32.0 kg/d). All three treatments received ten bST injections, PC responded to six, pTMR to four, and TMR to nine administration of bST during the 21 wk trial; milk response averaged 13.8, 15.6, and 11.8% for PC, pTMR, and TMR, respectively. Cows fed pTMR and TMR had higher milk fat (3.33 vs. 3.13%) and true protein (2.97 vs. 2.82%) percentage than cows fed PC. Cows on PC gained less body weight and lost more body condition compared to cows on pTMR and TMR. Initial concentrations of plasma nonesterified fatty acids (NEFA) were higher in PC (302 μ eq/L) than in pTMR (130 μ eq/L) and TMR (225 μ eq/L). Plasma urea nitrogen (PUN) was lower in both pTMR and TMR treatments (13.8 mg/dl) than in PC treatment (17.2 mg/dl). Performance was improved combining pasture and TMR compared to pasture plus concentrate, resulting in higher milk production, milk production persistency, milk fat and protein percentage, and no loss in body condition score.

In the fourth study, six multiparous Holstein cows fitted with rumen cannulas were used to study the effect of these three feeding systems combining pasture and total mixed ration (TMR) on ruminal digestion in a 21-wk repeated measures experiment. Rumen ammonia nitrogen concentration was lower in both the pTMR and TMR treatments (10.2 mg/dl) than in

the PC treatment (19.9 mg/dl). Rumen pH was not affected by treatments and averaged 5.87. Neither total volatile fatty acids concentration (137.5 mmol/L) nor main individual volatile fatty acids proportions (64.4, 20.6, and 12.0 mol/100 mol for acetate, propionate, and butyrate, respectively) differed among treatments. The combination of pasture with TMR reduced the total potentially degradable fraction of DM (85.5 vs. 82.3%) of pasture, and increased the lag time (5.4 vs. 6.5 h) and reduced the potentially digestible fraction (82.1 vs. 74.9%) of pasture NDF. Ruminal losses of ammonia nitrogen were reduced combining pasture and TMR, however no positive effects on rumen pH were observed. The combination of pasture and TMR also decreased the rumen digestion of pasture, indicating the presence of negative associative rumen effects.

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Chapter 1

Introduction

Most dairy farms in the USA use a confinement feeding system based on conserved forages (e.g. corn silage, hay, or haylage), and concentrates composed by grains (e.g. corn), protein sources (e.g. soybean meal), by-products (e.g. nonforage fiber sources) and minerals and vitamins. This feeding system maximizes milk production per cow of high genetic merit dairy cattle. Well-managed dairy herds average over 10,000 kg of milk per lactation and feed costs typically represent over 50%.

Low milk prices and narrow profit margins during the last 15 years has caused some dairy farmers to look for alternative and more profitable feeding systems. A grazing system offered the opportunity of reducing cost of forage during the typical 6 to 7 month pasture season in the Northeast and Midwest USA (Muller and Fales, 1998). Research in the USA has focused on the understanding of feeding strategies combining pasture and supplements for high producing dairy cows. Over the last 10 years research in the USA on animal performance and nutrient utilization of high producing dairy cows on pasture has increased (Berzaghi et al., 1996; Delahoy, 2000; Hoffman et al., 1993; Holden et al., 1994a; Holden et al., 1994b; Holden et al., 1995; Hongerholt and Muller, 1998; Jones-Endsley et al., 1997; Kolver and Muller, 1998; Kolver et al., 1998; McCormick et al., 1999; McCormick et al., 2001a; McCormick et al., 2001b; Reis and Combs, 2000a; Reis and Combs, 2000b; Reis et al., 2001; Ruiz et al., 2001a; Soriano et al., 2000; Soriano et al., 2001; Vazquez and Smith, 2000; Vazquez and Smith, 2001; White, 2000; White et al., 2001; Wu et al., 2001).

The challenge of managing the high producing dairy cow on pasture is to achieve high levels of pasture and total DMI by manipulating pasture management and supplementation strategies to maintain high milk production (McGilloway and Mayne, 1996). High producing dairy cows on pasture need supplemental energy to reach their genetic potential for intake and milk production (Kolver and Muller, 1998). When grazing cows are supplemented, pasture intake decrease, which is known as substitution rate (Kellaway and Porta, 1993). Substitution rate is a major factor contributing to the variation in milk response to concentrate supplementation (Kellaway and Porta, 1993). Among the several factors influencing the substitution rate and milk response to concentrate supplementation, the amount of pasture offered per day, or pasture allowance, is of major importance.

Studies with low producing dairy cows have reported a high substitution rate and low milk response to concentrate supplementation at a high pasture allowance (Kellaway and Porta, 1993). There is a lack of information on substitution rate and milk response to concentrate supplementation with high producing dairy cows grazing at different pasture allowances, and reasons for the substitution rate are not well understood either. It has been suggested that substitution rate is produced by negative associative effects in the rumen (Dixon and Stockdale, 1999) and reduction in grazing time (McGilloway and Mayne, 1996) with concentrate supplementation.

Grazing time, biting rate and bite mass explain pasture DMI of grazing cows. Both concentrate supplementation and pasture allowance may also influence grazing behavior of dairy cows. Information on grazing behavior of high producing dairy cows in the USA is lacking. A good understanding of grazing behavior is required to design efficient grazing management

systems (Rook, 2000). Pattern of grazing time may also determine daily variations of ruminal pH and ammonia nitrogen concentration, and therefore rumen digestion by cows on pasture.

Another feeding strategy for high producing cows on pasture is the supplementation of pasture with a TMR, which may be called partial TMR because the pasture grazed by the cows is not physically part of the TMR. Some of the advantages of a partial TMR may include the provision of a more uniform ration throughout the year and the grazing season, a more accurate monitor of pasture and total DMI, less chance of rumen digestive problems due to slug feeding of grain because some forage is fed with the concentrate rather than separate, and higher milk production and higher milk fat and protein production. There are no published studies that have compared animal performance and rumen digestion of pasture as the only forage plus concentrate, a total TMR diet, and the combination of both pasture and TMR.

The objectives of this research were: 1) to determine the substitution rate and milk response to concentrate supplementation of high producing dairy cows grazing at two pasture allowances and to identify the factors related to the substitution rate, 2) to quantify the effects of pasture allowance and concentrate supplementation on grazing behavior and to evaluate the relation between grazing pattern and rumen pH and ammonia nitrogen concentration, 3) to compare pasture and total DMI, milk production and composition, body weight and body condition score of high producing dairy cows on three feeding systems combining pasture and TMR during the entire grazing season, and 4) to evaluate the effect of three feeding systems combining pasture and TMR on rumen fermentation and in situ pasture rumen digestion of high producing dairy cows.

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Chapter 2

Literature Review: Supplementation for High Producing Dairy Cows on Pasture

2.1 INTRODUCTION

The use of pasture in feeding systems for dairy cows results in low cost feeding systems because grazed forage is one of the cheapest sources of nutrients (Clark and Kanneganti, 1998). The type of feeding system will be mainly determined by the milk price/feed cost ratio (Clark and Kanneganti, 1998). Pasture-based systems (based on pasture grazed in situ) are characterized by high milk output per unit of land, while confinement systems (based on conserved forages and concentrates) are characterized by high milk output per cow (Clark and Kanneganti, 1998).

The type of pastures used for dairy cows are commonly based on temperate species and described as high quality pastures or young and leafy pastures 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 (Clark and Kanneganti, 1998). Muller and Fales (1998) reported a range of 18 to 25% of CP, 40 to 55% of NDF, and 1.55 to 1.70 Mcal/kg DM of NE_L for well-managed grass pastures typically grazed in the Northeast part of USA.

In the USA, the dairy industry over the last 50 years has been characterized by a favorable milk price/feed cost ratio and therefore dairy systems tended to focus on high milk production per cow (Clark and Kanneganti, 1998; Muller and Fales, 1998). Average milk production per cow in the USA increased from 3200 kg in 1960 to 7300 kg in 1994 (Clark and

Kanneganti, 1998). Besides the high milk production per cow, climatic conditions (i.e. snow cover during 6 or 7 months of the year) in many important dairy areas in the USA (e.g. Midwest and Northeast) do not allow the implementation of year-round grazing systems (Clark and Kanneganti, 1998). Therefore, in countries such as USA the use of feeding systems combining the pasture grazed in situ plus other feed supplements (e.g. concentrates and conserved forage) is required.

The main objective to supplementation of grazing dairy cows is to increase total DMI and total energy intake relative to those reached on only pasture diets (Stockdale, 2000b). Considering the entire system of production, a primary goal of supplementation is to obtain both high milk production per cow and milk production per unit of land (Kellaway and Porta, 1993). Objectives of supplementation include among others (Kellaway and Porta, 1993): 1) increase milk production per cow, 2) increase stocking rate and therefore milk production per unit of land, 3) improve the use of pasture because of the higher stocking rate, 4) accelerate the growth of heifers in order to reach mature size earlier and enhance the genetic potential, 5) maintain or improve body condition score during pasture shortage in order to maintain or improve reproductive performance, 6) increase length of lactation during periods of pasture shortage, and 7) increase milk protein content through energy supplementation.

Previous reviews on grazing studies covering different animal performance and digestion aspects have been reported (Doyle et al., 1996; Kellaway and Porta, 1993; Leaver, 1985; Leaver, 1986; Stockdale, 2000b), however most have focused on low producing dairy cows. In order to develop appropriate strategies for supplementation of high producing dairy cows, the effect of different types of supplements on DMI, animal performance and digestion needs to be well understood.

The objective of this review is to summarize the effects of feeding concentrate supplements on grazing behavior, pasture and total DMI, milk production and composition, and rumen and post-ruminal digestion of high producing dairy cows on pasture. For the purpose of this review, high producing dairy cows are defined as those producing more than 25 kg/d of milk in early lactation or about 20 kg/d in late lactation. Although this review focuses on high producing dairy cows, data from low producing dairy cows are included in those areas where information with high producing dairy cows is not available. This review focuses on data from the USA, but also includes information from other countries where grazing systems are important (e.g. New Zealand, Australia, Ireland, UK, France, Argentina and others).

2.2 DRY MATTER INTAKE OF GRAZING COWS

2.2.1 Dry Matter Intake of Grazing Cows on Only Pasture Diets

Different theories about the factors controlling pasture DMI in ruminants has been presented (Baile and Della-Ferra, 1988; Dulphy and Demarquilly, 1994; Dynes, 1996; Forbes, 1995a; Forbes, 1995b; Forbes, 1995c; Forbes, 1996), detailed description of these theories exceeds the objectives of this review. Hodgson and Brookes (1999) described three factors affecting pasture DMI of grazing cows: 1) “feeding drive” or nutrient requirements of the cow; 2) “physical satiety” or factors associated with distension of the alimentary tract and amount, digestibility and rate of digestion and passage of the feed; and 3) “behavioral constraints” or

limits to the potential pasture DMI resulting from the combination of pasture and animal factors affecting grazing behavior.

Low pasture DMI has been identified as a major factor limiting milk production of high producing cows under grazing conditions (Kolver and Muller, 1998; Leaver, 1985; Mayne and Gordon, 1995; McGilloway and Mayne, 1996). In his review, Leaver (1985) suggested that high producing dairy cows fed only pasture could reach total DMI of 3.25% of BW. However, the intake is usually less than 3% of BW indicating that grazing dairy cows do not reach their potential maximum DMI. Mayne and Wright (1988) estimated when no pasture quantity and quality restrictions exist, pasture DMI of high yielding dairy cows can reach 3.5% of BW.

Beever and Thorp (1997) proposed that total DMI of high producing cows fed only pasture is lower than with other diets which include concentrates because of physical constraints (a maximum amount of time per day for grazing without interfering with other essential processes such as rumination and resting), rate of forage removal from the rumen (including the processes of digestion and passage), and water consumption associated with pasture (70 to 90 kg/d approximately, for a 600 kg cow with a total DMI around 21 kg/d).

Research studies in the USA with high producing cows fed only pasture are limited. Kolver and Muller (1998) found that early lactation cows grazing high quality grass pasture in the spring had a pasture DMI of 19.1 kg of DM, or 3.4% of BW. However, when compared with cows on a nutritionally balanced TMR ration, cows consumed 4.5 kg less DM. The intakes of DM and NE_L were significantly lower with the pasture fed cows, however the intake of CP and NDF did not differ between pasture and TMR fed cows. Kolver and Muller (1998) concluded that the difference in DMI, rather than energy content of pasture, appeared to be the major factor responsible for the lower total energy intake and milk production.

One pasture management alternative to increasing pasture DMI is increasing the frequency of feeding through a more frequent allocation of new pasture paddocks (Dalley et al., 2001). With high producing dairy cows (39 DIM, 31 kg milk/d at the beginning of the experiment) grazing a ryegrass (*Lolium perenne*) pasture at two pasture allowances (40 or 65 kg DM/cow/d) offered once daily (at 0900 h) or six times daily (at 0600, 0900, 1100, 1330, 1800, and 2000 h), pasture DMI (15.6 vs. 15.9 kg/d) and milk production (25.4 and 25.2 kg/d) were similar (Dalley et al., 2001). In a second experiment where cows grazed at a pasture allowance of 50 kg DM/cow/d, pasture DMI did not differ (15.2 and 16.3 kg/d) when pasture was offered once daily (0900 h) or 6 times per day (0800, 1130, 1700, 2030, 2400, and 0330 h). Milk production was higher for the once daily time pasture allocation (26.7 vs. 25.7 kg/d) (Dalley et al., 2001).

2.2.2 Effect of Pasture Allowance

Many factors affect pasture DMI (Hodgson and Brookes, 1999; Poppi et al., 1987). Those include, among others, pre-grazing pasture mass (amount of pasture per unit area; kg DM/ha) and pasture allowance (amount of pasture offered per cow; kg DM/cow/d) (Poppi et al., 1987). Pasture allowance is calculated by dividing the pre-grazing pasture mass by the number of cows per unit area (Poppi et al., 1987). The effect of pasture allowance on pasture DMI has received a lot of attention. Leaver (1986) proposed that high yielding cows could obtain all their nutrient requirements from pasture, but this would involve a high pasture allowance, and a high energy content and low sward contamination; conditions that occur only for short periods of time in the spring (Clark et al., 1997).

Several researchers (Dalley et al., 1999; Holmes and Wilson, 1984; Stockdale, 1985) have reported that pasture DMI is closely related to pasture allowance. The relationships between pasture DMI and pasture allowance has been described as asymptotic (Dalley et al., 1999; Minson, 1990; Peyraud et al., 1996; Poppi et al., 1987). In his review, Leaver (1985) proposed a maximum DMI at pasture allowance between 45 to 55 g DM/kg BW or 27 to 33 kg DM/cow/d for a 600 kg BW cow. Pasture DMI increases as pasture allowance increases, but at a declining rate reaching the plateau when pasture allowance is 10 to 12% of BW or 60 to 72 kg DM/cow/d for a 600 kg BW cow (Hodgson and Brookes, 1999). Data from Australia (Doyle et al., 1996) reported that pasture DMI continues to increase as pasture allowance increases up to 15 kg DM/100 kg BW or 90 kg DM/cow/day for a 600 kg BW dairy cow.

Dalley et al. (1999) evaluated the effect of pasture allowance on pasture DMI of dairy cows grazing a ryegrass pasture as the only feed. Pasture DMI increased curvilinearly (11.2, 13.2, 15.4, 16.5, 17.2, and 18.5 kg DM/cow/d) with increasing pasture allowance (20, 30, 40, 50, 60, and 70 kg DM/cow/d) and a plateau at a pasture allowance of 55.2 kg DM/cow/d. On average, pasture DMI increased 0.14 kg/kg DM increase in pasture allowance for the 20 to 70 kg DM/cow/d range. However, pasture DMI increased 0.18 kg/kg DM as pasture allowance increased from 20 to 50 kg DM/cow/d (Dalley et al., 1999). Pasture utilization (defined as pasture DMI/pasture allowance) decreased from 54% to 26% with the increase in pasture allowance.

Peyraud et al. (1996) also reported a curvilinear relationship between pasture DMI and pasture allowance (20, 30, and 40 kg DM/cow/d), with pasture DMI reaching a plateau at the medium pasture allowance (32.6 kg DM/cow/d). Pasture DMI increased 2.7 kg/cow/d between

the low and medium pasture allowance, but only increased 0.6 kg/cow/d between the medium and the high pasture allowance (Peyraud et al., 1996).

The effect of pasture allowance on pasture DMI is affected by other pasture factors such as pre-grazing mass, species, and quality (Poppi et al., 1987). Wales et al. (1999) reported that as pasture allowance increased from 20 to 70 kg DM/cow/d, pasture DMI increased linearly from 7.1 to 16.2 kg DM/cow/d at a pre-grazing pasture mass of 3100 kg DM/ha, and from 9.9 to 19.3 kg DM/cow/d at a pre-grazing pasture mass of 4900 kg DM/ha. Regardless of pre-grazing pasture mass, pasture DMI increased 0.18 kg DM/kg DM increase in pasture allowance.

Recent studies have reported the effect of pasture allowance on pasture DMI of high producing dairy cows fed only pasture (Bargo et al., 2002; Dalley et al., 2001; Delaby et al., 2001; Kolver and Muller, 1998; Stockdale, 2000a; Wales et al., 2001). Kolver and Muller (1998) reported a pasture DMI of 19.1 kg/cow/d when high producing dairy cows grazed a grass based pasture (> 50% of ryegrass) at a pasture allowance of 38.5 kg DM/cow/d (i.e. 49.6% of pasture utilization). High producing dairy cows grazing an orchardgrass (*Dactylis glomerata*) based pasture had a pasture DMI of 17.5 and 20.6 kg DM/cow/d at low (25 kg DM/cow/d) and high (40 kg DM/cow/d) pasture allowance, respectively (Bargo et al., 2002). Pasture DMI increased 0.21 kg DM/kg DM increase in pasture allowance (Bargo et al., 2002). Pasture DMI increased with pasture allowance (measured at a 5 cm cutting height) in 2 of 3 experiments conducted with high producing dairy cows grazing a ryegrass pasture (Delaby et al., 2001). In the first experiment, pasture DMI increased from 11.3 to 13.0 kg/cow/d when pasture allowance increased from 12.1 to 15.8 kg DM/cow/d. In the second experiment, pasture DMI did not differ (12.6 kg/cow/d) when pasture allowance was increased from 16.6 to 19.6 kg DM/cow/d. In the third experiment, pasture DMI increased from 12.9 to 15.0 kg/cow/d when pasture allowance increased from 16.5

to 21.0 kg DM/cow/d (Delaby et al., 2001). Stockdale (2000a) reported an increase in pasture DMI from 14.3 to 19.3 kg/d when pasture allowance of a ryegrass-based pasture was increased from 26.7 to 53.5 kg DM/cow/d. Dalley et al. (2001) also reported higher pasture DMI (17.9 vs. 13.6 kg/d) when high producing dairy cows grazed a ryegrass pasture offered at a higher pasture allowance (65 vs. 40 kg DM/cow/d). Pasture DMI by high producing dairy cows in early lactation increased from 11.2 to 15.6 kg/d when pasture allowance of a ryegrass pasture was increased from 19 to 37 kg DM/cow/d (Wales et al., 2001).

If the goal is to maximize pasture DMI of high producing dairy cows pasture management must ensure unrestrictive pasture conditions both in terms of quality and quantity. However, availability of pasture of high nutritive value in unrestrictive quantities is only found for short periods of time during the spring (Clark et al., 1997). Unrestrictive pasture conditions (i.e. high pasture allowance) also imply low pasture utilization (pasture DMI/pasture allowance lower than 50%) and therefore poor utilization of pasture per area (McGilloway and Mayne, 1996). The results of the studies reviewed indicate that maximum pasture DMI is achieved when pasture allowance is between 3 to 4 times the pasture actually consumed. Hodgson and Brookes (1999) suggested pasture allowance between 3 to 5 times. However, even under unrestrictive pasture conditions, total DMI levels achieved by high producing dairy cows are lower than those by cows consuming TMR diets (Kolver and Muller, 1998) or pasture diets including supplements (Bargo et al., 2002; Beever and Thorp, 1997). Therefore high producing dairy cows on pasture need to be supplemented to obtain total DMI which approaches their genetic potential.

2.2.3 Methods to Estimate Dry Matter Intake in Grazing Cows

Estimation of DMI in grazing cows is more difficult and less accurate when compared with determination of DMI with cows fed in confinement. An extensive review of the different methods or techniques to estimate DMI in grazing cows was reported by Leaver (1982). Techniques may be classified in two categories: pasture-based or animal-based techniques (Meijs et al., 1982).

Pasture-based techniques estimate DMI by difference (pasture offered – pasture refused). Advantages and disadvantages of these methods have been discussed in detailed by Meijs et al. (1982). These techniques require estimation of pasture mass before and after the grazing period and the difference is calculated for the group of cows and then divided by the number of animals. Pasture mass can be estimated using cutting techniques, cutting to the ground level or to a specific pasture height (e.g. 4 or 5 cm). Non-destructive methods can be also used to estimate pasture mass such as the rising plate meter which is calibrated to correlate pasture height with pasture mass (Earle and McGowan, 1979). More extensive reviews of pasture measurement techniques can be found in Hodgson et al. (1999) and 't Mannetje (2000). The main disadvantage of these techniques is that pasture DMI is estimated as a group and not individually (Reeves et al., 1996).

Animal-based techniques for DMI estimation in grazing trials allow individual DMI estimation, and therefore the use of individual cows as experimental units. The most common animal-based technique used is based on the estimation of fecal production and diet digestibility (Le Du and Penning, 1982; Peyraud, 1998). The estimation of DMI is based on the following equation: $DMI = \text{fecal production} / (1 - \text{digestibility of the diet})$. Fecal production is estimated using external markers, with chromium oxide being the most common marker used (Peyraud,

1998). Although one of the main errors with this technique is the unknown recovery of this marker, Peyraud (1998) suggested that there is no systematic discrepancy in recovery rate between cows or periods of measurements; therefore it can be used to estimate fecal flow. Fecal samples can be taken from the ground (global sampling) or directly from the rectum (grab sampling) (Peyraud, 1998). Digestibility of the diet can be determined by laboratory procedures (e.g. in vitro DM digestibility by the procedure of Tilley and Terry (1963)), and individual values of digestibility are not available for each cow. Peyraud (1998) proposed the determination of digestibility through equations, based on the relationship between digestibility and N and ADF in feces, which allows the estimation of individual cow digestibility. It is also very important that pasture samples for digestibility analysis represent the pasture actually eaten by the cows.

The use of alkanes is another technique commonly used for DMI estimation in grazing cows (Dove and Mayes, 1991; Dove and Mayes, 1996). This technique is based on the use of plant cuticular wax alkanes as markers. Indigestible odd-chain alkanes from pasture in combination with orally dosed even chain alkanes are used to estimate DMI (Dove and Mayes, 1991). A major advantage of this technique is that it is independent of marker recovery in feces and accounts for the level of digestibility of individual animals (Dove and Mayes, 1996). A major disadvantage of this technique is the important variability in the content of n-alkanes in pastures composed of several species (Malossini et al., 1994).

Malossini et al. (1996) compared the alkane and chromium oxide techniques for estimating pasture DMI in grazing dairy cows producing 21 kg/d of 4% FCM and supplemented with 1.5 to 8 kg/d of concentrate. Assuming a 100% marker recovery, the chromium oxide technique overestimated pasture DMI by 5.1% in comparison with the n-alkane method. The highest sources of variations in each technique were between-days variation (54.6%) for the

alkane technique, and within-days variation (67%) for the chromium oxide technique. It was concluded that pasture DMI was similar between methods if a 95.5% of recovery is assumed for chromium oxide and that the choice of one method or the other must be based on the work and difficulties involved (Malossini et al., 1996).

Ruiz et al. (2001) evaluated the use of chromium mordanted neutral detergent residue (Cr-NDr) and cobalt EDTA (Co-EDTA) as predictors of DMI with high producing grazing dairy cows. Two 12-d field trials were conducted with 15 cows (571 kg BW, 41.2 kg of milk/d, 124 DIM) and 14 cows (589 kg BW, 32.2 kg of milk/d, 123 DIM), respectively, that grazed an orchardgrass/white clover (*Trifolium repens*) based pasture and were supplemented with a 8.3 kg/d of a corn-based concentrate and 0.8 kg/d of mostly mixed grasses hay (average of both trials). Pasture and total DMI was 24.8 and 34.0 kg/d (5.95% of BW) in trial 1, and 23.2 and 32.4 kg/d (5.52% of BW) in trial 2, respectively. Ruiz et al. (2001) concluded that the unrealistically high total DMI was due to two possible reasons: 1) diurnal variations in fecal excretion of the marker used, and 2) failure in collecting representative pasture samples.

2.2.4 Equations to Predict Dry Matter Intake of Grazing Cows

Because DMI estimation of grazing cows demand the use of labor-intensive and indirect (i.e. external or internal markers) techniques that carry several sources of error, equations based on animal and pasture variables to predict DMI of grazing cows have been developed (Caird and Holmes, 1986; Vazquez and Smith, 2000; Vazquez and Smith, 2001). Prediction of DMI by grazing dairy cows can be done using either empirical models based on regression equations (e.g. Caird and Holmes, 1986; Vazquez and Smith, 2000) or conceptual or mechanistic models based

on the description of long-term mechanisms of intake regulation (e.g. Vazquez and Smith, 2001). For a detailed description on empirical and mechanistic models see McNamara et al. (2000). For models in pasture-based systems for dairy cows see Cherney and Mertens (1998) and Rickert et al. (2000).

Caird and Holmes (1986) used data from 9 experiments including 203 rotationally and 154 continuously grazed cows to develop equations to predict total DMI. Cows grazed ryegrass pastures, and milk production and supplementation level averaged 21.5 kg/d and 1.2 kg/d of concentrate, respectively. Animal variables used in the regression analysis included: total organic matter (OM) intake (TOMI, kg/d), herbage OM intake, concentrate supplementation (C, kg/d), live weight (LW, kg), milk yield (MY, kg/d), herbage OM digestibility, week of lactation (WL), and week after January 1 (WEEK). Pasture variables used in the regression included herbage mass (HM, ton OM/ha), herbage allowance (HAL, kg OM/cow/d) and sward height (SHT, cm).

For rotationally grazed cows the best equation ($R^2 = 0.68$) was:

$$\text{TOMI} = 0.323 + 0.177\text{MY} + 0.010\text{LW} + 1.636\text{C} - 1.008\text{HM} + 0.540\text{HAL} - 0.006\text{HAL}^2 - 0.048\text{HAL} \times \text{C}$$

For continuously grazed cows the best equation ($R^2 = 0.54$) was:

$$\text{TOMI} = 8.228 + 0.208\text{MY} + 0.004\text{LW} + 0.069\text{WL} - 0.118\text{C} - 0.289\text{SHT} + 0.133\text{C} \times \text{SHT} - 0.011\text{WEEK}$$

Vazquez and Smith (2000) used data from 163 observations from 27 published grazing studies with dairy cows to obtain equations to predict total and pasture DMI by regression analysis. Mean milk production and supplementation level were 15.9 kg/d and 1.9 kg/d, respectively. Independent variables used to develop the models included 4% FCM (FCM, kg/d), days since calving, pasture allowance (PA, kg DM), NDF in pasture available (NDFp, % DM),

NDF in pasture selected (NDFs, % DM), percentage of legumes in pasture (LEG, %), amount of concentrate supplemented (kg DM), amount of forage supplemented (kg DM), total supplementation (SUP, kg DM), pasture allowance and total supplementation interaction (PASUP), BW (kg), and change in BW (CBW, kg/d).

Best equation ($R^2 = 0.95$) developed for total DMI estimation was:

$$\text{DMI} = 4.47 + 0.14\text{FCM} + 0.024\text{BW} + 2.00\text{CBW} + 0.04\text{PA} + 0.022\text{PASUP} + 0.10\text{SUP} - 0.13\text{NDFp} - 0.037\text{LEG}$$

When only animal variables were included in the model the R^2 decreased to 0.71:

$$\text{DMI} = -1.84 + 0.38\text{FCM} + 0.018\text{BW} + 2.82\text{CBW}$$

The best equation to estimate pasture DMI (PDMI) presented a R^2 of 0.91:

$$\text{PDMI} = 4.47 + 0.14\text{FCM} + 0.024\text{BW} + 2.00\text{CBW} + 0.04\text{PA} + 0.022\text{PASUP} - 0.90\text{SUP} - 0.13\text{NDFp} - 0.037\text{LEG}$$

2.3 GRAZING BEHAVIOR

Pasture DMI in grazing cows can be expressed as the product of the time spent grazing or grazing time (min/d) and the rate of pasture DMI, which is the product of the biting rate (bites/min) and the amount of pasture harvested in each bite or bite mass (g DM/bite) (Hodgson and Brookes, 1999; Rook, 2000). Bite mass can be expressed as the product of bite volume and the density of pasture grazed; and bite volume can be expressed as the product of bite area and bite depth (Rook, 2000). Factors affecting grazing time, biting rate, and bite mass have been reviewed, among others, by Hodgson (1982) and Rook (2000).

Few grazing behavior studies have been conducted with high producing dairy cows under practical or production conditions, probably because of methodological limitations. The methods to measure grazing time, biting rate, and bite mass have been reviewed by Forbes (1988) and Rook (2000). Bite mass can be measured directly by using esophageally fistulated animals or indirectly dividing pasture DMI by the total number of bites per day (Forbes, 1988; Rook, 2000). Because the use of esophageally fistulated animals is expensive, and may compromise animal welfare and normal behavior, bite mass is often calculated indirectly (Rook, 2000). Forbes (1988) concluded that indirect bite mass calculation underestimated bite size in comparison with direct bite mass estimations. Both grazing time and biting rate can be measured visually or automatically (Forbes, 1988). Visual estimation of biting rate requires the recording of head movements and sound associated with pasture prehension. Automatic methods for biting rate are based on recording jaw and sometimes head movements. Visual grazing time measurements are based on recording grazing activity at different intervals of time (e.g. 5 to 10 minutes), and has the disadvantages of being labor-intensive and limited by daylight (Rook, 2000). Traditionally, automatic grazing time measurements are conducted using vibracorders (Forbes, 1988; e. g. Kienzle Vibracorders as reported by Arriaga-Jordan and Holmes, 1986). Recently an automatic method for grazing behavior estimation was developed (Rutter et al., 1997). This equipment presents several advantages over the traditional manual methods, such as lower number of people required, less operator-associated errors, and more detailed behavior information (Champion et al., 1998).

Bite mass has the greatest influence on pasture DMI among the three grazing behavior variables (Forbes, 1988; Laca et al., 1992; McGilloway and Mayne, 1996). Although bite mass is also affected by animal's anatomy characteristics (e.g. mouth; Rook, 2000), it is principally

determined by pasture-related characteristics (Hodgson and Brookes, 1999), such as pasture height (McGilloway et al., 1999; Phillips, 1993) and density (Rook, 2000). Pasture height is the major constraint on bite mass in temperate pastures, with the effect primarily on bite depth rather than on bite area (Rook, 2000). It has been reported that dairy cows consistently remove around one-third of the height of pasture marked tillers, regardless of tiller height (Wade et al., 1989). It has been also reported that bite mass decreases with a reduction in pasture height both in unsupplemented (Gibb et al., 1997; McGilloway et al., 1999) and supplemented (Rook et al., 1994) dairy cows.

In many grazing behavior studies, pasture height is expressed as sward surface height, which refers to the height of the top surface of the leaf canopy on an undisturbed sward (Hodgson and Brookes, 1999). Gibb et al. (1997) studied (using short-term measurement periods of 1 h) the effect of three sward surface heights (5, 7, and 9 cm) on grazing behavior of dairy cows on a continuously grazed ryegrass pasture. Bite mass decreased from 0.31 g OM/bite at 7 or 9 cm to 0.23 g OM/bite at 5 cm. Neither biting rate (76 bites/min) nor grazing time (604 min/d, automatically measured over 24 h period) were affected by bite mass. McGilloway et al. (1999) conducted three experiments using short-term measurements (1 h) to study the effect of sward surface height (21, 13, 10 and 7 cm in experiment 1; 11, 9 and 6 cm in experiment 2; 12 and 6 cm in experiment 3) on bite mass and biting rate of dairy cows grazing a ryegrass pasture without supplementation. Bite mass was calculated dividing pasture DMI (estimated from changes in pre- and post-grazing BW with a correction for insensible BW loss) by the total number of bites (estimated by visual observations). Bite mass decreased with reductions in sward surface height (from 1.28 g DM to 0.85 g DM in experiment 1, and from 1 g DM to 0.66 g DM in experiment 2), while biting rate was not affected (55.5 bites/min in experiment 1; 61.8

bites/min in experiment 2). A significant interaction was found in experiment 3 between sward surface height and density; the reduction in bite mass was higher at low pasture density (from 1.02 to 0.47 g DM/bite) than at high pasture density (from 0.97 to 0.63 g DM/bite) (McGilloway et al., 1999).

Grazing time and biting rate are influenced by animal-related characteristics such as genetic merit and level of production. Both grazing time and biting rate act as compensatory factors to avoid reductions in pasture DMI when bite mass decreases, however this compensatory mechanism is partial. For example, the upper limit of grazing time to compensate for a reduction in bite mass is determined for the time required for other activities such as ruminating (Rook, 2000). Under poor pasture conditions (e.g. very short pasture), all three variables decline (Hodgson and Brookes, 1999). High genetic cows (32.0 kg/d of milk at the beginning of the trial) had significantly higher grazing time and biting rate than low genetic cows (24.8 kg/d at the beginning of the trial) supplemented with concentrate (Bao et al., 1992). Grazing time of cows fed a ryegrass pasture was measured visually for a period of 7 h between morning and evening. The high genetic cows grazed more time (218 vs. 204 min) and at a higher biting rate (63.5 vs. 60.8 bites/min) than the low genetic cows.

2.3.1 Effect of Concentrate Supplementation on Grazing Behavior

Studies evaluating the effect of level (Arriaga-Jordan and Holmes, 1986; Rook et al., 1994), or both level and type (Kibon and Holmes, 1987; Sayers, 1999 (exp. 2)) of energy supplementation on grazing behavior of dairy cows are presented in *Table 1*. Increasing the amount of supplement reduced grazing time but did not affect biting rate (Arriaga-Jordan and

Holmes, 1986; Kibon and Holmes, 1987; Rook et al., 1994). Arriaga-Jordan and Holmes (1986) reported data from dairy cows grazing rotationally or continuously a ryegrass pasture and supplemented either with 1 or 6 kg/d of a barley-based concentrate. Grazing time was reduced 54 min/d in the continuously grazing system and 43 min/d in the rotationally grazing system when cows were supplemented with 6 kg/d of concentrate. Biting rate was not affected by supplementation level but was higher in the continuously grazing system (75 vs. 63 bites/min). The smallest bite mass was found when cows grazed continuously and received 1 kg/d of concentrate (Arriaga-Jordan and Holmes, 1986).

Rook et al. (1994) studied the effect of pasture height (4, 6 or 8 cm; measured by a rising plate meter that compressed the sward before measurements; Hodgson et al., 1999) and concentrate supplementation (0 or 4 kg/d) on grazing behavior of cows grazing a ryegrass/white clover pasture. Concentrate supplementation, but not pasture height, reduced grazing time (recorded automatically) from 685 to 606 min/d (20 min/kg concentrate). A significant interaction between pasture height and supplementation level was found for biting rate and bite mass. Biting rate was not affected neither by pasture height or concentrate supplementation (53.2 bites/min). Bite mass decreased as pasture height decreased (0.56, 0.43, and 0.40 for 8, 6, and 4 cm, respectively), while the supplementation level had no effect on bite mass (Rook et al., 1994).

Level (0 vs. 3 kg/d) but not type of energy supplement (cereal vs. beet pulp) reduced grazing time of dairy cows grazing a ryegrass at two pasture heights (5 or 6.5 cm; measured by a rising plate meter) (Kibon and Holmes, 1987). Biting rate was not affected by supplementation level, type of supplement or pasture height, while bite mass was significantly lower at the low pasture height (0.34 vs. 0.39 g DM/bite) (Kibon and Holmes, 1987). Recently, Sayers (1999 (exp. 2)) found that both type (starch or fiber-based concentrate) and level (5 or 10 kg/d) of

concentrate affected grazing time and total number of bites of dairy cows grazing a ryegrass pasture, while bite mass was not affected by these factors (0.60 g DM). Total number of bites per day and grazing time were higher when cows were supplemented with a fiber-based concentrate (20285 bites/d and 439 min/d) than with starch-based concentrate (18673 bites/d and 408 min/d). Grazing time was reduced by 16 min/kg of fiber-based concentrate and 20 min/kg of starch-based concentrate (Sayers, 1999 (exp. 2)). When the amount of concentrate was increased from 5 to 10 kg/d, total number of bites per day decreased from 22023 to 16933 and grazing time decreased from 469 to 378 min/d (Sayers, 1999 (exp. 2)).

In summary, the main effect of energy supplementation on grazing behavior is a reduction in grazing time (Arriaga-Jordan and Holmes, 1986; Kibon and Holmes, 1987; Rook et al., 1994; Sayers, 1999). When summarizing *Table 1*, there is a negative relationship between grazing time (GT, min/d) and concentrate DMI (CDMI, kg/d). For both types of energy concentrates (starch and fiber-based), the significant negative regression found was: $GT = 628 (\pm 22) - 25 (\pm 5) CDMI$ ($P < 0.0001$; $R^2 = 0.58$). When only starch-based concentrates were considered: $GT = 572 (\pm 21) - 20 (\pm 4) CDMI$ ($P < 0.002$; $R^2 = 0.72$). When only fiber-based concentrates were considered: $GT = 657 (\pm 26) - 27 (\pm 6) CDMI$ ($P < 0.002$; $R^2 = 0.64$). Based on these equations, supplementation with energy concentrates reduce grazing time between 20 to 27 min/kg of DM.

2.4 SUBSTITUTION RATE AND MILK RESPONSE TO SUPPLEMENTATION

When grazing cows are supplemented, pasture DMI decreased, which is known as substitution rate; i.e. substitution rate is the decrease in pasture intake (kg/d) per kg of supplement fed (Kellaway and Porta, 1993). Grazing experiments having the goal to estimate substitution rate require the use of unsupplemented or control treatments. Substitution rate (SR) is calculated as: $SR \text{ (kg/kg)} = (\text{Pasture DMI in unsupplemented treatment} - \text{Pasture DMI in supplemented treatment}) / \text{supplement DMI}$. A substitution rate lower than one means that total DMI on the supplemented treatment is higher than total DMI on the unsupplemented treatment; a substitution rate equal to one means that total DMI on the supplemented treatment is equal to total DMI on the supplemented treatment; and a substitution rate higher than one means that total DMI in the supplemented treatment is lower than total DMI in the unsupplemented treatment.

Substitution rate is one of the main factors contributing to the variation seen in milk response to supplementation (Kellaway and Porta, 1993; Stockdale, 2000a). Although milk response to supplementation is always expressed as kg milk/kg supplement, two different types of milk response will be discussed in this review: 1) overall milk response, or the increase in kg of milk per kg of supplement DMI, which is calculated relative to an unsupplemented treatment; and 2) marginal milk response, or the increase in kg of milk per kg of incremental increase in supplement DMI, which may be calculated in those studies that evaluate different levels of supplementation (Walker et al., 2001).

There is usually a negative relationship between substitution rate and milk response: when substitution rate is high (i.e. high reduction in pasture DMI with supplementation, resulting in only a low increase in total DMI), milk response is low. Because milk response is expressed as kg milk/kg supplement, this expression is usually used to establish if the short-term

supplementation is profitable or not based on milk and concentrate price. However, additional long-term and whole system factors should also be considered in the economic evaluation, e.g. increase in stocking rates of the farm, improvement in pasture utilization, positive effects on body condition score and reproduction performance, increase in lactation length and positive changes in milk composition (Kellaway and Porta, 1993).

Since substitution rate and milk response are closely related, factors affecting these variables will be discussed together. Substitution rate and milk response to supplementation are affected by several pasture, animal, supplement, and season factors (Kellaway and Porta, 1993; Stockdale, 2000a; Stockdale, 2000b). Some of the most important factors are pasture allowance, pasture height, pasture species, pasture mass and pasture quality for the pasture-related factors; type and level of supplementation for the supplement-related factors; and genetic merit of cows, level of production and stage of lactation for the animal-related factors (Kellaway and Porta, 1993; Stockdale, 2000a; Stockdale, 2000b). This discussion will focus on pasture allowance, level of supplementation, and type of supplement.

2.4.1 Pasture Allowance

Several studies have reported that substitution rate increases as pasture allowance increases (Bargo et al., 2002; Grainger and Mathews, 1989; Meijs and Hoekstra, 1984; Robaina et al., 1998; Stakelum, 1986a; Stakelum, 1986b; Stockdale and Trigg, 1985). Most of these studies were conducted with low producing cows supplemented with less than 5 kg/d of concentrate (Grainger and Mathews, 1989; Meijs and Hoekstra, 1984; Robaina et al., 1998; Stakelum, 1986a; Stakelum, 1986b; Stockdale and Trigg, 1985), and one study used high

producing cows fed more than 7 kg/d of concentrate (Bargo et al., 2002). Meijs and Hoekstra (1984) reported an increase in substitution rate from 0.10 to 0.69 kg pasture/kg concentrate when pasture allowance increased from 16.6 to 33.3 kg DM/cow/d. Pre-grazing pasture mass was measured at 4 cm cutting height, therefore pasture allowance values are lower than those from studies that measured pre-grazing pasture mass at ground level. Two experiments conducted in Ireland (Stakelum, 1986a; Stakelum, 1986b) found substitution rate in the range of 0.24 to 0.28 and 0.59 to 0.61 for pasture allowance of 16 and 24 kg DM/cow/d, respectively. Grainger and Mathews (1989) reported that substitution rate increased from 0 to 0.25 to 0.69 kg pasture/kg concentrate when pasture allowance increased from 7.6 to 17.1 to 33.1 kg DM/cow/d, respectively. Stockdale and Trigg (1985) also reported substitution rate of zero kg pasture/kg concentrate for very low pasture allowance (14.9 kg DM/cow/d). Robaina et al. (1998) found a substitution rate of 0.57 kg pasture/kg grain at pasture allowance of 42.3 kg DM/cow/d, and 0.31 kg pasture/kg grain at pasture allowance of 20 kg DM/cow/d. For similar levels of pasture allowance (25 and 40 kg DM/cow/d), Bargo et al. (2002) reported substitution rate of 0.26 and 0.55 kg pasture/kg concentrate, respectively.

To summarize, these studies (Bargo et al., 2002; Grainger and Mathews, 1989; Meijs and Hoekstra, 1984; Robaina et al., 1998; Stakelum, 1986a; Stakelum, 1986b; Stockdale and Trigg, 1985) were divided in low pasture allowance (< 25 kg DM/cow/d) treatments and high pasture allowance (> 25 kg DM/cow/d) treatments. At a low pasture allowance (7.6 to 25 kg DM/cow/d), substitution rate averaged 0.19 kg pasture/kg concentrate (range from 0 to 0.31). At a high pasture allowance (24 to 42.3 kg DM/cow/d), substitution rate averaged 0.58 kg pasture/kg concentrate (range from 0.43 to 0.69). A significant linear regression was found between substitution rate (SR, kg pasture/kg concentrate) and pasture allowance (PA, kg DM/cow/d) as

described by the following equation: $SR = -0.1 (\pm 0.09) + 0.0198 (\pm 0.0036) PA$ ($R^2 = 0.67$; $P < 0.01$). When the quadratic expression of pasture allowance was included in the model, the R^2 improved to 0.75: $SR = -0.476 (\pm 0.196) + 0.053 (\pm 0.016) PA - 0.00065 (\pm 0.00031) PA^2$ ($R^2 = 0.75$; $P < 0.01$).

Grazing studies evaluating the effect of pasture allowance on substitution rate and milk response of high producing dairy cows (> 25 kg/d of milk in early lactation or around 20 kg/d in late lactation at the start of the study) reported that the substitution rate increased and milk response decreased when pasture allowance increased (Bargo et al., 2002; Robaina et al., 1998; Stockdale, 1999a; *Table 2*). Bargo et al. (2002) reported substitution rates of 0.26 and 0.55 kg pasture/kg concentrate for high producing dairy cows in mid-lactation when grazing an orchardgrass-based pasture at a pasture allowance of 25 and 40 kg DM/cow/d (measured cutting at ground level), respectively. Milk response was 1.36 and 0.96 kg milk/kg concentrate for the 25 and 40 kg DM/cow/d pasture allowance level, respectively (Bargo et al., 2002). Dairy cows in late lactation had substitution rate of 0.31 kg pasture/kg grain and a milk response of 0.98 kg milk/kg grain, and substitution rate of 0.57 kg pasture/kg grain and milk response of 0.54 kg milk/kg grain when grazed a ryegrass/white clover pasture at a pasture allowance of 21.1 or 42.3 kg DM/cow/d, respectively (Robaina et al., 1998). Stockdale (1999a) summarized data from 7 experiments conducted in Victoria (Australia) and found that at a low pasture allowance (30 kg DM/cow/d), substitution rate tended to be lower (average 0.37 kg pasture/kg concentrate) and milk response higher (average 0.83 kg milk/kg concentrate) than at high pasture allowance (40 kg DM/cow/d; substitution rate = 0.40 kg pasture/kg; milk response = 0.80 kg milk/kg concentrate).

Figure 1 shows the relationships between milk response and substitution rate in grazing studies presented in *Table 2* (Bargo et al., 2002; Robaina et al., 1998; Stockdale, 1999a). Each of these studies showed a negative relationship between milk response and substitution rate. When analyzing together, the data from the three experiments presented in *Table 2* found a significant negative relationship between milk response (MR) and substitution rate (SR), explained by the following equation: $MR = 1.63 (\pm 0.29) - 1.92 (\pm 0.73) SR$ ($P < 0.03$; $R^2 = 0.43$), indicating that the lower the substitution rate the higher the milk response expected.

The negative relationship between milk response and substitution rate described in *Figure 1* is in agreement with Stockdale (2000b), who summarized data from 20 grazing experiments conducted at Australia and found that milk response (MR) was negatively related with substitution rate (SR), season (spring, autumn, summer), and body condition score (BCS). The regression equation obtained in Stockdale (2000b) review was: $MR = 2.62 - 0.80SR - 0.28\text{season} - 0.34\text{BCS}$ ($P < 0.01$; $R^2 = 0.63$).

Higher substitution rates were observed when cows grazed at high pasture allowance may be partially explained by the higher quality of pasture actually eaten. Dixon and Stockdale (1999) suggested that substitution rate is generally higher when cows graze a high quality pasture than when they graze a medium quality pasture. Because cows grazing at high pasture allowance have the opportunity to graze more selectively, pasture actually eaten has higher digestibility than at low pasture allowance (Mayne and Wright, 1988). Kellaway and Porta (1993) in their review concluded that the milk response increased from 0 to 0.6 kg milk/kg concentrate when pasture management changed from ad libitum (i.e. high pasture allowance) to restricted offer (i.e. low pasture allowance).

2.4.2 Level of Supplementation

Results are inconsistent from grazing studies conducted with high producing dairy cows that have evaluated the effect of level of supplementation on substitution rate and milk response (Dillon et al., 1997; Reis and Combs, 2000b; Robaina et al., 1998; Walker et al., 2001; *Table 3*; *Figure 2*). Kellaway and Porta (1993) and Tamminga and Hof (2000) suggested that substitution rate increases with the level of concentrate.

Of the 4 studies summarized in *Table 3* and *Figure 2*, three studies had a negative relationship between milk response and substitution rate. *Figure 2* illustrates the relationship between milk response and substitution rate when level of concentrate was increased. Robaina et al. (1998) found substitution rate of 0.44, 0.65, and 0.58 kg pasture/kg concentrate and milk response of 1.56, 0.94, and 0.82 kg milk/kg concentrate when dairy cows in late lactation grazing a ryegrass/white clover pasture were supplemented with 1.8, 3.4, and 6.7 kg DM/d, respectively. A recent study (Reis and Combs, 2000b) conducted with high producing dairy cows (> 40 kg/d milk at the beginning of the trial) reported that substitution rate increased from 0.24 to 0.41 kg pasture/kg concentrate and milk response decreased from 1.00 to 0.86 kg milk/kg concentrate as the amount of a corn-based concentrate increased (0, 5, and 10 kg DM/d, respectively). Walker et al. (2001) found that substitution rate increased from 0.02 to 0.28 kg pasture/kg concentrate and milk response decreased from 1.07 to 0.91 kg milk/kg concentrate when concentrate level was increased from 3 to 10.4 kg DM/d. In contrast, Dillon et al. (1997) reported results from two years showing reductions in substitution rate (0.10 kg pasture/kg concentrate) and milk response (0.09 kg milk/kg concentrate) for cows grazing ryegrass pasture when level of supplementation was increased from 2 to 4 kg/d.

Combining the data from the 4 experiments presented in *Table 3*, no general relationships was found between milk response (MR) and substitution rate (SR), as indicated with the following non-significant linear equation: $MR = 0.75 (\pm 0.19) + 0.29 (\pm 0.57) SR$ ($P > 0.61$; $R^2 = 0.02$). Based on these 4 studies, the type of relationship is not clear between milk response and substitution rate when high producing dairy cows on pasture are supplemented with increasing level of concentrate.

2.4.3 Level of Supplementation and Marginal and Overall Milk Response

The marginal milk response to increasing level of concentrate has been described as curvilinear; i.e. the marginal increase in milk per kg of concentrate decreases as the level of concentrate increases (Kellaway and Porta, 1993). The response in milk production of high producing dairy cows grazing pasture and supplemented with different levels of concentrate is shown in *Figure 3* (Delaby et al., 2001; Reis and Combs, 2000b; Robaina et al., 1998; Sayers, 1999 (exp. 1); Sayers, 1999 (exp. 2); Walker et al., 2001). For this summary, two different groups of studies are established: 1) those conducted with dairy cows producing more than 28 kg milk/d regardless of stage of lactation (Delaby et al., 2001; Reis and Combs, 2000b) or with less than 100 DIM (Sayers, 1999 (exp. 2)), and 2) those conducted with dairy cows producing less than 23 kg milk/d and more than 100 DIM (Robaina et al., 1998; Sayers, 1999 (exp. 1); Walker et al., 2001).

Delaby et al. (2001) conducted three experiments with high producing dairy cows (174 to 182 DIM, 28.3 to 30.7 kg milk/d at the beginning of the study) grazing a ryegrass pasture (15.3 to 17.0% CP, 48.9 to 54.7% NDF, 75.9 to 81.5% OM digestibility) and supplemented with

different amounts of concentrate (0 to 5.37 kg DM/cow/d) and found an overall milk response of 1.04 kg milk/kg concentrate. Reis and Combs (2000b) studied the effect of increasing the amount of a corn-based concentrate (0, 5, and 10 kg DM/cow/d) on milk production of dairy cows (84 DIM; 41.6 kg milk/d at the beginning of the experiment) grazing a legume/grass pasture (39.8 to 42.7% NDF; 18.7 to 21.2% CP; 1.29 to 1.34 Mcal/kg DM of estimated NE_L). They found that marginal milk response decreased from 1.00 to 0.72 kg milk/kg concentrate when concentrate was increased from 5 to 10 kg DM/cow/d. However, when milk production was analyzed testing for linear and quadratic effect by contrast, the linear effect was significant ($P < 0.01$), while the quadratic effect was not ($P < 0.23$). Sayers (1999 (exp. 2)) used early lactation cows (40 DIM) grazing a ryegrass pasture (23.9% CP, 54.1% NDF) and reported that milk production increased from 31.2 to 34.6 kg/d when supplementation level was increased from 5 to 10 kg/d of starch based concentrate (marginal milk response = 0.60 kg milk/kg concentrate), and from 30.9 to 35.2 kg/d when supplementation level was increased from 5 to 10 kg/d of an fiber based concentrate (marginal milk response = 0.86 kg milk/kg concentrate).

Combining these three studies with “higher” producing cows (Delaby et al., 2001; Reis and Combs, 2000b, Sayers, 1999 (exp. 2); $n = 27$), the linear regression between milk production (MP, kg/d) and concentrate DMI (CDMI, kg/d) was: $MP = 22.14 (\pm 0.43) + 1.14 (\pm 0.09) CDMI$ ($P < 0.01$; $R^2 = 0.86$), indicating an overall milk response of 1.14 kg milk/kg concentrate (*Figure 3*).

Robaina et al. (1998) reported an study where late lactation dairy cows (180 DIM, 21.4 kg milk/d at the beginning of the study) grazing a ryegrass/white clover pasture (15.7% CP, 53.7% NDF, 71.5% in vitro DM digestibility) were supplemented with 0, 1.8, 3.4 and 6.7 kg DM/d of a barley-based concentrate. Overall milk response was 0.76 kg milk/kg concentrate.

Sayers (1999 (exp. 1)) reported a study where dairy cows in late lactation (207 DIM, and milk production not reported) grazing a ryegrass pasture (23.1% CP, 58.2% NDF, 25.1% ADF) were supplemented with 0, 2.6 or 5.6 kg DM/d of a barley/corn/sugar beet pulp based concentrate with different CP levels (10, 18, 26, and 34 % CP). The marginal milk response was 1.02 kg/kg concentrate when supplementation level increased from 0 to 2.6 kg DM/d, and 1.09 kg milk/kg supplement when supplementation level increased from 2.6 to 5.2 kg DM/d (Sayers, 1999 (exp. 1)). Walker et al. (2001) concluded from 3 experiments conducted with dairy cows in late lactation (166 to 211 DIM; 19.4 to 22.3 kg milk/d, at the beginning of the experiments) grazing a paspalum (*Paspalum dilatatum*)-based pasture (63.3 to 64.8% NDF; 11.9 to 12.1% CP; 59.2 to 62.2% in vitro DM digestibility, 1.94 to 2.06 Mcal/kg DM of estimated metabolizable energy) that marginal milk response decreased when level of a barley plus wheat-based concentrate (0, 3, 5, 7, 9, 10.4 kg DM/cow/d) supplementation was higher than 3 kg DM/cow/d. Walker et al. (2001) also concluded that the reduction in marginal milk response with increasing concentrate level beyond 3 kg/d depends on pasture allowance and therefore pasture DMI.

Combining these three studies (Robaina et al., 1998; Sayers, 1999 (exp. 1); Walker et al., 2001; n = 19), the following significant linear regression was found between milk production (MP, kg/d) and concentrate DMI (CDMI, kg/d): $MP = 13.48 (\pm 0.31) + 0.88 (\pm 0.06) CDMI$ ($P < 0.01$; $R^2 = 0.92$), indicating an overall milk response of 0.88 kg milk/kg concentrate (*Figure 3*).

Two important points can be stated from the two groups of studies presented in *Figure 3*. First, both lines are linear, suggesting that regardless of the grouping and over a wide range of amount of concentrate supplementation (0 to 10 kg DM/cow/d), the marginal milk response does not decrease by increasing the level of concentrate supplementation. Furthermore, since it would may not be practical to supplement with more than 10 kg DM/cow/d (or > 50% of the total diet

DMI) in order to avoid metabolic health problems such as acidosis or sub acidosis, it is probable that for high producing dairy cows on pasture the decreasing marginal milk response traditionally described (Kellaway and Porta, 1993) does not occur.

Second, comparing the first group (Delaby et al., 2001; Reis and Combs, 2000b; Sayers, 1999 (exp. 2)) against the second group (Robaina et al., 1998; Sayers, 1999 (exp. 1); Walker et al., 2001), the first group shows a higher level of production under zero supplement situations (i.e. different intercept) and also a greater overall milk response (i.e. different slope) than the second group. To confirm these inferences, tests were run using Dummy variables (PROC REG, SAS, 1999) to test if the intercept to the Y-axis and the slope of the regression lines differed. The first group had a significantly greater milk production with only pasture (zero supplement) (22.1 vs. 13.5 kg/d; $P < 0.05$) and a significantly greater milk response (1.14 vs. 0.88 kg milk/kg concentrate; $P < 0.05$). These differences could be related to differences in pasture quality and quantity, and stage of lactation and genetic merit of cows among the studies.

Tamminga and Hof (2000) suggested that the higher the milk production potential the lower the substitution rate. The substitution rate of cows producing 6000 kg of milk per year is 0.04 to 0.05 higher than with cows producing 8000 kg of milk per year with different pasture qualities and levels of supplementation (Tamminga and Hof, 2000). Dixon and Stockdale (1999) suggested that substitution rate is high if energy intake is high in relation to the cow requirements. Therefore, it can be expected that high producing cows have lower substitution rate than low producing cows.

A greater response to supplementation may be expected in high genetic merit cows because they partition more nutrients to milk production and lose more BW in early lactation than low genetic merit cows (Kellaway and Porta, 1993). Cows producing 25, 30 or 35 kg/d at

the start of the grazing season that were supplemented at a rate of 1 kg concentrate/3.3 kg of milk had milk responses of 0.50, 0.70, and 0.75 kg of milk/kg concentrate, respectively (Hoden et al., 1991).

Stage of lactation also influences lactational responses to concentrate supplements (Dixon and Stockdale, 1999). In early lactation, cows partition more nutrients toward milk production, thus milk response to supplementation may be higher than in late lactation when more nutrients are directed to BW (Kellaway and Porta, 1993). The average milk yield response to concentrate supplementation of grazing cows supplemented with 3 kg/d of concentrate was 0.7, 0.4, 0.5, and 0 kg milk/kg concentrate when they were between 86 to 114, 115 to 133, 134 to 187, and 188 to 243 DIM, respectively (O'Brien et al., 1999). Over five experiments with supplement intakes in the range from 0 to 7 kg/cow/d, marginal milk response was 1.3, 1.1, and 0.7 kg milk/kg supplement in early, mid, and late lactation (Stockdale et al., 1987).

2.4.4 Type of Supplement

Type of supplement (e.g. forage vs. concentrate supplements, main energy source in concentrate supplements, etc.) influences substitution rate (Stockdale, 2000b). It has been reported that forage supplementation decreases pasture DMI more than concentrates (Mayne and Wright, 1988). Including both low and high pasture allowances, substitution rate ranged from 0.84 to 1.02 for grass silage supplementation and from 0.11 to 0.50 for concentrate supplementation (Mayne and Wright, 1988). Recently, Stockdale (2000b) reviewed 39 sets of data from grazing studies and concluded that supplementation with forages, such as hay or corn silage, resulted in a 0.08 kg/kg higher substitution rate than supplementation with concentrates.

Stockdale (1999b) found, however, that substitution rates were similar when dairy cows grazing a ryegrass/white clover pasture were supplemented with grain (barley plus wheat), a protein supplement (lupins-grain) or hay. These opposite results could be explained by the fact that the effects of supplement type on substitution may be not expressed if both pasture allowance and level of supplementation are low as used by Stockdale (1999b).

The type of energy source in the concentrate may also affect the substitution rate. Meijis (1986) reported that substitution rate was reduced from 0.45 kg/kg with a high-starch concentrate to 0.21 kg/kg with a fiber-based concentrate when cows grazed a ryegrass pasture. Studies with grazing cows (Sayers, 1999 (exp. 2)) and cows fed pasture indoors (Schwarz et al., 1995; Spörndly, 1991) evaluated the effect of type of concentrate (starch vs. fiber based) on substitution rate and milk response on high producing dairy cows (> 25 kg/d of milk in early lactation or about 20 kg/d in late lactation) are presented in *Table 4* and *Figure 4*. Two studies (Sayers, 1999 (exp. 2); Schwarz et al., 1995) had a negative relationship between milk response and substitution rate, while the third study (Spörndly, 1991) had a positive of relationship. Sayers (1999 (exp. 2)) reported a substitution rate of 0.62 and 0.50 kg pasture/kg concentrate and milk response of 0.68 and 0.86 kg milk/kg concentrate when dairy cows in early lactation grazing a ryegrass pasture were supplemented with 10 kg DM/d of a starch or a fiber-based concentrate, respectively. The higher milk response with the fiber-based concentrate was associated with a lower substitution rate. In contrast, Schwarz et al. (1995) reported from two indoor experiments that substitution rate was slightly higher with fiber-based concentrates than with starch-based concentrates (0.42 vs. 0.38 kg/kg), which was associated with lower milk response (0.40 kg milk/kg fiber-based concentrate and 0.75 kg milk/kg starch-based concentrate). In another indoor study (Spörndly, 1991), however, lower substitution rate were obtained with starch-based

supplements (0.57 vs. 0.69 kg/kg) and were associated with lower milk responses (0.32 vs. 0.24 kg milk/kg concentrate) in comparison with fiber-based supplements. In these last studies (Schwarz et al., 1995; Spörndly, 1991) the effect of type of concentrates is confounded with the effect of level because two levels of each type of concentrate were fed. They also were conducted indoors, a condition where substitution rate may behave differently than in grazing studies.

Analyzing together the data from the 3 experiments presented in *Table 4*, no general relationships was found between milk response (MR) and substitution rate (SR), as indicated with the following non-significant linear equation: $MR = 0.87 (\pm 0.32) + 0.73 (\pm 0.59) SR$ ($P > 25$; $R^2 = 0.16$). Based on these 3 studies, there is no enough information available in the literature to conclude what type of relationship might be expected between milk response and substitution rate when high producing dairy cows on pasture are supplemented with starch or fiber-based concentrates.

2.4.5 Causes of Substitution Rate

Although the reasons for the substitution rate are not clear, it has been hypothesized that substitution rate is caused by: 1) negative associative effects (Dixon and Stockdale, 1999), or 2) reduction in grazing time (McGilloway and Mayne, 1996).

2.4.5.1 Associative Effects

When concentrate supplements are included in pasture diets, associative effects may occur if digestive and metabolic interactions between them change the intake of energy (Dixon

and Stockdale, 1999). Positive associative effects occur when energy intake is greater than that expected from the pasture and concentrate if they were fed alone, while negative effects occur when energy intake is lower (Dixon and Stockdale, 1999). An increase in total digestibility may be expected with the inclusion of concentrates in the diet because they are usually higher in digestibility than pasture. However, interactions between the digestion of concentrates and pasture may reduce fiber digestion (Dixon and Stockdale, 1999). The energy provided by the concentrate into the rumen (fermentable carbohydrates) may lead to reductions in rumen pH, which may decrease the activity or number of cellulolytic bacteria and reduce the rate of fiber digestion of pasture (Dixon and Stockdale, 1999). A reduction in rate of fiber digestion is potentially compensated by an increase in retention time, but this affects pasture DMI (Dixon and Stockdale, 1999). Therefore, a reduction in pasture DMI observed with supplementation could be a consequence of a reduction in digestion rate of pasture fiber. Negative associative effects would be expected more often if the amount of concentrate in the diet is large and pasture quality is low (Dixon and Stockdale, 1999).

The hypothesis on the associative effects could be tested by experiments planned to study the effects of different types and levels of concentrate supplementation. Based in this hypothesis, lower levels of concentrate supplementation or supplementation with concentrates of slow rate of degradation would produce a lower substitution rates (Kellaway and Porta, 1993; Tamminga and Hof, 2000). As shown above in *Table 3* studies on the effect of level of concentrate supplementation on substitution rate (Dillon et al., 1997; Reis and Combs, 2000b; Robaina et al., 1998; Walker et al., 2001) reported greater substitution rate (0.33 kg/kg; range: 0.19 to 0.58 kg/kg) at high levels of concentrate supplementation (≥ 5 kg DM/d), and smaller substitution rate (0.28 kg/kg; range: 0.02 to 0.65 kg/kg) at low levels of concentrate supplementation (< 5 kg

DM/d). However, the range of variation in these data is important. More information is needed in this area, especially for high levels of supplementation (> 8 kg/d), which are commonly used for high producing cows.

Under the same hypothesis, concentrates with a slow rate of carbohydrate degradation in the rumen would minimize substitution rate, especially at high levels of supplementation. The concentrates most rapidly degraded in the rumen, such as cereal grains (starch), would have a greater effect on substitution rate compared to more slowly degraded carbohydrate, such as fibrous by-products. As presented (*Table 4*), the effect of the type of concentrate on substitution rate has primarily compared starch-based vs. fiber-based concentrates (Sayers, 1999 (exp. 2), Schwarz et al., 1995; Spörndly, 1991) with inconsistent results. The inconsistent results may be related to the different sources of starch and fiber used in those studies that differ in the rate of rumen degradation. The ingredient composition of concentrates (proportion of starch or fiber carbohydrates and protein) varied also. In conclusion, more information is needed related to the type and level of concentrate supplementation and their interaction on substitution rate of high producing dairy on pasture.

2.4.5.2 Reduction in Grazing Time

The second hypothesis proposed to explain substitution rate is related to grazing time. It has been suggested that reductions in grazing time by supplementation would explain substitution rate (Mayne and Wright, 1988; McGilloway and Mayne, 1996). Pasture DMI of grazing cows can be defined as the product of time per day spent grazing and the rate of pasture DMI. Therefore, if supplementation reduces grazing time, and assuming that the rate of DMI

stays constant, pasture DMI decreases. McGilloway and Mayne (1996) reported reductions in grazing time from 15 to 22 min/kg concentrate. Other studies (Pulido and Leaver, 1991; Pulido and Leaver, 2000; Sayers, 1999) found shorter reductions (4 min/kg concentrate and 6 min/kg concentrate) when concentrate levels were increased from 0 to 6 kg/day. For concentrate supplementation levels of 0, 2 or 4 kg/d, Rook and Huckle (1996) reported reductions in grazing time from 4 to 16 min/kg. In a later study, Sayers (1999 (exp. 2)) found a reduction in grazing time of 16 min/kg of fiber-based concentrate and 20 min/kg of starch-based concentrate when level of supplementation was increased from 5 to 10 kg/d.

In summary, there is nonconclusive information to confirm or reject either of these hypotheses proposed to explain substitution rate. On the other hand, it would be logical that substitution rate could be related to both.

2.5 EFFECT OF ENERGY SUPPLEMENTATION ON DRY MATTER INTAKE, MILK PRODUCTION AND MILK COMPOSITION

2.5.1 Level of supplementation

Studies conducted with high producing dairy cows on pasture-based diets that have evaluated the effect of level of concentrate supplementation on DMI, and milk production and composition are presented in *Table 5* (Arriaga-Jordan and Holmes, 1986; Dillon et al., 1997; Hoden et al., 1991; Reis and Combs, 2000b; Robaina et al. (1998); Sayers, 1999 (exp. 2); Spörndly, 1991; Valentine et al., 2000; Walker et al., 2001; Wilkins et al., 1994).

Pasture DMI decreased and total DMI increased by increasing the amount of concentrate. Pasture DMI was numerically (Arriaga-Jordan and Holmes, 1986; Dillon et al., 1997) or significantly (Reis and Combs, 2000b; Robaina et al., 1998; Sayers, 1999 (exp. 2); Spörndly, 1991; Walker et al., 2001) reduced when level of concentrate increased, which is related to the substitution rate effect discussed in previous sections of this review. For the range of concentrate supplementation (0 to 10.4 kg DM/cow/d) reported in studies that measured pasture DMI (Arriaga-Jordan and Holmes, 1986; Dillon et al., 1997; Reis and Combs, 2000b; Robaina et al., 1998; Sayers, 1999 (exp. 2); Spörndly, 1991; Walker et al., 2001; *Table 5*), pasture DMI decreased 2.5 kg/cow/d (range: 0.6 to 4.1 kg/cow/d). Total DMI was numerically (Arriaga-Jordan and Holmes, 1986) or significantly (Dillon et al., 1994; Reis and Combs, 2000b; Robaina et al., 1998; Sayers, 1999 (exp. 2); Spörndly, 1991; Walker et al., 2001) increased 3.7 kg/cow/d (range: 1.8 to 7.5 kg/cow/d) with level of concentrate.

All the studies presented in *Table 5* reported that milk production increased with level of supplementation. Some studies (Valentine et al., 2000; Wilkins et al., 1994) found that milk production increased curvilinearly with level of supplementation. Others (Dillon et al., 1997; Reis and Combs, 2000b; Robaina et al., 1998; Spörndly, 1991; Walker et al., 2001) reported linear increases, even up to high levels of concentrate supplementation such as 10 kg/d of concentrate (Reis and Combs, 2000b; Walker et al., 2001).

Most of the studies showed that milk fat percentage decreased when level of concentrate supplementation was increased (Arriaga-Jordan and Holmes, 1986; Reis and Combs, 2000b; Sayers, 1999; Spörndly, 1991; Valentine et al., 2000; Walker et al., 2001). Other studies, however, showed no changes (Dillon et al., 1997; Hoden et al., 1991; Robaina et al., 1998; Wilkins et al., 1994). Several authors reported that increasing the level of concentrate

supplementation increased milk protein percentage (Hoden et al., 1991; Reis and Combs, 2000b; Sayers, 1999; Spörndly, 1991; Valentine et al., 2000; Wilkins et al., 1994). Linear increases in milk protein percentage have been reported for a wide range of levels of supplementation, including 0 to 4 kg DM/d (Hoden et al., 1991; Wilkins et al., 1994), 0 to 10 kg DM/d (Reis and Combs, 2000b), and 7 to 13 kg DM/d (Valentine et al., 2000). Others studies, however, found no changes in milk protein percentage within a range of supplementation from 0 to 3.6 kg DM/d (Dillon et al., 1997) and 0 to 10.4 kg DM/d (Walker et al., 2001).

2.5.2 Starch vs. Fiber based Concentrates

Energy supplementation for grazing cows is typically based on cereal grains such as corn, barley, sorghum or wheat, which are high in starch. Starch content of cereal grains varies from 70 to 80% of DM depending on type (Rooney and Pflugfelder, 1986). In a review by Huntington (1997), the rank of the starch content of common grains was wheat (77%), corn and sorghum (72%), and barley and oats (58%). Other less traditional starch sources are cassava and tapioca, which contain 50% and 20% starch, respectively. Nonforage fiber sources such as soy hulls, beet pulp, wheat middling and citrus pulp are high in structural carbohydrates but provide a highly fermentable fiber in the rumen. Due to their relatively high NE_L concentration and availability, nonforage fibers have been used to replace cereal grains as a concentrate source (Firkins, 1997).

Studies comparing starch or fiber-based concentrate supplements for high producing dairy cows on pasture-based diets are presented in *Table 6*. Some studies were grazing trials (Delahoy, 2000 (exp. 2); Meijjs, 1986; Sayers, 1999 (exp. 2)), while others were conducted under

non-grazing conditions with cows fed fresh chopped pasture (Schwarz et al., 1995; Spörndly, 1991; Valk et al., 1990).

Supplementation with fiber-based concentrates increased pasture DMI and total DMI, and therefore milk production in some grazing studies (Meijs, 1986; Sayers, 1999 (exp. 2), but not in another (Delahoy, 2000). Meijs (1986) compared starch (cassava) vs. fiber (beet pulp)-based concentrate as supplement for dairy cows grazing a ryegrass pasture. Pasture and total DMI was increased with the fiber-based concentrate. In a companion study, milk production was increased with fiber-based concentrate supplementation (26.9 kg/d) compared to starch-based concentrate supplementation (25.6 kg/d) (Meijs, 1986). Neither fat nor protein milk percentages were affected by treatments. More recently, Sayers (1999 (exp. 2)) conducted a study with high producing dairy cows grazing ryegrass supplemented with starch or fiber-based concentrate at two levels (5 or 10 kg/d). Regardless of the supplementation levels, fiber-based concentrate supplementation increased pasture and total DMI. However, milk production was not affected by type of concentrate. Level of supplementation increased milk production (31.1 vs. 34.9 kg/d for 5 and 10 kg/d, respectively). The fiber-based concentrate increased fat percentage, but decreased protein percentage in comparison with starch-based concentrate. In contrast, neither pasture or total DMI, nor milk production were affected when high producing dairy cows in late lactation grazing an orchardgrass pasture were supplemented with starch (corn) or fiber (beet pulp plus soy hulls) based concentrates (Delahoy, 2000 (exp. 2)). In this last study, the starch-based concentrate increased milk protein percentage in agreement with Sayers (1999 (exp. 2)).

Studies conducted under non-grazing conditions with pasture-fed cows reported similar (Schwarz et al., 1995; Spörndly, 1991) or lower (Valk et al., 1990) pasture and total DMI when starch-based concentrates were replaced for fiber-based concentrates. Milk production was not

affected (Schwarz et al., 1995; Spörndly, 1991) or was reduced (Valk et al., 1990) by fiber-based concentrates. Spörndly (1991) conducted a study where cows were fed *ad libitum* with fresh cut grass plus 2 kg/d of hay and supplemented with either starch (barley) or fiber (beet pulp) concentrates offered at two levels (3.3 and 5.7 kg/d). Type of supplement did not affect pasture DMI or milk yield (Spörndly, 1991). Fat and protein percentages were increased with the starch-based concentrate at the low and high level of supplementation, respectively (Spörndly, 1991). Schwarz et al. (1995) also found that neither pasture nor total DMI nor milk production differ when pasture-fed cows received either a starch (corn) or fiber (beet pulp)-based concentrate. Milk composition was not affected by type of concentrate supplementation. Valk et al. (1990) conducted two studies to determine the effects of concentrate types on DMI, herbage digestibility, milk yield, and milk composition for high producing dairy cows fed fresh forages. In experiment 1, cows were fed fresh pasture and supplemented with a starch (ground corn) or a fiber (beet pulp and soy hulls) concentrate. Supplements were offered at equal dry matter content. In experiment 2, cows were fed a starch or fiber supplement and an additional mix of fiber and starch grain supplement. Supplements had equal energy content. In experiment 1, total DMI tended to be higher for cows receiving the starch supplement, resulting in higher energy intake. Milk production was higher for the starch-based diet (28.4 vs. 25.8 kg/d), which was attributed to the increased energy intake (Valk et al. 1990). In experiment 2, total DMI was higher for the fiber-based diet and resulted in similar energy intake for all treatments. Milk yield was not different between treatments (31.1 kg/d), which was attributed to similar energy intakes.

Most of the studies found no response in milk production to fiber-based concentrate (Delahoy, 2000 (exp. 2); Sayers, 1999 (exp. 2); Schwarz et al., 1995; Spörndly, 1991). On the other hand, others found higher (Meijs, 1986) or lower milk production (Valk et al., 1990). Some

studies showed no changes in milk composition (Meijs, 1986; Schwarz et al., 1995; Valk et al., 1990). Compared to fiber-based concentrates, starch-based concentrates increased milk protein in some studies (Delahoy, 2000 (exp. 2); Sayers, 1999 (exp. 2); Spörndly, 1991). Only one study (Sayers, 1999 (exp. 2)) reported significantly lower milk fat percentage with corn-based concentrate. However, several studies (Delahoy, 2000 (exp. 2); Meijs, 1986; Schwarz et al., 1995; Valk et al., 1990) reported a numerically trend for lower milk fat percentage with corn-based concentrate.

Differences in results among the studies can be attributed to differences in the source of starch or fiber used in the concentrate, type of pasture and other components included in the diet. Starch sources included corn (Delahoy, 2000 (exp. 2); Schwarz et al., 1995; Valk et al., 2000), barley (Spörndly, 1991), cassava (Meijs, 1986), or the combination of barley, wheat plus corn (Sayers, 1999). The pasture used also differed among studies and included orchardgrass (Delahoy, 2000 (exp.2)), ryegrass (Meijs, 1986; Sayers, 1999) or grass/legumes mix (Schwarz et al., 1995). Source of starch and pasture specie can potentially affect rumen pH, and may explain variability in herbage DMI among studies. Meijs (1986) suggested that feeding a fermentable starch supplement in conjunction with highly degradable fresh forage reduces rumen pH causing a decrease in herbage digestion, a longer retention of feed in the rumen, and therefore a reduction in pasture and total DMI. Replacing starch-based concentrates with fiber-based concentrates would maintain higher pH in the rumen, enhancing herbage digestion and resulting in higher DMI. In the studies of Meijs (1986) and Sayers (1999 (exp. 2)) both pasture (ryegrass) and starch (cassava or barley plus wheat) are highly degradable in the rumen and could explain the response in DMI when starch was replaced for fiber. The inclusion of hay in the diet may help maintain pH in the rumen and therefore explain similar DMI even with highly degradable starch source

such as barley (Spörndly, 1991). The use of a starch source with lower degradability than barley such as corn (Delahoy, 2000 (exp. 2); Schwarz et al., 1995) may not be as detrimental to rumen pH and explain similar pasture and total DMI.

2.5.3 Processed Grain

Processing methods for grains used for dairy cattle have been review extensively by Theurer (1986) and Theurer et al. (1999). Grains are generally processed to some extent to provide ruminal microbial access to the nutrients protected from digestion by the pericarp (McAllister et al., 1990). Grain processing can be categorized in: 1) wet processing (e.g. soaking, steam-rolling, steam-flaking, exploding, pressure-cooking, early harvest, and reconstitution), and 2) dry processing (e.g. grinding, dry rolling, cracking, popping, roasting and pelleting) (Hale, 1973).

Studies evaluating the effect of processing methods, specifically steam and high moisture methods, of grain supplements such as corn or sorghum on DMI, and milk production and composition of dairy cows are presented in *Table 7*. Steam-rolling is a process in which grain is steamed for 15 min or less, grain moisture is increased to approximately 15% and then crushed with rollers of various sizes to produce a thick flake to usually a density of between 438 to 540 g/L (Theurer et al., 1999). Steam-flaking is a process in which grain is exposed to steam for 30 to 60 minutes in a stainless steel chamber to increase grain moisture to approximately 20% and then flaked through heated rollers to a specific density which range from 309 to 389 g/L (Theurer et al., 1999). The extent of processing increases as density decreases (Theurer et al., 1999). Optimal

density for steam-flaked corn appears to be between 309 to 386 g/L and for steam-flaked sorghum appears to be between 360 to 438 g/L (Theurer et al., 1999).

High moisture corn has higher ruminal degradability than dry corn because of internal characteristics of its starch (Nocek and Tamminga, 1991). This makes high moisture corn a potential advantageous energy supplement over dry corn because of the high RDP content in pasture typically grazed by dairy cows.

Except for Wu et al. (2001), none of the studies showed an increase in milk production of dairy cows on pasture-based diets when steam processed or high moisture grains replaced dry grains as supplement (Alvarez et al., 2001; Bargo et al., 1998; Delahoy, 2000 (exp. 1); Pieroni et al., 1999; Reis and Combs, 2000a; Reis et al., 2001; Soriano et al., 2000). Delahoy (2000 (exp. 1)) compared dry cracked corn with steam-flaked corn (density 360 g/L) as supplement for late lactation dairy cows grazing an orchardgrass-based pasture. Pasture and total DMI were similar between treatments. Steam-flaking did not modify milk production (24.3 kg/d) or milk composition. Similar results were reported by Reis and Combs (2000a) but for early lactation dairy cows supplemented with dry ground corn or steam-rolled corn (density 591 g/L). Supplementation with 9 kg/d of these two different forms of processed corn did not affected milk production (32.1 kg/d) or composition (3.35% fat and 2.97% protein).

Studies conducted with low producing dairy cows on pasture found no response on milk production or composition to processed corn (Bargo et al., 1998) or sorghum (Pieroni et al., 1999). Pieroni et al. (1999) showed that supplementation with steam-flaked sorghum (density 480 g/L) increased pasture and total DMI (+1.9 kg/d) in comparison with dry ground sorghum. However, neither milk production (20.5 kg/d) nor milk composition were affected by treatments.

Steam-flaked corn supplementation did not affect pasture or total DMI, milk production (20.6 kg/d) or composition of cows grazing a grass-legume pasture (Bargo et al., 1998).

Soriano et al. (2000) compared dry corn finely or coarsely ground and high moisture corn as supplement for high producing dairy cows grazing an orchardgrass pasture. Milk production (30.3 kg/d) and composition were not affected by corn processing. No pasture or total DMI data were reported in this study. In a study conducted with dairy cows in late lactation grazing annual pastures (Alvarez et al., 2001), the replacement of dry corn by high moisture corn (with the goal of providing equal amounts of starch by the supplement) did not affect DMI, milk production, or milk fat percentage. However, milk protein percentage was increased by supplementing with high moisture corn (3.39 vs. 3.30; $P < 0.10$). This agrees with results of Wu et al. (2001), who also reported greater milk protein content (3.26 vs. 3.15%; $P < 0.10$) when high moisture corn replaced dry corn as supplement for dairy cows on pasture. Wu et al. (2001) also reported a significant response in milk production to high moisture corn supplementation (22.9 vs. 20.5 kg/d), which could be likely related to the fact that corn DMI was the same (6.7 kg DM/cow/d) and therefore the amount of available starch provided by the corn supplement was higher with the high moisture corn.

Reis et al. (2001) reported two indoor experiments where high producing cows were supplemented with high levels (9 kg DM/d approx.) of dry corn or high moisture corn either finely or coarsely ground. The first experiment included a control (unsupplemented) treatment. Regardless of corn form, supplementation decreased pasture DMI (11.0 vs. 17.8 kg/d), increased total DMI (20.5 vs. 17.8 kg/d), milk production (30.3 vs. 25.6 kg/d) and protein percentage (3.36 vs. 3.14%), and decreased fat percentage (3.77 vs. 3.20%) compared to the control treatment. In the second experiment, supplementation with finely or coarsely ground dry or high moisture corn

did not affect total DMI (20.2 kg/d), milk production (26.5 kg/d) or milk composition (3.63% fat, 3.54% protein).

2.6 RUMEN DIGESTION OF GRAZING COWS

2.6.1 Level of Concentrate Supplementation

Studies that have reported daily means of ruminal fermentation parameters (pH, $\text{NH}_3\text{-N}$, and VFA) of dairy cows grazing or indoors on pasture-based diets supplemented with different levels of energy supplements are summarized in *Table 8* (Bargo et al., 2002; Berzaghi et al., 1996; Carruthers and Neil, 1997; Carruthers et al., 1997; García et al., 2000; Jones-Endsley et al., 1997; Khalili and Sairanen, 2000; Reis and Combs, 2000b; Sayers 1999 (exp. 2); Van Vuuren et al., 1986). Supplementation levels ranged from 0 to 10 kg DM/cow/d. Unsupplemented treatments (< 1 kg DM/d) from all studies averaged a ruminal pH of 6.27 (range from 6 to 6.57), a $\text{NH}_3\text{-N}$ concentration of 24.7 mg/dl (range from 12.8 to 39.1 mg/dl) and a total VFA concentration of 125.2 mmol/L (range 96.7 to 150 mmol/L). Supplemented treatments (1.1 to 10 kg DM/d) from all studies averaged a ruminal pH of 6.10 (range from 5.75 to 6.29), a $\text{NH}_3\text{-N}$ concentration of 18.3 (8.7 to 32.2 mg/dl), and a total VFA concentration of 120.9 (range from 90.3 to 151.4).

Studies summarized in *Table 8* can be divided in two categories. First, those studies that included unsupplemented treatments (< 1 kg/d of supplement) (Bargo et al. 2002; Berzaghi et al., 1996; Carruthers and Neil, 1997; Carruthers et al., 1997; García et al., 2000; Khalili and

Sairanen, 2000; Reis and Combs, 2000b; Van Vuuren et al., 1986;), and second, those studies that compared different levels of supplemented treatments (Jones-Endsley et al., 1997; Sayers, 1999 (exp. 2)).

Within the first category (unsupplemented vs. supplemented), concentrate supplementation reduced ruminal pH in some studies (Bargo et al., 2002; Carruthers and Neil, 1997; Carruthers et al., 1997) but not in others (Berzaghi et al., 1996; García et al., 2000; Khalili and Sairanen, 2000; Reis and Combs, 2000b; Van Vuuren et al., 1986). High levels of supplementation (> 8 kg/d) reduced ruminal pH of high producing dairy cows grazing orchardgrass pasture at two pasture allowances (25 and 40 kg DM/cow/d), with the pH reduction higher at low pasture allowance (6.57 (unsupplemented) vs. 6.25 (supplemented) at low pasture allowance, 6.40 (unsupplemented) vs. 6.29 (supplemented) at high pasture allowance) (Bargo et al., 2002). In two experiments where cows were fed fresh pasture indoors (Carruthers and Neil, 1997; Carruthers et al., 1997), the addition of low amounts (< 1.5 kg/d) of non-structural carbohydrates (50:50 cornflour and dextrose monohydrate) resulted in reduction of rumen pH in comparison with pasture-only treatments (6.15 vs. 6.05). Van Vuuren et al. (1986) reported similar rumen pH (5.93) for dairy cows on ryegrass pasture only or ryegrass pasture plus 5.3 kg DM/d of a high-starch or low-starch based concentrate diets. A similar result (rumen pH 6.10), was reported by Khalili and Sairanen (2000) with dairy cows grazing a meadow fescue (*Festuca pratense*)/timothy (*Phleum pratense*) pasture as the only feed or supplemented with 4 kg/d of concentrate. Supplementation with 5.4 kg DM/d of cracked corn did not affect rumen pH (6.3) of cows grazing a grass-legume pasture (Berzaghi et al., 1996). Supplementation of winter oats (*Avena sativa*) fed in confinement with approximately 2.5 kg/d of barley or corn did not affect rumen pH (6.06) compared to the control (unsupplemented) treatment (6.10) (García et al.,

2000). Compared to unsupplemented cows, supplementation with 5 or 10 kg/d of a corn-based concentrate did not affect rumen pH (6.68) of dairy cows grazing a legume-grass pasture (Reis and Combs, 2000b).

In the second category, increasing the amount of concentrate supplemented reduced rumen pH in one study (Sayers, 1999 (exp. 1)) but not in other (Jones-Endsley et al., 1997). Rumen pH decreased from 6.00 to 5.75 as concentrate increased from 5 to 10 kg DM/d in dairy cows grazing a ryegrass pasture (Sayers, 1999 (exp. 2)). Rumen pH did not differ (5.86) when dairy cows grazing an alfalfa (*Medicago sativa*)/orchardgrass pasture were supplemented with either 5.6 or 8.4 kg/d (Jones-Endsley et al., 1997).

The lack of consistency in the effect of level of concentrate supplementation on ruminal pH of grazing cows suggests that there is not a simple relation between amount of concentrate supplemented and rumen pH. Other factors such as pasture quality (e.g. stage of maturity when grazed, NDF content, etc), and the interaction between the amount and type of concentrate supplemented and pasture DMI may have a key role. The timing of rumen sample collection in relation to feeding can affect these results.

Reductions in rumen pH with supplementation were associated with higher total VFA concentrations in some studies (Bargo et al., 2002; Sayers, 1999 (exp. 2)). However, others found similar total VFA concentrations even with ruminal pH reduction with supplementation (Carruthers and Neil, 1997; Carruthers et al., 1997). Most of the studies did not report higher total VFA concentration when pasture-fed cows were supplemented (Berzaghi et al., 1996; Carruthers and Neil, 1997; Carruthers et al., 1997; García et al., 2000; Jones-Endsley et al., 1997; Khalili and Sairanen, 2000; Sayers, 1999 (exp. 2); Reis and Combs, 2000b; Van Vuuren et al., 1986). An increase in total VFA concentration was reported, however, when cows grazing at low

pasture allowance were supplemented with a corn-based concentrate (116.3 vs. 130.0 mmol/L) (Bargo et al., 2002). Energy supplements reduced molar proportion of acetate and increased the molar proportion of propionate in some studies (Bargo et al., 2002; García et al., 2000; Sayers, 1999 (exp. 2)). Some studies reported only a reduction in the acetate molar proportion (Khalili and Sairanen, 2000) or an increase in the propionate molar proportion (Jones-Endsley et al., 1997).

The most consistent effect of energy supplementation on rumen fermentation is a reduction in rumen $\text{NH}_3\text{-N}$ concentration. Over the ten studies summarized in *Table 8*, rumen $\text{NH}_3\text{-N}$ concentration of grazing dairy cows was significantly reduced by supplementation in six of the studies (Bargo et al., 2002; Carruthers and Neil, 1997; Carruthers et al., 1997; García et al., 2000; Reis and Combs, 2000b; Van Vuuren et al., 1986) and numerically in three (Berzaghi et al., 1996; Jones-Endsley et al., 1997; Sayers, 1999). Rumen $\text{NH}_3\text{-N}$ concentration reduction could be associated with a higher capture of $\text{NH}_3\text{-N}$ originated by the highly rumen degradable CP of pasture, but also to a reduction in total CP intake because energy supplement are usually low in CP.

2.6.2 Type of Energy Supplementation

The effect of type of energy supplementation on ruminal fermentation parameters of grazing or indoors pasture fed dairy cows is summarized in *Table 9* (Alvarez et al., 2001; Bargo et al., 1998; García et al., 2000; Khalili and Sairanen, 2000; Kolver et al., 1998; Reis and Combs, 2000a; Sayers, 1999 (exp. 2); Soriano et al., 2000; Van Vuuren et al., 1986). The studies presented in *Table 9* can be divided in three categories. First, studies comparing different type of

carbohydrates (starch vs. fiber based concentrates) (Khalili and Sairanen, 2000; Sayers, 1999 (exp. 2); Van Vuuren et al., 1986). Starch sources included corn and tapioca (Van Vuuren et al., 1986), barley, wheat and corn (Sayers, 1999 (exp. 2)), and barley and oats (Khalili and Sairanen, 2000). Fiber sources included beet and citrus pulp (Sayers, 1999 (exp. 2)) and beet pulp (Khalili and Sairanen, 2000; Van Vuuren et al., 1986). Second, studies comparing cereal grains differing in rumen degradability (barley vs. corn) (García et al., 2000). Third, studies comparing different processed corn (high moisture, steam-rolling, steam-flaking, grinding) (Alvarez et al., 2001; Bargo et al., 1998; Reis and Combs, 2000a; Soriano et al., 2000).

Compared to starch-based concentrates, supplementation with fiber-based concentrates did not affect ruminal pH (5.96) of grazing dairy cows receiving moderate (approximately 5 kg/d, Khalili and Sairanen, 2000; Van Vuuren et al., 1986) or high (approximately 10 kg/d, Sayers, 1999 (exp. 2)) levels of supplementation. No changes in rumen $\text{NH}_3\text{-N}$ concentration were reported by Van Vuuren et al. (1986) and Sayers (1999), while Khalili and Sairanen (2000) showed a reduction in this variable with the fiber-based concentrate (32.1 vs. 21.8 kg/d). All three studies reported high total VFA concentrations (121.6 to 132.0 mmol/L), however the type of carbohydrate did not affect it. Sayers (1999 (exp. 2)) found that supplementation with fiber-based concentrates increased the molar proportion of acetate (56.1 vs. 60.3 mol/100 mol) and butyrate (20.7 vs. 26.1 mol/100 mol), and decreased the molar proportion of propionate (26.1 vs. 20.7 mol/100 mol). Khalili and Sairanen (2000) reported no changes in the molar proportion of any of the three major VFAs.

García et al. (2000) evaluated two cereal grains (barley vs. corn) that differ in rumen degradability as a supplement for cows fed fresh winter oats. The amount of each grain provided 24% of starch in the total diet. Supplementation with a highly rumen degradable cereal (barley)

significantly reduced rumen $\text{NH}_3\text{-N}$ concentration compared to a more slowly rumen degradable (corn) (26.9 vs. 19.4 mg/dl). No differences were found in rumen pH (6.06) or total VFA concentration (93.8 mmol/L). Barley supplementation also resulted in a higher molar proportion of propionate (24.9 vs. 21.4 mol/100 mol) and a lower proportion of butyrate (11.9 vs. 13.7 mol/100 mol).

Some studies compared the effect of different corn processing methods on rumen fermentation of grazing dairy cows (Alvarez et al., 2001; Bargo et al., 1998; Reis and Combs, 2000a; Soriano et al., 2000). Rumen pH and total VFA concentrations were unaffected when dry corn was substituted for high moisture corn (Alvarez et al., 2001; Soriano et al., 2000), steam-rolled corn (Reis and Combs, 2000a) or steam-flaked corn (Bargo et al., 1998). Mean rumen $\text{NH}_3\text{-N}$ concentration was numerically (Soriano et al., 2000) or significantly (Alvarez et al., 2001) lower when cows grazing were supplemented with high moisture corn. Reis and Combs (2000a) found no changes in pH (6.53), $\text{NH}_3\text{-N}$ (12.9 mg/dl) or total VFA concentration (118.9 mmol/L) when grazing cows were supplemented with dry ground corn or steam rolled corn. However, steam-flaked corn reduced $\text{NH}_3\text{-N}$ concentration of cows grazing a grass-legume pasture (19.1 vs. 13.8 mg/dl) (Bargo et al., 1998).

Kolver et al. (1998) studied the rumen fermentation of dairy cows fed fresh cut orchardgrass and supplemented with 9.1 kg/d of a corn-based concentrate fed together with the pasture or fed 4 hours later. Ruminal pH was significantly lower when the concentrate was fed together with the pasture than when it was fed four hours later (6.06 vs. 6.17). Rumen $\text{NH}_3\text{-N}$ concentration was not affected by treatments and averaged 17.4 mg/dl. Total VFA concentration, molar proportion of acetate and propionate did not differ between treatments.

2.7 PASTURE IN SITU DIGESTION

Extensive reviews on the in situ technique have been published (Firkins et al., 1998; Nocek, 1988; Noziere and Michalet-Doreau, 2000; Vanzant et al., 1998). The main factors influencing in situ studies are animal characteristics, substrate characteristics, bag characteristics, temporal characteristics, other procedural aspects, and mathematical components (Vanzant et al., 1998).

Of the several factors affecting the in situ degradation of DM, NDF, and CP of pasture, this review focuses on the basal diet and form of sample incubation. Basal diet influences the microbial population in the rumen and can potentially affect the rate and extent of digestion of feedstuff incubated in the bags (Vanzant et al., 1998). Even for forage diets, the type of forage can affect in situ degradation results (Vanzant et al., 1998). It has been proposed that because of the many dietary factors affecting in situ degradation, in situ incubations ideally should be conducted in the rumen of animals that are consuming the diet of interest (Vanzant et al., 1998). Sample processing, particularly the grind size used for samples, is also one of the main factors affecting in situ results (Vanzant et al., 1998).

Several studies (Bargo et al., 2001; Reis and Combs, 2000a; Reis and Combs, 2000b; Sayers, 1999 (exp. 1); Sayers, 1999 (exp. 2)) have reported the effect of supplementation on in situ pasture digestion. Pasture species in those studies included among others legumes such as alfalfa and red clover, and grasses such as brome grass, orchardgrass, ryegrass, and winter oats (*Avena sativa* L.). Some studies were conducted indoors with cows consuming as a basal diet fresh forage (Sayers, 1999 (exp. 1)); others were conducted with cows grazing ryegrass (Sayers, 1999 (exp. 2)), winter oats (Bargo et al., 2001; Elizalde et al., 1992), or legume/grass pastures (Reis and Combs, 2000a; Reis and Combs, 2000b). Form and processing of pasture samples

included fresh-meat mincer (Bargo et al., 2001; Elizalde et al., 1992), frozen masticate-ground (Reis and Combs, 2000a; Reis and Combs, 2000b) and fresh-cut to 1 cm size (Sayers, 1999 (exp. 1); Sayers, 1999 (exp. 2)).

López et al. (1999) compared different mathematical models to analyze in situ data. For DM and CP fraction most of the studies reviewed here, used the model described by Ørskov and MacDonald (1979) based on a soluble fraction (a) and an insoluble potentially degradable fraction (b) at a constant rate (c) to describe the potentially degradable fraction (PD): $PD = a + b(1 - e^{-ct})$ where t refers to time. The effective degradability (ED) of DM and CP fractions was generally estimated using the following equation: $ED = a + b(c / (c + kp))$, where kp corresponds to rate of passage assumed. For comparison purposes, all effective degradability data presented in the following tables were calculated assuming a rate of passage of 6 %/h. In some cases for DM and in most cases for NDF, the models included a lag time.

2.7.1 Effect of Supplementation on In Situ Pasture Digestion

Studies (Bargo et al., 2001; Elizalde et al., 1992; Reis and Combs, 2000a; Reis and Combs, 2000b; Sayers, 1999 (exp.1), Sayers, 1999 (exp. 2)) reporting the effect of supplementation on DM, CP, and NDF in situ degradation of pasture are summarized in *Table 10*, *Table 11* and *Table 12*, respectively. In situ degradation of DM and CP of ryegrass was not affected by protein or energy supplementation (Sayers, 1999 (exp. 1), Sayers, 1999 (exp. 2)). Sayers (1999 (exp. 1)) reported that neither soluble (26.6%), insoluble potentially degradable (63.5%) fractions, nor rate of degradation (6.9 %/h) of DM were affected by the amount (2.6 vs.

5.6 kg DM/d) or CP content (10 vs. 34 %) of concentrate supplementation. Similar results were found for the CP fraction.

Sayers (1999 (exp. 2)) found that supplementation with 5 or 10 kg DM/d of a starch or a fiber-based concentrate did not affect rate or extent of degradation of DM or CP degradation of ryegrass. Soluble, insoluble potentially degradable fraction and rate of degradation of DM averaged 22.6%, 68.2%, and 6.9%/h, respectively (Sayers, 1999 (exp. 2)). Soluble, insoluble potentially degradable fraction and rate of degradation of CP averaged 9.9%, 88.7%, and 9.1%/h, respectively (Sayers, 1999 (exp. 2)).

Slight reductions in the insoluble potentially degradable and rate of DM degradation of legume/grasses pasture were reported when grazing cows were supplemented with high levels of corn-based concentrates (Reis and Combs, 2000a; Reis and Combs, 2000b). Supplementation with 9 kg DM/d of dry or steam-rolled corn with or without 3.2 kg/d of hay to grazing cows did not affect the soluble (47.8%), insoluble potentially degradable (40.7%), or effective degradability (74.6%) of DM of pasture; however the rate of DM degradation was lower with steam-rolled corn than with dry corn supplementation (11.4 vs. 12.2%/h) (Reis and Combs, 2000a). Compared to the unsupplemented treatment, the insoluble potentially degradable DM fraction of pasture decreased with the supplementation of 10 kg DM/d of corn (38.5 to 36.3%), while the soluble fraction (48.4%), rate of degradation (10.6%/h), and the effective degradability (72.5%) of DM were not affected by supplementation (Reis and Combs, 2000b).

Neither soluble (38.3%), insoluble potentially degradable (61.8%) fractions, nor the rate of degradation (4.4%/h) of CP of winter oats pasture were affected when grazing cows were supplemented with 5 kg DM/d of corn silage (Elizalde et al., 1992). However, corn silage

supplementation increased the lag time of NDF pasture degradation from 1.4 to 5.8 h (Elizalde et al., 1992).

Bargo et al. (2001) reported DM, CP and NDF in situ degradation of winter oats grazed by dairy cows supplemented with 6.3 kg DM/d of a low CP-sunflower meal-based concentrate, a high CP-sunflower meal-based concentrate, and a high CP-feather meal-based concentrate. Soluble fraction, insoluble potentially degradable fraction, rate of degradation and effective degradability of DM averaged 57.7%, 32.6%, 8.4%/h, and 76.6%, respectively, and were not affected by the treatments. Soluble fraction, insoluble potentially degradable fraction, rate of degradation and effective degradability of CP averaged 62.6%, 31.3%, 9.9%/h, and 81.9%, respectively, and were not affected by the treatments. In situ degradation of the winter oats NDF was not affected either by supplementation.

2.8 POST-RUMINAL DIGESTION OF GRAZING DAIRY COWS

The feeding value and digestion of pasture and pasture products (e.g. silage) for ruminants have been recent and extensively reviewed by Beever et al. (2000). Few studies have reported post-ruminal digestion of nutrients of supplemented dairy cows on pasture-based diets, probably because of methodology problems in grazing situations (Galyean and Owens, 1991). Some studies evaluated site and extent of digestion of pasture with grazing cows (Berzaghi et al., 1996; Delagarde et al., 1997; Jones-Endsley et al., 1997), while others were conducted in confinement with cows fed fresh cut pasture (García et al., 2000; Van Vuuren et al., 1993).

With the purpose of comparing the different studies, common parameters to express OM, NDF and N digestion were chosen. For OM, total tract apparent digestibility (**TTAD**, %), rumen apparent digestibility as proportion of OM intake (**RAD**, %) and rumen apparent digestibility as proportion of total OM digested (**RADD**, %). For NDF digestion, total tract apparent digestibility (**TTAD**, %) and rumen apparent digestibility as proportion of total NDF digested (**RADD**, %). For N digestion, flows of nonammonia N (**NAN**, in g/d and as % of N intake), nonammonia nonmicrobial nitrogen (**NANMN**, in g/d and as % of N intake), microbial N (**MN**, g/d) and efficiency of bacterial protein synthesis either per kg of OM apparently digested in the rumen (**AD**, g of bacterial N/kg of OM apparently digested in rumen) or per kg of OM truly digested in the rumen (**TD**, g of bacterial N/kg of OM truly digested in rumen).

2.8.1 Effect of Supplementation on Post-Ruminal Digestion of Dairy Cows on Pasture

The effects of supplementation on OM, NDF and N site of digestion of dairy cows on pasture-based diets are summarized in *Table 13*, *Table 14* and *Table 15*, respectively.

Berzaghi et al. (1996) studied the site and extent of nutrient digestion of dairy cows grazing a mixed grass-legume pasture unsupplement or supplemented with 6.4 kg/d of cracked corn. Level of significance in this study was 0.10. Pasture OM intake was lower for the supplemented cows (9.8 vs. 13.0 kg/d; $P < 0.07$), while total OM did not differ between treatments (14.1 kg/d). Substitution rate was 0.6 kg OM pasture/kg OM supplement. Corn supplementation reduced total tract OM digestibility (69.9 vs. 71.9%) and OM apparently (43.3 vs. 48.9%), and truly digested in the rumen. However, the percentage of OM digested in the rumen over the total tract OM digestibility did not differ between treatments (65.2%). Neutral

detergent fiber (NDF) intake was similar between treatments (6.8 kg/d). Ruminal NDF and total tract NDF (64.5 vs. 70.4%) digestibility were depressed by supplementation. Supplementation reduced total N intake (522 vs. 391 g/d), however there were no significant differences in duodenal flows of total N, NAN (384 g/d), NANMN (126 g/d), or MN (258 g/d). The recovery of N at the duodenum (total N flow/N intake, %) was increased from 75% for the pasture-only fed cows to 87% for the supplemented cows indicating higher losses of N in the rumen in the form of $\text{NH}_3\text{-N}$ for the unsupplemented cows. There were no differences in NANMN as percentage of total N intake (25.5%) indicating high rumen degradability of CP (74.5%) in both treatments (Berzaghi et al., 1996).

García et al. (2000) studied the effect of supplementation with corn or barley on nutrient digestion of dairy cows fed indoors with winter oats. Supplements replaced winter oats on a 1:1 DM basis and provided 24% of starch in the total supplemented diets. Intake and TTAD of OM did not differ among treatments, and averaged 7.1 kg/d and 81.4%, respectively. The proportion of OM intake digested in the rumen was similar among treatments (56%), but when expressed as percentage of total OM digested cows fed pasture-only tended to have higher digestibility of OM than supplemented cows (72.7% vs. 66.7; $P < 0.09$). The substitution on a 1:1 DM basis of pasture by corn or barley reduced NDF intake (3.2 vs. 2.6 kg/d). Total tract NDF digestibility (58.8% TTAD) and proportion of NDF digested in the rumen (77.1% RADD) were not affected by substitution of winter oats by corn or barley, probably because of the low level of supplementation (García et al., 2000). Substitution of forage by either corn or barley grain resulted in lower N intake (320 vs. 248 g/d), however flows to duodenum of total N, NAN (155 g/d) and NANMN (54 g/d) were not different. Total N flow, expressed as percent of N intake, was significantly higher for both grains in comparison with the only forage diet (68.1 vs. 60.2%),

showing that ruminal $\text{NH}_3\text{-N}$ losses can be reduced with energy supplementation but there were no significant differences between grains (García et al., 2000). No differences in NANMN were found among treatments (20.4% of N intake), reflecting high degradability of CP of pasture (79.6%). Neither bacterial N flow (100 g/d) nor efficiency of bacterial protein synthesis (25 g microbial/kg of OM apparently digested in the rumen, and 20 g microbial/kg of OM truly digested in the rumen) differed among treatments.

Van Vuuren et al. (1993) studied the effect of replacement of ryegrass pasture by 7.1 kg DM/cow/d by starch-based (corn) or fiber-based (beet pulp) concentrates. Total OM intake was 14.7 kg/d and TTAD of the OM did not differ among treatments (78.5%); however, supplementation with the starch-based concentrate decreased the RADD of the OM from 76.2 to 65.7%. Compared with the unsupplemented treatment, intake of NDF was reduced by the starch-based concentrate supplementation (6.61 vs. 5.73 kg/d), but was increased by the fiber-based concentrate supplementation (5.73 vs. 6.72 kg/d). The starch-based supplement reduced both the TTAD (from 79.1 to 74.5%) and the RADD (from 94.4 to 85.6% of NDF). Intake of N was reduced by supplementation (459 g/d to 441 g/d for the starch-based concentrate, and to 428 g/d for the fiber-based concentrate). Although duodenal flow of NAN did not differ (409 g/d), the proportion of NAN/N intake was lower for the unsupplemented treatment (89.1 vs. 96.5%) indicating higher losses of $\text{NH}_3\text{-N}$.

Jones-Endsley et al. (1997) studied the effect of CP content (12 vs. 16% CP) and level of concentrate offered (6.4 vs. 9.6 kg/d) on nutrient digestion of lactating dairy cows grazing a mixed grass-legume pasture. The level of significance in this study was 0.10. Total OM and NDF intake increased as CP content (16.7 to 18.2 kg OM/d, 9.1 to 10.1 kg NDF/d) and amount of concentrate (16.5 to 18.4 kg OM/d, and 9.1 to 10.1 kg NDF/d) increased. Total tract digestibility

of OM was significantly higher as CP content in the concentrate increased (73.4 vs. 75.7% for the 12 and 16% CP concentrate, respectively), while amount of concentrate did not affect it (74.5%). Total tract NDF digestibility increased as CP content and amount of concentrate increased from 65.2 to 68.1%. Ruminant digestibility of NDF as percentage of intake was not affected by treatments and averaged 67.8%. Intake of N was increased as CP in the concentrate increased (587 to 690 g/d), but was not as the amount of concentrate offered (638 g/d). Total N and NAN flow to the duodenum increased as CP content in the concentrate increased but did not change as the amount of concentrate offered increased. When expressed as percentage of N intake, NAN was similar among treatments and averaged 74.5%. Flow of NANMN increased as content of CP (from 144 to 187 g/d) and amount offered (from 157 to 174 g/d) of supplement increased. However, when expressed as percentage of N intake, NANMN did not differ among treatments and averaged 26.4%. The efficiency of microbial N synthesis, either expressed per kg of apparently (72.5) or truly (42.2) OM digested, did not differ among treatments.

Delagarde et al. (1997) studied the effect of supplementation with 2 kg/d of soybean meal on nutrient digestion of dairy cows grazing a ryegrass pasture fertilized at two N levels. Organic matter intake and total tract OM digestibility increased both with supplementation and level of N fertilization. Rumen apparent digestibility, as percentage of OM digested, was not affected by treatments and averaged 67.2%. Intake of N increased both with protein supplementation and fertilization, which was accompanied by increases in flow of NAN. At the low N pasture, protein supplementation reduced the proportion of NAN over N intake, indicating higher losses of ammonia. At the high N pasture, supplementation did not affect the proportion of NAN over N intake but the lowest values were found suggesting important losses of ammonia.

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Table 1: Effect of concentrate supplementation on grazing time (GT), biting rate (BR) and bite mass (BM) of dairy cows.

Reference	Cows ¹		Pasture ²	Supplement		GT min/d	BR bites/min	Total bites bites/d	BM g DM/bite
	DIM	Milk		Type	DMI, kg/d				
Arriaga-Jordan and Holmes, 1986	72	37.8	RG (rotational) ³	Barley	1	467 ^a	64 ^a	30000	0.53 g OM/bite
					6	424 ^b	62 ^a	26100	0.58
			RG (continuous) ³	Barley	1	525 ^a	75 ^b	39400	0.47
					6	471 ^b	75 ^b	35100	0.48
Kibon and Holmes, 1987	75	32.4	RG (5 cm) ⁴	Control	0	596 ^a	78	47000 ^a	0.32 ^a
				Cereal	3	571 ^b	77	44000 ^b	0.36 ^a
				Beet-pulp	3	559 ^b	77	43000 ^b	0.35 ^a
			RG (6.5 cm) ⁴	Control	0	585	76	45000 ^a	0.39 ^b
				Cereal	3	550	76	42000 ^b	0.39 ^b
				Beet-pulp	3	560	76	43000 ^b	0.40 ^b
Rook et al., 1994	48	NA ⁵	RG/WC (4 cm) ⁴	Control	0	765 ^a	62	NA	0.28
				Gluten corn/beet pulp	4	553 ^b	45	NA	0.51
			RG/WC (6 cm) ⁴	Control	0	651	47	NA	0.52
				Gluten corn/beet pulp	4	660	61	NA	0.33
			RG/WC (8 cm) ⁴	Control	0	639 ^a	53	NA	0.54
				Gluten corn/beet pulp	4	606 ^b	52	NA	0.58
Sayers, 1999 (exp. 2)	40	NA	RG	Barley/wheat/corn	5	458 ^b	NA	21302 ^b	0.61
				Barley/wheat/corn	10	358 ^d	NA	16041 ^d	0.58
				Beet pulp/citrus pulp	5	480 ^a	NA	22744 ^a	0.60
				Beet pulp/citrus pulp	10	398 ^c	NA	17825 ^c	0.61

^{a, b, c, d}Values within a reference followed by a different superscript letter differ ($P < 0.05$; unless other stated).

¹Pre-experimental DIM and milk (kg/d).

²Pasture species: RG = perennial ryegrass (*Lolium perenne*); WC = white clover (*Trifolium repens*).

³Grazing system.

⁴Pasture height.

⁵NA = not available.

Table 2: Effect of pasture allowance (PA) on substitution rate (SR) and milk response (MR) in grazing studies using high producing dairy cows supplemented with concentrate and including unsupplemented treatments.

Reference	Cows ¹		Pasture ²	Supplement		PA	SR ³	MR ³
	DIM	Milk		type	DMI, kg/d	kg DM/cow/d	Kg pasture/kg concentrate	kg milk/kg concentrate
Bargo et al., 2002	101	45.8	OG	Corn	7.9	25.0	0.26	1.36
						40.0	0.55	0.96
Robaina et al., 1998 (Experiment 1)	180	20.5	RG/WC	Barley/lupin	4.3	21.1	0.31	0.98
						42.3	0.57	0.54
Stockdale, 1999a ⁴	106 to 229	19.3 to 30.6	RG/WC/P	Barley/wheat	3 to 5	30	0.43	0.43
						30	0.45	0.55
						30	0.29	1.18
						30	0.30	1.17
						40	0.43	0.49
						40	0.46	0.98
						40	0.31	0.94

¹Pre-experimental DIM and milk (kg/d).

²Pasture species: OG = orchardgrass (*Dactylis glomerata*); P (*Paspalum dilatatum*); RG = perennial ryegrass (*Lolium perenne*); WC = white clover (*Trifolium repens*).

³Calculated relative to the unsupplemented treatment.

⁴Data from 7 experiments.

Table 3: Effect of level of concentrate on substitution rate (SR) and milk response (MR) in grazing studies using high producing dairy cows supplemented with concentrate and including unsupplemented treatments.

Reference	Cows ¹		Pasture ²	Concentrate Type ³	DMI, kg/d	SR ⁴ kg pasture/kg concentrate	MR ⁴ kg milk/kg concentrate
	DIM	Milk					
Dillon et al., 1997	31	27.0	RG	Corn/beet pulp (year 1)	2.0	0.46	0.50
					4.0	0.18	0.31
				Corn/beet pulp (year 2)	2.0	0.14	0.30
					4.0	0.21	0.35
Reis and Combs, 2000b	84	41.6	A/RC/RG	Corn	5.0	0.24	1.00
					10.0	0.41	0.86
Robaina et al., 1998 (Experiment 2)	180	21.4	RG/WC	Barley	1.8	0.44	1.56
					3.4	0.65	0.94
					6.7	0.58	0.82
Walker et al. 2001 (Experiment 1)	167	22.3	P/RG	Barley/wheat	3.0	0.02	1.07
					5.0	0.18	1.18
					7.0	0.21	1.07
					9.0	0.19	0.92
					10.4	0.28	0.91

¹Pre-experiment DIM and milk (kg/d).

²Pasture species: A = alfalfa (*Medicago sativa*); P = paspalum (*Paspalum dilatatum*); RC= red clover (*Trifolium pratense*); RG = perennial ryegrass (*Lolium perenne*); WC = white clover (*Trifolium repens*).

³Main energy source.

⁴Calculated relative to the unsupplemented treatment.

Table 4: Effect of type (starch vs. fiber-based) of concentrate on substitution rate (SR) and milk response (MR) in grazing or indoors studies using high producing dairy cows supplemented with concentrate and including unsupplemented treatments.

Reference	Cows ¹		Pasture ²	Supplement		SR ⁴		MR ⁴
	DIM	Milk		Type ³	DMI, kg/d	kg pasture/kg supplement	kg milk/kg concentrate	
Sayers, 1999 (Experiment 2)	40	NA	RG	Starch (barley/wheat/corn)	10.0	0.62		0.68
				Fiber (beet pulp/citrus pulp)	10.0	0.50		0.86
Schwarz et al., 1995 (Indoors) (Experiments 2 and 3)	42 to 56	25.3 to 28.2	Grass/legume	Starch (corn)	6.1	0.49		0.74
				Fiber (molassed sugar beet pulp)	7.1	0.52		0.37
				Starch (corn)	4.5	0.27		0.76
				Fiber (molassed sugar beet pulp)	5.2	0.31		0.42
Spörndly, 1991 (Indoors)	175	25.7	Grass	Starch (barley)	3.3	0.54		0.24
				Fiber (beet pulp)	3.3	0.64		0.06
				Starch (barley)	5.8	0.60		0.40
				Fiber (beet pulp)	5.6	0.71		0.41

¹Pre-experiment DIM and milk (kg/d).

²Pasture species: RG = perennial ryegrass (*Lolium perenne*).

³Main energy source.

⁴Calculated relative to the unsupplemented treatment or to the lower level of concentrate supplementation if there was no unsupplemented treatment in the study (e.g. Sayer, 1999).

⁵Not available.

Table 5: Effect of level of concentrate supplementation on DMI and milk production and composition of dairy cows on pasture-based diets.

Reference	Cow ¹		Pasture ²	Concentrate ³	DMI, kg/d			Milk kg/d	Fat %	Protein %	Fat kg/d	Protein kg/d
	DIM	Milk			Supplement	Pasture	Total					
Arriaga-Jordan and Holmes, 1986 ⁴	72	37.8	RG (rotational) ⁵	Barley	0.7	15.3	16.0	29.4	3.73 ^a	NA	1.10	NA
					4.7	14.7	19.4	31.9	3.51 ^a	NA	1.12	NA
			RG (continuous) ⁵	Barley	0.7	18.1	18.8	30.2 ^a	3.64 ^a	NA	1.10	NA
					4.7	16.5	21.2	32.8 ^b	3.40 ^b	NA	1.12	NA
Hoden et al., 1991	49	30.2	RG	Corn/beet pulp	0.5	NA	NA	20.7 ^a	3.78	3.03 ^a	0.78 ^a	0.62 ^a
					3.7	NA	NA	22.6 ^b	3.76	3.09 ^b	0.85 ^b	0.69 ^b
Dillon et al., 1997	31	27	RG	Corn/beet pulp	0.0	17.1	17.1 ^a	24.0 ^a	3.71	3.25	0.88	0.77 ^a
					1.8	16.5	18.3 ^b	25.0 ^b	3.68	3.28	0.90	0.82 ^b
					3.6	16.8	20.4 ^c	26.6 ^c	3.55	3.26	0.93	0.86 ^c
Reis and Combs, 2000b	84	41.6	A/RC/RG	Corn	0.0	13.9 ^a	13.9 ^a	21.8 ^a	3.89 ^a	2.85 ^a	0.88 ^a	0.62 ^a
					5.0	12.7 ^b	17.7 ^b	26.8 ^b	3.50 ^b	2.95 ^b	0.83 ^{ab}	0.79 ^b
					10.0	9.8 ^c	19.8 ^c	30.4 ^c	3.08 ^c	3.05 ^c	0.75 ^b	0.93 ^c
Robaina et al., 1998 (Experiment 2)	180	21.4	RG/WC	Barley	0.0	14.3 ^a	14.3 ^a	12.9 ^a	4.33	3.10 ^a	0.55	0.39
					1.8	13.5 ^b	15.3 ^b	15.7 ^b	4.33	3.19 ^b	0.67	0.49
					3.4	12.1 ^c	15.5 ^c	16.1 ^c	4.36	3.17 ^b	0.68	0.50
					6.7	10.4 ^d	17.1 ^d	18.4 ^d	4.36	3.29 ^b	0.78	0.59
Sayers, 1999 (Experiment 2)	40	NA ⁶	RG	Barley/wheat/corn	5.0	12.6 ^a	17.6 ^a	31.2 ^a	3.66 ^a	3.37 ^a	1.12	1.04 ^a
					10.0	9.5 ^b	19.4 ^b	34.6 ^b	2.99 ^b	3.55 ^b	1.04	1.22 ^b
Spörndly, 1991 (Indoors)	175	25.7	Grass	Barley	0.0 + 2 (hay)	14.6 ^a	16.5 ^a	19.7 ^a	4.46 ^a	3.28 ^a	0.88	0.65
					3.3 + 2 (hay)	12.7 ^b	18.0 ^b	20.5 ^{ab}	4.68 ^b	3.39 ^b	0.96	0.69
					5.8 + 2 (hay)	11.0 ^c	18.8 ^c	22.0 ^b	4.44 ^a	3.50 ^c	0.98	0.77
Valentine et al., 2000	41	NA	RG/RC	Barley	7.0	NA	NA	26.4 ^a	3.59 ^a	2.97 ^a	0.93	0.78 ^a
					10.0	NA	NA	28.0 ^b	3.47 ^a	3.06 ^b	0.97	0.86 ^b
					13.0	NA	NA	28.7 ^b	3.20 ^b	3.10 ^c	0.92	0.88 ^b
Walker et al., 2001 (Experiment 1)	167	22.3	P/RG	Barley/wheat	0.0	12.1 ^d	12.1 ^a	12.4 ^a	4.48 ^b	3.16	0.56	0.39
					3.0	12.0 ^d	15.0 ^b	15.6 ^b	4.21 ^b	3.33	0.66	0.52
					5.0	11.2 ^c	16.2 ^c	18.3 ^c	4.09 ^b	3.33	0.75	0.61
					7.0	10.6 ^{bc}	17.6 ^d	19.9 ^{cd}	4.05 ^b	3.30	0.81	0.66

Table 5 continued

					9.0	10.4 ^b	19.3 ^e	20.7 ^d	4.18 ^b	3.35	0.87	0.69
					10.4	9.2 ^a	19.6 ^e	21.9 ^d	3.25 ^a	3.41	0.71	0.75
Wilkins et al., 1994	41	NA	RG/WC	NA	0.0	NA	NA	22.9 ^a	3.69	2.79 ^a	0.83 ^a	0.64 ^a
					2.0	NA	NA	25.9 ^b	3.78	2.85 ^b	0.98 ^b	0.73 ^b
					4.0	NA	NA	26.0 ^b	3.87	2.94 ^c	1.00 ^b	0.76 ^c

^{a, b, c}Means within reference with different superscripts differ ($P < 0.05$).

¹Pre-experimental DIM and milk production (kg/d).

²Pasture species: A= alfalfa (*Medicago sativa*); P = paspalum (*Paspalum dilatatum*); RC = red clover (*Trifolium pratense*); RG = perennial ryegrass (*Lolium perenne*); WC = white clover (*Trifolium repens*).

³Main energy source in the concentrate.

⁴OM intake.

⁵Grazing system.

⁶Not available.

Table 6: Effect of starch (S) or fiber (F) based concentrate supplementation on DMI and milk production and composition of dairy cows on pasture-based diets.

Reference	Cow ¹		Pasture ²	Concentrate type ³	DMI, kg/d			Milk kg/d	Fat %	Protein %	Fat kg/d	Protein kg/d
	DIM	Milk			Supplement	Pasture	Total					
Delahoy, 2000 (Experiment 2)	182	35.5	OG	S (corn)	8.2	12.1	20.3	27.6	3.53	3.23 ^a	1.05	0.96
				F (beet pulp/soy hulls)	8.2	12.0	20.2	27.4	3.63	3.19 ^b	1.08	0.95
Meijs, 1986 ⁴	60	28.9	RG	S (cassava/corn)	5.5	11.7 ^a	17.2 ^a	25.6 ^a	3.96	3.40	1.01 ^a	0.87
				F (beet pulp/soybean hulls)	5.4	12.4 ^b	17.8 ^b	26.9 ^b	4.10	3.37	1.09 ^b	0.90
Sayers, 1999 (Experiment 2)	40	NA ⁵	RG	S (barley/wheat/corn)	5.0	12.6 ^a	17.6 ^a	31.2 ^a	3.66 ^a	3.37 ^a	1.12 ^a	1.04 ^a
				F (beet pulp/critus pulp)	5.0	13.4 ^b	18.4 ^b	30.9 ^a	3.94 ^b	3.30 ^b	1.18 ^b	1.00 ^b
				S (barley/wheat/corn)	10.0	9.5 ^c	19.4 ^c	34.6 ^b	2.99 ^c	3.55 ^c	1.04 ^a	1.22 ^c
				F (beet pulp/critus pulp)	10.0	10.9 ^d	20.8 ^d	35.2 ^b	3.62 ^a	3.34 ^a	1.26 ^b	1.17 ^d
Schwarz et al., 1995 (Indoors; exp. 2)	56	28.2	Grass/legumes	Control	0.0+0.15 (minerals)	14.9 ^a	15.0 ^a	20.3 ^b	4.16	3.13	0.85	0.62 ^a
				S (corn)	6.1+0.15 (minerals)	11.9 ^b	18.2 ^b	24.8 ^a	3.69	3.23	0.90	0.79 ^b
				F (molassed sugar beet pulp)	7.1+0.15 (minerals)	11.2 ^b	18.5 ^b	22.9 ^{ab}	3.90	3.16	0.89	0.72 ^b
Spörndly, 1991 (Indoors)	175	25.7	Grass	Control	0.0 + 1.9 (hay)	14.6 ^a	16.5 ^a	19.7 ^a	4.46 ^{ac}	3.28 ^a	0.88	0.65
				S (barley)	3.3 + 2 (hay)	12.7 ^b	18.0 ^b	20.5 ^{ab}	4.68 ^b	3.39 ^b	0.96	0.69
				F (beet pulp)	3.3 + 2 (hay)	12.4 ^b	17.7 ^b	19.9 ^a	4.50 ^a	3.44 ^{bc}	0.90	0.68
				S (barley)	5.8 + 2 (hay)	11.0 ^c	18.8 ^c	22.0 ^b	4.44 ^{ac}	3.50 ^c	0.98	0.77
				F (beet pulp)	5.6 + 2 (hay)	10.5 ^c	18.1 ^{bc}	22.0 ^b	4.32 ^c	3.41 ^b	0.95	0.75
Valk et al., 1990 ⁴ (Indoors; exp. 1)	83	34.6	RG	S (corn)	5.6+1.5(Cr ₂ O ₃ pellets)	11.2	18.3 ^a	28.4 ^a	3.92	3.48	1.11	0.98 ^a
				F (beet pulp)	5.4+1.4(Cr ₂ O ₃ pellets)	10.9	17.7 ^b	25.8 ^b	4.15	3.43	1.07	0.89 ^b

^{a, b, c, d}Means within reference with different superscripts differ ($P < 0.05$; unless other stated: Meijs, 1986, $P < 0.10$ for milk; Sayers, 1999 (exp. 2) interaction significant for fat and protein content).

¹Pre-experimental DIM and milk production (kg/d).

²Pasture species: OG = orchardgrass (*Dactylis glomerata*); RG = perennial ryegrass (*Lolium perenne*).

³Between parentheses main starch or fiber source used in the concentrate.

⁴OM intake.

⁵Not available.

Table 7: Effect of processing method of corn or sorghum on DMI and milk production and composition of dairy cows on pasture-based diets.

Reference	Cow ¹		Pasture ²	Processing type ³	DMI, kg/d			Milk kg/d	Fat %	Protein %	Fat kg/d	Protein kg/d
	DIM	Milk			Supplement	Pasture	Total					
Alvarez et al., 2001	170	NA	ARG/WO	DC corn	5.7	14.8	20.5	17.2	3.13	3.30 ^a	0.57	0.57
				HM corn	6.4	14.6	21.0	17.6	3.29	3.39 ^b	0.57	0.60
Bargo et al., 1998	28	NA ⁴	A/OG	DG corn	5	NA	NA	20.2	3.90	3.11	0.79	0.63
				SF corn (290 g/L) ⁵	5	NA	NA	21.0	3.71	3.10	0.78	0.65
Delahoy, 2000 (exp. 1)	216	33.5	OG	DC corn	7.2	15.5	22.7	24.3	3.73	3.26	0.91	0.79
				SF corn (360 g/L)	7.2	14.6	21.8	24.3	3.58	3.34	0.87	0.81
Pieroni et al., 1999	113	NA	A/OG/WC	DG sorghum (650 g/L)	5 + 2 (SM pellets) ⁶	12.4 ^a	18.6 ^a	20.2	3.35	3.17	0.68	0.64
				SF sorghum (480 g/L)	5 + 2 (SM pellets)	14.3 ^b	20.5 ^b	20.8	3.23	3.20	0.67	0.67
Reis and Combs, 2000a	95	NA	A/RC/OG	DG corn (680 g/L)	9	10.8	20.0	32.3	3.29	2.94	1.07	0.94
				SR corn (591 g/L)	9	10.7	19.8	31.8	3.39	2.98	1.07	0.94
Reis et al., 2001 (Indoors, exp. 1)	102	NA	A/Q	Control	0	17.8 ^a	17.8 ^a	25.6 ^a	3.77 ^a	3.14 ^a	0.98	0.79 ^a
				Finely DG shelled corn	8.9 + 0.5 (SBM) ⁶	11.1 ^b	20.7 ^b	30.7 ^b	3.15 ^b	3.36 ^b	0.96	1.03 ^b
				Coarsely ground HM ear corn	8.5 + 0.7 (SBM)	10.9 ^b	20.3 ^b	29.9 ^b	3.25 ^b	3.35 ^b	0.98	1.02 ^b
Reis et al., 2001 (Indoors, exp. 2)	167	NA	A/Q	Finely DG shelled corn	9.1 + 0.5 (SBM)	NA	20.6	27.5	3.63	3.52	1.01	0.96
				Coarsely ground HM ear corn	9.1 + 0.7 (SBM)	NA	19.9	25.9	3.69	3.49	0.96	0.90
				Finely ground HM ear corn	9.1 + 0.4 (SBM)	NA	20.0	26.1	3.56	3.61	0.99	0.99
Soriano et al., 2000	107	36.4	OG	HM corn	6	NA	NA	30.8	3.13	2.96	1.02	0.89
				Finely DG corn	6	NA	NA	29.7	2.94	2.99	0.86	0.87
				Coarsely DG corn	6	NA	NA	30.1	3.23	2.96	0.99	0.87
				HM corn	4	NA	NA	30.5	3.10	2.95	0.94	0.88
Wu et al., 2001	247	>30	RC/Q/K	DC shelled corn	6.7 + 1 (CS) ⁶ + 0.95 (RS) ⁶	NA	NA	20.5 ^a	3.67 ^a	3.15 ^a	0.76	0.68 ^a
				Finely ground HM corn	6.7 + 1 (CS) + 0.95 (RS)	NA	NA	22.9 ^b	3.28 ^b	3.26 ^b	0.75	0.77 ^b

^{a, b, c, d}Means within reference with different superscripts differ ($P < 0.05$; unless other stated; $P < 0.10$ for protein content; Alvarez et al. (2001) and Wu et al. (2001)).

¹Pre-experimental DIM and milk production (kg/d).

²Pasture species: A = alfalfa (*Medicago sativa*); ARG = annual ryegrass (*Lolium multiflorum*); B = brome grass (*Bromus inermis*); OG = orchardgrass (*Dactylis glomerata*); Q = quackgrass (*Elytrigia repens*); RC = red clover (*Trifolium pratense*); WC = white clover (*Trifolium repens*); WO = winter oats (*Avena sativa*).

³DC = dry cracked, DG = dry ground, HM = high moisture, SF = steam-flaked, SRL = steam-rolled; between parentheses density (g/L). ⁴Not available. ⁵Density (g/L). ⁶CS = corn silage; SBM = soybean meal, RS = roasted soybean.

Table 8: Effect of level of energy supplementation on mean ruminal fermentation parameters (pH, NH₃-N, and VFA) of dairy cows on pasture-based diets.

Reference	Pasture ¹	Concentrate			NH ₃ -N mg/dl	VFA, mmol/L			
		Type	DMI, ² kg/d	pH		Total	Acetate ³	Propionate ³	Butyrate ³
Bargo et al., 2002	OG low PA	Mineral-vitamin mix	0.8 (5%)	6.57 ^a	15.2 ^a	116.3 ^a	79.3 ^a (68.2 ^a)	22.3 ^a (19.1 ^a)	10.7 ^a (9.2 ^a)
		Corn based	8.6 (35%)	6.25 ^c	9.1 ^b	130.0 ^b	85.1 ^a (65.8 ^b)	25.6 ^b (19.5 ^b)	14.8 ^b (11.3 ^b)
	OG high PA	Mineral-vitamin mix	0.7 (4%)	6.40 ^b	15.3 ^a	129.8 ^b	88.9 ^b (68.6 ^a)	24.1 ^a (18.5 ^a)	12.5 ^c (9.6 ^a)
		Corn based	8.7 (35%)	6.29 ^c	8.7 ^b	130.6 ^b	84.9 ^b (65.2 ^b)	26.2 ^a (19.9 ^b)	15.1 ^d (11.6 ^b)
Berzaghi et al., 1996	TF/OG/WC	Control	0.0	6.4	22.4	150.0	NA ⁴ (63.2)	NA (18.7)	NA (12.9)
		Cracked corn	5.4 (36%)	6.2	17.1	148.0	NA (62.4)	NA (19.1)	NA (13.5)
Carruthers and Neil, 1997 (Indoors)	RG high N	Control	0.0	6.19 ^a	29.9 ^a	132.0 ^a	50.4 (NA)	24.3 (NA)	17.3(NA)
		NSC ⁵ supplement	1.3 (8%)	6.05 ^c	22.4 ^b	136.0 ^a	51.7 (NA)	25.5 (NA)	15.0 (NA)
	RG low N	Control	0.0	6.17 ^a	12.8 ^c	125.0 ^b	52.0 (NA)	25.3 (NA)	15.9 (NA)
		NSC supplement	1.3 (8%)	6.11 ^b	9.2 ^d	126.0 ^b	52.0 (NA)	24.1 (NA)	18.5 (NA)
Carruthers et al., 1997 (Indoors)	RG	Control	0.0	6.08 ^a	39.1 ^a	149.4	65.1 (NA)	18.4 (NA)	12.5 (NA)
		NSC supplement	1.1 (7%)	6.00 ^b	32.3 ^b	151.4	64.7 (NA)	19.2 (NA)	12.1 (NA)
García et al., 2000 (Indoors)	WO	Control	0.0	6.10	29.0 ^a	96.7	NA (61.5 ^a)	NA (19.6 ^a)	NA. (13.7 ^{ab})
		Barley (24% starch diet)	2.6 (35%)	6.09	19.4 ^b	90.3	NA (57.8 ^b)	NA (24.9 ^b)	NA (11.9 ^a)
		Corn (24% starch diet)	2.4 (29%)	6.02	26.9 ^c	97.3	NA (60.1 ^b)	NA (21.4 ^c)	NA (13.7 ^b)
Jones-Endsley et al., 1997	A/OG	Corn based	5.6 (31%)	5.90	19.1	102.8	NA (63.5)	NA (21.2 ^a)	NA (11.9)
			8.4 (42%)	5.82	17.6	102.1	NA (62.9)	NA (22.0 ^b)	NA (11.8)
Khalili and Sairanen, 2000	F/T	Control	0	6.13	28.7 ^{ab}	127.0	NA (65.9 ^a)	NA. (19.0)	NA (11.1)
		Barley	4 (NA)	6.17	32.1 ^a	127.0	NA (64.1 ^b)	NA. (19.1)	NA (12.5)
		Barley/oats/beet pulp	4 (NA)	6.01	21.8 ^b	132.0	NA (64.0 ^b)	NA (20.5)	NA (11.6)
Reis and Combs, 2000b	A/RC/RG	Corn	0	6.63	22.4 ^a	98.7	65.7 (NA)	17.8 ^a (NA)	9.9 (NA)
			5 (28%)	6.72	17.7 ^b	99.0	63.1 (NA)	19.9 ^b (NA)	10.9 (NA)
			10 (51%)	6.69	8.1 ^c	104.0	64.2 (NA)	23.9 ^c (NA)	11.3 (NA)
Sayers, 1999 (exp. 2)	RG	Concentrate	5 (28%)	6.00 ^a	13.9	119.3	NA (60.1 ^a)	NA (21.5 ^a)	NA (14.8)

Table 8 continued

			10 (50%)	5.75 ^b	11.7	124.8	NA (56.3 ^b)	NA (25.3 ^b)	NA (14.2)
Van Vuuren et al., 1986	RG	High starch	0.8 (6%)	6.0	32.3 ^a	127.0	NA	NA	NA
		High starch	5.4 (32%)	5.9	22.1 ^b	127.0	NA	NA	NA
		Low starch	5.2 (29%)	5.9	20.4 ^b	130.0	NA	NA	NA

^{a, b, c, d}Means within reference with different superscripts differ ($P < 0.05$).

¹Pasture species: A = alfalfa (*Medicago sativa*); F = meadow fescue (*Festuca pratense*); OG = orchardgrass (*Dactylis glomerata*); RG = perennial ryegrass (*Lolium perenne*); T = timothy (*Phleum pratense*); TF = tall fescue (*Festuca arundinacea*); WC = white clover (*Trifolium repens*); WO = winter oats (*Avena sativa*).

²Between parentheses supplement DMI as percentage of the total DMI.

³Between parentheses individual VFA expressed as molar proportion (mol/100 mol).

⁴Not available.

⁵Non-structural carbohydrate.

Table 9: Effect of type of energy supplementation on mean ruminal fermentation parameters (pH, NH₃-N, and VFA) of dairy cows on pasture-based diets.

Reference	Pasture ¹	Concentrate		pH	NH ₃ -N mg/dl	VFA, mmol/L			
		Type	DMI, ² kg/d			Total	Acetate ³	Propionate ³	Butyrate ³
Alvarez et al., 2001	ARG/WO	Dry cracked corn	5.7 (28%)	5.97	19.1 ^a	90.6	49.5 (NA)	24.3 (NA)	12.2 (NA)
		High moisture corn	6.4 (30%)	6.01	12.9 ^b	86.3	48.7 (NA)	21.9 (NA)	11.4 (NA)
Bargo et al., 1998	A/OG	Dry ground corn	6.2 (35%)	5.70	19.1 ^a	76.1	NA ⁵ (63.7)	NA (20.1)	NA (11.6)
		Steam-flaked corn (290 g/L) ⁴	6.6 (40%)	5.63	13.8 ^b	72.0	NA (60.9)	NA (22.8)	NA (11.6)
García et al., 2000 (Indoors)	WO	Barley (24% starch diet)	2.6 (35%)	6.09	19.4 ^a	90.3	NA (57.8)	NA (24.9 ^a)	NA (11.9 ^a)
		Corn (24% starch diet)	2.4 (29%)	6.02	26.9 ^b	97.3	NA (60.1)	NA (21.4 ^b)	NA (13.7 ^b)
Khalili and Sairanen, 2000	F/T	Barley	4 (NA)	6.17	32.1 ^a	127.0	NA (64.1)	NA (19.1)	NA (12.5)
		Barley/oats/beet pulp	4 (NA)	6.01	21.8 ^b	132.0	NA (64.0)	NA (20.5)	NA (11.6)
Kolver et al., 1998 (Indoors)	OG	Corn based (fed with pasture)	9.2 (49%)	6.06 ^a	16.3	118.0	75.3	24.7	13.5 ^a
		Corn based (fed 4 h after pasture)	9.1 (47%)	6.17 ^b	18.4	114.3	72.5	24.6	12.6 ^b
Reis and Combs, 2000a	A/RC/OG/B	Dry ground corn (680 g/L) ⁴	9 (45%)	6.57	13.3	117.3	74.5	29.9	13.6 ^a
		Steam-rolled corn (591 g/L)	9 (45%)	6.48	12.6	120.5	72.7	32.0	15.8 ^b
Sayers, 1999 (exp. 2)	RG	Starch based	5 to 10 (28 to 50%)	5.80	12.0	121.6	NA (56.1 ^a)	NA (26.1 ^a)	NA (13.7 ^a)
		Fiber based	5 to 10 (28 to 50%)	5.96	13.6	122.5	NA (60.3 ^b)	NA (20.7 ^b)	NA (15.4 ^b)
Soriano et al., 2000	OG	Coarsely ground corn	6 (NA)	NA	31.0	NA	NA	NA	NA
		High moisture corn	6 (NA)	NA	26.2	NA	NA	NA	NA
Van Vuuren et al., 1986	RG	High starch concentrate	5.4 (32%)	5.90	22.1	127.0	NA	NA	NA
		Low starch concentrate	5.2 (29%)	5.90	20.4	130.0	NA	NA	NA

^{a, b, c, d}Means within reference with different superscripts differ ($P < 0.05$). ¹Pasture species: A = alfalfa (*Medicago sativa*); B = brome grass (*Bromus inermis*); F = meadow fescue (*Festuca pratense*); OG = orchardgrass (*Dactylis glomerata*); RC = red clover (*Trifolium pratense*); RG = perennial ryegrass (*Lolium perenne*); T = timothy (*Phleum pratense*); WO = winter oats (*Avena sativa*). ²Between parentheses supplement DMI as percentage of the total DMI.

³Between parentheses individual VFA expressed as molar proportion (mol/100 mol).

⁴Density.

⁵Not available.

Table 10: Effect of supplementation on in situ DM degradation kinetics of pasture.

Reference	Basal diet ²	Sample form	Supplement	DMI, kg/d	Parameters ¹			
			Type		a	b	kd	ED
Bargo et al., 2001	WO	Fresh	Corn/sunflower meal (15.3, 39.2) ³	6.2	57.3	33.3	7.8	76.1
		Meat mincer	Corn/sunflower meal (23.3, 35.8)	6.4	57.4	33.2	8.1	76.4
			Corn/feather meal (24.6, 46.3)	6.4	58.3	31.4	9.4	77.4
Reis and Combs, 2000a	A/RC/OG/B	Frozen masticates	Dry corn	9	47.5	40.8	12.1 ^a	74.8
		Ground	Dry corn + hay	9 + 3.2	49.0	38.8	12.2 ^a	75.0
			Steam-rolled corn	9	47.8	40.8	11.4 ^b	74.5
			Steam-rolled corn + hay	9 + 3.2	46.8	42.3	11.3 ^b	74.0
Reis and Combs, 2000b	A/RC/RG	Frozen masticates	Corn based	0	47.9	39.4 ^a	10.5	73.0
		Ground		5	48.8	37.6 ^{ab}	10.1	72.4
				10	48.6	36.3 ^b	11.1	72.2
Sayers, 1999 (exp. 1) (Indoors)	RG	Fresh 10 mm	Control	0	26.8	63.6	7.6	58.0
			Concentrate 10%CP	2.6	26.1	64.2	7.5	56.0
			Concentrate 34%CP	2.6	26.8	62.8	6.5	55.0
			Concentrate 10%CP	5.2	27.0	63.9	6.7	56.0
			Concentrate 34%CP	5.2	26.5	63.0	6.5	54.0
Sayers, 1999 (exp. 2)	RG	Fresh	Fiber based concentrate	5	22.5	68.4	7.8	55.0
		10 mm	Starch based concentrate	5	21.9	71.6	6.7	54.0
			Fiber based concentrate	10	21.9	67.2	6.8	52.0
			Starch based concentrate	10	23.9	65.4	6.1	50.0

^{a, b, c}Means within reference with different superscripts differ ($P < 0.05$).

¹a = soluble fraction (%); b = insoluble potentially degradable fraction (%); kd = rate of degradation (%/h) of the b fraction; ED = effective degradability (%): $ED = a + b \times (kd / (kd + kp))$, where kp = rate of passage

²Pasture species: A = alfalfa (*Medicago sativa*), RC = red clover (*Trifolium pratense*), RG: perennial ryegrass (*Lolium perenne*), WO = winter oats (*Avena sativa*).

³Between parentheses: % CP and %RUP/CP, respectively.

Table 11: Effect of supplementation on in situ CP degradation kinetics of pasture.

Reference	Basal diet ²	Sample form	Supplement	DMI, kg/d	Parameters ¹			
			Type		a	b	kd	ED
Bargo et al., 2001	WO	Fresh	Corn/sunflower meal (15.3, 39.2) ³	6.2	62.4	31.7	9.8	82.1
		Meat mincer	Corn/sunflower meal (23.3, 35.8)	6.4	61.7	32.9	10.2	82.4
			Corn/feather meal (24.6, 46.3)	6.4	63.6	29.3	9.8	81.8
Elizalde et al., 1992	WO	Fresh	Control	0.0	41.8	58.2	4.1	65.3
		Meat mincer	Corn silage	5.0	34.7	65.3	4.6	63.0
Sayers, 1999 (exp. 1) (Indoors)	RG	Fresh 10 mm	Control	0.0	31.8	64.5	9.9	67.0
			Concentrate 10%CP	2.6	30.2	65.6	10.1	66.0
			Concentrate 34%CP	2.6	29.9	66.2	9.0	65.0
			Concentrate 10%CP	5.2	30.5	65.1	9.4	65.0
			Concentrate 34%CP	5.2	29.7	66.3	8.4	63.0
Sayers, 1999 (exp. 2)	RG	Fresh 10 mm	Fiber based concentrate	5.0	8.1	90.5	9.9	57.0
			Starch based concentrate	5.0	7.3	92.0	9.4	57.0
			Fiber based concentrate	10.0	9.2	89.1	9.0	56.0
			Starch based concentrate	10.0	15.2	83.0	7.9	56.0

^{a, b, c} Means within reference with different superscripts differ ($P < 0.05$).

¹ a = soluble fraction (%); b = insoluble potentially degradable fraction (%); kd = rate of degradation (%/h) of b fraction; ED = effective degradability (%): $ED = A + B \times (kd / (kd + kp))$, where kp = rate of passage

² Pasture species: RG = perennial ryegrass (*Lolium perenne*); WO = winter oats (*Winter oats*).

³ Between parentheses: % CP and %RUP/CP, respectively.

Table 12: Effect of supplementation on in situ NDF degradation kinetics of pasture.

Reference	Basal diet ²	Sample form	Supplement	DMI, kg/d	Parameters ¹				
			Type		a	b	kd	Lag	ED
Bargo et al., 2001	WO	Fresh	Corn/sunflower meal (15.3, 39.2) ³	6.2	0.0	82.5	5.2	0.00	NA
		Meat mincer	Corn/sunflower meal (23.3, 35.8)	6.4	0.0	81.9	4.9	0.61	NA
			Corn/feather meal (24.6, 46.3)	6.4	0.0	80.7	5.3	0.00	NA
Elizalde et al., 1992	WO	Fresh	Control	0	0.0	68.3	4.4	1.4 ^a	28.9
		Meat mincer	Corn silage	5	0.0	67.6	5.3	5.8 ^b	31.7

^{a, b, c}Means within reference with different superscripts differ ($P < 0.05$).

¹ a = soluble fraction (%); b = insoluble potentially degradable fraction (%); Lag = lag time (h); kd = rate of degradation (%/h); ED = effective degradability (%): $ED = A + B \times (kd / (kd + kp))$, where kp = rate of passage

²Pasture species: WO: winter oats (*Avena sativa*).

³Between parentheses: % CP and %RUP/CP, respectively.

Table 13: Effect of supplementation on OM digestion of dairy cows on pasture-based diets.

Reference	Pasture ²	Supplement		Organic matter ¹			
		Type	DMI or OMI, ³ kg/d	OMI, kg/d	TTAD, %	RAD, %	RADD, %
Berzaghi et al., 1996 (Grazing)	TF/OG/WC	Control	0.0	13.0	71.9 ^a	48.9 ^a	68.1
		Cracked corn	5.4 (36%)	15.2	69.9 ^b	43.3 ^b	62.3
Delagarde et al., 1997 (Grazing)	RG (0 kg N/ha)	Control	0.0	13.0 ^a	77.8 ^a	NA ⁴	68.0
		SBM (48% CP)	2 (13%)	15.5 ^b	79.1 ^b	NA	66.9
	RG (60 kg N/ha)	Control	0	15.6 ^c	80.9 ^c	NA	67.7
		SBM (48% CP)	2 (12%)	16.9 ^d	81.8 ^d	NA	66.3
García et al., 2000 (Indoors)	WO	Control	0.0	7.0	81.5	59.4	72.7
		Barley (24% starch diet)	2.6 (35%)	6.7	81.2	54.1	66.5
		Corn (24% starch diet)	2.4 (29%)	7.5	81.6	54.6	66.9
Jones-Endsley et al., 1997 (Grazing)	A/OG	Concentrate (12% CP)	6.9 (38%)	16.7 ^a	73.4 ^a	NA	NA
		Concentrate (16% CP)	7.0 (35%)	18.2 ^b	75.7 ^b	NA	NA
		Offered 6.4 kg/d	5.6 (31%)	16.5 ^a	74.6 ^b	NA	NA
		Offered 9.6 kg/d	8.4 (42%)	18.4 ^b	74.5 ^b	NA	NA
Van Vuuren et al., 1993 (Indoors)	RG	Control	1.7 ^a (10%)	14.6	78.6	59.4 ^a	75.6 ^a
		Starch based concentrate	7.0 ^b (43%)	14.8	78.0	51.2 ^b	65.7 ^b
		Fiber based concentrate	7.2 ^c (44%)	14.8	78.9	60.6 ^a	76.7 ^a

^{a, b, c, d} Means within reference with different superscripts differ ($P < 0.05$; unless other stated: Berzaghi et al., 1996; Jones-Endsley et al., 1997; $P < 0.10$).

¹OMI = total organic matter intake; TTAD = total tract apparent digestibility; RAD = rumen apparent digestibility (% of intake); RADD = rumen apparent digestibility (% of total digested).

²Pasture species: A = alfalfa (*Medicago sativa*); OG = orchardgrass (*Dactylis glomerata*); RG = perennial ryegrass (*Lolium perenne*); TF = tall fescue (*Festuca arundinacea*); WC = white clover (*Trifolium repens*); WO = winter oats (*Avena sativa*).

³Between parentheses supplement DMI as percentage of the total DMI.

⁴Not available.

Table 14: Effect of supplementation on NDF digestion of dairy cows on pasture-based diets.

Reference	Pasture ²	Supplement		NDF ¹		
		Type	DMI or OMI, ³ kg/d	NDFI, kg/d	TTAD, %	RADD, %
Berzaghi et al., 1996 (Grazing)	TF/OG/WC	Control	0.0	7.4	70.4 ^a	88.1
		Cracked corn	5.4 (36%)	6.2	64.5 ^b	82.6
García et al., 2000 (Indoors)	WO	Control	0.0	3.2 ^a	60.0	71.5
		Barley (24% starch diet)	2.6 (35%)	2.4 ^b	56.2	75.5
		Corn (24% starch diet)	2.4 (29%)	2.8 ^b	60.1	84.4
Jones-Endsley et al., 1997 (Grazing)	A/OG	Concentrate (12% CP)	6.9 (38%)	9.1 ^a	65.2 ^a	NA ⁴
		Concentrate (16% CP)	7.0 (35%)	10.1 ^b	68.1 ^b	NA
		Offered 6.4 kg/d	5.6 (31%)	9.1 ^a	65.2 ^a	NA
		Offered 9.6 kg/d	8.4 (42%)	10.1 ^b	68.1 ^b	NA
Van Vuuren et al., 1993 (Indoors)	RG	Control	1.7 ^a (10%)	6.61 ^a	78.7 ^{ab}	95.0 ^a
		Starch based concentrate	7.0 ^b (43%)	5.73 ^b	74.5 ^b	85.6 ^b
		Fiber based concentrate	7.2 ^c (44%)	6.72 ^c	79.2 ^a	93.8 ^a

^{a, b, c, d}Means within reference with different superscripts differ ($P < 0.05$; unless other stated: Berzaghi et al., 1996; Jones-Endsley et al., 1997; $P < 0.10$).

¹NDFI = total organic matter intake; TTAD = total tract apparent digestibility; RADD = rumen apparent digestibility (% of total digested)

²Pasture species: A = alfalfa (*Medicago sativa*); OG = orchardgrass (*Dactylis glomerata*); RG = perennial ryegrass (*Lolium perenne*); TF = tall fescue (*Festuca arundinacea*); WC = white clover (*Trifolium repens*); WO = winter oats (*Avena sativa*).

³Between parentheses supplement DMI as percentage of the total DMI.

⁴Not available.

Table 15: Effect of supplementation on nitrogen (N) digestion of dairy cows on pasture-based diets.

Reference	Pasture ²	Supplement		N intake g/d	Flow to the duodenum ¹			Efficiency ²	
		Type	DMI or OMI, ³ kg/d		NAN	NANMN	MN	AD	TD
Berzaghi et al., 1996 (Grazing)	TF/OG/WC	Control	0.0	522 ^a	371 (71.1)	128 (24.9)	243	39	29
		Cracked corn	5.4 (36%)	391 ^b	396 (84.1)	123 (26.2)	273	40	31
Delagarde et al., 1997 (Grazing)	RG (0 kg N/ha)	Control	0	309 ^a	394 ^a (127.5 ^a)	NA	NA	NA	NA
		SBM (48% CP)	2 (13%)	466 ^b	518 ^b (111.2 ^b)	NA	NA	NA	NA
	RG (60 kg N/ha)	Control	0	584 ^c	472 ^c (80.8 ^c)	NA	NA	NA	NA
		SBM (48% CP)	2 (12%)	706 ^d	570 ^d (80.7 ^c)	NA	NA	NA	NA
García et al., 2000 (Indoors)	WO	Control	0	320 ^a	162 (53.0 ^a)	59 (19.1)	102	25	20
		Barley (24% starch of diet)	2.6 (35%)	222 ^b	143 (61.7 ^b)	40 (18.3)	103	28	22
		Corn (24% starch of diet)	2.4 (29%)	273 ^b	159 (59.0 ^b)	64 (23.7)	94	23	18
Jones-Endsley et al., 1997 (Grazing)	A/OG	Concentrate (12% CP)	6.9 (38%)	587 ^a	446 ^a (75.9)	144 ^a (25.4)	302	78	45
		Concentrate (16% CP)	7.0 (35%)	690 ^b	504 ^b (73.0)	187 ^b (27.4)	317	67	40
		Offered 6.4 kg/d	5.6 (31%)	620	461 (74.4)	157 ^a (26.4)	303	74	42
		Offered 9.6 kg/d	8.4 (42%)	656	489 (74.5)	174 ^b (26.3)	315	71	42
Van Vuuren et al., 1993 (Indoors)	RG	Control	1.7 ^a (10%)	459 ^a	409 (89.1)	NA	254	29.4 ^a	NA
		Starch based concentrate	7.0 ^b (43%)	441 ^b	424 (96.1)	NA	261	34.3 ^b	NA
		Fiber based concentrate	7.2 ^c (44%)	428 ^c	396 (96.8)	NA	243	27.2 ^a	NA

^{a, b, c, d} Means within reference with different superscripts differ ($P < 0.05$; unless other stated: Berzaghi et al., 1996; Jones-Endsley et al., 1997; $P < 0.10$).

¹NAN = nonammonia N (g/d, between parentheses % of N intake); NANMN = nonammonia nonmicrobial N (g/d, between parentheses as % of N intake); MN = microbial N (g/d).

²AD = efficiency of microbial protein synthesis (g microbial N/kg of OM apparently digested in the rumen); TD = efficiency of microbial protein synthesis (g microbial N/kg of OM truly digested in the rumen)

³Pasture species: A = alfalfa (*Medicago sativa*); OG = orchardgrass (*Dactylis glomerata*); RG = perennial ryegrass (*Lolium perenne*); TF = tall fescue (*Festuca arundinacea*); WC = white clover (*Trifolium repens*); WO = winter oats (*Avena sativa*).

³Between parentheses supplement DMI as percentage of the total DMI.

⁴Not available.

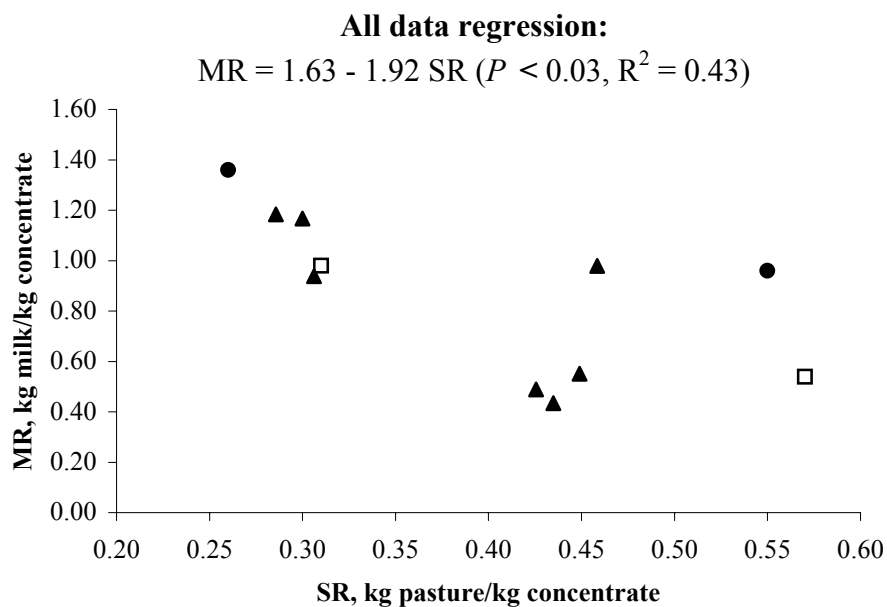


Figure 1: Relationship between milk response (MR) and substitution rate (SR) by grazing dairy cows supplemented with concentrate on studies evaluating the effect of pasture allowance (● Bargo et al., 2002; □ Robaina et al., 1998; ▲ Stockdale, 1999a).

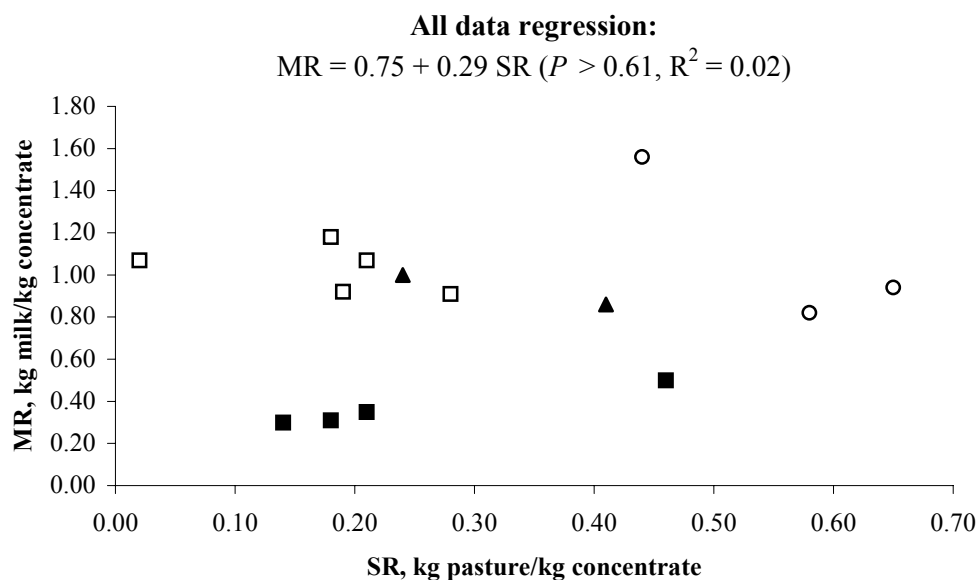


Figure 2: Relationship between milk response (MR) and substitution rate (SR) by grazing dairy cows supplemented with concentrate on studies evaluating the effect of concentrate level (■ Dillon et al., 1997; ▲ Reis and Combs, 2000b; ○ Robaina et al., 1998; □ Walker et al., 2001).

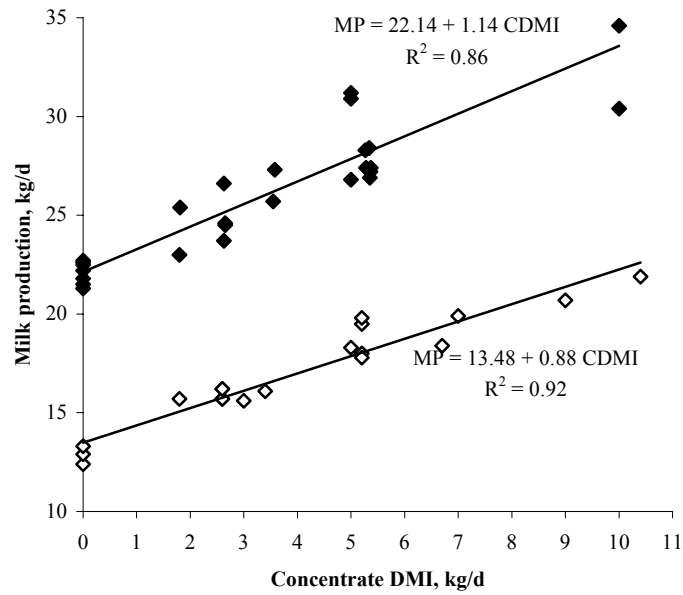


Figure 3: Relationship between milk production (MP) and concentrate DMI (CDMI) by grazing dairy cows supplemented with different levels of concentrate (♦ studies with cows < 90 DIM or > 28 kg milk/d at the beginning of the experiment; Delaby et al., 2001 (exp. 1, 2, and 3); Reis and Combs, 2000b; Sayers, 1999 (exp. 2); ◇ studies with cows > 160 DIM or < 23 kg milk/d at the beginning of the experiment; Robaina et al., 1998; Sayers, 1999 (exp. 1); Walker et al., 2001).

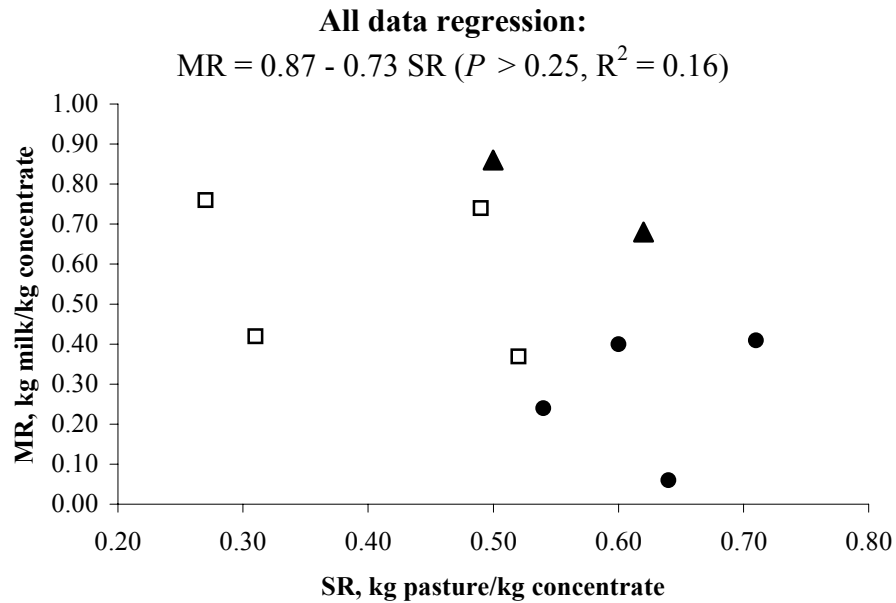


Figure 4: Relationship between milk response (MR) and substitution rate (SR) by grazing dairy cows supplemented with concentrate on studies evaluating the effect of type (starch or fiber-based) of concentrate (▲ Sayers, 1999 (exp. 2); □ Schwarz et al., 1995; ● Spörndly, 1991).

Chapter 3

Milk Response to Concentrate Supplementation of High Producing Dairy Cows Grazing at Two Pasture Allowances

3.1 ABSTRACT

Twenty multiparous Holstein cows (4 ruminally cannulated) in five 4 x 4 Latin squares with 21-d periods were used to study the effect of concentrate supplementation when grazed at two pasture allowances. The four dietary treatments resulted from the combination of two pasture allowance targets (low, 25 vs. high, 40 kg DM/cow per d) and two concentrate supplementation levels (zero vs. 1 kg concentrate/4 kg milk). Concentrate supplementation decreased pasture dry matter intake 2.0 kg/d at the low pasture allowance (17.5 vs. 15.5 kg/d) and 4.4 kg/d at the high pasture allowance (20.5 vs. 16.1 kg/d). Substitution rate was lower at the low pasture allowance (0.26 kg pasture/kg concentrate) than at the high pasture allowance (0.55 kg pasture/kg concentrate). Total DMI of both supplemented treatments averaged 24.4 kg/d. Milk production of both supplemented treatments averaged 29.8 kg/d, but was increased with higher pasture allowance in the unsupplemented treatments (19.1 vs. 22.2 kg/d). Milk response to concentrate supplementation was 1.36 and 0.96 kg milk/kg concentrate for the low and high pasture allowance, respectively. Concentrate supplementation reduced milk fat percentage but increased milk protein percentage. Rumen pH and $\text{NH}_3\text{-N}$ concentration were decreased by concentrate supplementation. Substitution rate was likely related to both negative associative effects in the rumen (reductions in rumen pH, rate of pasture digestion and NDF digestibility)

and reductions in grazing time. This latter was quantitatively more important, explaining between 80 to 100% of the reduction in pasture DMI observed.

(Key words: pasture allowance, concentrate supplementation, substitution rate, milk, rumen fermentation, grazing behavior)

Abbreviation key: CS = concentrate supplementation, **Low PA - U** = low pasture allowance – unsupplemented, **Low PA - CS** = low pasture allowance – concentrate supplementation, **High PA - U** = high pasture allowance – unsupplemented, **High PA - CS** = high pasture allowance – concentrate supplementation, **IVDMD** = in vitro dry matter digestibility, **NH₃-N** = ammonia N, **PA** = pasture allowance

3.2 INTRODUCTION

Energy is the first limiting nutrient for high producing cows grazing high quality pastures as the only feed (Kolver and Muller, 1998). Compared with cows on a nutritionally balanced TMR, early lactation cows grazing high quality pasture with no supplement had lower total DMI (19.0 vs. 23.4 kg/d) and milk production (29.6 vs. 44.1 kg/d). While the intake of CP and NDF did not differ between cows fed pasture and TMR, the intake of DM and NE_L was significantly lower in the pasture fed cows, suggesting that high producing dairy cows on pasture need supplemental energy to reach their genetic potential for intake and milk production.

Substitution rate is defined as the decrease in pasture intake per kg of supplemental feed (Kellaway and Porta, 1993). Several animal, pasture and supplement factors affect substitution

rate, including pasture allowance, amount of concentrate fed, digestibility of pasture, chemical and physical properties of the concentrate, and stage of lactation (Kellaway and Porta, 1993). Among these factors, the amount of pasture offered per cow daily or pasture allowance (**PA**) has a major effect on substitution rate. Several studies have reported that substitution rate is greater at high PA (Grainger and Mathews, 1989; Meijs and Hoekstra, 1984; Robaina et al., 1998; Stakelum, 1986). Research in Ireland (Stakelum, 1986) found substitution rates in the range of 0.24 to 0.28 and 0.59 to 0.61 for PA of 16 and 24 kg DM/cow/d, respectively. Robaina et al. (1998) reported a 0.57 kg reduction in pasture intake/kg grain at a PA of 42.3 kg DM/cow/d, and 0.31 kg pasture /kg supplement at a PA of 20 kg DM/cow/d. All of these studies were conducted with dairy cows producing less than 20 kg/d of milk.

Substitution rate is a major factor contributing to the variation seen in milk response to concentrate supplementation (**CS**) (Kellaway and Porta, 1993). The lower the substitution rate the greater the milk response obtained per kg of supplement. In the review of Kellaway and Porta (1993), which summarized research primarily with cows producing less than 20 kg/d of milk, milk response averaged 0.6 kg milk/kg concentrate when cows grazed restricted pasture (low PA) and almost 0 kg milk/kg concentrate when cows grazed pasture ad libitum (high PA). There is a lack of information on substitution rate and milk response to CS of high producing dairy cows grazing at different PA. We hypothesized that high producing dairy cows have a lower substitution rate and higher milk response to CS than reported with low producing dairy cows.

The causes of substitution rate are not well understood. Some authors (Dixon and Stockdale, 1999) suggested that substitution rate is caused by negative associative effects in the rumen. The energy provided by the concentrate (fermentable carbohydrates) may lead to reductions in rumen pH, reducing the rate of pasture fiber digestion, and resulting in a reduced

pasture intake (Dixon and Stockdale, 1999). Others (McGilloway and Mayne, 1996) have suggested that substitution rate is produced by a reduction in grazing time when cows are fed supplemental concentrate. McGilloway and Mayne (1996) reported reductions in grazing time of 15 to 22 min/kg of concentrate. Recently, Sayers (1999) found a reduction in grazing time between 16 to 20 min/kg of concentrate when amount of concentrate was increased from 5 to 10 kg/d.

The objectives of this study were to determine the substitution rate and milk response to CS of high producing dairy cows grazing at two PA, and to identify the factors related to the substitution rate.

3.3 MATERIALS AND METHODS

3.3.1 Cows and treatments

Twenty multiparous Holstein cows (4 fitted with ruminal cannulas) [BW, 631 ± 71 kg; milk yield, 45.8 ± 6.6 kg/d; parity, 2.8 ± 0.8 ; DIM, 101 ± 35 (mean $\pm$ SD)] were blocked by days in milk (one block of ruminally cannulated cows) and randomly assigned to four dietary treatments within five 4×4 Latin squares with 21-d periods. Cows were selected from The Pennsylvania State University Dairy Cattle Research and Education Center (University Park, PA), which averaged 11436 kg of milk and 363 kg of protein per lactation in 1999. The first two periods were conducted during the spring (period 1 from May 10 to May 30; period 2 from May 31 to June 20), and the last two periods were conducted during the fall (period 3 from September

3 to September 23; period 4 from September 24 to October 14) of 1999. Between the spring periods and fall periods, all cows were kept indoors in a tie-stall and fed a nutritionally balanced TMR with the objective of delete any carryover effect of treatments. This period of time also helped to avoid the drought period that usually occurs during the summer, which decreases both quality of pasture fed and quantity of pasture availability.

The four dietary treatments were arranged in a 2 x 2 factorial. Factors evaluated were PA (low vs. high) and CS (unsupplemented vs. supplemented). The four dietary treatments resulted from the combination of the two levels of these two factors: low PA – unsupplemented (Low PA - U); low PA – concentrate supplementation (Low PA - CS); high PA – unsupplemented (High PA - U); and high PA – concentrate supplementation (High PA - CS).

Pasture allowance targets were 25 and 40 kg DM/cow per d to the ground level for the low and high PA treatments, respectively. To achieve these targets, pre-grazing pasture mass was measured every two days to adjust the size of the paddock and the amount of pasture offered per cow on a daily basis (PA). Pre-grazing pasture mass (kg DM/ha) was measured by cutting ten quadrants (0.124 m²/quadrant) of pasture to ground level, and drying at 55 °C in a forced air oven. A new paddock was constructed daily using a temporary polywire. A second polywire fence was used to prevent back-grazing. New paddocks were given to the cows each morning at approximately 0700 h after the morning milking. Half of the cows (n = 10) grazed at low PA and half of the cows (n = 10) grazed at high PA divided by a temporary polywire. Pasture botanical composition was measured at the end of each of the four periods and averaged 50% smooth brome grass (*Bromus inermis* L.), 33% orchardgrass (*Dactylis glomerata* L.), 7% Kentucky bluegrass (*Poa pratensis* L.), and 10% weeds and dead material. Pasture was fertilized with

nitrogen before the beginning of the first period (April 25) and the beginning of the third period (August 18) at a rate of 50 kg N/ha.

Unsupplemented cows received no CS but were fed a mineral-vitamin mix at a rate of 1 kg/d per cow to avoid mineral and vitamin deficiencies. The actual amount of concentrate offered per cow in the supplemented group was 1 kg of concentrate/4 kg of milk at the beginning of period 1 using the pre-trial milk production. An upper limit of 10 kg DM/cow per d was fixed for those cows producing more than 40 kg/d of milk. The amount of concentrate offered was also adjusted before the beginning of period 3 using the milk production level prior to that period. Both the mineral-vitamin and the concentrate mixes were fed individually in two equal feedings after each milking. The ingredient and chemical composition of the mineral-vitamin and the concentrate mixes used for the unsupplemented and supplemented cows, respectively, is shown in *Table 16*. Cows were milked twice daily at 0600 and 1730. Walking distance from pasture to the milking parlor averaged 0.9 km (range: 0.75 to 1.20 km).

3.3.2 Experimental Measures and Sample Analyses

During each of the four 21-d periods, the first 10 d were used to adjust the cows to the different dietary treatments and the last 11 d were used for experimental measures.

Intake was estimated from d 15 to 19 during each period using Cr_2O_3 as an indigestible fecal marker. The indigestible fecal marker Cr_2O_3 was dosed twice daily (10 g Cr_2O_3 /d) after each milking (0630 h and 1800 h, approximately) for ten days beginning on d 10 of each period. Fecal grab samples were collected at 0630 h and 1800 h from d 15 to d 19 to estimate DMI, and immediately frozen (- 20 °C). After d 19, fecal grab samples were collected at the same hours for

three additional days but only in the four rumen cannulated cows to estimate rate of passage (Grovum and Williams, 1973), and immediately frozen (- 20 °C).

During the same days that fecal samples were taken (d 15 to 19), samples of the mineral-vitamin and the concentrate mixes were collected daily and pasture samples were plucked by hand twice daily after each milking to the approximate height to which cows grazed. Samples were dried at 55 °C in a forced air oven and ground through a 1-mm screen (Wiley Mill, Thomas Scientific, Philadelphia, PA). Mineral-vitamin mix and concentrate samples were composited by period, while hand-plucked pasture samples were composited by day. Composited mineral-vitamin mix, concentrate and hand-plucked pasture samples were analyzed for DM, CP, and ash (AOAC, 1990), soluble CP (Krishnamoorthy et al., 1982), ADF and NDF (Ankom Daisy II, ANKOM Technology Corp., Fairport, NY), total nonstructural carbohydrate (TNC) (Smith, 1981; modified to use potassium ferricyanide as the colorimetric indicator), and in vitro DM digestibility (IVDMD) (Ankom Daisy II, ANKOM Technology Corp., Fairport, NY).

Fecal samples were thawed, dried at 55 °C in a forced air oven, and ground through a 1-mm screen (Wiley Mill, Thomas Scientific, Philadelphia, PA). A composited sample per cow was made for each period. Fecal samples were analyzed for CP (AOAC, 1990), ADF and NDF (Ankom Daisy II, ANKOM Technology Corp., Fairport, NY), and Cr (Parker et al., 1989). Fecal Cr was used to calculate fecal output, and then pasture and total DMI as described by Kolver et al. (1998). Apparent digestibilities of DM, NDF, and CP were determined with the fecal output estimated using Cr₂O₃ as marker and DMI intake, and the nutrient concentration in feces and feed.

Ruminal fluid samples from the four cannulated cows were collected during d 20 at 0, 4, 8, 12, 16, and 20 h beginning at 0530 h. At 0 h (0530 h) and 12 h (1730 h), cows were sampled

indoors because these two sampling times coincided with the a.m. and p.m. milking, respectively. At the other sampling times, cows were sampled in the pasture using a temporary construction made with mobile gates in the paddock. Ruminal digesta samples were taken from the dorsal, ventral and caudal area of the rumen, and squeezed through four layers of cheesecloth. The pH of the filtered ruminal fluid was measured immediately using a portable digital Cole Palmer pH-meter, and 15-ml aliquot preserved with 3 ml of 25% metaphosphoric acid and 3 ml of 0.6% 2-ethyl butyric acid (internal standard). These samples were stored at -20°C and subsequently analyzed for $\text{NH}_3\text{-N}$ and VFA (Yang and Varga, 1989).

The in situ technique was used to estimate ruminal disappearance of DM, CP and NDF of pasture in the different treatments. Hand-plucked pasture samples were taken the day before the bags were incubated (d 19), cut into lengths of approximately 1 cm and placed in polyester bags (Marvelier White, Strauss Co., New York, NY) with a mean pore size of $52\text{ }\mu\text{m}$ as described by Lykos and Varga (1995). Bags were incubated in the rumen of the four cannulated cows for 0, 4, 8, 12, 16, 20, 24 and 48 h after being soaked in 39°C distilled water for 15 min beginning at 0530 h on d 20. Bags were inserted in reverse order and the 24, 20, 16, 12, 8 and 4 h incubation times coincided with rumen fluid sampling times.

Blood samples were collected at 0630 h on d 14 (before the cows receive the mineral-vitamin mix or concentrate) from the coccygeal vessels as described by Kolver and Muller (1998). Plasma was analyzed for glucose (Glucose Kit no. 510, Sigma Chemical Co., St. Louis, MO), urea nitrogen (Stanbio Urea Nitrogen Kit 580, Stanbio Laboratory, Inc., San Antonio, TX), and non-esterified fatty acids (NEFA) (Wako NEFA C-Kit no. 990-75401, Wako Chemicals USA, Inc., Richmond, VA).

Spot urine samples were taken by vulval stimulation twice daily after each milking on two consecutive days (d 14 and 15). Samples were acidified with HCl to maintain pH below 2 and stored at -20°C . Urine samples were thawed, a composited sample per cow was made for each period, and analyzed for allantoin (Chen, 1989) and creatinine (Sigma Kit no. 555-A; Sigma Chemical Co., St. Louis, MO).

Between periods 2 and 3, when all cows were on a TMR diet, total urine was collected from eight cows (two of the Latin squares) with the purpose of estimating daily creatinine excretion as an internal marker to estimate the total urine excretion (Valadares et al., 1999) during the grazing periods. Cows were kept and milked in metabolic stalls using individual portable milking units and total urine was collected during 4 consecutive days with indwelling Folley catheters (24 French, 75-ml balloons) as described by Valadares et al. (1999). One mean daily creatinine excretion value (mg/kg of BW per d) was computed for each of the eight cows (Valadares et al., 1999). Total urine volume in grazing Periods 1, 2, 3 and 4 were estimated using the daily creatinine excretion value and the creatinine concentration in the spot urine samples taken in each period as: $\text{urine (L/d)} = \text{BW} \left(\frac{\text{total creatinine excretion in mg/kg of BW per d}}{\text{creatinine concentration in the spot urine samples in mg/L}} \right)$ (Valadares et al., 1999). Total urine in each of the periods was used to estimate the total excretion of N in urine and, together with the N excretion in feces and milk, efficiency of N utilization was calculated.

Grazing behavior and ruminating time were measured in 16 cows from d 10 to 16 using the automatic IGER behavior recorders (Rutter et al., 1997). Each day recorders were placed on one cow per treatment, twice daily after each milking and before cows were moved to the pasture. During the morning, recorders were placed on cows from 0730 h to 1730 h (10 hours). Before the evening milking, recorders were removed from the cows to avoid damages during

milking and feeding time, and also to download the information recorded during that period of time. During the night recorders were placed on cows from 1830 h to 0600 h (12 hours and 30 minutes). Before the morning milking, recorders were removed and the information downloaded. In average, total time of recorders on the cows was 22 hours and 30 minutes. Recorders were placed on cows during each of the four periods, however, because of problems with the equipment, no data was recorded during period 1. Data from periods 2, 3 and 4 were analyzed as a completed randomized experimental design with treatments as the only variable. A total final number of nine records per treatment were used in the analysis using the software IGER GRAZETM (GRAZE User's guide, documentation version 1.0, program version 0.74).

Milk production was recorded daily from d 11 to 21 during each period. Milk samples were collected three times on d 13, 16 and 19 and preserved with 2-bromo-2-nitropropane-1,3 diol. Milk fat, total protein, true protein and lactose were analyzed by infrared spectrophotometry (Foss 605B Milk-Scan; Foss Electric, Hillerod, Denmark) by the Pennsylvania DHIA milk testing laboratory.

Cows were weighed after evening milking on two consecutive days at the beginning (d 1 and 2) and at the end (d 20 and 21) of each period. The same days, the body condition of the cows was scored by two experimented independent observers using the five-point body condition score (BCS) scale (1 = thin; 5 = fat) (Wildman et al., 1982).

3.3.3 Statistical Analyses

A total of 20 cows were initially assigned to the trial. One cow (rumen cannulated) on the treatment Low PA - U was removed from the experiment during period 1 because of health

problems not related with the treatment. One cow on treatments Low PA - U and High PA - U during periods 1 and 2 died after the end of the period 2. Before period 4, one cow assigned to treatment High PA - U in this period was dried off because of advanced stage of lactation (> 300 DIM). The final numbers of cows per treatment was 19 because of one missing cow during period 1 in treatment Low PA - U, one missing cow during period 3 in treatment Low PA - CS, one missing cow during period 4 in treatment High PA - CS, and one missing cow during period 4 in treatment High PA - U.

Performance data (intake, milk production and milk composition) were analyzed as a 4×4 Latin square, replicated five times with a 2×2 factorial arrangement of treatments by the general linear models procedure of SAS (1999). The model included square, cow-within-square, period, main effect of pasture allowance, main effect of CS, interaction between PA and CS, plus the interactions between square and the other factors (square by period, square by PA, square by CS, and square by PA by CS). Both square and cow-within-square were considered random effects, while the others were considered fixed effects.

Rumen data (rumen fermentation and in situ data) were analyzed as a 4×4 Latin square with a 2×2 factorial arrangement of treatments by the general linear models procedure of SAS (1999). The model included cow, period, main effect of PA, main effect of CS, interaction between PA and CS, hour, plus the interactions between hour and the other factors (hour by cow, hour by period, hour by PA, hour by CS, and hour by PA by CS). Cow effect was considered random, while the others were considered fixed effects.

When significant ($P < 0.05$) effects due to dietary treatments were detected, mean separation was conducted by the PDIFF option in SAS (1999). All means presented are least squares means.

3.4 RESULTS AND DISCUSSION

3.4.1 Weather data

Mean precipitations and the average high and low temperatures for each period and the average of the previous ten years (1988-1998) are summarized in *Table 17*. Only period 2 had lower precipitations compared with the previous ten years average precipitations (a shortage of 29 mm), which could affected pasture quality. On the first day of the experiment and period 1 (May 10), sunrise occurred at 0500 h and sunset at 1917 h, while the last day of the period 2 (June 20) sunrise occurred at 0440 h and sunset at 1946 h. On average, day length during periods 1 and 2 was 14 h 41 min. The first day of period 3 (September 3), sunrise occurred at 0540 h and sunset at 1843 h; the last day of the experiment and period 4 (October 14), sunrise occurred at 0621 h and sunset at 1733 h. On average, day length during periods 3 and 4 was 12 h 08 min.

3.4.2 Pasture Management and Quality

Pasture management variables are presented in *Table 18*. The targeted PA for the low and high PA treatments were 25 and 40 kg DM/cow per d, respectively. Actual PA for the low and high PA treatments averaged 27 and 49 kg DM/cow per d, respectively ($P < 0.05$). The higher amount of pasture offered was provided by adjusting the paddock size (102 vs. 179 m²/cow per d, for low and high PA, respectively), because the pre-grazing pasture mass between pasture treatments did not differ and averaged 2761 kg DM/ha ($P > 0.05$). Cows grazing at low PA had a lower average post-grazing pasture mass (1013 vs. 1575 kg DM/ha; $P < 0.05$). The efficiency of harvesting, defined as pasture consumed/pasture offered, was significantly greater at low pasture allowance (62 vs. 42%; $P < 0.05$). Dalley et al. (1999) found decreased efficiency of pasture harvesting from 54% to 26% when PA increased from 20 to 70 kg DM/cow per d. In that study, pasture was offered at 20, 30, 40, 50, 60, and 70 kg DM/cow per d. Pasture DMI reached a plateau of maximum intake at a PA of 55.2 kg DM/cow per d (Dalley et al., 1999), a value that is comparable to the average PA used in this study (49 kg DM/cow per d) suggesting that pasture intake was likely maximized for the high PA.

Pasture quality parameters (*Table 19*) correspond to hand-plucked pasture samples and represent the pasture selected by the cows. On average, pasture quality was 20% CP, 15% TNC and 56% NDF, and did not differ between low and high PA treatments. The high PA treatment had a higher IVDMD ($P < 0.05$), reflecting that pasture selection by cows was enhanced when higher amount of pasture was offered. Pasture quality parameters were within the ranges summarized by Muller and Fales (1993) for cool-season grass during the spring and summer in Pennsylvania. Values of the IVDMD of pasture ($> 70\%$) were similar to those reported by Kolver and Muller (1998) for cool season grasses in Pennsylvania.

Significant period effects were detected for all parameters ($P < 0.05$), indicating that pasture quality varied among periods. Comparing the spring periods (periods 1 and 2) versus the fall periods (periods 3 and 4), pasture had higher DM (26.9 vs. 16.1%), NDF (59.4 vs. 51.9%), ADF (28.7 vs. 26.8%), and lower TNC (13.9 vs. 15.5%) and IVDMD (70.7 vs. 72.6%) during the spring. This is attributed to the fact that spring pasture was grazed at a more advanced stage of maturity with the rapid rate of pasture growth.

3.4.3 Intake of Pasture and Substitution Rate

Intake and apparent digestibility of nutrients are shown in *Table 20*. Dry matter intake of the mineral-vitamin mix averaged 0.74 kg/d in both unsupplemented treatments and DMI of concentrate averaged 8.63 kg/d in both supplemented treatments. As previously mentioned, the amount of concentrate offered to the supplemented cows was adjusted based on the milk production of cows before the period 1, and was readjusted before period 3. Concentrate DMI for the spring (periods 1 and 2) and fall (periods 3 and 4) periods was significantly different ($P < 0.05$) and averaged 9.7 and 7.3 kg/d, respectively.

A significant interaction between PA and CS was found for pasture and total DMI ($P < 0.05$; *Table 20*). Concentrate supplementation decreased pasture DMI at both PA, however the decrease in pasture DMI was higher when cows grazed at high vs. low PA (4.4 vs. 2.0 kg DM/d), indicating that the substitution rate was greater at the high PA. Based on concentrate DMI and the reduction in pasture DMI with supplementation, the substitution rate was 0.26 and 0.55 kg pasture/kg concentrate at the low and high PA, respectively. Low substitution rates have been reported previously when cows grazed at low PA by Grainger and Mathews (1989), Meijs and

Hoekstra (1984), Robaina et al. (1998), Stakelum (1986a) and Stakelum (1986b). Summarizing these studies, at low PA (7.6 to 22.2 kg DM/cow per d) the substitution rate averaged 0.19 kg pasture/kg concentrate (range from 0 to 0.31 kg/kg). At high PA (24 to 42.3 kg DM/cow per d), the substitution rate averaged 0.58 kg pasture/kg concentrate (range from 0.43 to 0.69 kg/kg). Kellaway and Porta (1993) concluded that as PA increased, substitution rate increased.

The lower substitution rate at the low PA resulted in a greater increase in total DMI. Concentrate supplementation increased total DMI, but the increase was greater when cows grazed at low vs. high PA (5.8 vs. 3.6 kg/d). Total DMI of both supplemented treatments did not differ (24.4 kg/d; $P > 0.05$). This number may be overestimated by the Cr_2O_3 method used to determine total DMI. Holden et al. (1994) reported that the use of Cr_2O_3 as fecal marker overestimated DMI of silage and hay by 10% in comparison with DMI measured directly.

Apparent digestibility of DM was increased with supplementation, but the increase was higher at the low PA (CS x PA; *Table 20*; $P < 0.05$). This is in agreement with results of Reis and Combs (2000) that reported an increase in DM digestibility when cows were supplemented with 5 or 10 kg/d of a corn based concentrate. Reis et al. (2001) also found a significantly higher DM digestibility in dairy cows fed a direct-cut grass-legume forage diet supplemented with 10 kg/d of dry or high moisture corn when compared with unsupplemented forage (69.4 vs. 60.6%). Apparent digestibility of CP was decreased with CS from 75.6 to 72.6% ($P < 0.05$), although it was not affected by PA ($P > 0.05$; *Table 20*).

Intake of pasture and total diet NDF had a significant interaction between PA and CS ($P < 0.05$; *Table 20*). Concentrate supplementation reduced intake of pasture NDF by 1.2 kg/d at low PA (9.9 vs. 8.7 kg/d) and 2.5 kg/d at high PA (11.5 vs. 8.9 kg/d) ($P < 0.05$). Total intake of

NDF was similar in both treatments that grazed at the low PA (10.1 kg/d), but was reduced by 1.1 kg/d with CS at the high PA ($P < 0.05$). Total NDF expressed as a percent of dietary DM averaged 54.7% (54% from the forage) in the unsupplemented treatments and averaged 42.2% (36.1% from the forage) in the supplemented treatments. These values exceed those recommended as minimals for high producing dairy cows fed TMR diets (25% of NDF of dietary DM, 19% of NDF from forage) (NRC, 2001). However, specific recommendations for an optimal NDF concentration in diets of grazing cows are not defined (NRC, 2001). At the low PA, CS slightly reduced the apparent digestibility of NDF (1.1% points) while at the high PA, the reduction in apparent digestibility of NDF was 4.3% ($P < 0.05$). This suggests a negative associative effect with CS, and explain some of the different rate of substitution between the low and the high PA.

Total intake of NE_L (*Table 20*) was increased significantly by CS, however the increase was greater at the low PA than at the high PA (11.4 vs. 7.7 Mcal/d). This was a result of an increase in 14.8 Mcal/d of NE_L from CS because pasture NE_L intake was reduced by supplementation (3.3 vs. 7.3 Mcal/d at the low and high PA, respectively). Total intake of NE_L in both supplemented treatments averaged 41.6 Mcal/d.

The total diet composition averaged 19.5% CP, 54.2% NDF, 27.2% ADF and 1.63 Mcal/kg DM of NE_L for the two unsupplemented treatments, and averaged 17.7% CP, 42.2% NDF, 20.2% ADF and 1.70 Mcal/kg DM of NE_L for the two supplemented treatments (*Table 20*).

3.4.4 Milk Production and Milk Composition

A significant interaction was found for milk and 3.5% FCM production between PA and CS ($P < 0.05$; *Table 21*). Supplementation increased milk production, however the increase was greater when cows grazed at the low vs. the high PA (10.6 vs. 7.7 kg/d). The PA by CS interaction for milk production is consistent with the interaction between PA and CS found for total DMI. Because of this interaction, milk response to CS was greater when cows grazed at low vs. high PA (1.36 vs. 0.96 kg of milk/kg of concentrate). Previous studies have reported higher milk responses to CS for cows grazing at low PA (Grainger and Mathews, 1989; Robaina et al., 1998; Stakelum, 1986a). Stakelum (1986a) reported a milk response of 0.61 and 0.22 kg milk/kg concentrate when low producing cows grazed ryegrass pasture at PA of 16 or 24 kg DM/cow per d. For PA of 7.6, 17.1 and 33.6 kg DM/cow per d, Grainger and Mathews (1989) reported greater milk responses for cows grazing ryegrass pasture with lower PA (0.97, 0.69 and 0.28 kg/kg, respectively) when cows produced less than 25 kg/d of milk. More recently, Robaina et al. (1998) reported milk responses of 0.98 and 0.54 kg/kg concentrate for low and high PA (21.1 kg and 42.3 kg DM/cow per d, respectively) with cows producing less than 18 kg/d of milk. Summarizing these studies, at low PA (mean 15.5 kg DM/cow per d; range: 7.6 to 21.1 kg DM/cow per d) the response to supplementation averaged 0.81 kg milk/kg concentrate (range: 0.61 to 0.98 kg milk/kg concentrate). At high PA (mean 33.3 kg DM/cow per d; range: 24 to 42.3 kg DM/cow per d) the response to supplementation averaged 0.35 kg milk/kg concentrate.

In our study, the milk response to CS at both low and high PA were higher (0.96 and 1.36 kg milk/kg concentrate, respectively) compared to these studies. Kellaway and Porta (1993) summarized grazing studies conducted with low producing dairy cows and reported an average milk response to CS of 0.6 kg milk/kg concentrate when pasture was restricted, and no response

when high quality pasture was available *ad libitum*. The higher milk responses found in our study are likely related to the high genetic merit and milk production of the Holstein cows used. Grazing studies conducted in USA with high producing dairy cows (Reis and Combs, 2000) reported a response of 1.00 and 0.72 kg milk/kg concentrate when cows grazing an alfalfa – ryegrass pasture were supplemented with 5 or 10 kg/d of a corn based concentrate, respectively. Recent studies from Europe (Delaby et al., 2001) also reported high milk responses to CS for high producing dairy cows grazing ryegrass pasture at different PA: 1.14, 0.98, 1.06, 0.82, 1.09, and 1.06 kg milk/kg concentrate for 12.1, 15.8, 16.6, 19.6, 16.5, and 21.0 kg DM/cow/d (measured at 5 cm cutting height), respectively. In this study, however, the interaction between CS and PA (i.e. higher milk response at low PA) was not clear as it was in our study.

Significant period effects were found for milk production ($P < 0.05$). During periods 1 and 2 (spring periods), milk production averaged 24.2 kg/d and 34.9 kg/d for the unsupplemented and supplemented cows grazing at the low PA, respectively; and 25.7 kg/d and 35.4 kg/d for the unsupplemented and supplemented cows grazing at the high PA, respectively. These results show that milk production of high producing dairy cows in early-mid lactation (101 to 142 DIM) grazing high quality pasture as the only feedstuff can produce 24.2 to 25.7 kg/d of milk, depending on the PA. Supplementation with 9.7 kg DM/d of concentrate resulted in a milk yield of 35.2 kg/d, regardless of PA. During periods 3 and 4 (fall periods), milk production averaged 15.2 kg/d and 23.7 kg/d for unsupplemented and supplemented cows at the low PA, and 18.9 kg/d and 24.5 kg/d for unsupplemented and supplemented cows at the high PA, respectively. Therefore, high producing dairy cows in late lactation (212 to 252 DIM) fed pasture as the only feedstuff can reach milk production levels of 15.2 to 18.9 kg/d depending on the PA, and 24.1 kg/d when supplemented with 7.3 kg DM/d of concentrate, regardless of PA.

Concentrate supplementation reduced milk fat percentage in milk at both PA ($P < 0.05$) but increased milk fat yield ($P < 0.05$; *Table 21*). Unsupplemented cows averaged 3.81% milk fat, while supplemented cows averaged 3.31% milk fat. The reduction in milk fat percentage is in agreement with other grazing studies where cows were supplemented with concentrates above 5 kg/cow/d (Arriaga-Jordan and Holmes, 1986; Delaby et al., 2001; Reis and Combs, 2000; Reis et al., 2001; Sayers, 1999). In agreement with our study, reductions in milk fat content have been reported dairy cows grazing a ryegrass pasture regardless of PA (i.e. no significant interaction between CS and PA) (Delaby et al., 2001). Arriaga-Jordan and Holmes (1986) reported a decrease in milk fat content from 3.73 to 3.51% when cows were supplemented with 1 or 6 kg/d of a barley based concentrate. Studies with high producing dairy cows showed linear reductions in milk fat content with a linear increase in CS: 0, 5 and 10 kg/d (Reis and Combs, 2000), and 0, 3, and 6 kg/d or 0, 2, 4, 6 kg/d (Delaby et al., 2001). Sayers (1999) also reported a reduction in milk fat from 3.66 to 2.99% with high producing dairy cows grazing ryegrass pasture when the supplementation was increased from 5 to 10 kg/d. Supplementation with 10 kg/d of dry shelled or high moisture ear corn to dairy cows fed with direct-cut grass-legume forage reduced milk fat percentage from 3.77 to 3.20% (Reis et al., 2001).

Concentrate supplementation increased the total protein and true protein percentage in milk, and the milk protein yield at both PA ($P < 0.05$; *Table 21*). The increase in both total and true protein is related to the higher total energy intake (*Table 20*) in the supplemented cows. Total protein content in unsupplemented and supplemented cows averaged 2.96% and 3.10%, respectively. Previous grazing studies showed that CS increased milk protein (Delaby et al., 2001; Petch et al., 1997; Sayers, 1999; Reis and Combs, 2000; Reis et al., 2001). Supplementation with 5 kg/d of cereal concentrate increased total and true protein in dairy cows

grazing at a pasture allowance of 23 kg DM/cow per d (Petch et al., 1997). Increasing the CS from 5 to 10 kg/d increased milk protein from 3.37 to 3.55% for cows grazing ryegrass pasture (Sayers, 1999). Milk protein percentage was linearly increased (2.85, 2.95 and 3.05%) with 0, 5 or 10 kg/d of a corn based concentrate with alfalfa – ryegrass pasture (Reis and Combs, 2000). Delaby et al. (2001) also reported a linear increase in milk protein with linear increase in CS (0, 3, 6 kg/d or 0, 2, 4, 6 kg/d) for dairy cows grazing at different PA. Milk protein was increased from 3.14 to 3.36% when high producing dairy cows fed direct-cut grass-legume forage were supplemented with 10 kg/d of dry or high moisture corn (Reis et al., 2001).

Concentrate supplementation reduced MUN concentration regardless of the PA (14.1 vs. 11.3 mg/dl) ($P < 0.05$; *Table 21*). This is in agreement with Reis and Combs (2000) who reported a linear reduction in MUN (20.2, 16.9 and 13.4 mg/dl) in cows supplemented with 0, 5 or 10 kg/d of a corn based concentrate. The lower MUN values in the unsupplemented cows reported in our study compared to that of Reis and Combs (2000) may be related to a grass pasture compared to a pasture including alfalfa. Cows consuming a direct-cut grass-legume forage had a higher MUN concentration (10.0 vs. 14.7 mg/dl) when fed 10 kg/d of dry or high moisture corn compared to no supplementation (Reis et al., 2001). Supplementation increased the proportion of true protein over total protein and reduced the proportion of non-protein nitrogen and MUN in total protein ($P < 0.05$; *Table 21*). A previous study where cows grazed at a PA of 23 kg DM/cow/d reported a lower milk non-protein nitrogen and urea nitrogen when supplemented with 5 kg/d of concentrate (Petch et al., 1997).

3.4.5 Body Weight, Body Condition Score, Blood and Urine Metabolites

Neither initial nor final BW and BCS were affected by treatments ($P > 0.05$; *Table 22*). However the type of non-continuous design (Latin square with 21 d periods) used in our study does not allow evaluating these long-term variables. Data are presented to give information of body size of cows used in the experiment.

Blood metabolites (glucose, blood urea nitrogen and NEFA) concentrations are shown in *Table 22*. Concentrate supplementation decreased blood urea nitrogen (BUN) at both PA (16.1 vs. 12.5 mg/dl; $P < 0.05$), and is consistent with the reduction in MUN concentration suggesting that supplementation improved the efficiency of dietary nitrogen utilization.

Concentrate supplementation increased blood glucose concentration at both PA (66.7 vs. 62.9 mg/dl; $P < 0.05$). Although this difference was significant, the glucose level in unsupplemented cows was within normal physiological levels (45 to 70 mg/dl). Opatpatanakit et al. (1993) reported increased glucose concentrations with supplementation of 0, 4 and 8 kg/d of barley to cows grazing a ryegrass – white clover pasture, although these differences (65.0, 68.1 and 68.5 mg/dl) were not significant. Lower glucose concentrations have been reported for high producing dairy cows fed pasture as the only feed compared with a nutritionally balanced TMR (59.8 vs. 65.4 mg/dl) (Kolver and Muller, 1998).

Unsupplemented cows had higher concentrations of NEFA than supplemented cows at both PA ($P < 0.05$; *Table 22*), however the unsupplemented cows grazing at the low PA had the highest numerical concentrations (380 μ eq/L). The higher NEFA concentrations in the unsupplemented cows suggest higher mobilization of fat reserves in comparison with the supplemented cows. Gagliostro and Lavandera (1997) found no changes in NEFA concentrations

with supplementation (6 kg/d of corn or barley based concentrate) in a short-term study with dairy cows producing 20 kg/d of milk.

The concentrations of purine derivatives (allantoin, uric acid, xanthine and hypoxanthine) in urine have been proposed as a non-invasive method for the estimation of the microbial protein flow to the duodenum in intact animals (Gonda, 1995). Among the purine derivatives, allantoin is the most important in cattle and is excreted as a constant proportion to the other purine derivatives. Therefore, the use of allantoin concentration would be sufficient to estimate rumen microbial protein (Gonda, 1995). Creatinine, the end product of phosphocreatinine degradation, can be used as an internal marker to predict metabolic processes in intact animals. Urinary excretion of creatinine is not affected by energy or protein intake changes and is excreted in proportion to body weight (Gonda, 1995). The allantoin/creatinine ratio in spot urine samples was constant during the day and followed the same trend as allantoin excretion in urine, supporting the idea that allantoin/creatinine ratio could be used as a useful index of total allantoin excretion in urine, and therefore avoiding the total collection of urine (Gonda, 1995).

A PA by CS interaction was found for allantoin concentration in urine spot samples ($P < 0.05$; *Table 22*). Concentrate supplementation increased the allantoin concentration in urine spot samples, but the increase was higher at low PA (1414 vs. 1878 mg/L) compared to the higher PA (1617 vs. 1908 mg/L). Creatinine concentration in urine spot samples was increased by CS at both PA ($P < 0.05$; *Table 22*). Similarly, the allantoin/creatinine ratio in spot samples of urine was increased by CS at both PA ($P < 0.05$; *Table 22*), indicating that CS increased rumen microbial protein supply. These results are in agreement with the increased allantoin/creatinine ratio reported by Reis et al. (2001) with corn supplementation in high producing dairy cows fed direct-cut grass-legume forage.

3.4.6 Ruminal Fermentation

A significant PA by CS interaction was detected for rumen pH ($P < 0.05$; *Table 23*). Concentrate supplementation reduced rumen pH, however the reduction was greater at the low PA (6.57 vs. 6.25) compared with the high PA (6.40 vs. 6.29) ($P < 0.05$). Both supplemented groups had similar rumen pH (average 6.27; $P > 0.05$). Previous grazing studies have reported no modification in rumen pH with medium (Van Vuuren, 1993) or high (Reis and Combs, 2000) levels of CS. In agreement with our study, rumen pH decreased as concentrate increased from 5 to 10 kg/d in dairy cows grazing a ryegrass pasture (Sayers, 1999). Supplementation with 10 kg/d of dry or high moisture corn to dairy cows fed direct-cut grass-legume forage reduced rumen pH from 6.06 to 5.69 (Reis et al., 2001).

Ruminal pH oscillation at 4 h intervals during a 24-h period is shown in *Figure 5*. Unsupplemented cows grazing at low PA (Low PA - U) had higher pH throughout the day, especially during the morning. Unsupplemented cows grazing at high PA (High PA - U) had intermediate pH values, that were lower than unsupplemented cows grazing at low PA but higher than both supplemented treatments, indicating the interaction between PA and CS for the mean daily values (*Table 23*). Both supplemented cows had the lowest rumen pH values during the 24-h which did not differ between PA (Low PA - CS and High PA - CS). The pattern of rumen pH variations differed between the unsupplemented and supplemented treatments. Both supplemented treatments had the same pattern with important reductions in rumen pH after cows were fed concentrate and initiated grazing. The highest rumen pH values in supplemented cows were found after the periods of lower grazing activity at 1330 h and between 0130 h and 0530 h. Unsupplemented cows had lower rumen pH at 1730 h, probably because of differences in grazing behavior patterns.

Concentrate supplementation reduced significantly the concentration of $\text{NH}_3\text{-N}$ at both PA (15.3 vs. 8.9 mg/dl; *Table 23*; $P < 0.05$). The reduction in rumen $\text{NH}_3\text{-N}$ concentration with supplementation is consistent with the reductions in BUN and MUN, and with other research (Reis and Combs, 2000; Reis et al., 2001; Van Vuuren, 1993;). Reis and Combs (2000) reported a linear decreased in rumen $\text{NH}_3\text{-N}$ (22.4, 17.7 and 8.1 mg/dl) for supplementation levels of 0, 5 or 10 kg/d with high producing cows. Supplementation with 10 kg/d of dry or high moisture corn to dairy cows fed direct-cut grass-legume forage reduced rumen $\text{NH}_3\text{-N}$ concentration (from 35.1 to 17.9 mg/dl) (Reis et al., 2001). The reduction in rumen $\text{NH}_3\text{-N}$ with CS is likely associated with a improved utilization of $\text{NH}_3\text{-N}$ as a result of the high rumen degradability of pasture proteins, or to a reduction in total CP intake because energy supplements are lower in CP than pasture. The reduction in rumen $\text{NH}_3\text{-N}$ concentration in our study is likely related to a higher utilization of ammonia because microbial protein synthesis was increased (higher allantoin/creatinine ratio) (*Table 22*) and total CP intake was not reduced with supplementation (*Table 20*). This would suggest a higher efficiency in dietary protein utilization in both supplemented treatments.

Rumen $\text{NH}_3\text{-N}$ concentrations during a 24 h period were higher at most hours in the unsupplemented cows (*Figure 6*). Regardless of PA, both unsupplemented groups had higher $\text{NH}_3\text{-N}$ values than both supplemented groups. The patterns of rumen $\text{NH}_3\text{-N}$ concentrations in both unsupplemented treatments and both supplemented treatments were similar. Unsupplemented cows had a peak in rumen $\text{NH}_3\text{-N}$ around 1330 h indicating rumen proteolysis of pasture after a period of high grazing activity following the morning milking. On the other hand, supplemented cows had a much more constant pattern of $\text{NH}_3\text{-N}$ in the rumen, indicating the improved utilization of ammonia by the energy provided ruminally with the concentrate.

A significant PA by CS interaction was detected for total VFA concentration ($P < 0.05$; *Table 23*). At a low PA, CS increased total VFA concentration by 13.7 mmol/L, while at high PA, total VFA concentration did not change. The interaction in total VFA followed the interaction found with rumen pH (*Table 23*) and total DMI (*Table 20*). Propionate concentration was increased by CS ($P < 0.05$). Reis and Combs (2000) reported a linear increase in propionate concentration when grazing cows were supplemented with 0, 5 and 10 kg/d of concentrate. Concentrate supplementation also significantly increased butyrate concentration ($P < 0.05$), which agrees with results from Reis and Combs (2000) with high producing cows. A significant PA by CS interaction for both the acetate/propionate ratio and the (acetate + butyrate)/propionate ratio ($P < 0.05$), and agrees with the reduction in milk fat percentage in the supplemented cows. A linear reduction in acetate/propionate ratio was found in grazing cows supplemented with 0, 5 or 10 kg/d (Reis and Combs, 2000).

Neither the volume nor the wet fill of rumen contents were affected by PA or CS ($P > 0.05$; *Table 23*). The wet fill of rumen contents averaged 83.3 kg, a value higher than the 72.4 kg reported by Reis and Combs (2000). The DM percentage of the rumen content was significantly higher in both supplemented treatments (14.2 vs. 12.5%; $P < 0.05$). The higher DM content in the supplemented treatments resulted in a tendency for a higher dry fill of rumen content ($P < 0.08$). Concentrate supplementation tended to also increase disappearance rate and reduce retention time, calculated based on the dry fill and total DMI ($P < 0.14$).

3.4.7 In Situ Pasture Rumen Digestion

The kinetics of pasture DM and NDF rumen digestion is in *Table 24*. For DM degradation, the lag time was not affected by the treatments and averaged 2.2 h ($P > 0.05$). Neither the soluble nor the insoluble potentially degradable fractions of DM were affected by PA or CS ($P > 0.05$). The soluble fraction averaged 24.2%, a lower value than the 48.4% reported previously for alfalfa based pasture (Reis and Combs, 2000). Elizalde et al. (1999) reported that alfalfa had a higher soluble fraction of DM than grasses. The insoluble potentially degradable fraction averaged 56.5% ($P > 0.05$). The degradation rate of DM was reduced by CS at both PA from 6.8 to 5.4 %/h ($P < 0.05$). The negative effect of CS on degradation rate of pasture DM may indicate the existence of negative associate effects in the rumen of the supplemented cows. Degradation rates found in our study were lower than those reported by Reis and Combs (2000), and may be related to use of alfalfa-ryegrass pasture. The rate of DM degradation in alfalfa was twofold higher than values reported for grasses (13.7 and 6.7%/h, respectively; Elizalde et al., 1999).

Neither lag time (4.3 h) nor the soluble fraction (8.9 %) of NDF were affected by treatments ($P > 0.05$; *Table 24*). Concentrate supplementation increased the insoluble potentially degradable fraction of NDF at both PA ($P > 0.05$). Concentrate supplementation decreased the rate of degradation of NDF from 5.5 to 4.5 %/h at low PA and from 4.7 to 3.6 %/h at high PA ($P < 0.05$). This suggests the existence of minor negative associative effects in the rumen when cows were fed supplements and rumen pH is reduced. A recent continuous culture fermenters study (de Veth and Kolver, 2001) reported that large reduction in DM and NDF digestibility of high quality pasture occurred when pH was lower than 5.8, suggesting that within the pH range of 5.8 to 6.6 elevated fiber digestibility can be maintained.

3.4.8 Efficiency of N Utilization

Total N intake was not affected by treatments (643 g/d; $P > 0.05$; *Table 25*). Pasture N intake was numerically reduced in 125 g/d by CS. Daily N excretion in feces was significantly increased by CS (158 vs. 190 g/d; $P < 0.05$), while daily N excretion in urine was significantly reduced by CS (229 vs. 178 g/d; $P < 0.05$). Concentrate supplementation increased excretion of N in milk (97 vs. 135 g/d; $P < 0.06$) as expected because supplemented cows produced more milk and with a higher protein content (*Table 21*).

Nitrogen partitioning among feces, urine and milk is also given in *Table 25*. The percentage of N intake excreted in feces was not affected by treatments and averaged 27% ($P > 0.05$). Kolver et al. (1998) reported a 24% of N intake partitioned to feces in high producing cows consuming grass pasture and supplemented with 9 kg/d of concentrate. Higher feces N excretion (percentage of N intake) was reported by Van Vuuren (1993) for dairy cows on grass-based diets supplemented with starch based concentrates (35%). A significant PA by CS interaction was found for N excreted in urine as proportion of total N intake ($P < 0.05$). At low PA, CS reduced the percentage of urinary N from 42 to 27%. At high PA, the reduction in urine N as proportion of total N intake was from 33 to 28%. Values of urine N as proportion of total N intake found in our study for the supplemented cows were similar to those previously reported for supplemented dairy cows consuming grass pasture (30%) (Kolver et al., 1998). Van Vuuren (1993) found in dairy cows fed with ryegrass pasture and 6.6 kg/d of a starch based concentrate that 38% of the N intake was eliminated in urine. For unsupplemented cows, Van Vuuren (1993) found higher values (54 to 66%) than in our study (33 to 42%). Concentrate supplementation increased the proportion of total N partitioned to milk from 15.8 to 20.3% at both PA ($P < 0.05$). Milk N as proportion of total N intake for supplemented cows were lower than those reported by

Kolver et al. (1998) (25%) for high producing cows supplemented with similar levels of concentrate (9.2 kg DM/d) and consuming similar pastures. Van Vuuren (1993) reported for dairy cows consuming ryegrass fertilized with different levels of N as only feed that between 16 to 23% of the N intake was excreted in milk. In agreement with our results, Carruthers and Neil (1997) reported an increase in fecal N and milk N, and a reduction in urine N as proportions of N intake for dairy cows consuming ryegrass pasture and supplemented with non-structural carbohydrate compared to cows without non-structural carbohydrate supplementation.

3.4.9 Grazing Behavior and Ruminating time

The components of grazing behavior are given in *Table 26*. Pasture DMI can be described as the product of grazing time (min/d), biting rate (bites/min) and bite mass (g DM/bite). Concentrate supplementation reduced grazing time at both PA ($P < 0.05$). In two experiments with dairy cows grazing a ryegrass pasture, Pulido and Leaver (2001) reported lower grazing time when unsupplemented (531 to 540 min/d) but similar grazing time when supplemented with 6 kg/d of concentrate (515 to 526 min/d). Although the interaction between PA and CS was not significant, the reduction in grazing time was less at the low PA (75 min/d) than at the high PA (104 min/d). The reduction in grazing time with CS is consistent with the reduction in pasture DMI previously described (*Table 20*). The significantly greater reduction in pasture DMI at the high PA is in agreement with the numerically greater reduction in grazing time at the high PA.

When expressed per kg of concentrate supplemented, grazing time was reduced by 10 min/kg of concentrate at low PA, and by 13 min/kg of concentrate at high PA. McGilloway and

Mayne (1996) reported reductions in grazing time of 15 to 22 min/kg concentrate. In a later study, Sayers (1999) reported a reduction in grazing time of 16 to 20 min/kg of concentrate when amount of supplementation was increased from 5 to 10 kg/d. In agreement with our results, Pulido and Leaver (2001) reported shorter reduction in grazing time per kg of concentrate (4 to 10 min) for grazing cows producing more than 30 kg/d of milk. Therefore, reduction in grazing time is a common with CS and has been proposed as an explanation for substitution rate (McGilloway and Mayne, 1996).

Biting rate was not affected by PA or CS (55 bites/min; $P > 0.05$; *Table 26*). Previous grazing studies reported no effect of CS on biting rate (Arriaga-Jordan and Holmes, 1986; Rook et al., 1994). Arriaga-Jordan and Holmes (1986) reported data from cows grazing a ryegrass pasture and supplemented either with 1 or 6 kg/d of a barley based concentrate and found that level of supplementation did not affect biting rate (63 bites/min). Concentrate supplementation did not affect biting rate (53 bites/min) of dairy cows grazing a ryegrass – white clover pasture (Rook et al., 1994).

Bite mass was not altered by PA or CS and averaged 0.57 g DM/bite ($P > 0.05$; *Table 26*). No differences were anticipated because cows of all treatments grazed a pasture that was offered on a daily basis at similar pre-grazing pasture mass and pasture height (*Table 18*). It is well known that bite mass is principally determined by pasture characteristics such as pre-grazing pasture mass and pasture height (McGilloway et al., 1999).

Ruminating time values are for 22 hours and 30 minutes per day because the recorders were removed from the cows during milking time (*Table 26*). Concentrate supplementation reduced the time that cows expended ruminating by 87 min/d ($P < 0.05$) or by 10 min/kg

concentrate at the low PA and by 12 min/kg concentrate at the high PA. The reduction in ruminating time with CS is in agreement with the reduction in rumen pH observed when cows were fed supplement (*Table 23*). Ruminating time values in our study are well below those reported by previous studies with grazing cows (around 400 min/d; Sayers, 1999). Although our lower values may be related with the fact that recorders were off the cows before and during milking times (time of day when cows had high ruminating activity based on visual observations), even assuming that during that time cows were ruminating, ruminating time would be lower 400 min/d. Ruminating time was not affected by level of concentrate supplementation (0, 3, or 6 kg/d) and averaged 444 min/d in dairy cows grazing a ryegrass pasture (Sayers, 1999). Pulido and Leaver (2001) also reported no effect of concentrate level (0, 3, or 6 kg/d in experiment 1, and 0 or 6 kg/d in experiment 2) on ruminating time of dairy cows grazing a ryegrass pasture (average: 389 min/d). In agreement with our results, Sayers (1999) reported a reduction in ruminating time when amount of concentrate was increased from 5 to 10 kg/d in high producing cows grazing ryegrass pasture (433 vs. 405 min/d or 6 min/kg concentrate) that produced also a reduction in rumen pH (6.00 vs. 5.75).

3.4.10 Causes of Substitution Rate

Two hypotheses have been proposed to explain the substitution rate of forage for concentrate. First, the substitution rate may be produced by negative associative effects in the rumen of grazing cows supplemented with concentrates (Dixon and Stockdale, 1999). Second, substitution rate may be related to reductions in grazing time when cows on pasture are fed supplement (McGilloway and Mayne, 1996).

Based on the results, both negative associative effects in the rumen and the reduction in grazing time influenced the substitution rate. Both supplemented treatments had lower rumen pH (*Table 23*), lower rumen degradation rates of pasture (*Table 24*) and fiber digestibility (*Table 20*). Supplemented cows also had lower grazing time (*Table 26*). Those effects were more important when cows grazed at the high PA, since a higher substitution rate found with lower PA (0.55 vs. 0.26 kg pasture/kg concentrate).

We analyzed the relative importance of rumen fermentation and grazing behavior in the reduction of pasture intake and the substitution rate. At the low PA, grazing time was reduced 75 min/d with supplementation (*Table 26*). With the biting rate (55 bites/min) and bite mass (0.55 g DM/bite), the reduction in grazing time would result in a reduction in pasture DMI of 2.3 kg/d. Pasture DMI when measured by the fecal marker was 2.0 kg/d lower with CS (*Table 20*). Therefore, at a low PA the reduction in grazing time explained all of the reduction in pasture intake. This is supported by the fact that negative associative effects in the rumen were quite small. Rumen pH was reduced with supplementation from 6.57 to 6.25 (*Table 23*), however the pH of 6.25 is not low enough to have negative effects on the cellulolytic population in the rumen. The decrease in apparent digestibility of NDF was small (*Table 20*). The most important negative associative effect was the reduction in the digestion rate of pasture DM (7.6 to 5.3 %/h) and NDF (5.5 to 4.5 %/h) (*Table 24*).

At the high PA (where higher substitution rate was higher), CS reduced grazing time 104 min/d (*Table 26*). This reduction in grazing time, with a biting rate of 56 bites/min and bite mass of 0.60 g DM/bite, is estimated to reduce pasture DMI by 3.5 kg/d. Pasture DMI, measured by the fecal marker, was reduced 4.4 kg/d by supplementation. Therefore, at a high PA, the reduction in grazing time explained about 80% of the reduced pasture intake (3.5 kg/4.4 kg). The

remaining 20% may be related to negative associative effects in the rumen. At a high PA, those negative effects were likely more important than at a low PA. The reduction in rumen pH (6.40 to 6.29; *Table 23*), the reduction in digestion rates of pasture DM (6.0 to 5.4 %/h) and NDF (4.7 to 3.6 %/h) (*Table 24*), and the greater reductions in apparent digestibility of NDF (from 70.4 to 66.1%; *Table 20*) likely contributed to the reduced pasture intake.

3.5 CONCLUSIONS

Concentrate supplementation reduced pasture DMI, but the reduction in pasture DMI was significantly greater when cows grazed at the high PA (4.4 kg/d) than at the low PA (2.0 kg/d). Therefore, the substitution of pasture for concentrate was greater at the high PA (0.55 kg pasture/kg concentrate) than at the low PA (0.26 kg pasture/kg concentrate). Total DMI was increased by CS with the increase higher at the low PA (5.8 kg/d) than at the high PA (3.6 kg/d). Both supplemented group had similar total DMI (24.4 kg/d).

Milk production was increased by CS, but the increase was greater at a low PA (from 19.1 to 29.7 kg/d) than at a high PA (from 22.2 to 29.9 kg/d). The milk response to CS was greater at low pasture allowance (1.36 vs. 0.96 kg milk/kg concentrate). Concentrate supplementation decreased milk fat percentage (3.81 vs. 3.31%) but increased total protein (2.96 vs. 3.10%).

Concentrate supplementation decreased rumen pH and increased total VFA concentration, however the reduction in rumen pH and the increase in total VFA concentrations were greater at the low PA than at the high PA. Concentrations of rumen $\text{NH}_3\text{-N}$ were reduced

by CS (15.3 vs. 8.9 mg/dl), indicating that with the reduction in BUN and MUN and the increase in the allantoin/creatinine ratio (indicator of microbial protein synthesis), a higher efficiency of dietary nitrogen utilization occurred when grazing cows were supplemented with concentrate.

Grazing time was reduced 75 min/d at the low PA and 104 min/d at the high PA with CS. Neither PA nor CS affected biting rate (55 bites/min) or bite mass (0.57 g DM/bite). Substitution rate was related to negative associative effects in the rumen including a rumen pH reduction, a lower rate rumen degradation of pasture, a decrease in apparent digestibility of NDF, and also by a reduction in grazing time with supplementation.

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Table 16: Ingredient and chemical composition (mean $\pm$ SD) of the mineral-vitamin mix and the concentrate used for the unsupplemented and supplemented dairy cows, respectively.

	Mineral-vitamin mix	Concentrate
Ingredient composition, % DM		
Shell dry corn	29.3	62.3
Wheat midds	...	10.4
Barley grain	...	7.8
Roasted soybeans	...	5.2
Molasses	24.4	5.2
Corn gluten meal	...	2.6
Limestone	...	1.3
Dicalcium phosphate	19.5	2.1
Salt	11.7	1.4
Magnesium oxide	6.8	0.8
Dynamate	4.9	0.6
Selenium premix	1.5	0.15
PS TM premix #4	1.4	0.1
Vitamins A, D, E	0.5	0.05
Chemical composition		
DM, %	95.1 $\pm$ 1.4	90.8 $\pm$ 0.5
% DM		
OM, %	57.4 $\pm$ 6.0	91.6 $\pm$ 0.6
CP, %	9.5 $\pm$ 0.2	13.4 $\pm$ 0.9
TNC, %	17.3 $\pm$ 2.9	59.2 $\pm$ 2.7
NDF, %	17.9 $\pm$ 2.7	17.4 $\pm$ 1.1
ADF, %	11.4 $\pm$ 1.5	6.5 $\pm$ 0.6
IVDMD, %	87.7 $\pm$ 2.6	91.6 $\pm$ 6.2

Table 17: Weather data during the experimental periods and in the previous ten years.

	Period 1 (5/10 to 5/30)	Period 2 (5/31 to 6/20)	Period 3 (9/3 to 9/23)	Period 4 (9/24 to 10/14)
Precipitations, mm	58	45	96	70
1988-1998 mean, mm	62	74	96	47
Average high temperature, °C	22 (13 to 29)	25 (12 to 32)	23 (13 to 29)	18 (15 to 24)
1988-1998 mean, °C	21	25	23	18
Average low temperature, °C	10 (6 to 15)	14 (7 to 20)	13 (6 to 21)	8 (1 to 18)
1988-1998 mean, °C	9	13	12	7

Table 18: Pasture management of low and high pasture allowance treatments.

	Treatments ¹		SEM	<i>P</i> < ²	
	Low PA	High PA		PA	Period
Pasture allowance, kg DM/cow per d	26.7	48.9	1.4	<0.01	0.43
Area, m ² /cow per d	102	179	5	<0.01	<0.01
Pre-grazing herbage mass, kg DM/ha	2712	2809	99	0.49	<0.01
Post-grazing herbage mass, kg DM/ha	1013	1575	43	<0.01	0.01
Efficiency of harvesting, %	62	42	2	<0.01	0.02

¹Low PA = low pasture allowance (25 kg DM/cow/day); High PA = high pasture allowance (40 kg DM/cow/day).

²PA = effect of pasture allowance; Period = effect of period.

Table 19: Chemical composition of hand-plucked pasture samples from the low and high pasture allowance treatments.

	Treatments ¹		SEM	<i>P</i> < ²	
	Low PA	High PA		PA	Period
DM, %	21.7	21.4	0.5	0.69	<0.01
% DM					
OM, %	92.0	92.2	0.1	0.28	<0.01
CP, %	20.3	19.6	0.4	0.29	<0.01
Soluble CP, %	4.8	4.7	0.3	0.81	<0.01
Soluble CP, % CP	23.7	24.1	1.1	0.81	<0.01
TNC, %	14.2	15.3	0.3	0.03	<0.01
NDF, %	56.1	55.2	0.5	0.26	<0.01
ADF, %	28.3	27.3	0.3	0.04	<0.01
IVDMD, %	70.3	73.0	0.7	0.01	0.01

¹Low PA = low pasture allowance (25 kg DM/cow/day); High PA = high pasture allowance (40 kg DM/cow/day).

²PA = effect of pasture allowance; Period = effect of period.

Table 20: Intake and apparent digestibility of DM, CP, and NDF, and intake of NE_L of unsupplemented or supplemented dairy cows grazing at two pasture allowances.

	Low PA ¹		High PA ²		SEM	P < ³		
	U	CS	U	CS		CS	PA	CS x PA
DM intake, kg/d								
Supplement	0.8	8.6	0.7	8.7	0.1	<0.01	0.56	0.36
Pasture	17.5	15.5	20.5	16.1	0.4	<0.01	<0.01	0.01
Total	18.3	24.1	21.2	24.8	0.4	<0.01	<0.01	0.01
Total, % BW	2.91	3.85	3.37	3.97	0.06	<0.01	<0.01	0.01
DM AD, ⁴ %	66.2	70.9	70.2	70.9	0.3	<0.01	<0.01	<0.01
CP intake, kg/d								
Supplement	0.1	1.1	0.1	1.1	0.01	<0.01	0.64	0.40
Pasture	3.5	3.2	4.0	3.2	0.1	0.02	0.01	<0.01
Total	3.6	4.3	4.1	4.3	0.1	0.03	0.01	<0.01
CP AD, ⁴ %	74.4	72.9	76.8	72.3	0.6	<0.01	0.11	0.09
NDF intake, kg/d								
Supplement	0.2	1.5	0.1	1.5	0.02	<0.01	0.68	0.27
Pasture	9.9	8.7	11.4	8.9	0.2	<0.01	<0.01	<0.01
Total	10.1	10.2	11.5	10.4	0.2	0.03	<0.01	<0.01
Total, % BW	1.61	1.65	1.83	1.68	0.03	0.01	<0.01	<0.01
Forage, % BW	1.59	1.41	1.81	1.44	0.03	<0.01	<0.01	<0.01
NDF AD, ⁴ %	66.2	65.1	70.4	66.1	0.5	0.01	<0.01	<0.01
NE _L intake, ⁵ Mcal/d								
Supplement	0.71	15.41	0.69	15.67	0.17	<0.01	0.47	0.42
Pasture	28.93	25.59	33.87	26.58	0.67	<0.01	<0.01	<0.01
Total	29.64	41.00	34.56	42.25	0.65	<0.01	<0.01	<0.01
Total diet, %								
CP	19.7	17.9	19.3	17.5	0.11	<0.01	<0.01	0.51
NDF	54.4	42.4	54.0	41.9	0.29	<0.01	0.15	0.83
ADF	27.5	20.5	26.8	19.9	0.14	<0.01	<0.01	0.69
NE _L	1.62	1.70	1.63	1.70	0.001	<0.01	0.02	0.02

¹Low PA - U = low pasture allowance – unsupplemented; Low PA - CS = low pasture allowance – concentrate supplementation.

²High PA - U = high pasture allowance – unsupplemented; High PA - CS = high pasture allowance – concentrate supplementation.

³CS = main effect of concentrate supplementation; PA = main effect of pasture allowance; CS x PA = concentrate supplementation by pasture allowance interaction.

⁴Total tract apparent digestibility (AD).

⁵Calculated using NRC (1989) NE_L values for the mineral-vitamin mix, concentrate and pasture: 0.97, 1.80 and 1.65 Mcal/kg DM, respectively.

Table 21: Milk production and composition of unsupplemented or supplemented dairy cows grazing at two pasture allowances.

	Low PA ¹		High PA ²		SEM	<i>P</i> < ³		
	U	CS	U	CS		CS	PA	CS x PA
Milk, kg/d	19.1	29.7	22.2	29.9	0.8	<0.01	0.04	0.03
3.5% FCM, kg/d	20.3	28.4	23.3	28.9	0.9	<0.01	0.05	0.05
Fat								
%	3.82	3.29	3.79	3.32	0.07	<0.01	0.96	0.53
kg/d	0.74	0.96	0.84	0.98	0.04	0.01	0.08	0.01
Total protein (TP)								
%	2.98	3.08	2.93	3.11	0.04	<0.01	0.71	0.27
kg/d	0.55	0.89	0.64	0.90	0.02	<0.01	0.03	0.05
True protein								
%	2.76	2.89	2.73	2.92	0.04	<0.01	0.98	0.42
kg/d	0.50	0.83	0.59	0.84	0.02	<0.01	0.03	0.05
MUN, mg/dl	13.9	11.6	14.2	11.1	0.4	<0.01	0.74	0.49
N fractions, %								
True Protein/TP	92	94	93	94	0.3	0.01	0.12	0.20
NPN/TP	8	6	7	6	0.3	0.01	0.12	0.20
MUN/TP	5	4	5	4	0.1	<0.01	0.93	0.36
MUN/NPN	70	65	76	61	2.8	0.01	0.81	0.24

¹Low PA - U = low pasture allowance – unsupplemented; Low PA - CS = low pasture allowance – concentrate supplementation.

²High PA - U = high pasture allowance – unsupplemented; High PA - CS = high pasture allowance – concentrate supplementation.

³CS = main effect of concentrate supplementation; PA = main effect of pasture allowance; CS x PA = concentrate supplementation by pasture allowance interaction.

Table 22: Body weight (BW), body condition score (BCS), blood metabolites and urine metabolites of unsupplemented or supplemented dairy cows grazing at two pasture allowances.

	Low PA ¹		High PA ²		SEM	<i>P</i> < ³		
	U	CS	U	CS		CS	PA	CS x PA
BW, kg								
Start	635	633	637	633	5	0.63	0.85	0.85
End	629	632	633	633	4	0.73	0.50	0.67
Change	-5	-1	-3	0	4	0.40	0.67	0.91
BCS								
Start	2.73	2.73	2.73	2.74	0.03	0.94	0.95	0.79
End	2.56	2.69	2.64	2.69	0.03	0.22	0.75	0.75
Change	-0.08	-0.04	-0.09	-0.05	0.02	0.14	0.61	0.93
Blood metabolites								
PUN, ⁴ mg/dl	16.43	12.54	15.72	12.48	0.44	<0.01	0.38	0.45
Glucose,mg/dl	62.96	65.76	62.84	67.65	1.05	<0.01	0.40	0.34
NEFA, µeq/L	380.36	282.48	308.19	243.73	22.3	<0.01	0.02	0.45
Urine metabolites								
Allantoin (A), mg/L	1414	1878	1617	1908	41	<0.01	0.01	0.05
Creatinine (C), mg/L	505	585	524	587	18	<0.01	0.57	0.64
A/C ratio	2.89	3.26	3.17	3.35	0.11	0.02	0.09	0.39

¹Low PA - U = low pasture allowance – unsupplemented; Low PA - CS = low pasture allowance – concentrate supplementation.

²High PA - U = high pasture allowance – unsupplemented; High PA - CS = high pasture allowance – concentrate supplementation.

³CS = main effect of concentrate supplementation; PA = main effect of pasture allowance; CS x PA = concentrate supplementation by pasture allowance interaction.

⁴PUN = plasma urea nitrogen.

Table 23: Ruminal fermentation parameters and rumen content characteristics of unsupplemented or supplemented dairy cows grazing at two pasture allowances.

	Low PA ¹		High PA ²		SEM	<i>P</i> < ³		
	U	CS	U	CS		CS	PA	CS x PA
pH	6.57	6.25	6.40	6.29	0.04	<0.01	0.14	0.03
NH ₃ -N, mg/dl	15.22	9.14	15.33	8.71	0.54	<0.01	0.79	0.64
VFA, mmol/L								
Total	116.3	130.0	129.8	130.6	2.3	0.01	0.01	0.02
Acetate (A)	79.3	85.1	88.9	84.9	1.5	0.55	0.01	0.01
Propionate (P)	22.3	25.6	24.1	26.2	0.6	<0.01	0.08	0.38
Butyrate (B)	10.7	14.8	12.5	15.1	0.4	<0.01	0.03	0.13
iso-Butyrate	1.2	1.1	1.2	1.0	0.03	<0.01	0.74	0.68
Valerate	1.5	1.9	1.7	1.9	0.04	<0.01	0.02	0.14
iso-Valerate	1.3	1.5	1.4	1.4	0.1	0.15	0.42	0.01
A/P	3.6	3.4	3.7	3.3	0.1	<0.01	0.68	0.02
(A+B)/P	4.1	3.9	4.3	3.9	0.1	<0.01	0.48	0.03
Rumen content								
Volume, L	97.16	95.65	108.22	100.33	5.73	0.48	0.26	0.63
Wet fill, kg	80.64	79.39	89.82	83.28	4.75	0.48	0.26	0.63
Dry fill, kg DM	9.77	11.97	11.81	12.35	0.58	0.08	0.11	0.24
DM content, %	12.01	14.55	12.99	13.93	0.40	0.01	0.69	0.12
DR, ⁴ %/h	9.9	12.6	9.8	12.7	1.9	0.23	0.99	0.97
Retention time, ⁵ h	14.1	12.2	13.9	12.7	0.8	0.14	0.86	0.69
Rate of passage, %/h	4.56	5.74	5.59	5.65	0.28	0.09	0.18	0.12

¹Low PA - U = low pasture allowance – unsupplemented; Low PA - CS = low pasture allowance – concentrate supplementation.

²High PA - U = high pasture allowance – unsupplemented; High PA - CS = high pasture allowance – concentrate supplementation.

³CS = main effect of concentrate supplementation; PA = main effect of pasture allowance; CS x PA = concentrate supplementation by pasture allowance interaction.

⁴Disappearance rate = (DMI/24 h)/ dry fill.

⁵Retention time = dry fill / (DMI/24 h) (Poppi et al., 1987).

Table 24: In situ degradation kinetics of pasture of unsupplemented or supplemented dairy cows grazing at two pasture allowances.

	Low PA ¹		High PA ²		SEM	<i>P</i> < ³		
	U	CS	U	CS		CS	PA	CS x PA
DM								
Lag time, h	2.36	1.06	3.17	2.03	1.35	0.44	0.57	0.96
Soluble, %	20.7	26.3	27.7	22.1	4.4	0.99	0.78	0.29
Degradable, %	57.7	56.4	52.9	59.0	3.4	0.55	0.79	0.36
Rate, %/h	7.6	5.3	6.0	5.4	0.4	0.01	0.12	0.09
ED, ⁴ %	46.7	50.3	47.4	45.1	0.9	0.62	0.13	0.06
NDF								
Lag time, h	3.33	4.88	4.75	4.38	0.63	0.39	0.50	0.19
Soluble, %	6.6	10.6	11.5	6.9	2.6	0.93	0.83	0.17
Degradable, %	64.6	68.8	65.9	74.7	2.4	0.05	0.21	0.39
Rate, %/h	5.5	4.5	4.7	3.6	0.2	<0.01	0.01	0.92
ED, ⁴ %	28.3	28.7	28.4	24.6	0.9	0.15	0.10	0.09

¹Low PA - U = low pasture allowance – unsupplemented; Low PA - CS = low pasture allowance – concentrate supplementation.

²High PA - U = high pasture allowance – unsupplemented; High PA - CS = high pasture allowance – concentrate supplementation.

³CS = main effect of concentrate supplementation; PA = main effect of pasture allowance; CS x PA = concentrate supplementation by pasture allowance interaction.

⁴ED = effective degradability at a rate of passage assumed of 6 %/h.

Table 25: Efficiency of N utilization of unsupplemented or supplemented dairy cows grazing at two pasture allowances.

	Low PA ¹		High PA ²		SEM	<i>P</i> < ³		
	U	CS	U	CS		CS	PA	CS x PA
N intake , g/d								
Supplement	11.8	170.4	10.9	178.4	2.8	0.06	0.49	0.47
Pasture	579.3	483.8	646.9	492.4	20.4	0.12	0.28	0.22
Total	591.1	654.2	657.8	670.8	19.4	0.12	0.22	0.16
Fecal N, g/d	156.9	184.3	158.7	196.6	4.6	0.05	0.18	0.17
Urine N, ⁴ g/d	244.5	172.9	213.4	182.6	9.3	0.04	0.37	0.20
Milk N, g/d	92.3	131.2	100.9	138.0	4.9	0.06	0.08	0.58
N balance, g/d	97.4	165.8	184.8	153.6	20.9	0.47	0.32	0.03
N dig, %	73.3	71.9	75.7	70.6	0.6	0.08	0.20	0.09
BV, ⁵ %	42.2	61.9	55.8	60.6	2.5	0.18	0.16	0.01
N, % N intake								
Fecal N	26.7	28.1	24.3	29.4	0.6	0.08	0.20	0.09
Urine N	41.8	27.3	33.2	27.8	1.7	0.16	0.17	0.03
Milk N	15.7	20.0	15.8	20.6	0.9	0.02	0.59	0.21

¹Low PA - U = low pasture allowance – unsupplemented; Low PA - CS = low pasture allowance – concentrate supplementation.

²High PA - U = high pasture allowance – unsupplemented; High PA - CS = high pasture allowance – concentrate supplementation.

³CS = main effect of concentrate supplementation; PA = main effect of pasture allowance; CS x PA = concentrate supplementation by pasture allowance interaction.

⁴Total urine volume estimated using daily creatinine excretion (mg/kg of BW per d) as internal marker (Valadares et al., 1999).

⁵Biological value = [(N intake – (fecal N output + urine N output))/(N intake – fecal output)] x 100.

Table 26: Grazing behavior and ruminating time of unsupplemented or supplemented dairy cows grazing at two pasture allowances.

	Low PA ¹		High PA ²		SEM	<i>P</i> < ³		
	U	CS	U	CS		CS	PA	CS x PA
Grazing time, min/d	609	534	626	522	37	0.02	0.94	0.70
Total bites, bites/d	34419	28501	35235	28563	2706	0.03	0.87	0.89
Biting rate, bites/min	56	54	56	55	2	0.55	0.74	0.72
Bite mass, ⁴ g DM/bite	0.55	0.55	0.60	0.59	0.05	0.97	0.34	0.96
Ruminating time, ⁵ min/d	263	183	210	116	41	0.04	0.16	0.87

¹Low PA - U = low pasture allowance – unsupplemented; Low PA - CS = low pasture allowance – concentrate supplementation.

²High PA - U = high pasture allowance – unsupplemented; High PA - CS = high pasture allowance – concentrate supplementation.

³CS = main effect of concentrate supplementation; PA = main effect of pasture allowance; CS x PA = concentrate supplementation by pasture allowance interaction.

⁴Bite size = pasture DMI/(grazing time x biting rate).

⁵Measured over 22 h and 30 min per d.

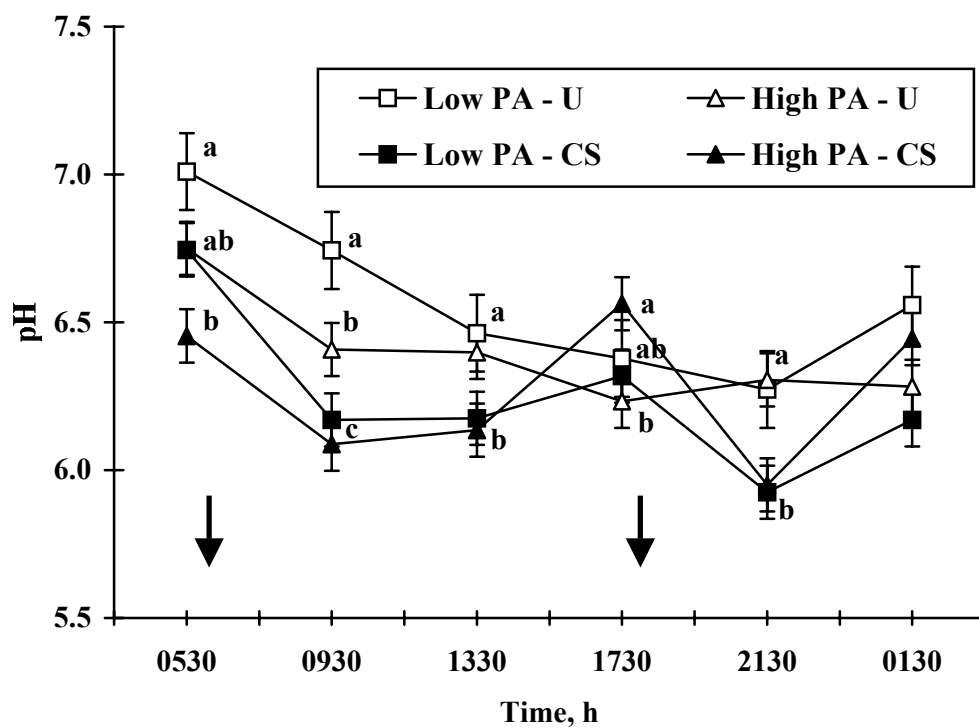


Figure 5: Daily rumen pH variations of unsupplemented or supplemented dairy cows grazing at two pasture allowances. Arrows indicate time of concentrate feeding for supplemented treatments after milkings.

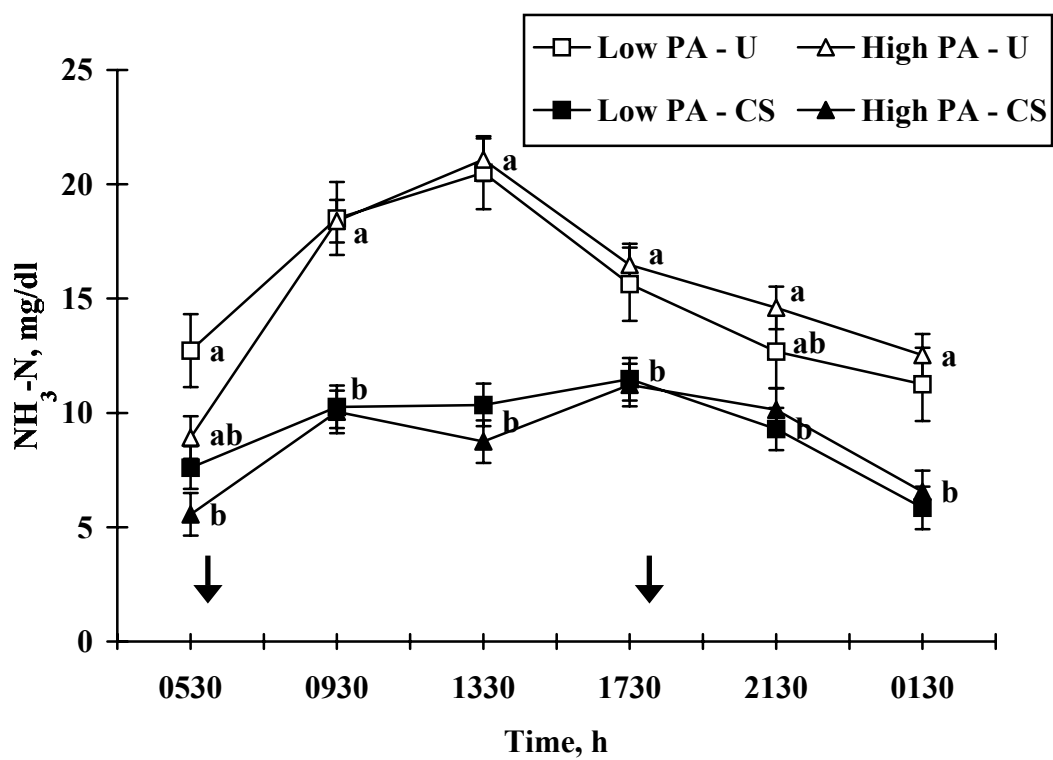


Figure 6: Daily rumen $\text{NH}_3\text{-N}$ concentration variations of unsupplemented or supplemented dairy cows grazing at two pasture allowances. Arrows indicate time of concentrate feeding for supplemented treatments after milkings.

Chapter 4

Effect of Pasture Allowance on Grazing Behavior of Unsupplemented or Supplemented High Producing Dairy Cows

ABSTRACT

The effect of pasture allowance on the grazing behavior of unsupplemented or supplemented high producing Holstein cows was studied in two single reversal experiments. Ten multiparous cows (2 ruminally cannulated) were unsupplemented, and six multiparous cows (2 ruminally cannulated) were supplemented with a corn-based concentrate. Within each supplementation group cows grazed a bromegrass/orchardgrass-based pasture offered at two pasture allowances: 1) 25 kg DM/cow/d, and 2) 40 kg DM/cow/d. Recorders were used to measure grazing time and bites per d. Bite mass was calculated dividing the pasture DMI, measured by chromic oxide, by the total number of bites per d. With unsupplemented cows, pasture DMI (18.8 vs. 15.6 kg/d) and milk production (17.6 vs. 15.2 kg/d) were higher at high pasture allowance. The higher pasture DMI was a result of a longer grazing time and a greater number of bites after the p.m. milking. Rumen pH was lower (6.33 vs. 6.60) and total VFA concentration was higher (134.5 vs. 119.5 mmol/L) at high pasture allowance. With supplemented cows, pasture and total DMI (14.7 and 22.8 kg/d) and milk production (27.8 kg/d) were not affected by pasture allowance. Grazing behavior of supplemented cows was not affected by pasture allowance. Neither rumen pH (6.05) nor total VFA concentration (148.4 mmol/L) differed between low and high pasture allowance. Pasture allowance had important effects on grazing behavior, pasture DMI and milk production of unsupplemented cows, however

neither grazing behavior nor performance of supplemented cows were affected by pasture allowance.

(Key words: pasture allowance, grazing behavior, rumen fermentation)

Abbreviation key: **IVDMD** = in vitro dry matter digestibility, **MUN** = milk urea nitrogen, **NH₃-N** = ammonia nitrogen, **PA** = pasture allowance, **PUN** = plasma urea nitrogen.

4.1 INTRODUCTION

The use of grazing systems on dairy farms has increased during the last 15 years (Muller and Fales, 1998), with approximately 16% of dairy farmers in Pennsylvania (Gripp et al., 1993) and 23% of dairy farmers in Wisconsin (Jackson-Smith et al. 1997) using pasture as forage for their herds. The use of a pasture system increases profit per cow because of reduced cost of feed, capital equipment, and labor, even with a decrease in milk production per cow (Weigel et al., 1999). The challenge of managing the high producing cow on pasture is to achieve high levels of pasture and total DMI by manipulating pasture management and supplementation strategies in order to obtain high milk production (McGilloway and Mayne, 1996).

Dry matter intake of pasture is related to three behavior variables: grazing time, biting rate, and bite mass (Aldden and Whittaker, 1970). Phillips and Leaver (1986) estimated for dairy cows a maximum grazing time of 10 h/d and biting rate of 66 bites/min, which results in a maximum total of 40000 bites/d. An understanding of grazing behavior is required to design efficient grazing management systems (Rook, 2000).

Grazing behavior depends on several factors including quantity and quality of pasture, physiological state and the nutrient requirements of animals, and the weather conditions (Woodward, 1997). The amount of pasture offered per cow on a daily basis, or pasture allowance (PA), is important for maximizing pasture DMI of dairy cows, and therefore influences the components of grazing behavior (Hodgson and Brookes, 1999). Bao et al. (1992) reported higher grazing time and biting rate in high (32.0 kg/d of milk) than in low (24.8 kg/d of milk) genetic merit dairy cows. This factor is particularly important to grazers in the USA because of the high genetic merit, with some well-managed pasture based systems reaching levels that exceed 9000 kg of milk/cow/year (Parker et al., 1993).

There is a lack of information on the grazing behavior of high producing dairy cows related to pasture type and management, supplementation strategies, and climatic conditions of the Northeast USA. Grazing behavior of dairy cows measured by methodology that does not affect production, such as the automatic behavior recorders (Rutter et al., 1997), has not been reported in the USA. Information on the grazing behavior of high producing dairy cows with or without concentrate supplementation grazing with a different PA has not been reported.

The objectives of this study were to quantify the effects of PA and concentrate supplementation on grazing behavior and rumen digestion of high producing dairy cows. One of the main objectives was to evaluate the relation between grazing pattern and fluctuations on rumen pH and ammonia nitrogen ($\text{NH}_3\text{-N}$) concentration.

4.2 MATERIALS AND METHODS

4.2.1 Cows and Treatments

Sixteen multiparous Holstein cows (4 fitted with rumen cannulas) [BW, 651 ± 76 kg; milk yield, 32.1 ± 7.1 kg/d; parity, 2.8 ± 0.8 ; DIM, 212 ± 35 (mean $\pm$ SD)] were selected from The Pennsylvania State University Dairy Cattle Research and Education Center (University Park, PA) (11436 kg of milk and 363 kg of protein per lactation in 1999), to study the grazing behavior. Ten cows (2 fitted with rumen cannulas) were unsupplemented, and six cows (2 fitted with rumen cannulas) were supplemented. Unsupplemented cows received no concentrate supplement and were fed a mineral-vitamin mix at a rate of 1 kg/d per cow to avoid mineral and vitamin deficiencies. The amount of concentrate offered per cow to the supplemented group was 1 kg of concentrate/4 kg of milk, based on the pre-trial milk production. The ingredient and chemical composition of the mineral-vitamin and the concentrate mixes used for the unsupplemented and supplemented cows, respectively, are shown in *Table 27*.

Within each supplementation group, cows were paired by DIM, BW, and milk production, and randomly assigned to two grazing groups. Cows grazed a grass pasture offered at two amounts per cow per d (PA): 1) low PA, or 2) high PA. Pasture allowance targets were 25 and 40 kg DM/cow/d (measured to the ground level) for the low and high PA treatments, respectively. To achieve these targets, pre-grazing pasture mass was measured every two days to adjust the size of the paddock. Pre-grazing pasture mass (kg DM/ha) was measured by cutting ten quadrants (0.124 m^2 /quadrant) of pasture to ground level, and drying at 55°C in a forced air oven. A new paddock was constructed daily using a temporary polywire. A second polywire

fence was used to prevent back-grazing. New paddocks were given to the cows each morning at approximately 0700 h. Pasture botanical composition averaged 50% smooth brome grass (*Bromus inermis* L.), 33% orchardgrass (*Dactylis glomerata* L.), 7% Kentucky bluegrass (*Poa pratensis* L.), and 10% weeds and dead material. Pasture was fertilized with nitrogen before the beginning of the first period (August 18) at a rate of 50 kg N/ha.

Two grazing periods of 21 days each were conducted during the fall of 1999 (period 1 from September 3 to September 23; period 2 from September 24 to October 14). Within each supplementation group, cows grazed at the two levels of PA in a single reversal design to determine a final number of observations of 10 (2 rumen cannulated) for each PA treatment in the unsupplemented group, and 6 (2 rumen cannulated) for each PA treatment in the supplemented group. Cows were milked twice daily at 0600 and 1730 h. Walking distance from pasture to the milking parlor averaged 0.9 km (range: 0.75 to 1.20 km).

4.2.2 Experimental Measures and Sample Analyses

During each of the two 21-d periods, the first 10 d were used to adjust the cows to the treatments and the last 11 d were used for experimental measures. Pasture DMI was estimated from d 15 to 19 during each period using Cr_2O_3 as an indigestible fecal marker. The indigestible fecal marker Cr_2O_3 was dosed twice daily (10 g Cr_2O_3 /d) after each milking (0630 h and 1800 h, approximately) for ten days beginning on d 10 of each period. Fecal grab samples were collected at 0630 h and 1800 h from d 15 to 19, and immediately frozen (-20°C).

During the same days that fecal samples were taken (d 15 to 19), samples of the mineral-vitamin and the concentrate mixes were collected daily, and pasture samples were plucked by

hand twice daily after each milking to the approximate height to which cows grazed. Samples were dried at 55 °C in a forced air oven and ground through a 1-mm screen (Wiley Mill, Thomas Scientific, Philadelphia, PA). Mineral-vitamin mix and concentrate samples were composited by period, while hand-plucked pasture samples were composited by day. Composited mineral-vitamin mix, concentrate and hand-plucked pasture samples were analyzed for DM, CP, and ash (AOAC, 1990), soluble CP (Krishnamoorthy et al., 1982), ADF and NDF (Ankom Daisy II, ANKOM Technology Corp., Fairport, NY), total nonstructural carbohydrate (TNC) (Smith, 1981; modified to use potassium ferricyanide as the colorimetric indicator), and in vitro DM digestibility (IVDMD) (Ankom Daisy II, ANKOM Technology Corp., Fairport, NY).

Fecal samples were thawed, dried at 55 °C in a forced air oven, and ground through a 1-mm screen (Wiley Mill, Thomas Scientific, Philadelphia, PA). A composited sample per cow was made for each period and analyzed for Cr (Parker et al., 1989). The mineral-vitamin mix DMI fed to the unsupplemented cows and the concentrate DMI fed to the supplemented cows were measured daily in every cow as the difference between offered and refused the same five days that fecal grab samples were taken. Pasture DMI was estimated in the unsupplemented cows using the equation: $\text{pasture DMI} = [(\text{g Cr/d})/(\text{g Cr/g fecal DM}) - \text{mineral-vitamin mix DMI} \times (1 - \text{IVDMD of mineral-vitamin mix})] / (1 - \text{IVDMD of pasture})$. Pasture DMI was estimated in the supplemented cows using the equation: $\text{pasture DMI} = [(\text{g Cr/d})/(\text{g Cr/g fecal DM}) - \text{concentrate DMI} \times (1 - \text{IVDMD of concentrate})] / (1 - \text{IVDMD of pasture})$. Total DMI in the unsupplemented cows was determined as mineral-vitamin mix DMI plus pasture DMI, and total DMI in the supplemented cows was determined as concentrate DMI plus pasture DMI.

Ruminal fluid samples from the four cannulated cows were collected during d 20 at 0, 4, 8, 12, 16, and 20 h beginning at 0530 h. At 0 h (0530 h) and 12 h (1730 h), cows were sampled

indoors because these two sampling times coincided with the a.m. and p.m. milking, respectively. At the other sampling times, cows were sampled in the pasture using a temporary construction made with mobile gates in the paddock. Ruminal digesta samples were taken from the dorsal, ventral and caudal area of the rumen, and squeezed through four layers of cheesecloth. The pH of the filtered ruminal fluid was measured immediately using a portable digital Cole Palmer pH-meter, and 15-ml aliquot preserved with 3 ml of 25% metaphosphoric acid and 3 ml of 0.6% 2-ethyl butyric acid (internal standard). These samples were stored at - 20 °C and subsequently analyzed for $\text{NH}_3\text{-N}$ and VFA (Yang and Varga, 1989).

Grazing behavior was measured from d 10 to 16 using the automatic IGER behavior recorders (Rutter et al., 1997). Each day recorders were placed on one cow per treatment, twice daily after each milking and before cows were moved to the pasture. Recorders were not placed on the rumen cannulated cows. The final number of recordings was 6 for the unsupplemented cows and 6 for the supplemented cows. During the morning, recorders were placed on cows from 0730 h to 1730 h (10 hours). Before the evening milking, recorders were removed from the cows to avoid damage during milking and feeding time, and also to download the information recorded during that period of time. During the night recorders were placed on cows from 1830 h to 0600 h (12 hours and 30 minutes). Before the morning milking, recorders were removed and the information downloaded. On average, the recorders were on the cows 22 hours and 30 minutes per day. Data recorded was analyzed using the software IGER GRAZETM (GRAZE User's guide, documentation version 1.0, program version 0.74).

Milk production was recorded daily from d 11 to 21 during each period. Milk samples were collected three times on d 13, 16 and 19 and preserved with 2-bromo-2-nitropropane-1,3 diol. Milk fat, total protein, true protein and lactose were analyzed by infrared spectrophotometry

(Foss 605B Milk-Scan; Foss Electric, Hillerød, Denmark) by the Pennsylvania DHIA milk-testing laboratory.

4.2.3 Statistical Analyses

Data from unsupplemented or supplemented cows were analyzed separately as two single reversal designs with two 21-d periods by the general linear models procedure of SAS (1999). For the animal performance data (DMI, milk production and composition), the model included treatment, cow and period. For the grazing behavior and rumen fermentation data the model included treatment, period, cow, hour, plus the interactions between hour and the other factors (hour by treatment, hour by cow, hour by period). Cow was considered a random effect, while the others were considered fixed effects. When significant ($P < 0.05$; unless otherwise indicated) effects due to treatments were detected, mean separation was conducted by the PDIFF option in SAS (1999). All means presented are least squares means.

4.3 RESULTS AND DISCUSSION

4.3.1 Weather Data and Pasture Management and Quality

Mean precipitations and the average high and low temperatures for each period and the average of the previous ten years (1988-1998) are summarized in *Table 28*. The first day of period 1 (September 3), sunrise occurred at 0540 h and sunset at 1843 h; the last day of the

experiment and period 2 (October 14), sunrise occurred at 0621 h and sunset at 1733 h. On average, day length during periods 1 and 2 was 12 h 08 min.

Pasture management variables are presented in *Table 29*. Actual PA for the low and high PA treatments averaged 27 and 47 kg DM/cow/d, respectively ($P < 0.05$). The low and high PA levels were determined adjusting the paddock size (95 vs. 170 m²/cow/d for low and high PA, respectively), because the pre-grazing pasture mass between treatments did not differ and averaged 2768 kg DM/ha ($P > 0.05$). Cows grazing at low PA had a lower average post-grazing pasture mass (1010 vs. 1476 kg DM/ha; $P < 0.05$). Pasture DMI reached a plateau of maximum intake at a PA of 55.2 kg DM/cow per d (Dalley et al., 1999), suggesting that pasture intake was likely maximized for the high PA treatment (47 kg DM/cow/d).

Pasture quality parameters (*Table 30*) are from hand-plucked pasture samples and represent the pasture selected by the cows. On average, pasture quality was 21% CP, 16% TNC and 52% NDF, and did not differ between low and high PA treatments. The high PA treatment had a higher IVDMD ($P < 0.05$). Pasture quality parameters were within the ranges summarized by Muller and Fales (1998) for cool-season grass-based pastures in Pennsylvania.

4.3.2 Dry Matter Intake, Milk production, and Milk Composition

Dry matter intake, milk production, and milk composition of unsupplemented and supplemented dairy cows grazing at low or high PA is shown in *Table 31*. Total (19.6 vs. 16.4 kg/d) and pasture (18.8 vs. 15.6 kg/d) DMI of unsupplemented cows was 21% higher ($P < 0.05$) at high PA. Total DMI, as percentage of BW, averaged 2.7% for the low PA and 3.1% for the high PA ($P < 0.05$). Several studies reported that pasture DMI of unsupplemented grazing dairy

cows increased as PA increased (Delaby et al., 2001; Grainger and Mathews, 1989; Robaina et al., 1998; Stakelum, 1986; Wales et al., 2001). Total DMI was increased from 16.9 to 18.2 kg/d in unsupplemented cows grazing a ryegrass pasture when PA was increased from 16 to 24 kg DM/cow/d (Stakelum, 1986). Robaina et al. (1998) reported that total DMI (measured using the alkane technique) of unsupplemented dairy cows grazing a ryegrass-white clover pasture increased from 12.1 to 14.9 kg/d as PA increased from 20 to 40 kg DM/cow/d. Pasture DMI (measured by pre and post grazing pasture mass difference) of unsupplemented dairy cows was 6.0, 11.8, and 15.9 kg/d at PA of 7.6, 17.1, and 33.1 kg DM/cow/d, respectively (Grainger and Mathews, 1989). Those previous studies were conducted with low producing dairy cows in early lactation producing between 7.3 to 23 kg/d of milk. Recent data from studies conducted with high producing dairy cows on pasture (Delaby et al., 2001; Wales et al., 2001) found the same tendency. Wales et al. (2001) reported that pasture DMI (estimated by difference between pre and post grazing pasture mass) increased from 11.2 to 15.6 kg/cow/d when PA of a perennial ryegrass pasture was increased from 19 to 37 kg DM/cow/d. Delaby et al. (2001) found that pasture DMI (measured by difference between pre and post pasture height) increased from 12.9 to 15.0 kg/d as PA increased from 16.5 to 21.0 kg DM/cow/d (at a 5 cm cutting height), respectively. Dry matter intake response to PA averaged 16% (Delaby et al., 2001) and 39% (Wales et al., 2001), compared to a 21% response in this study. Differences in pasture DMI between those studies and this study may be partially attributed to differences in milk production and stage of lactation of cows, pasture quality, and techniques for estimation of pasture DMI.

Neither concentrate (8.1 kg/d), nor pasture (14.7 kg/d), nor total (22.8 kg/d) DMI of supplemented cows differed between low and high PA ($P > 0.05$; *Table 31*). Total DMI, as percentage of BW, averaged 3.4%. Previous studies conducted in the USA with high producing

dairy cows on pasture reported total DMI between 19.8 to 20 kg/d when supplemented with approximately 8 kg/d of concentrate (Reis and Combs, 2000a; Reis and Combs, 2000b). In agreement with our results, previous studies reported similar total DMI for dairy cows grazing at different PA when supplemented with 3.2 kg/d (Grainger and Mathews, 1989) or 5 kg/d (Robaina et al., 1998) of concentrate. The similar total DMI by supplemented cows grazing at different PA in the current and previous studies is likely explained by the substitution rate (i.e. the reduction in pasture DMI with supplementation), which is higher when PA is increased, with the final result of a similar total DMI (Kellaway and Porta, 1993).

Milk production was 16% greater (17.6 vs. 15.2 kg/d; $P < 0.05$) when unsupplemented cows grazed at high PA (*Table 31*). Milk production obtained in this study (15.2 to 17.6 kg/d) represents the level of production of cows in late lactation (212 to 254 DIM) on only pasture diets with an intensive grazing management during the fall. In a previous study, Kolver and Muller (1998) reported a milk production of 29.6 kg/d in early lactation (59 DIM) cows grazing a grass pasture during the spring. Reis and Combs (2000b) reported that unsupplemented cows (83 DIM) grazing a legume-grass pasture in the summer produced 21.8 kg/d of milk. Differences in stage of lactation, season, year and pasture type explain the differences in milk production obtained between those studies and this current study. Several studies have evaluated the effect of PA on milk production in low producing dairy cows on only pasture diets (Grainger and Mathews, 1989; Robaina et al., 1998; Stakelum, 1986). Summarizing those studies, milk production of unsupplemented cows was increased 23% when PA was increased from 16 to 33 kg DM/cow/d. Working with high producing dairy cows, Delaby et al. (2001) found contradictory results on the effect of PA on milk production of unsupplemented cows. Over three experiments, only one showed a significant 4% increase in milk production (21.3 vs. 22.2 kg/d)

with PA, while the other two experiments showed no response (mean of 22.3 kg/d of milk) (Delaby et al., 2001). In agreement with this current study, Wales et al. (2001) found a 17% increased in milk production (24.2 vs. 20.7 kg/d) with PA.

Milk production of supplemented cows was not affected by PA and averaged 27.8 kg/d ($P > 0.05$; *Table 31*). This production level represents the potential milk production capable with high producing dairy cows in late lactation (212 to 254 DIM) grazing good quality pasture and supplemented with 8 kg/d of a corn based concentrate. Lower milk production levels (19.8 and 21.4 kg/d) were found by Wu et al. (2001) in two experiments with late lactation (220 and 203 DIM) cows grazing a legume-grass pasture during the summer and supplemented with 6.2 and 7.9 kg/d of a high moisture corn-based supplement. Differences between studies could be assigned to pasture type and management, weather conditions and between-year variations. Previous studies with low producing dairy cows reported that PA did not affect milk production when cows were supplemented with 3.2 kg/d (Grainger and Mathews, 1989; 23.6 kg milk/d) or 5 kg/d of concentrate (Robaina et al., 1998; 15.6 kg milk/d). A recent study including three experiments with high producing dairy cows in mid lactation grazing a ryegrass pasture (Delaby et al., 2001), reported that milk production was not affected by PA when cows were supplemented with 6 kg/d of concentrate. In the study of Delaby et al. (2001) overall milk production averaged 27.6 kg/d, which is similar to the milk production found in this study. The similar milk production reported by Delaby et al. (2001) with a lower level of supplementation than in this current study could be explained by the higher quality of the ryegrass pasture compared to the brome grass/orchard grass pasture.

Milk fat, total protein and true protein of unsupplemented cows were not affected by PA and averaged 3.73, 3.19 and 3.01%, respectively ($P > 0.05$; *Table 31*). Previous studies (Delaby

et al., 2001; Grainger and Mathews, 1989; Robaina et al., 1998; Wales et al., 2001) reported similar milk fat and protein content for unsupplemented cows grazing at different PA. Milk urea nitrogen (**MUN**) of unsupplemented cows averaged 13.4 mg/dl and was not affected by PA ($P > 0.05$). Wales et al. (2001) found no differences in MUN concentration between dairy cows grazing at low or high PA. Reis and Combs (2000b) reported a MUN of 20.2 mg/dl for unsupplemented high producing dairy cows grazing a legume-grass pasture. The lower MUN value in our study may be partially related to the fact that cows grazed a grass pasture, while in the study of Reis and Combs (2000b) cows grazed a pasture including alfalfa.

Milk composition of supplemented cows was not affected by PA ($P > 0.05$; *Table 31*). Fat, total protein, and true protein percentages averaged 3.44, 3.08, and 2.90%, respectively. Delaby et al. (2001) reported similar fat content for high producing dairy cows grazing a ryegrass pasture at different PA and supplemented with 6 kg/d of concentrate. Pasture allowance did not affect MUN of supplemented cows (12.4 mg/dl; $P > 0.05$). The current results agree with previous studies where PA did not affect milk composition of supplemented cows (Grainger and Mathews, 1989; Robaina et al., 1998).

4.3.3 Rumen Fermentation Parameters

Rumen fermentation parameters of unsupplemented and supplemented dairy cows grazing at low or high PA is shown in *Table 32*. Previous studies have reported rumen fermentation parameters of dairy cows grazing pasture without supplementation (Berzaghi et al., 1996; Delagarde et al., 1997; Khalili and Sairanen, 2000; Reis and Combs, 2000b; Van Vuuren

et al., 1986; Wales et al., 2001), however some do not report the PA (Berzaghi et al., 1996; Reis and Combs, 2000b) and only Wales et al. (2001) evaluated the effect of PA.

Mean rumen pH on unsupplemented cows was lower (6.33 vs. 6.60; $P < 0.05$; *Table 32*) at high PA, and may be partially related to the higher pasture DMI (*Table 31*). Berzaghi et al. (1996) reported a rumen pH of 6.4, and Reis and Combs (2000b) reported a rumen pH of 6.63 for unsupplemented cows grazing legume/grass-based pastures, similar to that found for the unsupplemented cows at low PA (6.60; *Table 6*). Khalili and Sairanen (2000) reported a rumen pH of 6.13 for unsupplemented dairy cows grazing at a high PA (> 40 kg DM/cow/d) similar to the one used in this study (47 kg DM/cow/d). Delagarde et al. (1997) reported a similar pH value (6.26) to the unsupplemented cows at high PA treatment (6.33) for unsupplemented dairy cows grazing a ryegrass pasture at high PA (19.9 kg OM/cow/d above 5 cm cutting height). In contrast with this current study, Wales et al. (2001) reported that PA (19 vs. 37 kg DM/cow/d) did not affect rumen pH (5.89) of unsupplemented dairy cows grazing a perennial ryegrass.

Total VFA concentration was higher (134.4 vs. 119.5 mmol/L) when unsupplemented cows grazed at high PA ($P < 0.05$; *Table 32*), in agreement with the lower rumen pH. Wales et al. (2001), however, did not find differences in total VFA concentration (149.5 mmol/L) when cows grazed at low or high PA. High values of total VFA concentrations (150 mmol/L) were reported for unsupplemented cows (Berzaghi et al., 1996). Others reported lower total VFA concentrations for unsupplemented cows (106 mmol/L, Delagarde et al., 1997; 127 mmol/L, Khalili and Sairanen, 2000; 98.7 mmol/L, Reis and Combs, 2000b).

Rumen $\text{NH}_3\text{-N}$ concentration of unsupplemented cows was not affected by PA (17.1 mg/dl, $P > 0.05$; *Table 32*). This agrees with Wales et al. (2001), who reported no differences in $\text{NH}_3\text{-N}$ concentration (16.1 mg/dl) for unsupplemented cows grazing a ryegrass pasture of 17.4%

CP at low or high PA. Delagarde et al. (1997) reported a similar rumen $\text{NH}_3\text{-N}$ concentration (17.7 mg/dl) to this current study for unsupplemented cows grazing a ryegrass pasture of 17.3% CP at a PA of 19.9 kg OM/cow/d (at a 5 cm cutting height). Higher rumen $\text{NH}_3\text{-N}$ concentrations (22.4 to 32.3 mg/dl) were reported by others (Berzaghi et al., 1996; Van Vuuren et al., 1986) for unsupplemented dairy cows grazing pastures with a higher CP level (24.4 to 25.1% CP) than the pasture grazed in this study (21.0% CP). Others studies (Khalili and Sairanen, 2000; Reis and Combs, 2000b), however, reported higher rumen $\text{NH}_3\text{-N}$ concentrations (22.4 to 28.7 mg/dl) for unsupplemented dairy cows grazing pasture with similar CP content (20.8 to 20.9%) to the pasture grazed in our study.

Rumen fermentation parameters of supplemented dairy cows grazing at two PA are also shown in *Table 32*. Mean rumen pH did not differ between treatments and averaged 6.05 ($P > 0.05$). No previous studies have evaluated the effect of PA on rumen fermentation of dairy cows supplemented with concentrate. High producing dairy cows grazing a ryegrass pasture at a PA of 23 kg DM/cow/d had a rumen pH of 6.00 or 5.75 when supplemented with 5 or 10 kg/d of concentrate, respectively (Sayers, 1999). Khalili and Sairanen (2000) reported a rumen pH of 6.01 and 6.17 for cows grazing at high PA (> 40 kg DM/cow/d) and supplemented with 4 kg/d of barley or commercial concentrate, respectively. Van Vuuren et al. (1986) reported a rumen pH of 5.9 for grazing dairy cows supplemented with 7 kg/d of a starch-based concentrate.

Total VFA concentration on supplemented cows averaged 148.4 mmol/L ($P > 0.05$; *Table 32*), similar to that reported by Berzaghi et al. (1996). Others reported lower VFA concentrations (127 mmol/L; Van Vuuren et al., 1986; 129.5 mmol/L, Khalili and Sairanen, 2000) for supplemented grazing cows, probably due to the lower levels of concentrate supplementation (Khalili and Sairanen, 2000). Reis and Combs (2000b) reported a total VFA

concentration of 99.0 and 104.0 mmol/L for supplementation levels of 5 or 10 kg/d of a corn-based concentrate, respectively. Sayers (1999) found a total VFA concentration of 119.3 or 124.8 mmol/L when high producing cows supplemented with 5 or 10 kg/d of concentrate, respectively. Iso-butyrate and iso-valerate concentrations were significantly higher for supplemented cows grazing at low PA ($P < 0.05$).

Rumen $\text{NH}_3\text{-N}$ concentration on supplemented cows did not differ between treatments (13.9 mg/dl; $P > 0.05$; *Table 32*). Khalili and Sairanen (2000) reported higher rumen $\text{NH}_3\text{-N}$ concentration (> 21 mg/dl), which could be explained by the lower level of concentrate supplementation. Reductions in rumen $\text{NH}_3\text{-N}$ concentration have been reported (from 13.9 to 11.7 mg/dl; Sayers, 1999; 17.7 to 13.8 mg/dl; Reis and Combs, 2000b) for grazing dairy cows when concentrate level was increased from 5 to 10 kg/d. Dairy cows supplemented with similar levels of concentrate but grazing pasture with higher CP than this study have reported rumen $\text{NH}_3\text{-N}$ concentrations higher than 17 mg/dl (Berzaghi et al., 1996; Van Vuuren et al., 1986).

4.3.4 Grazing Behavior

Grazing behavior parameters of unsupplemented or supplemented dairy cows grazing at low or high PA is presented in *Table 33*. Total grazing time was higher when unsupplemented cows grazed at high PA (638 vs. 461 min/d; $P < 0.05$; *Table 33*). The higher total daily grazing time was a result of a higher grazing time during the night (GT p.m.) (301 vs. 137 min; $P < 0.05$). Grazing time during the morning (GT a.m.) did not differ between treatments and averaged 331 min ($P > 0.05$).

Few studies (Delagarde et al., 1997; Pulido and Leaver, 2001; Wales et al., 2001) presented grazing behavior data of unsupplemented dairy cows on pasture, and only one (Wales et al., 2001) evaluated the effect of PA. In agreement with this current study, Wales et al. (2001) reported that grazing time (measured by visual observations every 10 min) increased significantly from 396 to 468 min/d as PA increased. Delagarde et al. (1997) reported grazing time (measured using a portable electronic device) of 546 min/d for unsupplemented dairy cows grazing a ryegrass pasture at a PA of 19.9 kg OM/cow/d (above 5 cm); while grazing time during the evening averaged 257 min. Pulido and Leaver (2001) reported a grazing time (measured by visual observations every 10 min) of 540 min/d for unsupplemented cows grazing a ryegrass pasture. The differences in grazing time between those studies and this current study could be partially explained by differences in size of cows, type of pasture, and method of measuring grazing behavior.

Total number of bites per d was significantly higher when unsupplemented cows grazed at high PA ($P < 0.05$; *Table 33*). The total number of bites per d of cows at high PA (> 36000 bites/d) was close to the maximum number of total bites (40000 bites/d) estimated by Phillips and Leaver (1986) for dairy cows. Delagarde et al. (1997) reported a total of 29000 bites/d for cows grazing at a PA of 19.9 kg OM/cow/d (at a 5 cm cutting height). The higher total bites per d at high PA resulted in a significantly higher number of bites during the night (bites p.m.) ($P < 0.05$).

Average daily biting rate and biting rate during the morning (BR a.m.) of unsupplemented cows averaged 55 bites/min ($P > 0.05$; *Table 33*). Unsupplemented cows grazing at high PA tended to have a higher biting rate during the night (BR p.m.) (57 vs. 52

bites/min; $P < 0.11$). Delagarde et al. (1997) reported similar biting rate (54 bites/min) for unsupplemented dairy cows on pasture.

Bite mass was significantly lower when unsupplemented cows grazed at high PA ($P < 0.05$; *Table 33*). The lower bite mass at the high PA may be explained by cows grazing the same paddock after the a.m. and the p.m. milkings. Unsupplemented cows at high PA had a significantly higher grazing time during the evening than cows at low PA, therefore it is likely that cows grazed a pasture with structure characteristics that did not maximize bite mass. Bite mass is influenced by pasture structure, accessibility of preferred plant components, and material mass than can be encompassed within a bite (Poppi et al., 1987). The bite mass found for the cows grazing at high PA in our study (0.51 g DM/bite) was similar to the values (0.53 g OM/bite) reported by Delagarde et al. (1997). It should be noted, however, that the use of long-term (e.g. daily) intakes compared to short-term (e.g. hourly) intakes to calculate bite mass may result in a bias because the calculation of bite mass assumes that bite mass is constant over that period of time (Rook, 2000).

None of the grazing behavior variables of the supplemented cows were affected by PA ($P > 0.05$; *Table 33*). Previous studies have not evaluated the effect of PA on grazing behavior of dairy cows supplemented with concentrate. Total daily grazing time averaged 598 min, grazing time between the a.m. and the p.m. milking averaged 351 min, and grazing time between the p.m. and the a.m. milking averaged 247 min ($P > 0.05$; *Table 33*). The total daily grazing time is close to the maximum daily grazing time of 10 h/d estimated by Phillips and Leaver (1986). Rook and Huckle (1996) reported a similar value of grazing time (597 min/d; measured using recorders) for dairy cows grazing a ryegrass/white clover and supplemented with 4 kg/d of concentrate. More recently, Pulido and Leaver (2001) reported a grazing time (measured by

visual observations every 10 min) of 515 min/d for high producing dairy cows grazing a ryegrass pasture and supplemented with 6 kg/d of a barley-based concentrate. Lower values of grazing time (measured using Kienzle Vibracorders) were reported for dairy cows grazing a ryegrass/white clover pasture and supplemented with 5 kg/d of a cereal-based concentrate (Arriaga-Jordan and Holmes, 1986). Sayers (1999) reported grazing time (measured by automatic behavior recorders) of 458 and 358 min/d for high producing dairy cows grazing a ryegrass pasture at a PA of 23 kg DM/cow/d and supplemented with 5 or 10 kg/d of a starch-based concentrate, respectively.

Total number of bites per day, number of bites during the morning (bites a.m.) and number of bites during the evening and night (bites p.m.) of supplemented cows were not affected by PA ($P > 0.05$; *Table 33*). Supplemented cows of both treatments averaged 32137 bites/d, a higher value than reported by Arriaga-Jordan and Holmes (1986) (26100 bites/d) and Sayers (1999) (21302 and 16041 bites/d for 5 and 10 kg/d of concentrate, respectively). Biting rate of supplemented cows averaged 54 bites/min and it was not affected by PA ($P > 0.05$; *Table 33*). Arriaga-Jordan and Holmes (1986) reported higher values of biting rate (63 bites/min).

Bite mass of supplemented cows was not affected by PA and averaged 0.46 g DM/bite ($P > 0.05$; *Table 33*). No differences between treatments were expected because cows grazed a pasture that was offered on a daily basis at similar pre-grazing pasture mass (*Table 29*) and pasture height, which determines bite mass (McGilloway et al., 1999; Phillips and Leaver, 1986). However, the estimation of bite mass using long-term intake estimation may be bias because it was assumed to be constant in a 24-h period (Rook, 2000). Higher values of bite mass have been previously found in high producing dairy cows supplemented with 5 kg/d of a cereal-based concentrate (Arriaga-Jordan and Holmes, 1986). Sayers (1999) reported bite mass of 0.61 and

0.58 g DM/bite for high producing dairy cows supplemented with 5 or 10 kg/d of a starch-based concentrate, respectively.

4.3.5 Pattern of Grazing Behavior and Its Relation with Rumen Digestion Variations

The grazing time pattern of unsupplemented dairy cows grazing at two PA and its relation with the daily fluctuations of rumen pH and $\text{NH}_3\text{-N}$ concentration is shown in *Figure 7*. The bottom part of the figure shows the grazing time in min/h for a 24-h period (e.g. at 0500 h cows on the high PA treatment grazed 12 min of the 60 min of 0500 h). In the middle and the top part of the figure, rumen pH and $\text{NH}_3\text{-N}$ concentration, respectively, are presented at each of the 6 sampling times during the 24-h period.

A significant treatment by hour interaction was found for both rumen pH and $\text{NH}_3\text{-N}$ concentration ($P < 0.05$). The morning grazing time was not affected by PA (*Table 33*), however cows grazing at high PA tended to graze more at 0900 h ($P < 0.12$). This tendency toward a higher grazing activity was related with numerically lower rumen pH of cows at high PA for the 0930 h ($P < 0.13$) and the 1330 h ($P > 0.15$) sampling time. Cows grazing at high PA had a higher grazing time during the evening and night than cows grazing at low PA (*Table 33*). Grazing time at high PA was numerically higher at 1900, 2000, and 2100 h, and significantly higher at 2200, 2300, 2400 and 0400 h ($P < 0.10$). The higher grazing activity of cows at high PA was related with significantly lower rumen pH at different sampling times. The higher grazing time between 2200 and 2400 h was associated with a significantly lower rumen pH at 0130 h ($P < 0.05$); the higher grazing time at 0400 h was associated with a trend toward a lower rumen pH at 0530 h ($P < 0.13$). Only at the 0530 h sampling time, did the rumen $\text{NH}_3\text{-N}$

concentration differ between treatments ($P < 0.10$). The higher rumen $\text{NH}_3\text{-N}$ concentration at low PA, however, was unexpected because cows grazed less time on that treatment. Of interest is the peak rumen $\text{NH}_3\text{-N}$ concentration above 20 mg/dl at 0930 h and 1330 h.

The grazing time pattern of supplemented cows grazing at two PA and the daily fluctuations of rumen pH and $\text{NH}_3\text{-N}$ concentration are shown in *Figure 8*. Although the mean rumen pH of supplemented cows did not differ with PA (*Table 32*), a significant treatment by hour interaction was detected for rumen pH ($P < 0.05$). Supplemented cows grazing at high PA had a higher grazing time at 1500 h ($P < 0.05$), which was associated with a lower rumen pH at 1730 h ($P < 0.10$). A lower rumen pH at 0130 h ($P < 0.10$) and a higher grazing time ($P < 0.10$) at 0200 h were observed for supplemented cows at high PA. For the rumen $\text{NH}_3\text{-N}$ concentration, the treatment by hour interaction was not significant ($P > 0.05$), and this parameter did not differ at any sampling time for supplemented cows, as expected because grazing time pattern was similar during the 24 h.

4.4 CONCLUSIONS

Pasture allowance had important effects on performance, rumen digestion and grazing behavior of unsupplemented dairy cows on pasture. Pasture DMI was increased when unsupplemented cows grazed at high PA, which resulted in a higher milk production. Milk composition of unsupplemented cows was not affected by PA. Rumen pH was lower when cows grazed at high PA, while neither rumen $\text{NH}_3\text{-N}$ nor total VFA concentrations were affected by PA. Unsupplemented cows grazing at high PA had a higher daily grazing time and number of

bites due to higher values of these two behavioral parameters during the evening. At high PA, however, unsupplemented cows had a lower bite mass.

Pasture allowance had no major effects on performance, rumen digestion and grazing behavior of supplemented dairy cows. Total DMI, milk production, and milk composition were similar in supplemented cows regardless of PA. Rumen fermentation parameters did not differ between PA treatments. None of the grazing behavior variables (grazing time, biting rate, and bite mass) of supplemented cows were affected by PA.

The pattern of grazing time of both unsupplemented and supplemented dairy cows grazing at two PA explained daily variations in rumen pH and $\text{NH}_3\text{-N}$ concentration, beyond the daily means observed. Lower rumen pH was observed when cows had higher grazing activity. Similar rumen $\text{NH}_3\text{-N}$ concentrations were associated with similar grazing time per h.

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Table 27: Ingredient and chemical composition (mean $\pm$ SD) of the mineral-vitamin mix and the concentrate used for the unsupplemented and supplemented dairy cows, respectively.

	Mineral-vitamin mix	Concentrate
Ingredient composition, % DM		
Shell dry corn	29.3	62.3
Wheat midds	...	10.4
Barley grain	...	7.8
Roasted soybeans	...	5.2
Molasses	24.4	5.2
Corn gluten meal	...	2.6
Limestone	...	1.3
Dicalcium phosphate	19.5	2.1
Salt	11.7	1.4
Magnesium oxide	6.8	0.8
Dynamate	4.9	0.6
Selenium premix	1.5	0.15
PS TM premix #4	1.4	0.1
Vitamins A, D, E	0.5	0.05
Chemical composition		
DM, %	93.9 $\pm$ 0.2	91.1 $\pm$ 0.5
% DM		
OM, %	62.4 $\pm$ 2.9	91.7 $\pm$ 0.5
CP, %	9.6 $\pm$ 0.3	14.1 $\pm$ 0.9
TNC, %	19.7 $\pm$ 1.5	57.1 $\pm$ 0.6
NDF, %	20.3 $\pm$ 0.7	18.2 $\pm$ 0.9
ADF, %	12.6 $\pm$ 0.2	6.9 $\pm$ 0.1
IVDMD, %	86.6 $\pm$ 2.4	89.5 $\pm$ 9.8

Table 28: Weather data during the experimental periods and in the previous ten years.

	Period 1 (9/3 to 9/23)	Period 2 (9/24 to 10/14)
Trial weather		
Precipitations, mm	96	70
Average high temperature, °C	23 (13 to 29)	18 (15 to 24)
Average low temperature, °C	13 (6 to 21)	8 (1 to 18)
1988-1998 weather		
Precipitations, mm	96	47
Average high temperature °C	23	18
Average low temperature, °C	12	7

Table 29: Pasture management of low and high pasture allowance (PA) treatments.

	Pasture allowance ¹		SEM	<i>P</i> < ²	
	Low PA	High PA		PA	Period
Pasture allowance, kg DM/cow per d	27	47	2	<0.01	0.94
Area, m ² /cow per d	95	170	6	<0.01	0.04
Pre-grazing herbage mass, kg DM/ha	2751	2784	135	0.86	0.03
Post-grazing herbage mass, kg DM/ha	1010	1476	57	<0.01	0.01
Efficiency of harvesting, %	63	46	3	<0.01	0.64

¹Pasture allowance targets: Low PA = 25 kg DM/cow/d; High PA = 40 kg DM/cow/d.

²PA = effect of pasture allowance; Period = effect of period.

Table 30: Chemical composition of hand-plucked pasture samples of low and high pasture allowance (PA) treatments.

	Pasture allowance ¹		SEM	$P <^2$	
	Low PA	High PA		PA	Period
DM, %	16.3	16.0	0.8	0.78	0.05
% DM					
OM, %	91.1	91.0	0.2	0.94	0.32
CP, %	21.3	20.7	0.5	0.46	0.02
Soluble CP, % CP	25.7	26.3	0.9	0.73	0.01
TNC, %	14.9	16.2	0.6	0.10	<0.01
NDF, %	52.9	50.8	0.9	0.11	0.03
ADF, %	27.4	26.2	0.6	0.08	0.33
IVDMD, %	70.3	74.9	1.1	0.01	0.18

¹Pasture allowance targets: Low PA = 25 kg DM/cow/d; High PA = 40 kg DM/cow/d.

²PA = effect of pasture allowance; Period = effect of period.

Table 31: Dry matter intake, and milk yield and composition of unsupplemented or supplemented high producing dairy cows grazing at two pasture allowances (PA).

	Unsupplemented cows			Supplemented cows		
	Low PA	High PA	SEM	Low PA	High PA	SEM
DMI, kg/d						
Supplement	0.8	0.8	0.03	8.1	8.1	0.1
Pasture	15.6 ^a	18.8 ^b	0.5	14.3	15.1	0.6
Total	16.4 ^a	19.6 ^b	0.5	22.4	23.2	0.6
DMI, %BW	2.69 ^a	3.14 ^b	0.07	3.32	3.41	0.1
Milk, kg/d ¹	15.2 ^a	17.6 ^b	0.4	27.7	27.9	0.5
Fat						
% ¹	3.73	3.72	0.07	3.32	3.56	0.13
kg/d ¹	0.56 ^a	0.66 ^b	0.02	0.89	0.99	0.04
Total protein						
%	3.21	3.16	0.03	3.07	3.09	0.04
kg/d ¹	0.47 ^a	0.55 ^b	0.01	0.82 ^a	0.85 ^b	0.01
True protein						
%	3.03	2.98	0.03	2.89	2.91	0.03
kg/d ¹	0.45 ^a	0.52 ^b	0.01	0.77	0.80	0.01
MUN, mg/dl ²	13.2	13.6	0.7	12.2	12.5	0.3

^{a, b}Least square means within unsupplemented or supplemented in the same row with different superscripts differ ($P < 0.05$).

¹Significant ($P < 0.05$) effect of period for unsupplemented cows.

²MUN = milk urea nitrogen.

Table 32: Rumen fermentation of unsupplemented or unsupplemented high producing dairy cows grazing at two pasture allowances (PA).

Item	Unsupplemented cows			Supplemented cows		
	Low PA	High PA	SEM	Low PA	High PA	SEM
pH ^{1, 2}	6.60 ^a	6.33 ^b	0.05	6.04	6.05	0.07
NH ₃ -N, mg/dl ^{1, 2}	17.09	17.20	0.97	14.01	13.69	1.17
VFA, mmol/L						
Total ^{1, 2}	119.46 ^a	134.39 ^b	3.02	150.84	146.02	4.38
Acetate (A) ^{1, 2}	82.24 ^a	92.58 ^b	2.13	99.05	94.07	2.79
Propionate (P) ^{1, 2}	22.51	24.05	0.59	29.69	29.95	1.04
Butyrate ^{1, 2}	10.35 ^a	13.14 ^b	0.28	16.36	16.79	0.53
Iso-Butyrate ^{1, 2}	1.34	1.32	0.03	1.44 ^a	1.29 ^b	0.04
Valerate ^{1, 2}	1.47 ^a	1.75 ^b	0.05	2.15	2.16	0.06
Iso-Valerate ^{1, 2}	1.55	1.55	0.05	2.14 ^a	1.74 ^b	0.05
A:P ²	3.70 ^a	3.88 ^b	0.03	3.35 ^a	3.23 ^b	0.03

^{a, b}Least square means within unsupplemented or supplemented in the same row with different superscripts differ ($P < 0.05$).

¹Significant ($P < 0.05$) effect of period for unsupplemented cows.

²Significant ($P < 0.05$) effect of period for supplemented cows.

Table 33: Grazing behavior of unsupplemented or supplemented high producing dairy cows grazing at two pasture allowances (PA).

Item	Unsupplemented cows			Supplemented cows		
	Low PA	High PA	SEM	Low PA	High PA	SEM
Grazing time (GT), min/d	461 ^a	638 ^b	46	614	581	33
GT a.m., min	324	337	27	364	338	15
GT p.m., min	137 ^a	301 ^b	34	251	242	24
Bites/d	24353 ^a	36663 ^b	2447	32918	31356	2236
Bites a.m.	17285	19401	1269	19777	18399	1114
Bites p.m.	7068 ^a	17262 ^b	2111	13141	12956	1472
Biting rate (BR), bites/min	53	57	2	54	54	3
BR a.m., bites/min ¹	53	57	2	54	54	3
BR p.m., bites/min	52	57	2	52	54	2
Bite mass, ² g DM/bite	0.64 ^a	0.51 ^b	0.03	0.43	0.48	0.05

^{a, b}Least square means within unsupplemented or supplemented in the same row with different superscripts differ ($P < 0.05$).

¹Significant ($P < 0.05$) effect of period for unsupplemented cows.

²Bite mass = pasture DMI / (bites/d).

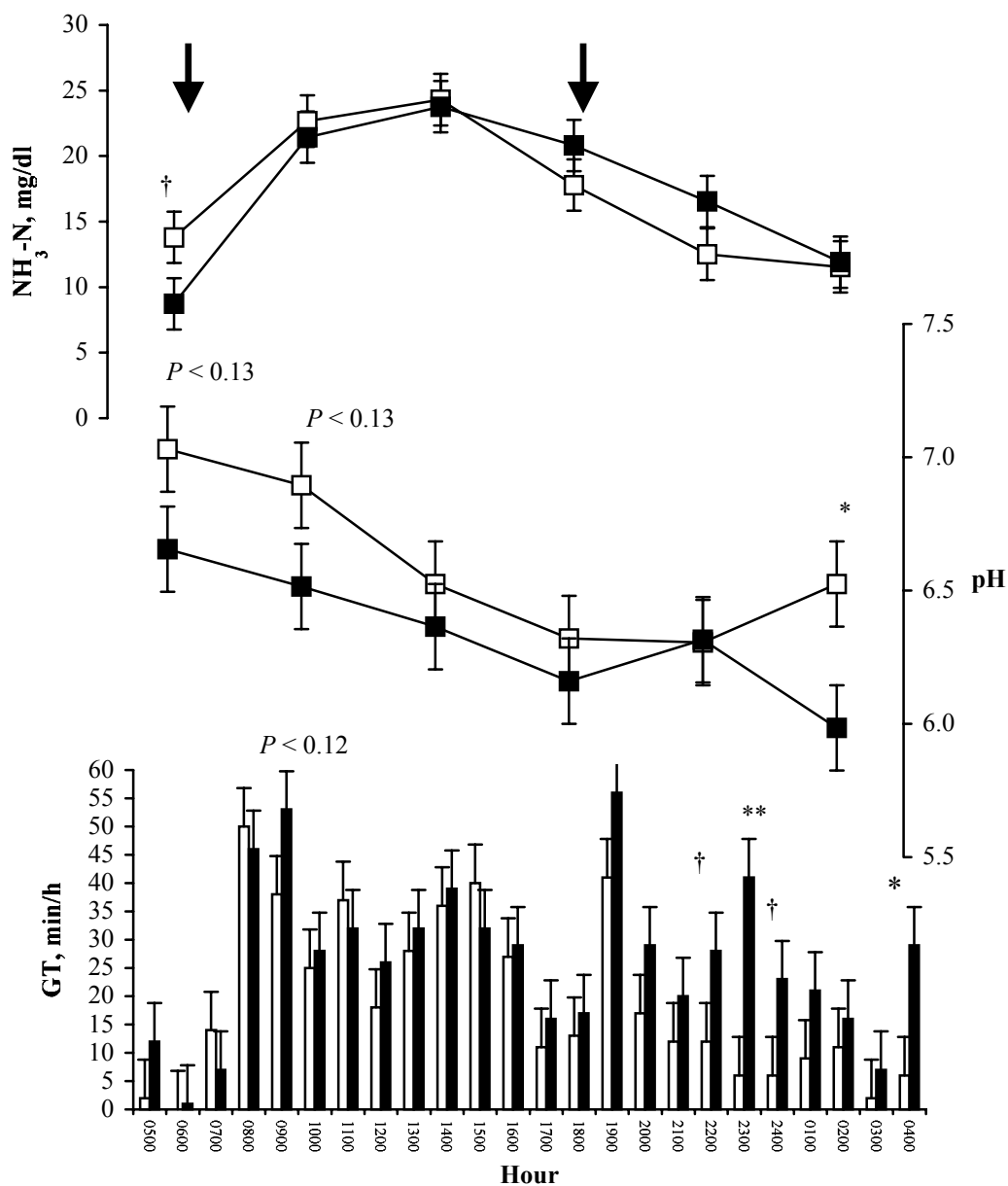


Figure 7: Daily rumen pH and $\text{NH}_3\text{-N}$ concentration and the grazing time pattern in min/h for a 24-h period for unsupplemented high producing dairy cows grazing at low (□) or high (■) pasture allowance. Arrows indicate milking times. † $P < 0.10$, * $P < 0.05$, ** $P < 0.01$.

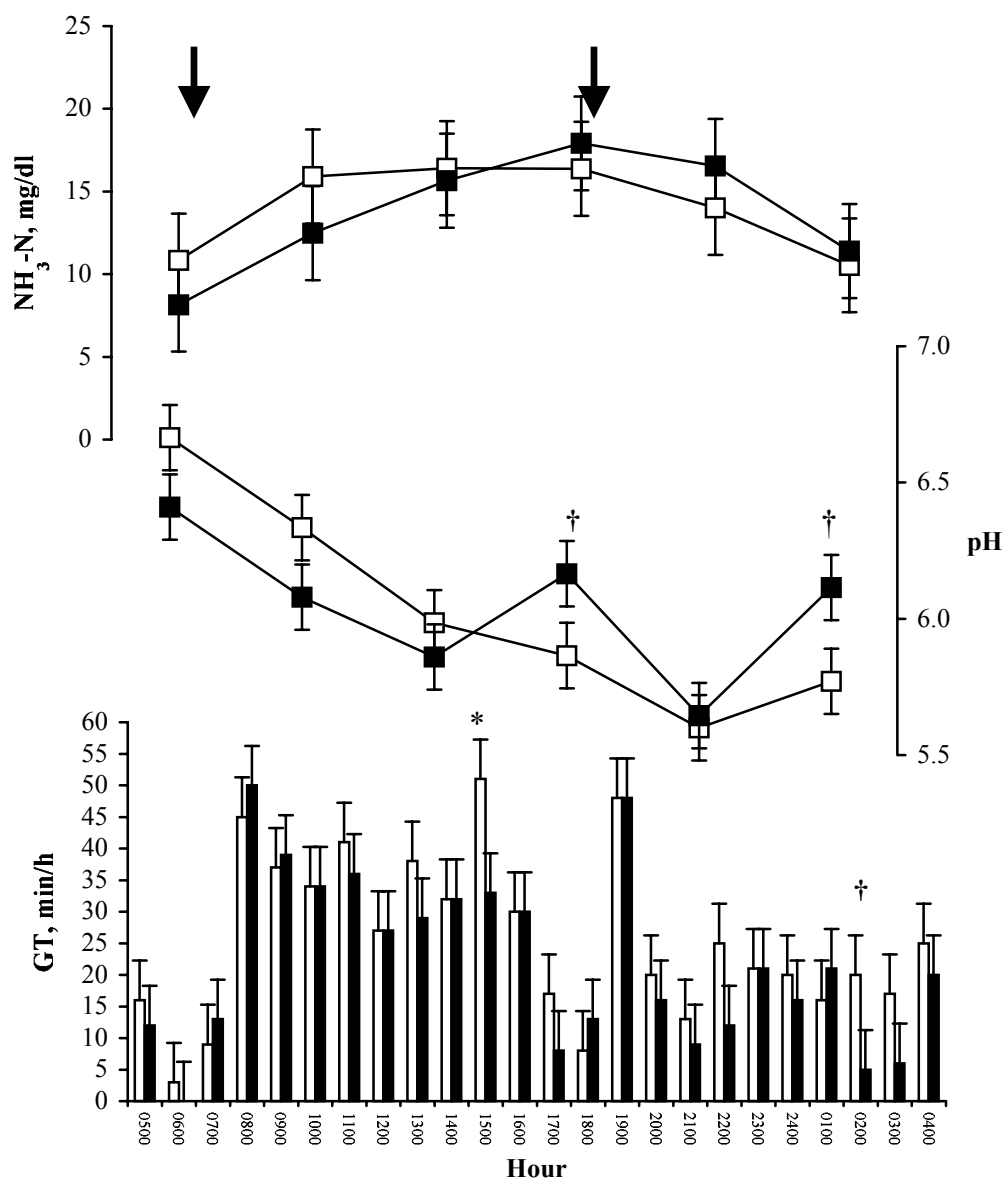


Figure 8: Daily rumen pH and NH₃-N concentration and the grazing time pattern in min/h for a 24-h period for supplemented high producing dairy cows grazing at low (□) or high (■) pasture allowance. Arrows indicate milking and concentrate feeding times. †*P* < 0.10, **P* < 0.05, ***P* < 0.01.

Chapter 5

Performance of High Producing Dairy Cows with Three Different Feeding Systems Combining Pasture or Total Mixed Rations

5.1 ABSTRACT

Forty-five Holsteins cows (15 primiparous, 30 multiparous) were used to compare three feeding systems combining pasture and total mixed ration (TMR) on animal performance in a 21-wk repeated measures experiment. The three treatments were: 1) pasture plus concentrate (PC), 2) pasture plus partial TMR (pTMR), and 3) TMR (non pasture). Total dry matter intake (DMI), using chromic oxide as a marker, was 21.6, 25.3, and 26.8 kg/d for PC, pTMR, and TMR, respectively. Milk production was highest for TMR (38.1 kg/d), lowest for PC (28.5 kg/d), and intermediate for pTMR (32.0 kg/d). All three treatments received ten bST injections, PC responded to six, pTMR to four, and TMR to nine administration of bST during the 21 wk trial; milk response averaged 13.8, 15.6, and 11.8% for PC, pTMR, and TMR, respectively. Cows fed pTMR and TMR had higher milk fat (3.33 vs. 3.13%) and true protein (2.97 vs. 2.82%) percentage than cows fed PC. Cows on PC gained less body weight and lost more body condition compared to cows on pTMR and TMR. Initial concentrations of plasma nonesterified fatty acids (NEFA) were higher in PC (302 $\mu\text{eq/L}$) than in pTMR (130 $\mu\text{eq/L}$) and TMR (225 $\mu\text{eq/L}$). Plasma urea nitrogen (PUN) was lower in both pTMR and TMR treatments (13.8 mg/dl) than in PC treatment (17.2 mg/dl). Performance was improved combining pasture and TMR compared to

pasture plus concentrate, resulting in higher milk production, milk production persistency, milk fat and protein percentage, and no loss in body condition score.

(Key words: pasture, total mixed rations, milk production, milk composition, body condition score, grazing behavior)

Abbreviation key: IVDMD = in vitro dry matter digestibility, MUN = milk urea nitrogen, TNC = total nonstructural carbohydrates.

5.2 INTRODUCTION

Energy is the first limiting nutrient for high producing dairy cows on pasture. Kolver and Muller (1998) reported that the lower milk production from high producing cows grazing high quality pasture as the only feed (29.6 kg/d) compared with cows consuming a nutritionally balanced TMR (44.1 kg/d) was due to a lower DMI that resulted in a lower energy intake. This study indicated that high producing cows on pasture need supplemental energy to reach the genetic potential for milk production. Recently, some studies conducted in the US with high producing cows grazing high quality pastures (Bargo et al., 2000; Reis and Combs, 2000a; Reis and Combs, 2000b; Soriano et al., 2000) reported that supplementation with 8 to 9 kg/d of corn based concentrates increased total DMI to approximately 21 to 22 kg/d and sustained milk production around 30 kg/d. These studies also reported that energy supplementation reduced milk fat percentage, which was associated with a high amount of starch and the lack of effective fiber of pasture. Although concentrate supplementation increased total DMI and milk production

relative to only pasture, both are still low compared to values when feeding a TMR. These studies also were relatively short (between 9 and 12 wk) or were conducted as non-continuous designed experiments (e.g. Latin square designs) where BW and BCS changes could not be evaluated.

Many dairy farmers in the US avoid using pasture because milk production per cow is lower than with a confinement feeding systems (Kolver and Muller, 1998; White, 2000; White et al., 2001). Some management challenges where all the forage is provided from pasture include low milk production, low milk fat and protein content, variations in production because of climate conditions, difficulty in estimating and budgeting pasture availability, and inaccurate estimation of total and pasture DMI (Muller and Fales, 1998). Supplementation of pasture with a TMR, a feeding system that is called partial TMR because the pasture grazed by the cows is not physically part of the TMR, may reduce these challenges. Many dairy producers of the Northeast and Midwest have the equipment and knowledge for TMR feeding systems because this system is used during the non-grazing season. Some of the advantages of feeding a partial TMR include the provision of a more uniform ration throughout the year and the grazing season, an easier and more accurate monitor of pasture and total DMI, less chance of rumen digestive problems due to slug feeding of grain because some forage is fed with the concentrate rather than separate, and likely a higher milk production per cow with a higher milk fat and protein content.

There are no studies that have compared pasture as the only forage plus concentrate, a total TMR diet, and the combination of both pasture and TMR. Previous studies have compared milk performance of high producing dairy cows consuming pasture as the only feed versus cows fed a TMR (Kolver and Muller, 1998; Kolver et al., 2000), TMR versus pasture plus concentrate (White, 2000; White et al., 2001), or TMR versus TMR plus pasture (Soriano et al., 2001).

However, these studies were either short-term studies of 4 to 6 wk (Kolver and Muller, 1998; Soriano et al., 2001) or long-term study that did not include a partial TMR treatment (White, 2000; White et al., 2001). Our objective was to compare three feeding systems combining pasture and a TMR during a 21 wk period on animal performance.

5.3 MATERIALS AND METHODS

5.3.1 Cows and Treatments

Forty-five Holstein cows (15 primiparous and 30 multiparous) [BW, 624 ± 56 kg; milk yield, 44.9 ± 7.5 kg/d; parity, 2.19 ± 1.24 ; DIM, 109 ± 39 (mean $\pm$ SD)] were used in a 21-wk trial to study the effect of three feeding systems on animal performance. Cows were selected from the dairy herd of The Pennsylvania State University Dairy Cattle Research and Education Center (University Park, PA), which averaged 11436 kg of milk and 363 kg of protein per cow per lactation in 1999. Cows were stratified in groups of three by lactation number and DIM, and randomly assigned to three dietary treatments: 1) pasture + concentrate (PC); 2) pasture + TMR (partial TMR; pTMR), and 3) TMR (non-pasture).

Cows on the PC treatment grazed a pasture with an average botanical composition of 50% smooth brome grass (*Bromus inermis* L.), 33% orchardgrass (*Dactylis glomerata* L.), 7% Kentucky bluegrass (*Poa pratensis* L.), and 10% weeds and dead material. Pasture was fertilized five times with 50 kg/ha of nitrogen before the start of the trial on April 20 and during the trial

on June 7 (wk 5), June 19 (wk 7), July 31 (wk 13), and September 5 (wk 18). The target amount of pasture offered per cow (pasture allowance) was 30 kg DM/d, measured to the ground level. Pre-grazing pasture mass was measured every week to adjust the size of the paddock to maintain the targeted pasture allowance. Pre-grazing pasture mass (kg DM/ha) was measured by cutting fifteen quadrants ($0.124 \text{ m}^2/\text{quadrant}$) of pasture to ground level, and drying at 55°C in a forced air oven. A new paddock was constructed daily using a temporary polywire, and divided by a polywire to offer a new portion after each milking. A second polywire fence was used to prevent back-grazing. New paddocks were provided each morning at approximately 0700 h. After the a.m. milking, 2/3 of the daily paddock was offered to 30 cows (15 of the PC treatment and 15 of the pTMR treatment) to ensure a pasture allowance of 15 kg DM/cow for a half day. After the p.m. milking, the other 1/3 portion of the paddock was offered to the 15 cows of the PC treatment with a pasture allowance of 15 kg DM/cow for a half day to complete the daily allowance of 30 kg DM/cow per d on this treatment. Cows on the PC treatment also received a corn based concentrate with the ingredient and chemical composition shown in *Table 34*. The amount of concentrate offered per cow was 1 kg/4 kg of milk at the beginning of the trial based on the pre-trial milk production. An upper limit of 10 kg DM/cow/d was established to reduce the risk metabolic problems in the rumen. The amount of concentrate offered was readjusted at the middle of the trial (wk 10) using the milk production from wk 9 and 10.

Cows on the pTMR treatment grazed between the a.m. and the p.m. milking with the cows on the PC treatment. After the p.m. milking, cows on the pTMR treatment were housed overnight in a free stall barn where they were fed 17 kg DM of the TMR (*Table 34*), which was the same TMR that was fed to the TMR, non-grazing treatment. After the a.m. milking, cows on the pTMR were moved to the pasture with the PC treatment to graze as explained above. Cows

on the pTMR treatment were fed 2 to 3 kg/d of the same concentrate fed to the PC concentrate (*Table 34*) with the purpose of having a similar forage:concentrate ratio in the total ration for the PC and the pTMR treatments.

Cows on the TMR treatment remained in a free-stall barn and were group fed the TMR (formulated according NRC, 1989; *Table 34*) once daily at 0730 h at an approximately rate of 28 kg DM/cow/d. Feed was pushed up three times per d.

Cows were milked twice daily at 0530 h and 1730 h. All cows received bST injections every two weeks. Walking distance from pasture to the milking parlor averaged 0.9 km (range: 0.75 to 1.20 km).

5.3.2 Experimental Measures and Sample Analyses

Dry matter intake was measured four times during wk 2 (May 15), 6 (June 12), 14 (August 7), and 19 (September 11). Cows fed the pTMR and the TMR treatments were placed in a tie-stall barn during these periods and fed individually to measure DMI of TMR. Total DMI was also estimated for the three treatments using Cr_2O_3 as an indigestible fecal marker. The indigestible fecal marker Cr_2O_3 was dosed twice daily (10 g/d) after each milking (0700 h and 1900 h, approximately) for eleven days. Fecal grab samples were collected at 0700 h and 1900 h from d 7 to 11, and immediately frozen (-20 °C).

During the same days that fecal samples were taken, samples of the concentrate and the TMR were collected, and pasture samples were plucked by hand to the approximate height to which cows grazed (Wallis de Vries, 1995). Hand-plucked pasture samples were also taken every week. Samples were dried at 55 °C in a forced air oven and ground through a 1-mm screen

(Wiley Mill, Thomas Scientific, Philadelphia, PA). Concentrate and TMR samples were composited by period, while hand-plucked pasture samples taken daily during the intake periods were kept as daily samples and hand-plucked pastures taken weekly were kept as weekly samples. Composited concentrate and TMR samples, and both daily (intake periods) and weekly hand-plucked pasture samples were analyzed for DM, CP, and ash (AOAC, 1990), soluble CP (Krishnamoorthy et al., 1982), ADF and NDF (Ankom Daisy II, ANKOM Technology Corp., Fairport, NY), total nonstructural carbohydrate (TNC) (Smith, 1981; modified to use potassium ferricyanide as the colorimetric indicator), and in vitro DM digestibility (IVDMD) (Tilley and Terry, 1963).

Fecal samples were thawed, dried at 55 °C in a forced air oven, and ground through a 1-mm screen (Wiley Mill, Thomas Scientific, Philadelphia, PA). A composited sample per cow was made for each period of intake measures. Fecal samples were analyzed for CP (AOAC, 1990), NDF (Ankom Daisy II, ANKOM Technology Corp., Fairport, NY), and Cr (Parker et al., 1989). The concentrate DMI in both the PC and the pTMR treatments, and the TMR DMI in both the pTMR and the TMR treatments were measured daily in every cow as the difference between offered and refused the same five days that fecal grab samples were taken. Total DMI in the TMR treatment was also determined by the equation: $\text{total DMI (TMR)} = [(g \text{ Cr/d}) / (g \text{ Cr/g fecal DM})] / (1 - \text{IVDMD of TMR})$. Pasture DMI was estimated in the PC treatment using the equation: $\text{pasture DMI} = [(g \text{ Cr/d}) / (g \text{ Cr/g fecal DM}) - \text{concentrate DMI} \times (1 - \text{IVDMD of concentrate})] / (1 - \text{IVDMD of pasture})$. Pasture DMI was estimated in the pTMR treatment using the equation: $\text{pasture DMI} = [(g \text{ Cr/d}) / (g \text{ Cr/g fecal DM}) - \text{concentrate DMI} \times (1 - \text{IVDMD of concentrate}) - \text{TMR DMI} \times (1 - \text{IVDMD of TMR})] / (1 - \text{IVDMD of pasture})$. Total DMI in the PC treatment was determined as concentrate DMI plus pasture DMI, in the pTMR

treatment as pasture DMI plus concentrate DMI plus TMR DMI, and in the TMR treatment as TMR DMI. Apparent digestibility of DM, NDF, and CP were determined with the fecal output estimated using Cr_2O_3 as marker and DMI, and nutrient concentration in feces and feed.

Milk production was recorded daily from d 1 (May 8) to 147 (October 1). Milk samples were collected weekly during the 21 wk of the experiment and preserved with 2-bromo-2-nitropropane-1,3 diol. Milk fat and true protein were analyzed by infrared spectrophotometry (Foss 605B Milk-Scan; Foss Electric, Hillerod, Denmark) by the Pennsylvania DHIA milk testing laboratory.

Cows were weighed after the p.m. milking on two consecutive days every two weeks (wk 1, 3, 5, 7, 9, 11, 13, 15, 17, 19, and 21). The same weeks, the body condition of the cows was scored by two experimented independent observers using the five-point BCS scale (1 = thin; 5 = fat) (Wildman et al., 1982).

Blood samples were collected before the cows received the concentrate or the TMR at 0600 h every two weeks (wk 1, 3, 5, 7, 9, 11, 13, 15, 17, 19, and 21) from the coccygeal vessels into one 20-ml evacuated tube containing sodium heparin, and one 10-ml evacuated tube containing potassium oxylate-sodium fluoride (glycolytic inhibitor). Blood was immediately placed on ice and transported to the laboratory. Samples were centrifuged at $3000 \times g$ for 15 minutes at 4 °C. Plasma was analyzed for glucose (Glucose Kit no. 510, Sigma Chemical Co., St. Louis, MO), urea nitrogen (Stanbio Urea Nitrogen Kit 580, Stanbio Laboratory, Inc., San Antonio, TX), and non-esterified fatty acids (NEFA) (Wako NEFA C-Kit no. 990-75401, Wako Chemicals USA, Inc., Richmond, VA).

Urine samples were taken four times by vulval stimulation twice daily after each milking on two consecutive days on May 18 and 19 (wk 2), June 14 and 15 (wk 6), August 9 and 10 (wk 14), and September 13 and 14 (wk 19). Samples were acidified with HCl to maintain pH below 2 and stored at -20°C . Urine samples were thawed, composited in one sample per cow per period, and analyzed for allantoin (Chen, 1989) and creatinine (Sigma Kit no. 555-A; Sigma Chemical Co., St. Louis, MO).

Grazing behavior was measured in the cows on the PC and the pTMR treatments from May 29 to June 13, from June 26 to July 11, and from August 21 to September 6 using the automatic IGER behaviour recorders (Rutter et al., 1997). Each day recorders were put on four cows, two of each treatment, after the a.m. milking and before cows were moved to the pasture. During the morning, recorders were installed from 0700 to 1730 h (10 hours and 30 minutes). Before the p.m. milking, recorders were removed from the cows to avoid damages during the milking and feeding, and also to download the information recorded during that period of time. During the night, recorders were installed on the same two cows of the PC treatment from 1830 h to 0600 h (11 hours and 30 minutes). Before the a.m. milking, recorders were again removed and the information downloaded. In average, total time recorders were on the cows was 22 hours. A total final number of 25 records per treatment were analyzed using the software IGERT GRAZETM (GRAZE User's guide, documentation version 1.0, program version 0.74).

5.3.3 Statistical Analyses

Data were analyzed as repeated measures using the PROC MIXED procedure of SAS (1999). The model included the fixed effects of treatments, parity (primiparous or multiparous),

week, treatment by week interaction, the random effect of cows nested within treatment, and the residual error. For each variable analyzed, cow nested within treatment was subjected to three covariance structures: compound symmetry, autoregressive order 1, and unstructured covariance. The covariance that resulted in the smallest Akaike's Information Criterion and Schwarz Bayesian Criterion was used. Least squares means and SEM are reported for all data. For all the variables the interaction between treatment and parity was not significant and therefore only treatments least squares means are presented. When significant ($P < 0.05$) effects due to dietary treatments were detected, mean separation was conducted by the PDIFF option in SAS (1999).

A total of 45 cows were originally assigned to the trial. The final number of cows used in the data analysis was 43. One cow on the PC treatment died for reasons not related to the treatment during wk 8. One cow belonging to the TMR treatment was removed for health problems not related with the treatment and did not complete 8 wk in the study, therefore the data were not used in the analysis.

5.4 RESULTS AND DISCUSSION

5.4.1 Weather Data

Mean precipitations were 82, 105, 87, 87 and 51 mm for the months of May, June, July, August and September, respectively. Average precipitations in the previous ten years period (1989-1999) for May, June, July, August and September were 82, 89, 90, 100 and 105 mm,

respectively. Only in September, there was a shortage of precipitation compared to the averages. Mean monthly temperatures during May, June, July, August and September were 16.8, 20.6, 20.2, 20.3 and 16.2 °C, respectively. Average low temperatures during May, June, July, August and September were 10.9, 15.7, 15.0, 15.5 and 10.7 °C, respectively. For the previous ten years period (1989-1999), average low temperatures for those months were 8.9, 14.2, 16.5, 15.2 and 11.0 °C, respectively. Average high temperatures during May, June, July, August and September were 22.6, 25.5, 25.3, 25.0 and 21.7 °C, respectively. For the previous ten years period (1989-1999), average high temperatures for those months were 20.6, 25.4, 27.4, 26.3 and 21.9 °C, respectively. The first day of the experiment (May 8), sunrise occurred at 0502 h and sunset at 1915 h, while the last day of the experiment (October 1) sunrise occurred at 0608 h and sunset at 1754 h. In average, day length during May, June, July, August and September was 14 h 25 min, 14 h 59 min, 14 h 42 min, 13 h 44 min, and 12 h 27 min, respectively.

5.4.2 Pasture Management and Quality

Pasture quality parameters are shown in *Table 35*. Those values are from hand-plucked pasture samples and, therefore represent the pasture selected and consumed by the cows. On average, pasture quality was approximately 26% CP, 14% TNC, 26% ADF and 50% NDF, and 58% IVDMD. Pasture quality parameters were within the ranges summarized by Muller and Fales (1998) for CP, soluble CP, TNC, ADF and NDF for cool-season grass during the spring and summer in Pennsylvania. The digestibility (IVDMD) of pasture was similar to that reported by Kolver et al. (1998) for an orchardgrass based pasture in Pennsylvania (60%).

Pasture grazed had important variations in CP content during the 21 wk of experiment (*Figure 9*). Those variations were related to climate and pasture factors, and to nitrogen fertilization during the experiment. Minimum levels of CP in pasture were about 20% during wk 3, 4 and 12. Four peaks of CP content were observed in the pasture during wk 6, 8, 14 and 19 (*Figure 9*), which coincided with the weeks after 50 kg/ha of nitrogen fertilizer was applied per hectare.

5.4.3 Intake and Apparent Digestibility of Nutrients

Intake and apparent digestibility of nutrients are shown in *Table 36*. The total diet of the PC treatment was 60% pasture and 40% concentrate; the pTMR treatment, 30% pasture, 61% TMR and 9% concentrate; and the TMR treatment, 100% TMR (DM basis). The forage:concentrate ratio (DM basis) was approximately 60:40 for both the PC and the pTMR treatments, and 50:50 for the TMR treatments. Compared to the PC treatment, total DMI was 5.1 kg/d higher (26.7 vs. 21.6 kg/d) for the TMR treatment, and 3.6 kg/d higher (25.2 vs. 21.6 kg/d) for the pTMR treatment ($P < 0.05$). The data reported in *Table 36* corresponds to the total DMI estimated using chromic oxide as fecal marker, recognizing that this technique may overestimate DMI. Intake of TMR on both the pTMR and the TMR treatments was also measured directly by individual feeding and refusals. The actual DMI on the pTMR treatment averaged 15.5 kg/d (*Table 36*). The DMI on the TMR treatment averaged 24.3 kg/d (23.9, 24.6, 23.9 and 24.9 kg/d for May, June, August and September, respectively), or 2.5 kg/d less than determined by the chromic oxide method. Based on these two methods, the indirect technique using of chromic oxide overestimated DMI on the TMR treatment by 10%, which agrees with the value reported

by Holden et al. (1994) for dry cows. If a 10% overestimation is assumed for all treatments, the total DMI is 19.5, 22.7, and 24.3 kg/d for the PC, pTMR, and TMR, respectively. Total DMI on the PC treatment was similar to that reported by Buckley et al. (2000) who found that high genetic merit dairy cows had higher total DMI than medium genetic merit dairy cows on pasture plus concentrate feeding systems (20.9 vs. 19.3 kg/d, measured using the n-alkane technique).

Total DMI expressed as percentage of the BW averaged 3.58, 3.99, and 4.15% for the PC, pTMR, and TMR treatments, respectively. If the intakes are discounted 10% due to the Cr₂O₃ method, DMI is 3.24, 3.59, and 3.73% of BW for the PC, pTMR, and TMR, respectively. Total tract apparent DM digestibility did not differ among treatments and averaged 63.3% ($P > 0.05$). Similar values for DM digestibility (62.7%) were reported for high producing dairy cows grazing grass-legume pasture and supplemented with 10 kg/d of a corn based concentrate (Reis and Combs, 2000b). Previous studies reported a total tract apparent DM digestibility of 61.3% for lactating dairy cows consuming a TMR similar to the one fed in this study (Dann et al., 1999), similar to the 62.3% for the TMR treatment.

A significant treatment by period interaction was found for total DMI, indicating that the difference in total DMI among treatments changed between periods (*Figure 10*). While the total DMI of the TMR treatment remained relatively constant with a minimum of 26.2 kg/d for May and a maximum of 27.3 kg/d for September, both the PC and the pTMR treatments had larger variations. Both treatments had a reduction in total DMI during June and August when compared with May and September (*Figure 10*), and was the result of a reduction in pasture DMI during these two periods (*Figure 11*). The reduction in total and pasture DMI in both the PC and pTMR treatments may be attributed to temperatures during June (25.5 °C) and August (25.0 °C). The average high temperatures during the 11 days of intake measures averaged 19.2, 25.3, 24.8 and

22.2 °C for the intake periods of May (5/15 to 5/27), June (6/12 to 6/24), August (8/7 to 8/19) and September (9/11 to 9/23), respectively. The similar DMI in May of 23.8, 26.6, and 26.2 kg/d for the PC, pTMR and TMR treatments, respectively, suggests that grazing management should be modified in the summer months by feeding supplemental TMR indoors during the day time hours and graze during the evening.

Total CP intake did not differ among treatments and averaged 4.7 kg/d, even with lower total DMI in both the PC and the pTMR treatments ($P > 0.05$; *Table 36*). The CP content in the total diets averaged 21.8, 19.1, and 16.9% in the PC, pTMR, and TMR treatments, respectively. The high CP content in the total diet of the PC treatment is a result of the high CP content of the pasture (23.9 to 29.5%). Total tract apparent CP digestibility was lower in the pTMR and the TMR treatments than in the PC treatment ($P < 0.05$). Kolver et al. (1998) reported CP digestibility of the total diet higher than 70% for high producing cows consuming high quality pasture and supplemented with 9.2 kg/d of a corn-based concentrate. Dann et al. (1999) reported similar CP digestibility values (58.7%) to the 57.9% for TMR diets for lactating dairy cows.

The highest total intake of NDF was found in the pTMR treatment, and the lowest in the PC treatment ($P < 0.05$; *Table 36*). Total diet NDF content averaged 36.5, 34.5 and 30.7% for the PC, the pTMR and the TMR treatments, respectively. Total NDF intake as percentage of BW was lower for both the PC and the TMR treatments than for the pTMR treatment ($P < 0.05$). The TMR treatment had lower NDF digestibility (41.4%) than both the PC (45.2%) and the pTMR (43.7%) treatments ($P < 0.05$). The inclusion of pasture in both treatments explains this difference because of the highly digestibility fiber in high quality pastures (NRC, 2001). Reis and Combs (2000b) reported a 45.9 and a 47.4% digestibility of NDF for grazing cows

supplemented with 5 or 10 kg/d of corn grain based concentrates, respectively. For TMR diets, Dann et al. (1999) found similar values of NDF digestibility (38.7%) to this study (41.4%).

The TMR treatment had a 23% and 9% significant higher NE_L intake than the PC and the pTMR treatments, respectively; and the pTMR treatment had a 14% higher NE_L intake than the PC treatment ($P < 0.05$; *Table 36*). The energy concentration of the PC, the pTMR and the TMR treatments total diet was 1.63, 1.60, and 1.64 Mcal/kg, respectively. The higher energy intake in the TMR treatment compared with the PC was a result of a higher total DMI because the NE_L content (1.63 Mcal/kg) was similar between treatments.

5.4.4 Milk Production

Milk production of the TMR treatment was significantly higher than that of the other two treatments ($P < 0.05$; *Table 37*). Cows on the TMR treatment produced 19% more milk (6.1 kg/d) than cows on the pTMR treatment and 33% more milk (9.6 kg/d) than cows of the PC treatment. Wu et al. (2001) reported a 7.7 kg/d of milk difference between fall calving cows grazing during the summer and supplemented with 7.9 kg/d of concentrate and the projected milk production if cows had remained on a TMR diet. In a four year study, White (2000) compared the milk production of the entire lactation of Holsteins cows fed a TMR in confinement or fed a pasture based feeding system supplemented with concentrate and hay or round-bale silage when pasture supply was limiting. Cows on the TMR diet produced significantly more milk per cow per day for the lactation than cows on the pasture-based diet (29.2 vs. 25.9 kg/d; White, 2000). Kolver et al. (2000) reported that North American and Dutch Holstein Friesian cows had a significantly higher milk production on a 100% TMR diet than on

100% pasture diet (22.6 vs. 14.9 kg/d). The combination of pasture with TMR in the pTMR treatment resulted in a significantly higher milk production (3.5 kg/d) than the PC treatment ($P < 0.05$).

The average milk production obtained for the TMR treatment for cows between 109 to 256 DIM (average DIM for this trial) represent a typical milk yield of high genetic merit dairy cows on a well managed indoor feeding system when fed a nutritionally balanced TMR diet and administered bST. This level of production through the entire lactation would result in 11621 kg per lactation (305 days x 38.1 kg/d), which is similar to the average herd milk production (11436 kg per lactation) for The Pennsylvania State University Dairy Cattle Research and Education Center. A similar milk production per lactation (11391 kg) is obtained when we consider that the cows averaged 40 kg/d during the first 108 days (0 to 108 DIM) prior to this experiment, 38.1 kg/d for the 147 days of the experiment (109 to 256 DIM), and 29.4 kg/d for the last 50 days of lactation (257 to 305 DIM) after this experiment.

The average milk production obtained in the PC treatment for cows between 109 to 256 DIM represents the production level of high producing dairy cows supplemented at a rate of 1 kg of concentrate/4 kg of milk on intensively managed pasture based feeding systems and administered bST. Bargo et al. (2000) found that high producing dairy cows grazing similar pastures (orchardgrass based) and supplemented with 8.6 kg/d of a corn based concentrate produced 29.8 kg/d of milk. The slightly higher milk production obtained in that study could be due to that study was of shorter duration (12 wk) than the current study (21 wk). Two previous long-term studies (24 to 25 wk) at The Pennsylvania State University reported lower milk yield for high producing dairy cows on pasture based feeding systems (Hoffman et al., 1993; Fales et al., 1995). Hoffman et al. (1993) reported milk yield of 24.1 kg/d for cows fed a corn-based

concentrate at a rate of 1 kg of concentrate/4 kg of milk with no bST. Fales et al. (1995) reported a milk yield of 25.5 kg/d for grazing cows supplemented with 6.1 kg/d of grain. Similar milk production levels (17.5 to 24.5 kg/d) were reported by Dhiman et al. (1999) for dairy cows consuming diets containing one-third or two-third of pasture during 20 wk. A four-year study (White, 2000) reported that cows fed pasture supplemented with concentrate and hay or round-bale silage produced 25.9 kg/d (daily average of entire lactation). The lower milk production between these last studies compared with our study may be explained by differences between years, stage of lactation, the use of bST, and the type of pasture. Overall, the cows of the PC treatment are estimated to produce about 9665 kg of milk per lactation assuming a milk production of 40 kg/d during the first 108 days (0 to 108 DIM) prior to this experiment, 28.5 kg/d for the 147 days of the experiment (109 to 256 DIM), and 21.1 kg/d for the last 50 days of the lactation (257 to 305 DIM) after this experiment. This would represent 85% of the lactation production of the TMR treatment.

There is limited published information on feeding systems combining pasture and TMR, therefore comparisons of the milk production obtained in the pTMR treatment for cows from 109 to 256 DIM are difficult. Milk production on the pTMR treatment was 3.5 kg/d higher than the PC treatment and 6.1 kg/d lower than the TMR treatment (*Table 37*), indicating that milk production in the systems combining both feedstuffs is closer to the pasture based feeding system than to the TMR based feeding system. In a 6 wk study, Soriano et al. (2001) found that milk yield was significantly greater (29.1 vs. 27.6 kg/d) for cows fed a TMR than cows fed a pasture in the morning and a TMR in the afternoon. Dhiman et al. (1999) compared the milk production of dairy cows on pasture diets where pasture constituted one-third, two-third or 100% of the total diet. The one-third pasture treatment received 11.6 kg DM/d of a supplement containing 25%

alfalfa hay, 48% high moisture corn, 18% roasted cracked soybeans, 6% soybean meal, and 2.7% mineral and vitamin mix and could be taken as equivalent to the pTMR treatment. The one-third pasture treatment produced 40% more milk (24.5 vs. 17.5 kg/d) than the two-third pasture treatment.

Lactation curves of the three treatments are shown in *Figure 12*. The high milk production (approximately 45 kg/d) for all the cows during the 6 wk prior to the experiment should be noted. Cows on both the PC and the pTMR treatments experienced a rapid decrease in milk production during the two weeks of adjustment (adj1 and adj2 in *Figure 12*) when pasture was gradually incorporated to the rations of these two treatments. The management of the PC and the pTMR cows during the adjustment period consisted of a gradual increase in the time that cows were allowed to graze during a 2 wk period. Cows on both treatments were moved to the pasture at 0800 h for a period of 2 h during the first two days, then for a period of 4 h during the subsequent two days, and then for a period of 8 h during the subsequent four days. During these first nine days of the adjustment period, cows were fed a TMR ad libitum and housed in a free-stall barn when not on pasture. During the last five days of the adjustment period, the cows on the PC treatment remained on pasture during morning and evening, whereas cows on the pTMR treatment were fed a TMR diet and housed in a free stall barn at night. During these two weeks, milk production by cows on the PC treatment decreased 18% from 45.7 to 37.7 kg (0.57 kg/d), and milk production by cows on the pTMR treatment decreased 15% from 44.9 to 38.4 kg (0.46 kg/d).

A rapid decrease in milk production during the first two weeks of pasture inclusion in the diet has been previously reported for high producing dairy cows (Fales et al., 1995; Hoffman et al., 1993; Jones-Endsley et al., 1997; Wu et al., 2001). Milk production declined about 25%

during the first 8 wk of a trial when cows grazed high quality grass pasture and were supplemented at a rate of 1 kg concentrate/4 kg of milk (Hoffman et al., 1993). A decrease in milk production from 43.0 to 30.4 kg occurred during the first 16 d (0.79 kg/d) when cows were supplemented with 6.4 to 9.6 kg/d of concentrate were moved to pasture (Jones-Endsley et al., 1997). Fall-calving dairy cows grazing during the summer and supplemented with concentrate had a reduction between 2.2 and 4.2 kg (0.31 and 0.60 kg/d) in the first week on pasture (Wu et al., 2001). The amount of milk reduction depends on the stage of lactation of cows, time of the year, and pasture quality. Possible reasons for this rapid decrease in milk production could be a short adjustment period (2 wk or less), changes in the rumen environment with a negative impact on rumen microbial populations, a nutritionally unbalanced diet during the transition period, and an increased maintenance requirements for energy because of walking and grazing activity.

When comparing wk 1 to 21, milk production decreased 16.7 kg from 37.8 to 21.1 kg/d on the PC treatment, 9.4 kg from 37.2 to 27.8 kg/d on the pTMR treatment, and 16.0 kg from 45.4 to 29.4 kg/d on the TMR treatment. The reduction in milk production within each treatment was -0.11 , -0.06 , and -0.11 kg/d for the PC, the pTMR, and the TMR treatments, respectively, indicating that both the PC and TMR treatments had a similar milk production persistency. The pTMR treatment had a higher milk production persistency during the 21 wk trial, some of which may be attributed to the unexplained increased milk production during wk 19, 20, and 21. Pulido and Leaver (2001) reported milk production decreases in the range of -0.10 to -0.18 kg/d for grazing dairy cows supplemented with concentrate and producing more than 30 kg/d of milk.

5.4.5 Discussion of Differences in Milk Production Among Treatments

The differences in milk production among treatments are likely related to differences in energy maintenance requirements for walking and grazing activity and energy intake.

The energy cost of grazing activity is attributed to the physical effort for eating grass from the pasture and the extra time selecting the grass (Agnew et al., 2000). Grazing cows also require walking a distance twice daily between pasture and milking parlor (Agnew et al., 2000). Using the equations developed by the NRC (2001) to account for the energy expended in these activities, the PC treatment had 5.4 Mcal/d increase in maintenance requirements. Based on the energy content of milk (0.64 Mcal of NE_L/kg of milk), this activity could account for 8.4 kg of milk/d or 88% of the difference in milk production obtained between the PC and the TMR treatments. The pTMR had an increase of 2.5 Mcal/d in the maintenance requirements. Based on the 0.67 Mcal of NE_L/kg of milk, this could account for 3.7 kg of milk/d or 61% of the difference in milk production between the pTMR and the TMR treatments.

The lower milk production in both the PC and the pTMR treatments compared to the TMR treatment may also be related to differences in energy intake (*Table 36*). The TMR treatment had 8.4 and 3.5 Mcal/d of NE_L higher intake than the PC and the pTMR treatments, respectively. This difference could account (based on the energy content of milk for the TMR treatment: 0.67 Mcal/kg) for 12.5 and 5.2 kg/d of milk of difference between the TMR treatment and the PC and the pTMR treatments, respectively. The difference in energy intake between the pTMR and the PC treatments (4.9 Mcal/d of NE_L) would account for a difference in 7.3 kg/d of milk between these two treatments.

5.4.6 Milk Composition

The use of TMR in both the pTMR and the TMR treatments increased milk fat percentage compared to the PC treatment (3.33 vs. 3.13%; $P < 0.05$; *Table 37*). The lower milk fat percentage in the PC treatment may be related to the highly digestible fiber in high quality pasture and the rapid consumption of concentrate due to twice daily feeding and feeding separately from forage (NRC, 2001). In a 4 year study that evaluated entire lactation performance of cows on a pasture plus concentrate diet or on a TMR diet, White (2000) also found a higher milk fat percentage on the TMR treatment than on the pasture treatment (3.71 vs. 3.45%). Variations in milk fat percentage in the three treatments (*Figure 13*) show that milk fat percent was lower in the PC treatment, particularly after wk 8, than in both the pTMR and the TMR treatments.

True protein percentage in milk was significantly higher in the TMR treatment than in the PC treatment (2.99 vs. 2.82%; $P < 0.05$; *Table 37*), while the true protein percentage in the pTMR treatment was numerically between those two treatments. The increase in true protein content in milk in the TMR treatment compared to the PC treatment is likely related to the higher energy intake (*Table 36*). There is a positive relationship between energy intake and milk protein percentage (DePeters and Cant, 1992). Milk true protein percentage variations in each treatment during the experiment are presented in *Figure 14*. The PC treatments had lower milk true protein percent during each week of the experiment compared to the TMR treatment, especially after wk 6.

Fat and true protein yield followed the results found for milk production and 3.5% FCM production (*Table 37*). Both fat and protein yield were significantly higher for the TMR treatment, with the lowest values for the PC treatment, and the pTMR treatment was intermediate

between these treatments ($P < 0.05$). Cows on the TMR treatment produced 18% more fat and 21% more true protein than the pTMR treatment, and 38% more fat and 41% more true protein than the PC treatment.

The concentration of milk urea nitrogen (MUN) decreased from 14.9 mg/dl on the PC treatment to 10.6 mg/dl on the TMR treatment ($P < 0.05$; *Table 37*). Weekly variations of MUN in the three treatments during the 21 wk of the experiment are shown in *Figure 15*. The PC treatment had larger variations in MUN during the experiments with peaks of more than 16 mg/dl occurring during wk 6, 8, 14 and 19 (16.5, 17.4, 23.2 and 18.6 mg/dl, respectively). These four MUN peaks on the PC treatment coincided with peaks in CP percent in the pasture grazed during wk 6, 8, 14 and 19 (*Figure 9*), which was the time of nitrogen fertilization. Weekly MUN for the pTMR treatment were intermediate between the PC and the TMR treatments during most weeks. Data from 82 dairy herds on pasture-based feeding systems during one year in Chile (Wittwer et al., 1999) reported a mean bulk MUN of 13.5 mg/dl, with a range from 4.1 to 32.1 mg/dl, and with high seasonal variation. The highest values were found during early spring (15.8 to 16.3 mg/dl) and the lowest during late summer (9.9 to 10.2 mg/dl), and peaks were correlated with CP content of pasture that in typical non-fertilized pasture in Chile averaged 16.5% in spring and 10.7% in the summer (Wittwer et al., 1999).

5.4.7 Milk Response to bST Injections

Cows on the three treatments received bST injections every two weeks starting on May 3. A total of 10 injections were given during the 21 wk experiment. To evaluate if a variable milk response to bST injections due to treatments, the daily milk response after each bST injection

was compared. For the 10 bST injections, milk production on the day of injection (day 1), the highest response day post-injection, and the percentage of milk increase is presented for the three treatments in *Table 38*. From the 43 cows used in the performance data analysis, three cows on the PC treatment, three cows on the pTMR treatment, and two cows on the TMR treatment responded less than five of the ten bST injection times. Some of these cows had health problems (e.g. mastitis) during those weeks, and therefore may not have responded to bST. A total of 11 cows on the PC treatment, 12 cows on the pTMR treatment and 12 cows on the TMR treatment were used to compared the milk production response to bST injections (*Table 38*).

For the first three bST injections (May 3, May 17 and May 31), a significant interaction existed among treatments, bST injection, and day ($P < 0.01$), which indicates that the response to bST injections differed among treatments. Daily milk production for the first three bST injections is presented in *Figure 16*. Neither cows on the PC or pTMR treatments responded to the first three bST injections ($P > 0.10$). Neither of these two treatments showed a milk response during any of the three 14 day periods as clearly shown in *Figure 16* and *Table 38*. Cows on the TMR treatment responded to bST injections and milk production was increased in 7.4 (18.5%), 3.7 (9.4%), and 3.5 (8.6%) kg/d during the first, second, and third bST injections, respectively. Peak of response was obtained in all the bST injections on day 8 of each 14 day period (*Table 38*).

During the second three bST injections (June 14, June 28 and July 12), a significant interaction among treatment, bST injection, and day was found ($P < 0.05$), although in this case a milk response was found for the three treatments and the interaction indicates that the level of response differed among treatments (*Table 38*). All three treatments presented a typical pattern of milk production after bST injections, with peak increase in milk production occurring between

6 and 10 days after each injection (*Figure 17*). Cows on the PC treatment had an increase in milk production of 3.5 (12.2%), 6.4 (23.9%), and 2.7 (9.2%) kg/d for the fourth, fifth and sixth bST injections, respectively. Cows on the pTMR treatment had an increase in milk production of 3.0 (9.3%), 4.8 (15.5%), and 1.9 (6.1%) kg/d for the fourth, fifth and sixth bST injections, respectively. Cows on the TMR treatment had an increase in milk production of 3.7 (9.9%), 6.4 (17.4%), and 3.0 (7.8%) kg/d for the fourth, fifth and sixth bST injections, respectively. On average, the milk response was 15.2% for the PC treatment, 10.3% for the pTMR treatment, and 11.7% for the TMR treatment for injections 4, 5, and 6. The peak milk response occurred between days 6 and 10 after each bST injection for all treatments. These results agree with previous studies with grazing dairy cows that reported that 93% of the milk response to bST injections were found seven days after injection (Michel et al., 1990). Others (Remond et al., 1991) reported peaks after bST injection on days 10 to 11.

Milk response to the last four bST injections (July 26, August 9, August 29 and September 6) are shown in *Figure 18* and *Table 38*. A significant treatment by bST injection by day interaction was found ($P < 0.05$). Cows on the PC treatment had a milk production response of 1.4 (4.9%), 3.4 (12.4%), and 4.4 (19.9%) kg/d for the seventh, eighth, and tenth bST injection. There was no response to the ninth bST injection. Cows on the pTMR treatment responded only to the tenth bST injection (7.0 kg/d of milk or 31.5%). Cows on the TMR treatment had a milk response of 2.8 (7.5%), 2.2 (6.4%) and 5.8 (20.3%) kg/d for the seventh, eighth and tenth bST injections, respectively, and had no response for the ninth bST injection. On average, the milk response was 12.4% for the PC treatment (average of three injections), 31.5% for the pTMR treatment (only for the tenth bST injection), and 11.4% for the TMR treatment (average of three

injections). Except for the seventh bST injection on the PC treatment, the highest response was reached between days 5 to 10 after bST injection in all treatments.

For the ten bST injection through the 21 wk of the experiment, cows on the PC treatment responded to six injections, cows on the pTMR to four injections, and cows on the TMR to nine injections, indicating clearly the significant interaction among treatment, bST injection and day detected. Milk response averaged 13.8% (4.9 to 23.9%) for the PC treatment, 15.6% (6.1 to 31.5%) for the pTMR treatment, and 11.8% (6.4 to 20.3%) for the TMR treatment.

Few studies are published on the milk response to bST injection with dairy cows on pasture, and all were conducted with cows producing less than 26 kg/d of milk (Fontes et al., 1997; Hoogendoorn et al., 1990; Kim et al., 1991; Peel et al., 1985; Remond et al., 1991; Valentine et al., 1990). Kim et al. (1991) reported no differences in milk production (25.5 kg/d) by dairy cows grazing ryegrass pasture when supplemented with 2 kg/d of concentrate without or with bST injections (slow-release bST every 4 wk at a dose of 640 mg). Dairy cows grazing a ryegrass-white clover pasture with no supplementation and injected daily with 50 mg/d of bST produced 4 kg/d more milk than dairy cows with no bST injections (control) (23.3 vs. 19.8 kg/d) (Peel et al., 1985). Dairy cows grazing a ryegrass-clover pasture and supplemented with 8 kg/d of a barley based concentrate had a significantly higher milk production with bST injections (every 14 days) compared to control cows (18.8 vs. 15.9 kg/d) in a 12 wk trial (Valentine et al., 1990). Remond et al. (1991) compared two amounts of concentrate (0.27 vs. 1.6 kg/d) without or with bST (500 mg injected at 14 days intervals) and reported a significant milk response to bST (18.8 vs. 17.1 kg/d) with grazing cows regardless of the concentrate level. In a 26 wk experiment, Hoogendoorn et al. (1990) reported a significant milk production response (14.8 vs. 13.5 kg/d) to two-weekly bST injections (dose equivalent to 25 mg/d) in dairy cows grazing a

ryegrass-white clover pasture and supplemented with green chopped corn during pasture shortage. In this study, however, a strong association between the degree of response to BST and supply of pasture was found, resulting in the highest response when pasture mass was greater than 2500 kg DM/ha and little response when pasture mass was lower than 2000 kg DM/ha (Hoogendoorn et al., 1990).

Compared to previous grazing studies, the lack of response to bST on the PC treatment during four of the injections and on the pTMR during six of the ten injections could be partially attributed to the genetic merit of the cows used in our study. Michel et al. (1990) compared the milk response to bST (14 days interval injections at a dose rate equivalent to 50 mg/d) with dairy cows on pasture of low or high genetic merit and found that low genetic merit cows had a higher response than high genetic merit cows (42.2 vs. 10.8% in fat yield). The authors suggested this may have occurred because high genetic merit cows had limited body reserves which could be mobilized to support the galactopoietic effects of bST (Michel et al. 1990). Oldenbroek et al. (1991) evaluated the effect of breed on milk response to bST and found that Friesian cows that had the highest milk yield prior to the trial had a lower response to bST compared to Jersey and Dutch Red and White cows. This same study compared the response to bST of dairy cows on different feeding systems including non-pasture diets fed ad libitum (50% concentrate:50% conserved forage) and pasture based diets (Oldenbroek et al., 1991). The response to bST was higher on a high concentrate indoor diets than on pasture based diets (Oldenbroek et al., 1991), which agrees with the higher number of responses by cows to the bST on the TMR treatment compared to the PC and the pTMR treatments.

Fontes et al. (1997) studied the response to bST (500 mg every 14 days) of Brazilian crossbred cows on a pasture plus TMR feeding system and reported a significant milk increase of

2.6 kg/d (14.2 vs. 11.6 kg/d). Although this last study had a feeding system relatively similar to the pTMR treatment of our study, differences in breed of cows and milk production level make the comparison between studies difficult.

Summarizing all the pasture studies that reported milk response to bST, milk production increased after bST injection and averaged 14.5% (9.6 to 22.4%) for dairy cows producing less than 24 kg/d of milk. In the only study that did not report a response to bST (Kim et al., 1991) milk production averaged 25.5 kg/d. In our study, cows on the PC treatment averaged 13.8% milk response to bST injections, but responded less to the injection than cows on the TMR treatment. More research is needed to understand the effect of bST on pasture-based and pasture plus TMR feeding systems for high producing dairy cows.

5.4.8 Body Weight and Body Condition Score

Initial BW did not differ among treatments and averaged 624 kg ($P > 0.05$; *Table 39*). The three treatments gained BW as shown (*Figure 19*) by the significantly higher final BW ($P < 0.05$). However, the cows on the TMR treatment had a significantly greater increase in BW in comparison with both the PC and the pTMR treatments (76 vs. 34 kg; $P < 0.05$).

Initial BCS did not differ among treatments and averaged 2.85 ($P > 0.05$; *Table 39*). Body condition score changes through the 21 wk are shown in *Figure 20*. Final BCS was significantly higher for the TMR treatment than for the PC treatment ($P < 0.05$), while final BCS in the pTMR did not differ from the PC and TMR treatments ($P > 0.05$). Comparing the initial and the final BCS within each treatment, cows on the PC treatment lost BCS (-0.20), cows on the pTMR treatment maintained BCS (0.01) and cows of the TMR treatment gained BCS (0.19).

Although, cows on the PC treatment lost BCS, the magnitude of that loss (-0.20 over 21-wk) was quite small. It is well known that BCS loss and the rate of BCS loss are good predictors of potential reproductive efficiencies (Staples et al., 1992). Kolver and Muller (1998) in a short term study of 4 wk reported losses of BCS (-0.50) for high producing dairy cows consuming only pasture while cows consuming a TMR diet in the same study maintained BCS. More recently, a four year study (White, 2000) found that over the entire lactation cows on a pasture based feeding system (concentrate and hay supplements provided during pasture shortage) had between 0.3 and 0.5 lower BCS than cows on a TMR based feeding system. However, reproductive performance (conception rate, first service conception, all services conceptions, overall pregnancy rate) did not differ between feeding systems (White, 2000).

5.4.9 Blood and Urine Metabolites

Blood and urine metabolites data are presented in *Table 40*. Glucose concentration was not affected by treatments and averaged 64.8 mg/dl ($P > 0.05$). Cows of the PC treatment had significantly higher plasma urea nitrogen (PUN) concentrations than both the pTMR and the TMR treatments (17.3 vs. 13.8 mg/dl) ($P < 0.05$). A significant interaction between treatment and week was observed for PUN concentration ($P < 0.05$). This interaction indicates that the pattern of PUN concentration differed among treatments as shown in *Figure 21*. The cows of the PC treatment had larger variations in PUN (maximum of 20.2 mg/dl in wk 19 and minimum of 13.9 mg/dl in wk 9). These variations reflect changes in CP content in the pasture (*Figure 9*) and the subsequent different intake of CP. The pTMR treatment had smaller variations in PUN concentration (maximum of 17.4 mg/dl in wk 13 and minimum of 12.1 mg/dl in wk 5) because

pasture was only 30% of the total diet (*Table 36*). The TMR treatment had the smallest variation in PUN concentration (maximum of 16.9 mg/dl in wk 13 and minimum of 12.4 mg/dl in wk 9) because 100% of the diet was a TMR with a more constant CP content.

Initial NEFA concentrations in both the PC and the TMR treatments were significantly higher than final NEFA concentrations, suggesting body fat mobilization at the beginning of the experiment in these two treatments ($P < 0.05$; *Table 40*). The NEFA concentration on the pTMR treatment remained relatively constant (i.e. initial concentration did not differ from final concentration; $P > 0.05$), indicating lower mobilization of body fat reserves during the initial weeks of the experiment. Final NEFA concentrations did not differ among treatments and averaged 115.3 $\mu\text{eq/L}$ ($P > 0.05$). A significant treatment by week interaction was found for NEFA concentration, which indicates that pattern of NEFA concentration through the experiment differed among treatments (*Figure 22*). Cows of the PC treatment had a drastic reduction in the NEFA concentration from the start until the end of the trial. High levels of NEFA concentration in the PC treatment are in agreement with a mobilization of body reserves by the loss of BCS and the lower gain of BW observed in this treatment (*Table 39*). Although the cows of the TMR treatment gained BCS and more BW than the cows of the PC treatment (*Table 39*), high levels of NEFA concentration were observed at the beginning of the trial with a lower decrease towards the end. This would indicate body reserves mobilization in order to sustain the high levels of milk production enhanced by the bST injections ($> 38 \text{ kg/d}$). The pTMR treatment had a relative stable concentration of NEFA during the trial, indicating less mobilization of body reserves, which is supported by the maintenance in BCS and the lower BW gain (*Table 39*) and milk production level (*Table 37*) than the TMR treatment.

Allantoin and creatinine concentrations, and allantoin/creatinine ratio in spot urine samples are shown in *Table 40*. Both allantoin and creatinine concentration were higher in the TMR treatment than in both the PC and the pTMR treatments ($P < 0.05$). Carruthers and Neil (1997) reported similar values for allantoin concentrations (1992.1 mg/L) for dairy cows consuming ryegrass pasture and supplemented with non-structural carbohydrates. The allantoin/creatinine ratio, an index of total allantoin excretion in urine and an indicator of rumen microbial protein synthesis (Gonda, 1995), was not affected by dietary treatments and averaged 2.92 ($P > 0.05$). Gonda and Lindberg (1994) reported an average allantoin/creatinine ratio of 3.02 for dairy cows between 98 to 112 DIM on a confinement feeding system. The lack of significance in the allantoin/creatinine ratio indicates that the rumen microbial protein synthesis was not affected by feeding system or that this technique was not sensitive enough to detect differences in microbial protein synthesis among treatments.

5.4.10 Grazing Behavior

The parameters of grazing behavior (grazing time, biting rate and bite mass) are presented in *Table 41*. Bite recorders were installed on the cows on the PC and the pTMR treatments from 0700 to 1730 h between the a.m. and the p.m. milking, and on the cows on the PC treatment from 1830 to 0600 h between the p.m. and the a.m. milking. Total grazing time averaged 9.5 h/d (572 min/d) for the PC treatment, and 4.2 h/half day (252 min/half day) for the pTMR treatment ($P < 0.05$). Research from Northern Ireland (Sayers, 1999) reported a grazing time of 7.9 h/d (473 min/d) for dairy cows grazing ryegrass pasture when supplemented with 6 kg/d of concentrate. In a second study, grazing time was 7 h/d (480 min/d) or 6.6 h/d (398 min/d)

when supplemented with 5 or 10 kg/d of a starch-based concentrate, respectively (Sayers, 1999). The high grazing time found in the PC treatment (> 9 h/d) is within the range cited by Rook (2000) who reported a maximum grazing time of 12 h/d for dairy cows. The higher values in our study compared to Sayers, (1999) could be partially explained by differences in daylight because grazing time may be constrained by available daylight (Rook, 2000).

Grazing time when the cows of both the PC and the pTMR treatments grazed together did not differ between treatments and averaged 4.3 h (259 min; $P > 0.05$; *Table 41*) during the 10 h and 30 min grazing period. This suggests that cows probably reached the maximum potential grazing time for that 10 h and 30 min period, and that the pasture DMI of 7.5 kg per cow obtained on the pTMR treatment may be near the maximum expected DMI during that number of hours. If feeding systems that combine pasture and TMR has the goal of increasing the proportion of pasture DMI in the diet (higher than the 30% of total DMI obtained in the pTMR treatment), cows need to graze in the evening to provide them the opportunity to increase grazing time per day. After the p.m. milking, cows on the PC treatment grazed 4.9 h (295 min). Rook (2000) concluded from several studies that grazing ruminants take a larger meal before sunset. For example, if cows on the pTMR treatment were allowed to graze after the p.m. milking for 90 min, assuming same biting rate (59 bites/min) and bite mass (0.55 g DM/bite) as obtained on the PC treatment, this would represent an extra 2.9 kg/d pasture DMI and result in a pasture DMI of 10.4 kg/d or 41% of the total diet.

The biting rate averaged 56 bites/min ($P > 0.05$; *Table 41*) and was expected not to be affected by treatments because is mainly affected by animal factors such as stage of lactation and body size (McGilloway and Mayne, 1996; Rook, 2000), which were similar in both treatments. The biting rate did not differ between treatments during the morning (55 bites/min; $P > 0.05$),

while the biting rates was higher in the PC treatment during the evening (59 bites/min). In his review, Rook (2000) concluded that biting rate of dairy cows is higher in the evening than in the morning. Gibb et al. (1998) reported that biting rate showed a quadratic effect of time during the day and reached a maximum value during the evening (52.6, 47.5, 51.6, and 59.4 bites/min at 0700, 1130, 1600, and 1900 h). Total bites were 31508 bites/d for the PC treatment, and 14316 bites/half day for the pTMR treatment ($P < 0.05$). Total daily bites on the PC treatment were below the maximum (40000 bites/d) estimated by Phillips and Leaver (1986). The number of bites between the a.m and p.m. milking were not affected by treatments and averaged 14294 bites ($P > 0.05$). During the evening, cows of the PC treatment had a higher number of bites than during daytime grazing (17236 vs. 14272).

For both the total number of bites and the biting rate, a significant period effect was found with a decrease in both parameters from period to period ($P < 0.05$; *Table 41*). Both parameters are determined by animal factors including stage of lactation, milk production, and body size (McGilloway and Mayne, 1996; Rook, 2000), which explains why number of bites and biting rate decreased with periods with the reduction in milk production associated with the advanced stage of lactation. A positive relationship was found between milk production and the number of bites per d for the PC treatment ($r^2 = 0.74$; *Figure 23*), which resulted in an increase of 5 kg/d of milk every 10000 bites. It should be noted that the highest producing cows exceeded 40000 bites/d compared to the average of 31508 bites/d. An increase in grazing time (min/d) and rate of pasture intake (g DM/min) has been previously reported for dairy cows on a ryegrass pasture producing between 16.9 to 35.5 kg/d of milk and supplemented with different levels of concentrate (from 0 to 6 kg/d) (Pulido and Leaver, 2001).

Bite mass was not affected by treatments and averaged 0.52 g DM/bite ($P > 0.05$; *Table 41*), as expected because this variable is mainly determined by pasture structural characteristics such as height and bulk density (Rook, 2000).

The grazing behavior parameters found in our study are within the range of values reported for high producing dairy cows grazing high quality pastures (McGilloway and Mayne, 1996; Rook, 2000). These typical values found in our study support the pasture DMI value of 12.9 kg/d for the PC treatment (572 min/d x 57 bites/min x 0.48 g DM/bite), and of 7.5 kg/d for the pTMR treatment (252 min/d x 54 bites/min x 0.55 g DM/bite) measured by the Cr_2O_3 method (*Table 36*).

5.5 CONCLUSIONS

The TMR feeding system maximized total DMI and milk production. The TMR treatment had the highest total DMI, the PC treatment the lowest, and the pTMR treatment intermediate. The TMR treatment produced 19% more milk than the pTMR treatment, and 33% more than the PC treatment. Both the pTMR and the TMR treatments had higher milk fat percentage. True protein percentage in milk was higher in the TMR treatment than in the PC treatment. Over the ten bST injections, cows on the PC treatment responded in six injections with a 13.8% milk increase, cows on the pTMR in four injections with a 15.6% milk increase, and cows on the TMR in nine injections with a 11.8% milk increase.

The three treatments gained BW after the 21 wk of the study, however, the cows of the TMR treatment had a higher increase in BW in comparison with both the PC and the pTMR

treatments. The PC treatment lost BCS, the pTMR treatment maintained BCS, and the TMR treatment gained BCS.

Animal performance was improved combining pasture and TMR compared to pasture plus concentrate. Milk production in the feeding system combining pasture and TMR was intermediate between the other feeding systems but closer to the pasture based feeding system than to the TMR based feeding system. The combination of pasture and TMR also resulted in higher milk fat and protein percentage, a higher body condition score, and lower PUN and NEFA concentrations than when pasture as the only forage plus concentrate.

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Table 34: Ingredient and chemical composition (mean $\pm$ SD) of the concentrate used to supplement the cows on the PC and pTMR treatments and the TMR fed to the cows on the pTMR and TMR treatments.

	Concentrate	TMR
Ingredient composition		
Dry corn	59.6	14.9
Wheat midds	20.7	...
Molasses	4.9	...
Animal protein blend ¹	4.6	...
Animal fat	2.3	...
Canola meal	1.5	3.4
Limestone	1.4	...
Dicalcium phosphate	1.3	...
Salt	1.3	...
Corn gluten meal	1.0	...
Dynamate 22S	0.57	...
Magnesium oxide	0.52	...
PS TM premix	0.16	...
Selenium premix	0.09	...
Vitamins A, D, E	0.06	...
Sodium bicarbonate	...	0.7
Bentonite	...	0.4
Commercial concentrate ²	...	18.8
Cookie by-products	...	8.8
Roasted soybean	...	2.6
Corn silage	...	27.1
Alfalfa haylage	...	13.4
Alfalfa hay	...	9.9
Chemical composition		
DM, %	89.4 $\pm$ 4.4	54.5 $\pm$ 1.9
% DM		
OM, %	91.4 $\pm$ 0.7	92.8 $\pm$ 0.2
CP, %	15.2 $\pm$ 0.4	16.9 $\pm$ 0.3
Soluble CP, %	2.8 $\pm$ 0.5	5.1 $\pm$ 0.4
TNC, %	53.7 $\pm$ 1.4	35.1 $\pm$ 1.1
NDF, %	15.7 $\pm$ 0.4	30.1 $\pm$ 1.5
ADF, %	6.5 $\pm$ 0.2	19.4 $\pm$ 1.2
IVDMD, %	72.2 $\pm$ 3.2	64.3 $\pm$ 1.4
NE _L , Mcal/kg ³	1.89	1.64

¹One-third of each fish meal, feather meal, and blood meal.

²Pellet composed by 44.2% distillers grain, 17.8% wheat midds, 17.8% soyhulls, 12.3% animal protein blend, 1.5% salt, 1.1% tallow, and 5.3% minerals and vitamins. The pellet had 27.7% CP, 1.85 Mcal of NE_L/kg, 1.92% Ca, 0.74% P, 1.16% Mg, 1.10% K, 0.41% S, 166.6 ppm Mn, 58.2 ppm Cu, 215.1 ppm Zn, 427.8 ppm Fe, 2.01 ppm Se, 2.57 ppm I, 1.03 ppm Co, 26795 UI/kg vitamin A, 6589 UI/kg vitamin D, and 176 UI/kg vitamin E.

³Estimated using values of NRC (2001).

Table 35: Chemical composition of the hand-plucked pasture samples during the four intake measurement periods.

	May	June	August	September	X	SEM
DM, %	19.9 ^a	17.0 ^{ab}	15.7 ^b	17.1 ^{ab}	17.4	1.0
% DM						
OM, %	93.2 ^a	92.9 ^a	90.9 ^b	91.1 ^b	92.1	0.2
CP, %	25.5 ^a	26.2 ^a	29.5 ^b	23.9 ^a	26.3	0.9
Soluble CP, %	6.4	6.3	7.2	6.2	6.5	0.5
TNC, %	13.0 ^a	14.1 ^{ab}	13.5 ^{ab}	14.9 ^b	13.9	0.6
NDF, %	53.5 ^a	52.1 ^a	46.5 ^b	47.5 ^b	49.9	1.0
ADF, %	25.9 ^{ab}	27.4 ^a	25.0 ^b	26.1 ^{ab}	26.1	0.6
IVDMD, %	57.6	57.5	58.3	58.5	58.0	1.2

^{a, b, c}Least square means in the same row with different superscripts differ ($P < 0.05$).

Table 36: Intake and apparent digestibility of DM, CP, and NDF, and intake of NE_L of dairy cows with three different feeding systems.

	Treatments ¹				Effects, ² <i>P</i> <		
	PC	pTMR	TMR	SEM	T	P	T x P
DM intake ³							
Concentrate, kg/d	8.7 ^a	2.2 ^b	-	0.1	<0.01	<0.01	<0.01
Pasture, kg/d	12.9 ^a	7.5 ^b	-	0.3	<0.01	<0.01	<0.01
TMR, kg/d	-	15.5 ^a	26.7 ^b	0.4	<0.01	0.29	<0.01
Total, kg/d	21.6 ^a	25.2 ^b	26.7 ^c	0.5	<0.01	<0.01	<0.01
Total, % BW	3.58 ^a	3.99 ^b	4.15 ^b	0.08	<0.01	<0.01	0.07
DM digestibility, ⁴ %	63.5	64.1	62.3	1.2	0.61	0.57	0.10
CP intake							
Concentrate, kg/d	1.3 ^a	0.3 ^b	-	0.02	<0.01	<0.01	<0.01
Pasture, kg/d	3.4 ^a	1.9 ^b	-	0.1	<0.01	<0.01	0.01
TMR, kg/d	-	2.6 ^a	4.5 ^b	0.1	<0.01	<0.01	<0.01
Total, kg/d	4.7	4.8	4.5	0.1	0.16	0.01	<0.01
CP digestibility, ⁴ %	70.5 ^a	64.8 ^b	57.9 ^c	0.5	<0.01	<0.01	0.48
NDF intake							
Concentrate, kg/d	1.4 ^a	0.4 ^b	-	0.02	<0.01	<0.01	<0.01
Pasture, kg/d	6.5 ^a	3.7 ^b	-	0.2	<0.01	<0.01	<0.01
TMR, kg/d	-	4.6 ^a	8.2 ^b	0.1	<0.01	<0.01	<0.01
Total, kg/d	7.9 ^a	8.7 ^b	8.2 ^{ab}	0.2	0.02	<0.01	<0.01
Total, % BW	1.30 ^a	1.38 ^b	1.25 ^a	0.03	0.01	<0.01	0.01
NDF digestibility, ⁴ %	45.2 ^a	43.7 ^a	41.4 ^b	0.7	<0.01	<0.01	0.18
NE _L intake, ⁵							
Concentrate, Mcal/d	16.4 ^a	4.2 ^b	-	0.2	<0.01	<0.01	<0.01
Pasture, Mcal/d	18.9 ^a	10.9 ^b	-	0.5	<0.01	<0.01	<0.01
TMR, Mcal/d	-	25.1 ^a	43.7 ^b	0.9	<0.01	<0.01	<0.01
Total, Mcal/d	35.3 ^a	40.2 ^b	43.7 ^c	0.7	<0.01	<0.01	0.97
Total diet							
F:C ⁶	60:40	60:40	50:50	...	...	...	...
CP, %	21.8 ^a	19.1 ^b	16.9 ^c	0.1	<0.01	<0.01	<0.01
NDF, %	36.5 ^a	34.5 ^b	30.7 ^c	0.2	<0.01	<0.01	<0.01
NE _L , Mcal/kg	1.63 ^a	1.60 ^b	1.64 ^a	0.01	<0.01	0.08	0.25

¹PC = pasture plus concentrate, pTMR = partial total mixed ration, TMR = total mixed ration.

²T = treatment, P = period, T x P = treatment x period interaction.

³Estimated using chromic oxide as fecal marker.

⁴Total tract apparent digestibility.

⁵NE_L for concentrate, pasture, and TMR: 1.89, 1.46 and 1.64 Mcal/kg DM, respectively (NRC, 2001). ⁶Forage:concentrate ratio.

^{a, b, c}Least square means in the same row with different superscripts differ (*P* < 0.05).

Table 37: Milk production and composition of dairy cows with three different feeding systems.

	Treatments ¹				Effects, ² $P <$		
	PC	pTMR	TMR	SEM	T	W	T x W
Milk, kg/d	28.5 ^a	32.0 ^b	38.1 ^c	1.2	<0.01	<0.01	<0.01
3.5% FCM, kg/d	26.9 ^a	30.9 ^b	36.6 ^c	1.0	<0.01	<0.01	<0.01
Fat							
%	3.13 ^a	3.35 ^b	3.30 ^b	0.05	0.01	0.01	<0.01
Kg/d	0.89 ^a	1.06 ^b	1.24 ^c	0.03	<0.01	<0.01	<0.01
True Protein							
%	2.82 ^a	2.95 ^{ab}	2.99 ^b	0.05	0.05	<0.01	<0.01
Kg/d	0.79 ^a	0.93 ^b	1.13 ^c	0.03	<0.01	<0.01	<0.01
MUN, mg/dl	14.9 ^a	12.0 ^b	10.6 ^c	0.4	<0.01	<0.01	<0.01
SCS ³	3.09	3.47	2.83	0.36	0.44	<0.01	<0.01

¹PC = pasture plus concentrate, pTMR = partial total mixed ration, TMR = total mixed ration.

²T = treatment, W = week, T x W = treatment x week interaction.

³SCS = $((\log_{10}(\text{SCC}/1000) - 2)/\log_{10}(2)) + 3$.

^{a, b, c}Least square means in the same row with different superscripts differ ($P < 0.05$).

Table 38: Milk production response to bST injections of dairy cows with three different feeding systems during 10 days injections periods.

Injection (date)	Treatment ¹	DIM ²	Day 1 kg/d	Highest response		
				Day	Milk, kg/d ($P <^3$)	Response, kg/d (%)
1 (5/3)	PC	121	36.9	4	37.5 (NS)	NS
	pTMR	114	37.2	7	38.1 (NS)	NS
	TMR	117	40.1	8	47.5 (0.03)	7.4 (18.5)
2 (5/17)	PC	135	33.9	7	36.3 (NS)	NS
	pTMR	128	34.9	10	36.7 (NS)	NS
	TMR	131	39.4	8	43.1 (0.10)	3.7 (9.4)
3 (5/31)	PC	149	32.6	5	33.0 (NS)	NS
	pTMR	142	35.1	9	38.1 (NS)	NS
	TMR	145	40.7	8	44.2 (0.08)	3.5 (8.6)
4 (6/14)	PC	163	28.8	10	32.3 (0.02)	3.5 (12.2)
	pTMR	156	32.3	9	35.3 (0.03)	3.0 (9.3)
	TMR	159	37.4	10	41.1 (0.01)	3.7 (9.9)
5 (6/28)	PC	177	26.8	7	33.2 (<0.01)	6.4 (23.9)
	pTMR	170	30.9	8	35.7 (<0.01)	4.8 (15.5)
	TMR	173	36.7	10	43.1 (<0.01)	6.4 (17.4)
6 (7/12)	PC	191	29.2	6	31.9 (0.04)	2.7 (9.2)
	pTMR	184	30.9	6	32.8 (0.10)	1.9 (6.1)
	TMR	187	38.3	7	41.3 (0.04)	3.0 (7.8)
7 (7/26)	PC	205	28.6	2	30.0 (0.01)	1.4 (4.9)
	pTMR	198	28.7	5	29.9 (NS)	NS
	TMR	201	37.1	6	39.9 (0.01)	2.8 (7.5)
8 (8/9)	PC	219	27.4	10	30.8 (0.01)	3.4 (12.4)
	pTMR	212	28.4	8	28.3 (NS)	NS
	TMR	215	34.4	10	36.6 (0.10)	2.2 (6.4)
9 (8/23)	PC	233	24.9	6	25.6 (NS)	NS
	pTMR	226	26.8	5	28.3 (NS)	NS
	TMR	229	35.2	7	36.1 (NS)	NS
10 (9/6)	PC	247	22.1	7	26.5 (<0.01)	4.4 (19.9)
	pTMR	240	22.2	6	29.2 (<0.01)	7.0 (31.5)
	TMR	243	28.6	5	34.4 (<0.01)	5.8 (20.3)

¹PC = pasture plus concentrate, pTMR = partial total mixed ration, TMR = total mixed ration.

²DIM at each injection time.

³Significantly different from day 1 ($P < 0.10$).

Table 39: Body weight and body condition score of dairy cows with three different feeding systems.

	Treatments ¹			SEM	Effects, ² $P <$		
	PC	pTMR	TMR		T	W	T x W
BW, kg							
Mean	620 ^a	631 ^a	675 ^b	16	0.05	<0.01	<0.01
Initial	612 ^A	613 ^A	646 ^A	16	...	...	...
Final	639 ^{B, a}	653 ^{B, a}	722 ^{B, b}	16	...	...	...
Change	27 ^a	40 ^a	76 ^b	11	...	...	...
BCS, 1 to 5							
Mean	2.61 ^a	2.76 ^{ab}	2.88 ^b	0.07	0.03	<0.01	0.01
Initial	2.81 ^A	2.89	2.86 ^A	0.08	...	...	...
Final	2.61 ^{B, a}	2.90 ^{ab}	3.05 ^{B, b}	0.08	...	...	...
Change	-0.20 ^a	0.01 ^{ab}	0.19 ^b	0.08	...	...	...

¹PC = pasture plus concentrate, pTMR = partial total mixed ration, TMR = total mixed ration.

²T = treatment, W = week, T x W = treatment x week interaction.

^{A, B}Least square means in the same column with different superscripts differ ($P < 0.05$).

^{a, b, c}Least square means in the same row with different superscripts differ ($P < 0.05$).

Table 40: Blood and urine metabolites of dairy cows with three different feeding systems.

	Treatments ¹			SEM	Effects, ² <i>P</i> <		
	PC	pTMR	TMR		T	W	T x W
Blood							
Glucose, mg/dl	64.3	65.6	64.5	0.6	0.26	<0.01	0.03
PUN, ³ mg/dl	17.2 ^a	13.8 ^b	13.8 ^b	0.3	<0.01	<0.01	<0.01
NEFA, µeq/L							
Mean	176.3 ^a	123.7 ^b	166.1 ^a	11.7	0.01	<0.01	<0.01
Initial, wk 1	301.6 ^{A, a}	129.6 ^b	224.9 ^{A, c}	24.4	...	...	...
Final, wk 21	93.5 ^B	126.2	126.4 ^B	23.4	...	...	...
					Effects, ⁴ <i>P</i> <		
Urine				SEM	T	P	T x P
Allantoin (A), mg/L	1695.9 ^a	1793.2 ^a	2102.2 ^b	84.5	<0.01	<0.01	<0.01
Creatinine (C), mg/L	594.3 ^a	633.3 ^a	750.8 ^b	35.5	<0.01	<0.01	<0.01
A/C ratio	3.00	2.90	2.86	0.08	0.46	<0.01	0.13

¹PC = pasture plus concentrate, pTMR = partial total mixed ration, TMR = total mixed ration.

²T = treatment, W = week, T x W = treatment x week interaction.

³PUN = plasma urea nitrogen.

⁴T = treatment; P = period; T x P = treatment x period interaction.

^{A, B}Least square means in the same column with different superscripts differ ($P < 0.05$).

^{a, b, c}Least square means in the same row with different superscripts differ ($P < 0.05$).

Table 41: Grazing behavior and ruminating time of dairy cows with two different feeding systems.

	Treatments ¹		SEM	Effects, ² <i>P</i> <		
	PC	pTMR		T	P	T x P
Grazing time (GT), min/d	572	252	2	<0.01	0.19	0.63
GT a.m., min	266	252	12	0.40	0.25	0.66
GT p.m., min	295	...	14	...	0.17	...
Biting rate (BR), bites/min	57	54	2	0.17	<0.01	0.32
BR a.m., bites/min	55	54	2	0.58	<0.01	0.29
BR p.m., bites/min	59	...	2	...	0.05	...
Total bites, bites/d	31508	14316	1285	<0.01	0.04	0.25
Bites a.m.	14272	14316	443	0.95	0.20	0.89
Bites p.m.	17236	...	1072	...	0.04	...
Bite mass, ³ g DM/bite	0.48	0.55	0.03	0.09	0.69	0.84

¹PC = pasture plus concentrate, pTMR = partial total mixed ration.

²T = treatment, P = period, T x P = treatment x period interaction.

³Bite mass = pasture DMI/(grazing time x biting rate).

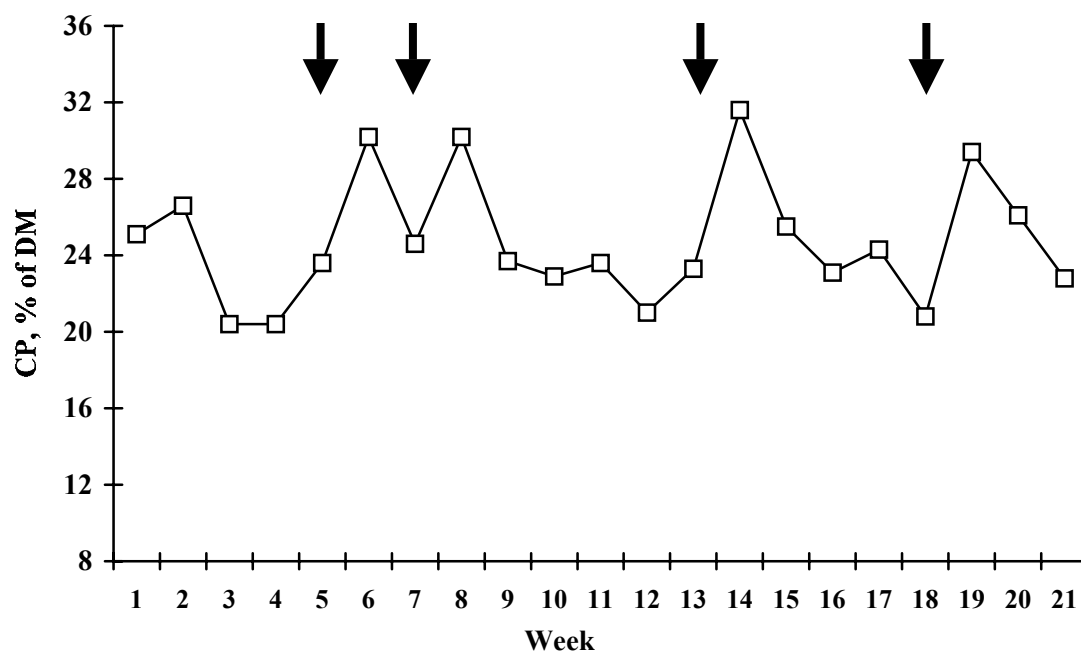


Figure 9: Variations of CP content in the pasture grazed for the cows on pasture plus concentrate (PC), and cows on partial total mixed ration (pTMR). Arrows indicate fertilization with 50 kg N/ha (wk 5, 7, 13 and 18).

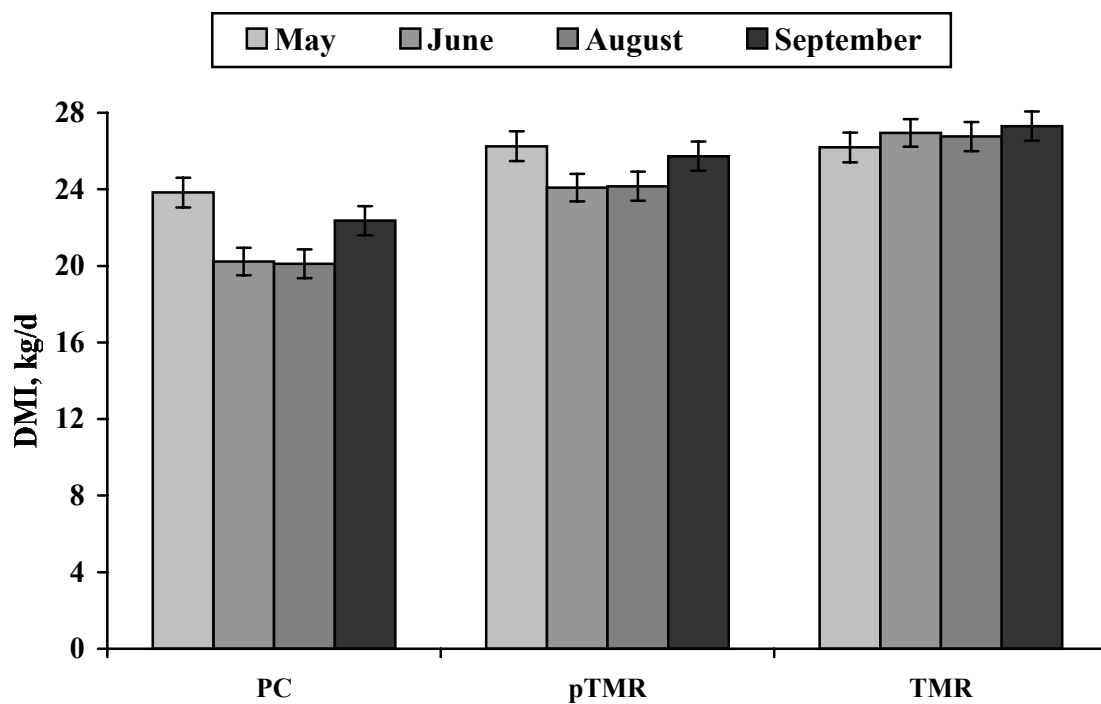


Figure 10: Total DMI in each of four periods by dairy cows with three different feeding systems: pasture plus concentrate (PC), partial total mixed ration (pTMR) or total mixed ration (TMR) as determined by Cr_2O_3 method.

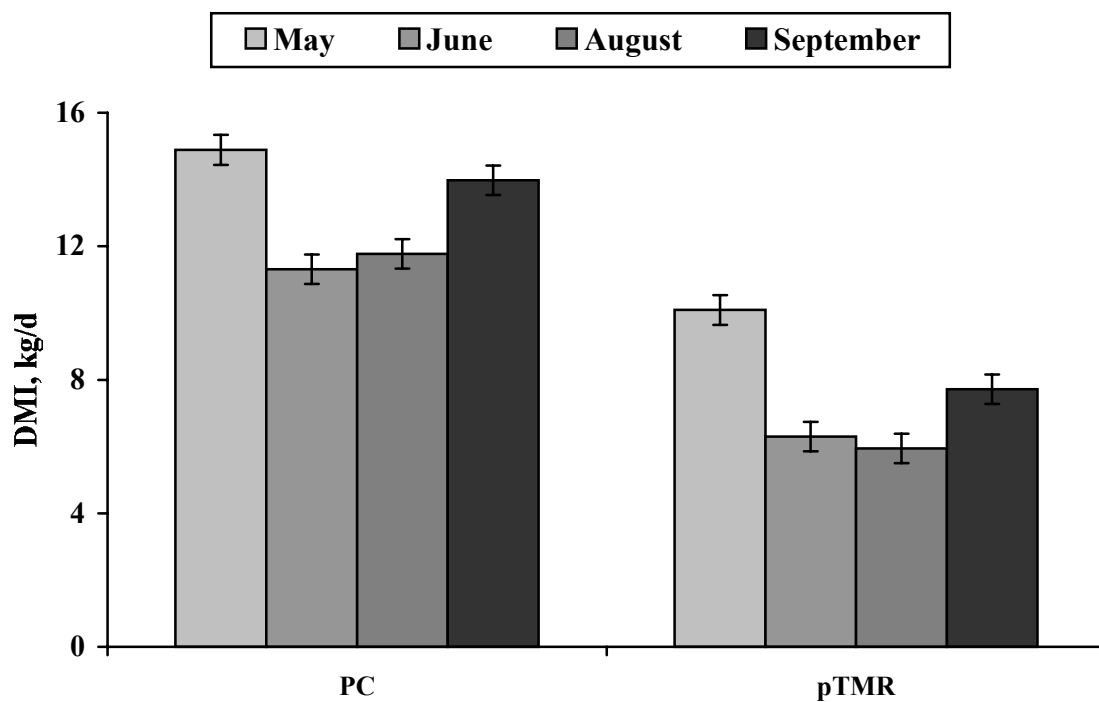


Figure 11: Pasture DMI in each of four periods by dairy cows with two different feeding systems: pasture plus concentrate (PC) or partial total mixed ration (pTMR) as determined by Cr₂O₃ method.

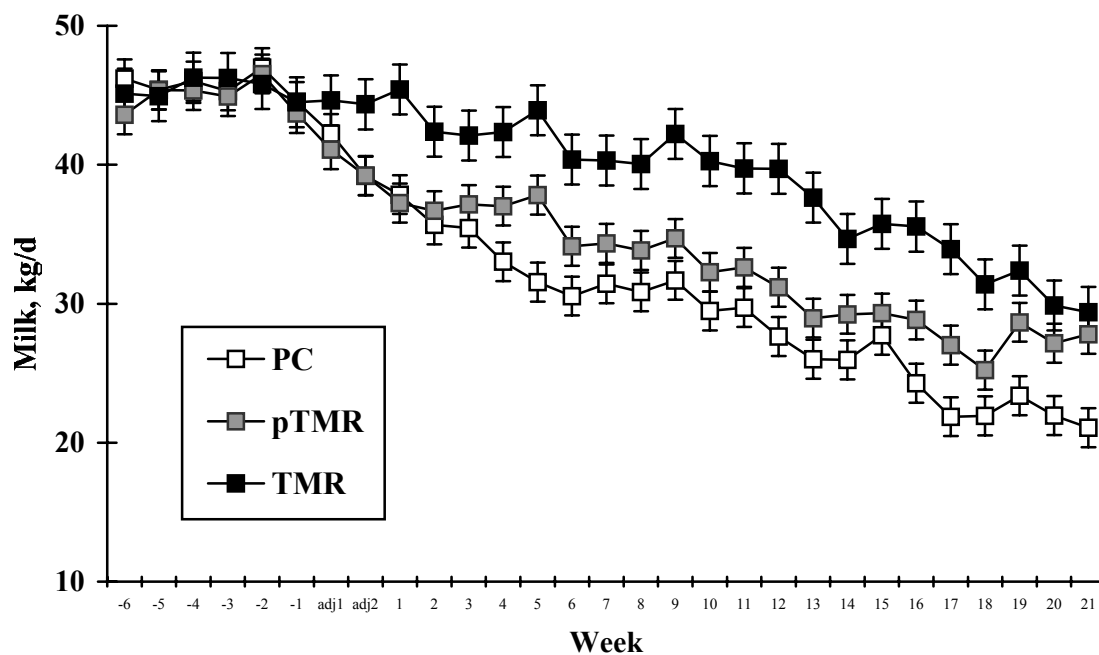


Figure 12: Average weekly milk production of dairy cows with three different feeding systems: pasture plus concentrate (PC), partial total mixed ration (pTMR) or total mixed ration (TMR) for 6 wk prior to the study, 2 wk of adjustment, and 21 wk of the trial.

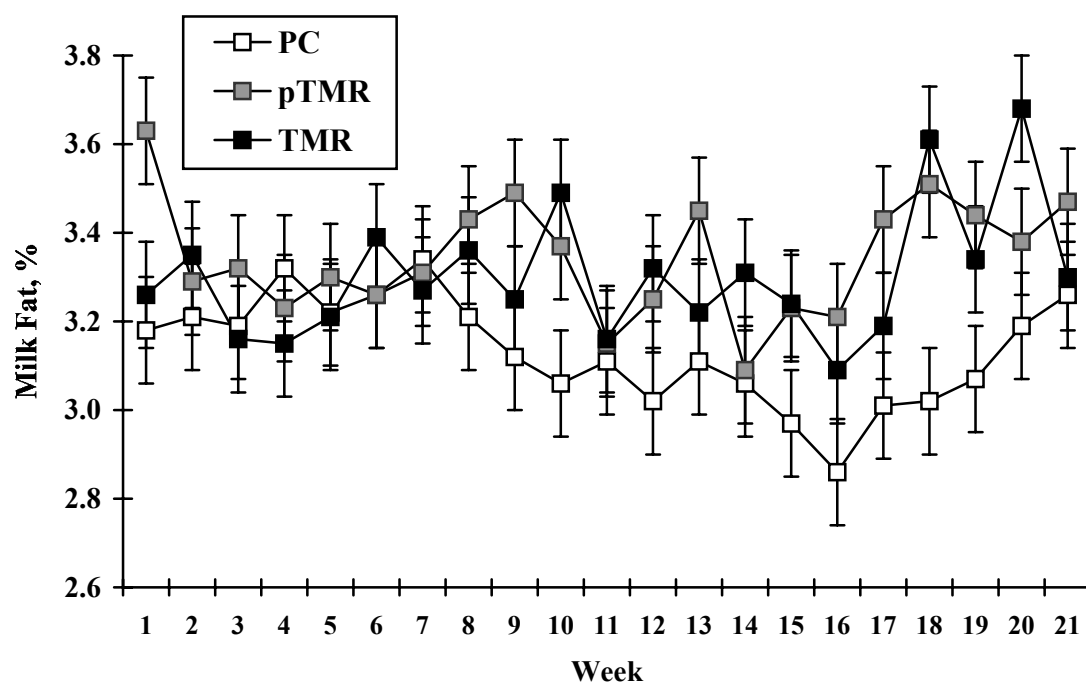


Figure 13: Average weekly milk fat percentage of dairy cows with three different feeding systems: pasture plus concentrate (PC), partial total mixed ration (pTMR) or total mixed ration (TMR).

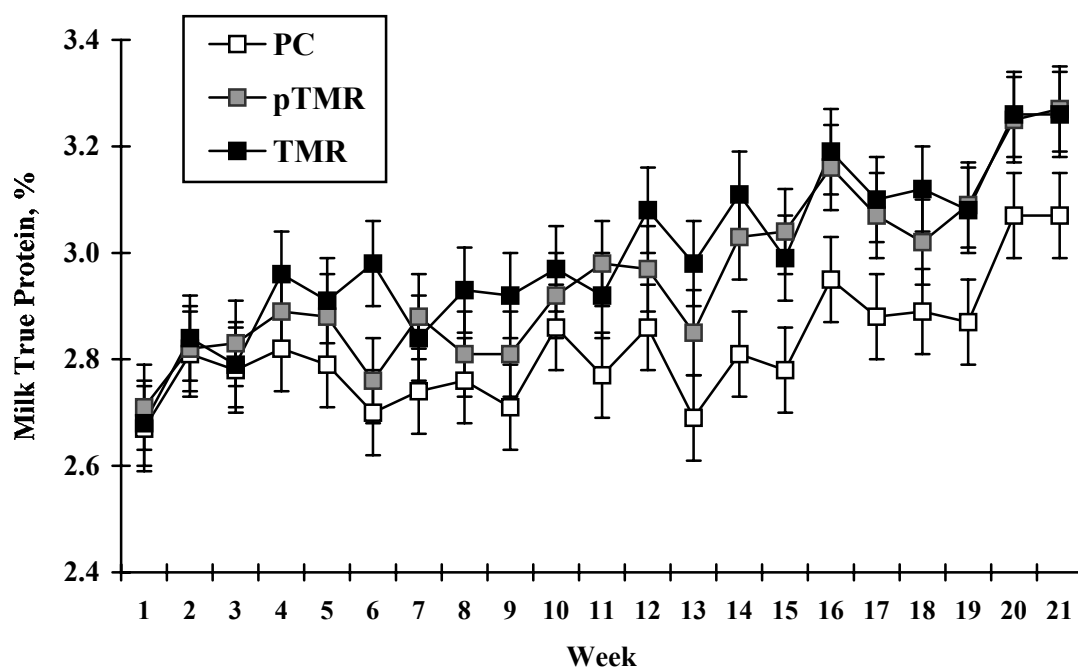


Figure 14: Average weekly milk true protein percentage of dairy cows with three different feeding systems: pasture plus concentrate (PC), partial total mixed ration (pTMR) or total mixed ration (TMR).

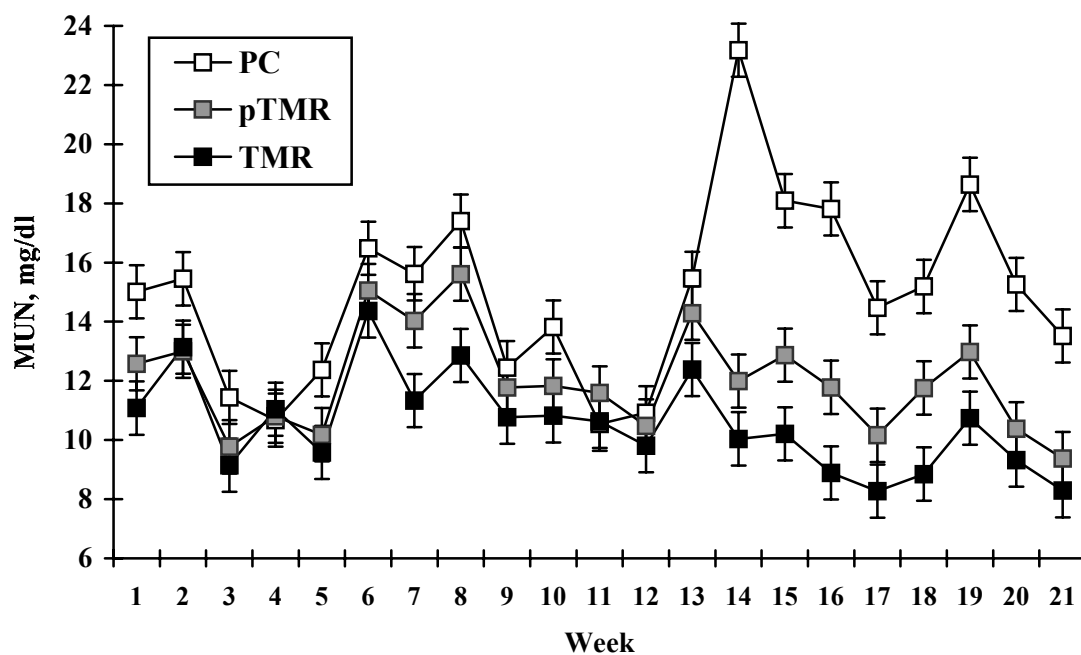


Figure 15: Average weekly milk urea nitrogen (MUN) of dairy cows with three different feeding systems: pasture plus concentrate (PC), partial total mixed ration (pTMR) or total mixed ration (TMR).

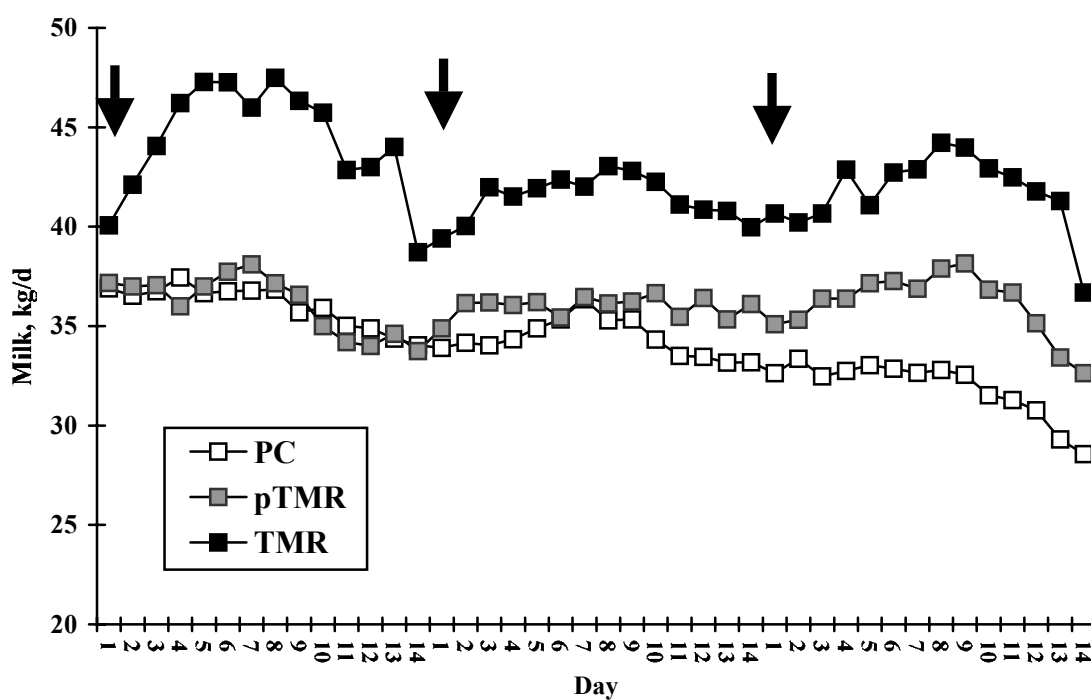


Figure 16: Milk response to first, second and third bST injections of dairy cows with three different feeding systems: pasture plus concentrate (PC), partial total mixed ration (pTMR) or total mixed ration (TMR). Black arrows indicate the days of injections (5/3, 5/17 and 5/31).

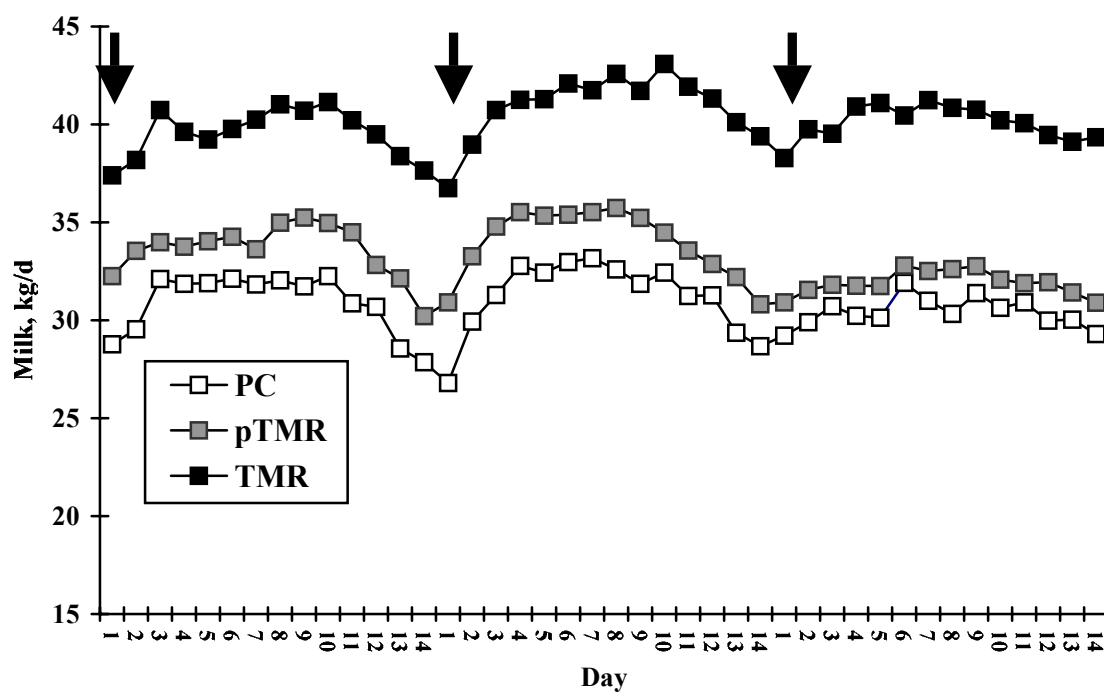


Figure 17: Milk response to fourth, fifth and sixth bST injections of dairy cows with three different feeding systems: pasture plus concentrate (PC), partial total mixed ration (pTMR) or total mixed ration (TMR). Black arrows indicate the days of injections (6/14, 6/28 and 7/12).

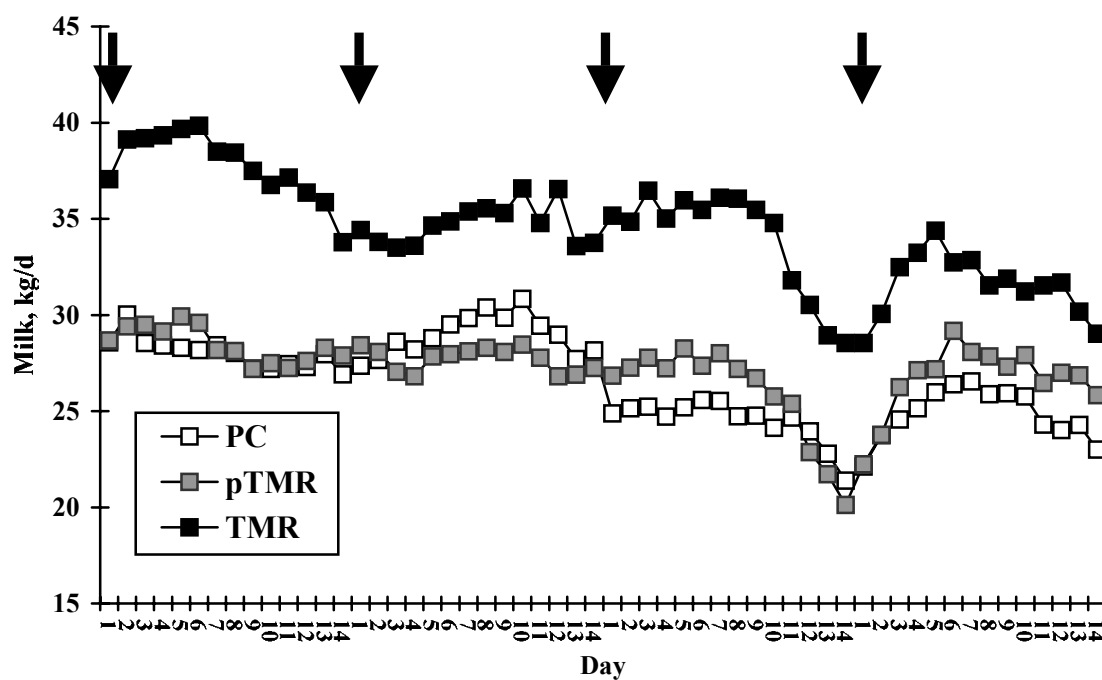


Figure 18: Milk response to seventh, eighth, ninth and tenth bST injections of dairy cows with three different feeding systems: pasture plus concentrate (PC), partial total mixed ration (pTMR) or total mixed ration (TMR). Black arrows indicate the days of injections (7/26, 8/9, 8/29 and 9/6).

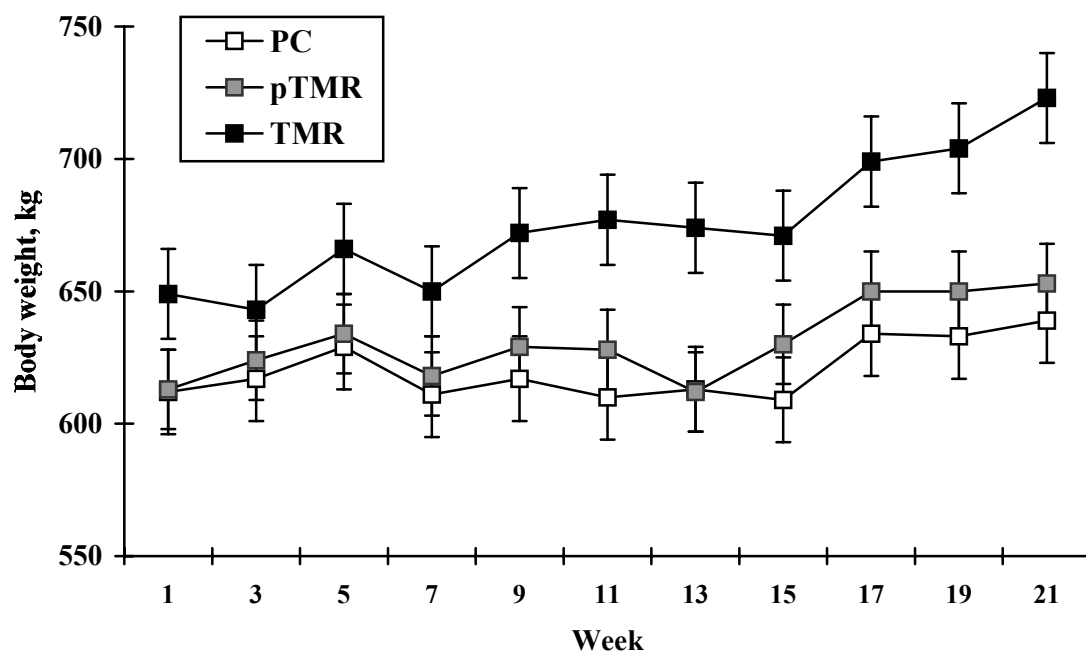


Figure 19: Body weight of dairy cows with three different feeding systems: pasture plus concentrate (PC), partial total mixed ration (pTMR) or total mixed ration (TMR).

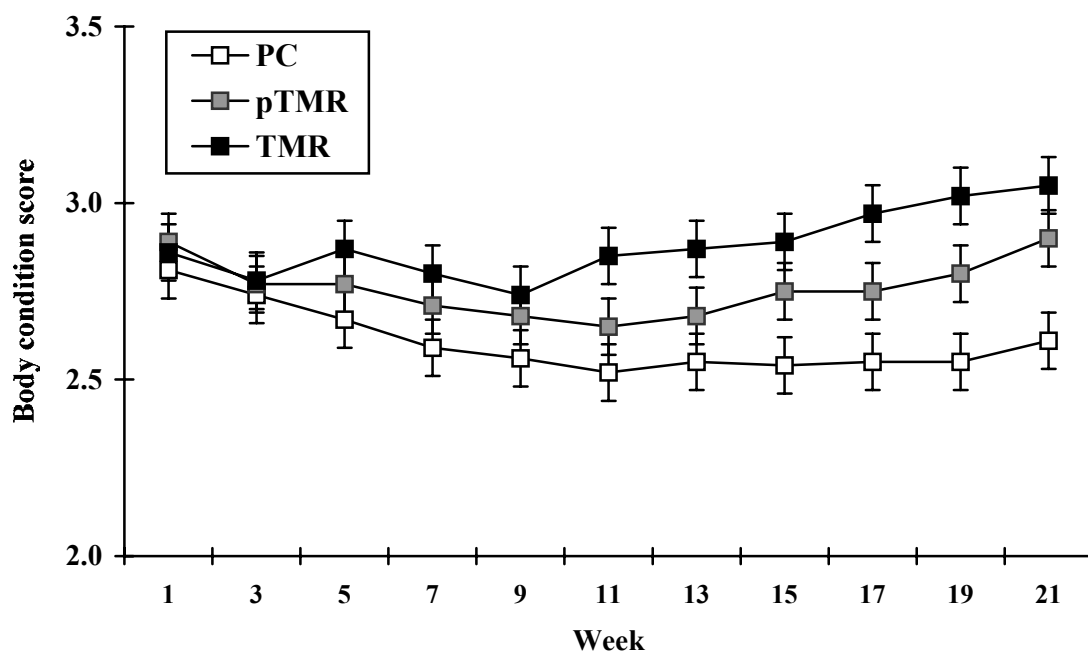


Figure 20: Body condition score of dairy cows with three different feeding systems: pasture plus concentrate (PC), partial total mixed ration (pTMR) or total mixed ration (TMR).

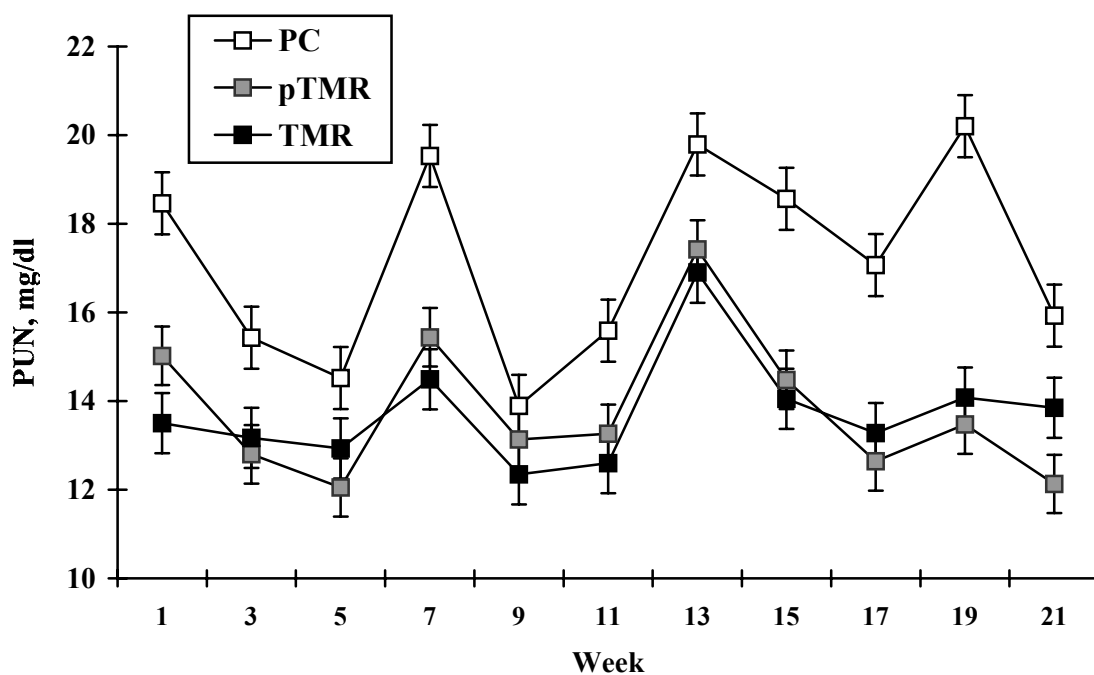


Figure 21: Plasma urea nitrogen (PUN) concentrations of dairy cows with three different feeding systems: pasture plus concentrate (PC), partial total mixed ration (pTMR) or total mixed ration (TMR).

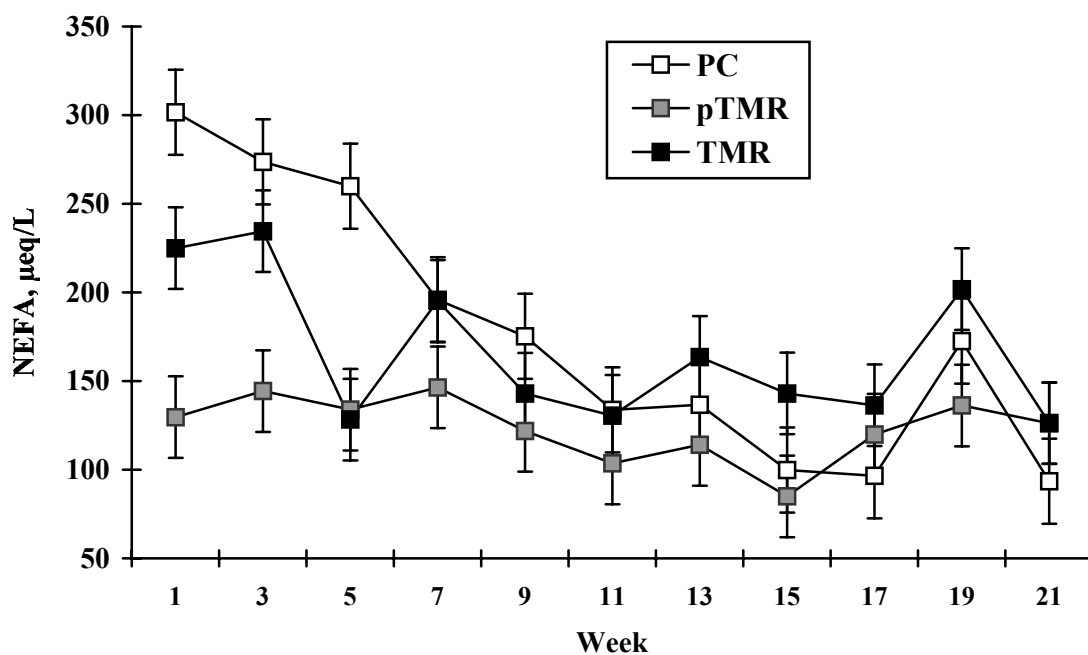


Figure 22: Non-esterified fatty acids (NEFA) concentrations of dairy cows with three different feeding systems: pasture plus concentrate (PC), partial total mixed ration (pTMR) or total mixed ration (TMR).

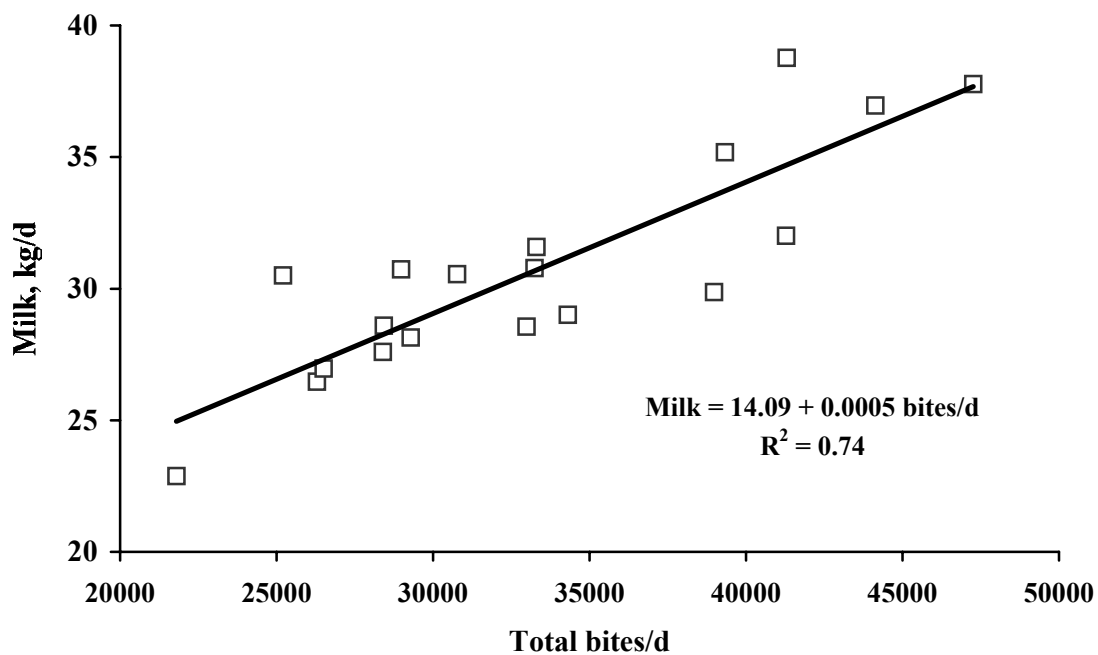


Figure 23: Relationship between milk production and number of bites per d on the pasture plus concentrate (PC) treatment.

Chapter 6

Ruminal Digestion of Three Different Feeding Systems Combining Pasture and Total Mixed Rations

6.1 ABSTRACT

Six multiparous Holsteins cows fitted with rumen cannulas were used to study the effect of three feeding systems combining pasture and a total mixed ration (TMR) on ruminal digestion in a 21-wk repeated measures experiment. The three treatments were: 1) pasture plus concentrate (PC), 2) pasture plus partial TMR (pTMR), and 3) all TMR. Ruminal ammonia nitrogen concentration was lower in both the pTMR and TMR treatments (10.2 ± 0.5 mg/dl) than in the PC treatment (19.9 ± 0.5 mg/dl). Ruminal pH was not affected by treatment and averaged 5.87. Neither total volatile fatty acids concentration (137.5 mmol/L) nor individual volatile fatty acids proportions (63.1, 20.6, and 12.0 mol/100 mol for acetate, propionate, and butyrate, respectively) differed among treatments. The pTMR treatment reduced the total potentially degradable fraction of DM (85.5 vs. 82.3%) and the potentially digestible fraction of NDF (82.1 vs. 74.9%) of pasture compared to the PC treatment. Ruminal losses of ammonia nitrogen were reduced combining pasture and TMR. The combination of pasture and TMR also decreased the ruminal DM and NDF digestion of pasture, indicating the presence of negative associative rumen effects.

(**Key words:** pasture, total mixed rations, rumen parameters, in situ digestion)

Abbreviation key: ED = effective degradability, IVDMD = in vitro dry matter digestibility, $\text{NH}_3\text{-N}$ = ammonia nitrogen, pTMR = partial TMR, TNC = total nonstructural carbohydrates.

6.2 INTRODUCTION

When pasture plus concentrate is fed to high genetic merit cows, milk production is lower than typically found in confinement feeding systems based on a nutritionally balanced TMR where DMI and milk production are maximized (White, 2000; White et al., 2001). Studies with high producing dairy cows on pasture (Bargo et al., 2000; Reis and Combs, 2000a; Reis and Combs, 2000b; Soriano et al., 2000) reported that energy supplementation with 8 to 9 kg/d of corn-based concentrates resulted in a total DMI of 22 kg/d and milk production of 30 kg/d. This lower animal performance may be related to changes in ruminal fermentation and digestion characteristics when cows grazed high quality pasture. Pasture-based diets are often characterized by reduced rumen pH (< 6.0), low acetate/propionate ratio, a high concentration of ammonia nitrogen ($\text{NH}_3\text{-N}$), and a high rate of passage (Bargo et al., 2001; Holden et al., 1994; Rearte and Santini, 1993).

Supplementing a TMR to a pasture-based feeding system may improve performance through changes in rumen digestion and fermentation. This system is called partial TMR (**pTMR**) because the pasture grazed by the cows is not physically part of the TMR. Feeding a pTMR could result in higher performance because of higher total DMI and milk production with a higher fat and protein content. Potential positive effects on rumen fermentation could include an increase in rumen pH and a reduction in rumen $\text{NH}_3\text{-N}$ because of the inclusion in the diet of forage sources higher in effective fiber and lower in CP content than pasture. However, with the

combination of pasture and TMR associative effects may occur in the rumen (Dixon and Stockdale, 1999).

Previous studies have compared milk performance of dairy cows fed only pasture or fed a TMR (Kolver and Muller, 1998; Kolver et al., 2000), or fed a TMR compared to pasture plus concentrate (White, 2000; White et al., 2001). However, these studies did not include a pTMR treatment or evaluate rumen digestion and fermentation. There are no published studies that have compared rumen digestion of pasture plus concentrate, a total TMR diet, and the combination of both pasture and TMR (pTMR). The objective was to evaluate these three feeding systems on rumen fermentation parameters and in situ pasture rumen digestion of high producing dairy cows.

6.3 MATERIALS AND METHODS

6.3.1 Cows and Treatments

Six multiparous Holstein cows fitted with ruminal cannulas [BW, 653 ± 79 kg; milk yield, 43.7 ± 9.2 kg/d; parity, 3.0 ± 0.9 ; DIM, 115 ± 24 (mean $\pm$ SD)] were used in a 21-wk trial to study the effect of three feeding systems on rumen digestion. Cows were selected from The Pennsylvania State University Dairy Cattle Research and Education Center (University Park, PA), which averaged 11436 kg of milk and 363 kg of protein per cow per lactation in 1999. Cows were grouped in two groups of three by lactation number and DIM, and randomly assigned

to three dietary treatments: 1) pasture + concentrate (PC); 2) pasture + TMR (partial TMR; pTMR), and 3) TMR (non-pasture).

Cows on the PC and the pTMR treatment grazed a pasture with an average botanical composition of 50% smooth brome grass (*Bromus inermis* L.), 33% orchardgrass (*Dactylis glomerata* L.), 7% Kentucky bluegrass (*Poa pratensis* L.), and 10% weeds and dead material. The chemical composition of the pasture is shown in *Table 42*. Pasture was fertilized five times with 50 kg/ha of nitrogen before the start of the trial on April 20 and during the trial on June 7 (wk 5), June 19 (wk 7), July 31 (wk 13), and September 5 (wk 18). The target pasture allowance was 30 kg DM/cow/d to ground level. Pre-grazing pasture mass was measured every week to adjust the size of the paddock to maintain the targeted pasture allowance. Pre-grazing pasture mass (kg DM/ha) was measured by cutting fifteen quadrants (0.124 m²/quadrant) of pasture to ground level, and drying at 55 °C in a forced air oven. A new paddock was constructed daily using a temporary polywire, and divided by a polywire to offer a new portion after each milking. A second polywire fence was used to prevent back grazing. New paddocks were given to the cows each morning at approximately 0700 h. After the a.m. milking, 2/3 of the daily paddock was offered to the cows on the PC and pTMR treatments to ensure a pasture allowance of 15 kg DM/cow for a half day. After the p.m. milking, the other 1/3 portion of the paddock was offered to the cows on the PC treatment with a pasture allowance of 15 kg DM/cow for a half day to complete the daily allowance of 30 kg DM/cow per d in this treatment. Cows on the PC treatment also received a corn-based concentrate with the chemical composition shown in *Table 43*. The amount of concentrate offered per cow was 1 kg/4 kg of milk at the beginning of the trial using the pre-trial milk production, with an upper limit of 10 kg DM/cow/d to avoid metabolic problems in the rumen (e.g. acidosis). The amount of concentrate offered was readjusted at the

middle of the trial (week 10) to keep the 1 kg/4 kg of milk ratio using the milk production from wk 9 and 10.

Cows on the pTMR treatment grazed between the a.m. and the p.m. milking with the cows on the PC treatment. After the p.m. milking, cows on the pTMR treatment were housed overnight in a free stall barn where they were fed 17 kg of DM of TMR (*Table 43*), which was the same TMR fed to the TMR treatment. Cows on the pTMR treatment were also fed approximately 3 kg DM of the same concentrate as fed to the PC treatment (*Table 43*) with the goal of having a similar forage:concentrate ratio in the total ration for the PC and the pTMR treatments.

Cows on the TMR treatment stayed in a free-stall barn and were fed the TMR treatment (formulated according NRC, 1989; *Table 43*). The TMR was group fed ad libitum once daily at 0730 h at a rate of approximately 28 kg DM/cow per day. Cows were milked twice daily at 0530 h and 1730 h. Cows received bST injections every two weeks. Walking distance from pasture to the milking parlor averaged 0.9 km (range: 0.75 to 1.20 km).

6.3.2 Experimental Measures and Sample Analyses

Dry matter intake was measured for eleven days during wk 2 (May 15), 6 (June 12), 14 (August 7), and 19 (September 11). Cows fed the pTMR and the TMR treatments were placed in a tie-stall barn during these periods and fed individually to measure DMI of TMR. Total DMI was also estimated for the three treatments using Cr_2O_3 as an indigestible fecal marker. The indigestible fecal marker Cr_2O_3 was dosed twice daily (10 g/d) after each milking (0700 h and

1900 h, approximately) for eleven days. Fecal grab samples to estimate DMI were collected at 0700 h and 1900 h from d 7 to 11, and immediately frozen (- 20 °C).

During the same days that fecal samples were taken, samples of the concentrate and the TMR were collected, and pasture samples were plucked by hand to the approximate height to which cows grazed (Wallis de Vries, 1995). Samples were dried at 55 °C in a forced air oven and ground through a 1-mm screen (Wiley Mill, Thomas Scientific, Philadelphia, PA). Concentrate and TMR samples were composited by period, while hand-plucked pasture samples were kept as daily samples. Composited concentrate and TMR samples, and daily pasture samples were analyzed for DM, CP, and ash (AOAC, 1990), soluble CP (Krishnamoorthy et al., 1982), ADF and NDF (Ankom Daisy II, ANKOM Technology Corp., Fairport, NY), total nonstructural carbohydrate (**TNC**) (Smith, 1981; modified to use potassium ferricyanide as the colorimetric indicator), and in vitro DM digestibility (**IVDMD**) (Tilley and Terry, 1963).

Fecal samples were thawed, dried at 55 °C in a forced air oven, and ground through a 1-mm screen (Wiley Mill, Thomas Scientific, Philadelphia, PA). A composited sample of feces per cow was made for each period of intake measures before grinding. Fecal samples were analyzed for CP (AOAC, 1990), NDF (Ankom Daisy II, ANKOM Technology Corp., Fairport, NY), and Cr (Parker et al., 1989). The concentrate DMI in both the PC and the pTMR treatments, and the TMR DMI in both the pTMR and the TMR treatments were measured daily in every cow as the difference between offered and refused the same five days that fecal grab samples were taken. Total DMI in the TMR treatment was also determined by the equation: total DMI (TMR) = [(g Cr/d)/(g Cr/g fecal DM)] / (1 – IVDMD of TMR). Pasture DMI was estimated in the PC treatment using the equation: pasture DMI = [(g Cr/d)/(g Cr/g fecal DM) – concentrate DMI x (1 – IVDMD of concentrate)] / (1 – IVDMD of pasture). Pasture DMI was estimated in the pTMR

treatment using the equation: pasture DMI = [(g Cr/d)/(g Cr/g fecal DM) – concentrate DMI x (1 – IVDMD of concentrate) – TMR DMI x (1 – IVDMD of TMR)] / (1 – IVDMD of pasture). Total DMI in the PC treatment was determined as concentrate DMI plus pasture DMI, in the pTMR treatment as pasture DMI plus concentrate DMI plus TMR DMI, and in the TMR treatment as TMR DMI.

Ruminal fluid samples were collected on May 26 (wk 3), June 23 (wk 7), August 18 (wk 15), and September 22 (wk 20) at 0, 4, 8, 12, 16, and 20 h after initiation at 0500 h on each of those four days. At the 4 h (0900 h) and 8 h (1300 h) sampling times, the four rumen cannulated cows on pasture (two of the PC and two of the pTMR treatments) and at the 16 h (2100 h) and 20 h (0100 h) sampling times the two rumen cannulated cows on the PC treatment were sampled in the pasture using a temporary construction made with mobile gates in the paddock. At 0 h (0500 h) and 12 h (1700 h) the two rumen cannulated cows on all treatments were sampled indoors because these two sampling times coincided with the time of milking. Cows on the TMR treatment were sampled indoors at all the sampling times. Ruminal digesta samples were taken from the dorsal, ventral and caudal area of the rumen, and squeezed through four layers of cheesecloth. The pH of the filtered ruminal fluid was measured immediately using a portable digital Cole Palmer pH-meter, and 15-ml aliquot preserved with 3 ml of 25% metaphosphoric acid and 3 ml of 0.6% 2-ethyl butyric acid (internal standard). These samples were stored at –20 °C and subsequently analyzed for NH₃-N and VFA (Yang and Varga, 1989).

The in situ technique was used four times during the 21-wk trial in May, June, August, and September to estimate ruminal disappearance of DM, CP and NDF of pasture, concentrate and TMR in the different treatments. Pasture and concentrate samples were incubated in the cows on the PC and the pTMR treatments. Samples of TMR were incubated in the cows on the

TMR treatment. Hand-plucked pasture, concentrate and TMR samples were taken the day before the bags were incubated. Fresh pasture samples were cut into lengths of approximately 1 cm, and fresh concentrate and TMR samples were processed using a meat-grinding machine for 1 min (Hobart Model) to simulate cows chewing. Approximately 5 g of DM of cut pasture, and processed concentrate and TMR samples were placed in polyester bags (22.86 cm x 10.16 cm; Marvelier White, Strauss Co., New York, NY) with a mean pore size of 52 μm . Prior to use, the bags were dried in a forced-air oven at 55 °C for 48 h. Filled bags were closed with a plastic tie 2 cm below the top, resulting in an effective surface area of 20.86 cm x 10.16 cm. Because of DM content changes in pasture, concentrate, and TMR samples from period to period, the final sample size to surface area ratio averaged 16, 25 and 37 mg DM/cm², respectively. Bags were tied to the end of a 100-cm fishing line and incubated in the rumen of the six rumen cannulated cows for 0, 4, 8, 12, 16, 20, 24 and 48 h after being soaked in 39 °C distilled water for 15 min beginning at 0500 h on May 25 (wk 3), June 22 (wk 7), August 17 (wk 15), and September 21 (wk 20). Bags were inserted in reverse order and the 24, 20, 16, 12, 8 and 4 h incubation times coincided with rumen fluid sampling times. Prior to incubation, the filled bags were maintained at 4 °C. Duplicate bags were incubated at all times, except at 24 and 48 h where triplicate bags were incubated. An alfalfa hay standard (4-mm ground) was incubated in duplicated bags at 4, 24 and 48 h and was used to monitor variation within and among cows. All standards were within a previously established range and no time points required correction. Duplicate bags with no sample (blanks) were incubated at 4, 24 and 48 h, and were used to adjust for material entering the bags. After retrieval at 0 h, bags were immediately washed in a washing machine (three cycles; 2-min fill and 2-min rinse per cycle, followed by a final spin; Lykos and Varga, 1995) and brought to the laboratory in a insulated thermos. Bags were then opened and the

contents were washed with cold running water inside the bags with gentle agitation for 1 min and squeezed. Bags were dried at 55 °C for 48 h, weighed to determine DM disappearance, and the residue ground through a 1-mm screen (Wiley Mill, Thomas Scientific, Philadelphia, PA). Duplicate (or triplicate for the 24 and 48 h bags) residues at each time point were composited within cow and analyzed for DM and CP (AOAC, 1990) and NDF (Ankom Daisy II, ANKOM Technology Corp., Fairport, NY). Unincubated samples of pasture, concentrate and TMR, used to fill the bags, were also analyzed for DM and CP (AOAC, 1990) and NDF (Ankom Daisy II, ANKOM Technology Corp., Fairport, NY).

Dry matter, CP and NDF disappearance of pasture and TMR samples, and DM and CP disappearance of concentrate at each incubation time were calculated from the DM, CP and NDF residues in each bag. Kinetic parameters of DM, CP and NDF in situ disappearance of pasture, concentrate and TMR were estimated by the Marquardt nonlinear procedure (NLIN) of SAS (1999). If a delay on the initiation of disappearance of DM, CP, or NDF was observed, a model including a lag time parameter was used.

No lag was observed in the disappearance of DM and CP in pasture, concentrate and TMR samples; therefore data were fitted to the model of Ørskov and McDonald (1979):

$$D = A + B (1 - e^{-kd t}), \text{ where}$$

D = DM or CP disappearance

A = soluble fraction, %

B = insoluble potentially degradable fraction, %

kd = fractional degradation rate, %/h

t = time, h

Effective degradability (**ED**) of DM and CP of forages was calculated in previous studies (Elizalde et al., 1999; Hoffman et al., 1993; Kolver et al., 1998) as $ED = A + B (kd / (kd + kp))$, assuming a single passage rate (kp) of 6 %/h. Others (Coblentz et al., 1998; Waldo et al., 1972) suggested that the used of a single passage rate for different forages is not appropriate; therefore in this study where cows received pasture and conserved forage from the TMR, ED of DM and CP was not calculated.

A lag was observed in the disappearance of NDF in pasture and TMR samples; therefore data were fitted to the model of Mertens and Lofton, (1980):

$FR = P e^{-kd(t-L)} + U$, when $t > L$; and $FR = P + U$, when $0 < t < L$, where

FR = NDF remaining at time t

P = potentially digested fraction ($100 - U$) at fractional rate kd, %

kd = fractional degradation rate, %/h

U = fraction undigested at 48 h, %

L = lag time, h

t = time of incubation, h

Milk production was recorded daily from d 1 (May 8) to 147 (October 1). Milk samples were collected weekly during the 21 weeks of the experiment and preserved with 2-bromo-2-nitropropane-1,3 diol. Milk fat, true protein and lactose were analyzed by infrared spectrophotometry (Foss 605B Milk-Scan; Foss Electric, Hillerod, Denmark) by the Pennsylvania DHIA milk testing laboratory.

Cows were weighed after the p.m. milking on two consecutive days every two weeks (wk 1, 3, 5, 7, 9, 11, 13, 15, 17, 19, and 21). Body condition scoring of the cows was conducted at the same time by two experienced independent observers using the five-point BCS scale (1 = thin; 5 = fat) (Wildman et al., 1982).

6.3.3 Statistical Analyses

Rumen digestion data were analyzed as repeated measures using the PROC MIXED procedure of SAS (1999). The model included the fixed effects of treatments, week, week by treatment interaction, hour, hour by treatment interaction, hour by week interaction, hour by week by treatment interaction, the random effect of cows nested within treatment, and the residual error.

For each variable analyzed, cow nested within treatment was subjected to three covariance structures: compound symmetry, autoregressive order 1, and unstructured covariance. The covariance that resulted in the smallest Akaike's Information Criterion and Schwarz Bayesian Criterion was used. Least squares means and SEM are reported for all data. When significant ($P < 0.05$) effects due to dietary treatments were detected, mean separation was conducted by the PDIFF option in SAS (1999).

6.4 RESULTS AND DISCUSSION

6.4.1 Animal Performance

The objective of presenting these data is to demonstrate that rumen cannulated cows were similar in performance to treatments as those cows from the larger companion study (Chapter 5). If the rumen cannulated cows responded similar trends in DMI, milk production and composition and BW, the extrapolation of rumen digestion data to the companion study with a greater number of cows ($n = 45$) is valid.

Cows on the TMR treatment consumed 17% more total DMI than cows on the PC treatment, and 2% more than cows on the pTMR treatment. On a DM basis, cows on the PC treatment consumed 22.6 ± 1.4 (mean $\pm$ SEM) kg/d of a diet composed of 39% concentrate and 61% pasture, cows on the pTMR treatment consumed 25.7 ± 1.4 kg/d of a diet composed of 10% concentrate, 29% pasture, and 61% TMR, and cows on the TMR treatment consumed 26.3 ± 1.4 kg/d of a diet composed of 100% TMR (50% forage and 50% concentrate). These total DMI and diet compositions were very similar to those reported in the performance study (Chapter 5).

Differences in milk production among treatments were similar to differences in milk production reported in Chapter 5. The TMR fed cows produced 39% more milk than those fed the PC treatment (39.7 vs. 28.5 kg/d; ± 4.2 SEM) and 25% more milk than cows fed the pTMR treatment (39.7 vs. 31.7 kg/d; ± 4.2 SEM). Similar trends were found for milk fat and true protein content to those reported in Chapter 5. The cows fed the pTMR and the TMR treatments had higher milk fat percentage compared to cows fed the PC treatment (3.33 vs. 3.10%; ± 0.10 SEM). The highest true protein was found for the TMR treatment (3.08%) and the lowest for the

PC treatment (2.76%), with an intermediate value for the pTMR treatment (2.94%). Milk urea nitrogen was higher for cows fed the PC treatment than either the pTMR or the TMR treatments (15.6 vs. 11.8 mg/dl; ± 2.0 SEM).

In agreement with the results reported in the performance study (Chapter 5), initial BW did not differ among treatment but with a high variation as expected because of the low number of cows (668 ± 56 kg/d; mean $\pm$ SEM). Numerically cows on the PC treatment gained less BW during the experiment than the cows on both the pTMR and TMR treatment, following the trend reported in Chapter 5.

Although no significant differences were detected among treatments for total DMI, milk production and composition in this study due to low number of cows, all these variables followed the same trends as reported in the companion animal performance study (Chapter 5).

6.4.2 Ruminal Fermentation

6.4.2.1 pH

Ruminal fermentation parameters are shown in *Table 44*. Mean rumen pH did not differ among treatments and averaged 5.87 ± 0.04 ($P > 0.05$). The similar rumen pH for the three treatments could be related to the lower forage:concentrate ratio (50:50) in the TMR treatment and the high rumen digestibility of the pasture in both the PC and the pTMR treatments even with a higher forage:concentrate ratio (60:40). Satter et al. (1999) reported that the rumen pH of high producing dairy cows fed diets with 50% of concentrate is often 5.7 or less. Similar values

(5.79 ± 0.05 , range: 5.68 to 5.87) to the PC treatment were reported for dairy cows consuming fresh grass-legume forage and supplemented with 10 kg/d of corn (Reis et al., 2001). For lactating dairy cows in early lactation fed a TMR with corn as the main energy source, Dann et al. (1999) reported a higher mean rumen pH (6.33 ± 0.08) than in our study. Although both studies fed a TMR with a 50:50 concentrate:forage ratio, the lower rumen pH found in the present study may be partially attributed to the higher DMI compared to the study of Dann et al. (1999) (26.3 vs. 21.0 kg/d).

A significant treatment by hour interaction was detected for rumen pH ($P < 0.05$). This indicates that although the mean rumen pH was similar, the daily pattern of rumen pH variation differed among treatments (*Figure 24*). Rumen pH of the PC treatment followed the typical pattern of grazing cows supplemented twice daily after milkings. The PC treatment had a significant ($P < 0.05$) reduction in rumen pH at 0900 h (5.80) and at 2100 h (5.76) after cows received the supplement and were moved to the pasture and grazed actively for the first 2 or 3 hours. Significant increases in rumen pH in this treatment were found at 1700 h (6.08) and at 0500 h (6.09), after noon and midnight when cows have a low grazing activity and concentrate was already digested. The pTMR treatment had significantly higher rumen pH than the PC treatment at 1300 h (6.14 vs. 5.79; $P < 0.05$) and 1700 h (6.46 vs. 6.08; $P < 0.05$). A substantial reduction in rumen pH was observed in the pTMR treatment after 1700 h time when cows stayed indoors and consumed the TMR (6.46 at 1700 h vs. 5.69 at 2100 h; $P < 0.05$). In the TMR treatment, after a rumen pH increase in the early morning hours (5.98 at 0500 h, 6.17 at 0900 h), a reduction in rumen pH (5.85 at 1300 h, 5.79 at 1700 h, and 5.57 at 2100 h) was observed when cows started to eat the TMR that was fed once daily around 0730 h. Among the three treatments, the pTMR resulted in the greatest variations in pH (minimum of 5.48 at 0100 h and maximum of

6.46 at 1700 h); while the TMR treatment presented a more constant rumen pH pattern with maximum of 6.17 at 0900 h and minimum of 5.57 at 2100 h.

A significant overall period effect was detected for rumen pH ($P < 0.05$; *Figure 25*; *Table 45*). Comparing rumen pH across periods and within treatments, cows on the PC treatment had a significantly lower pH in period 2, while cows fed the pTMR and the TMR exhibited a more constant pH (*Figure 25*). This could be attributed to a decreased forage:concentrate ratio during period 2 because of a lower pasture DMI during period 2 with a similar concentrate DMI as reported in Chapter 5. Cows on the PC treatment of the companion study (Chapter 5) had a lower forage:concentrate ratio during period 2 compared to periods 1, 2, and 3 (56:44 vs. 61:39). Comparing within periods and across treatments, cows on the PC treatments had higher rumen pH than cows on the pTMR and the TMR treatments during period 1. In the other three periods rumen pH did not significantly differ among treatments ($P > 0.05$).

6.4.2.2 VFA

Total VFA concentration did not differ among treatments and averaged 137.5 mmol/L ($P > 0.05$; *Table 44*). Previous grazing studies where cows were supplemented with corn based concentrate reported high total VFA concentration (> 130 mmol/L) (Bargo et al., 2000; Reis et al., 2001). For early lactation dairy cows fed a TMR of a 50:50 forage:concentrate ratio, Dann et al. (1999) reported a lower total VFA concentration (129 mmol/L) than that found in this study (141.6 mmol/L), which is in agreement with the higher rumen pH reported by Dann et al. (1999).

Individual VFA molar proportions did not differ among treatments ($P > 0.05$). The three principal individual VFA proportions averaged 63.1, 20.6 and 12.0 mol/100 mol for acetate,

propionate and butyrate, respectively. The acetate/propionate ratio and the (acetate + butyrate)/propionate ratio did not differ among treatments (3.11 and 3.71, respectively; $P > 0.05$).

6.4.2.3 $\text{NH}_3\text{-N}$

Rumen $\text{NH}_3\text{-N}$ concentration was significantly higher for the PC treatment than for both the pTMR and the TMR treatments (19.9 vs. 10.2 mg/dl; $P < 0.05$, *Table 44*). This result is consistent with the significantly higher MUN and PUN concentrations in the PC treatment reported in the animal performance study (Chapter 5). Similar rumen $\text{NH}_3\text{-N}$ concentration (16.9 mg/dl) was reported for high producing dairy cows supplemented with 10 kg/d of different types of corn and consuming a fresh cut grass-legume pasture with 21.4% of CP (Reis et al., 2001). Bargo et al. (2000) reported lower $\text{NH}_3\text{-N}$ concentration (8.9 mg/dl) for dairy cows grazing a grass pasture and supplemented with a corn based concentrate, however the pasture CP content in that study was lower (19.9%) than in this study (26.3%). Dann et al. (1999) reported a mean $\text{NH}_3\text{-N}$ concentration of 10.1 mg/dl for dairy cows on a TMR diet, a similar value to the one found for the TMR treatment (9.7 mg/dl).

A significant treatment by hour interaction was detected for $\text{NH}_3\text{-N}$ ($P < 0.05$), indicating that the pattern over a 24 h period differed among treatments (*Figure 26*). During the six sampling times, rumen $\text{NH}_3\text{-N}$ concentration was significantly higher for the PC treatment than both the pTMR and the TMR treatments (*Table 44*). The pattern of rumen $\text{NH}_3\text{-N}$ in the PC treatment followed a typical pattern for grazing cows with two daily peaks corresponding to ingestion of high CP pasture after cows were moved to the paddock (20.7 mg/dl at 1300 h, and

25.8 mg/dl at 2100 h). The two lowest rumen $\text{NH}_3\text{-N}$ concentrations in this treatment were found after midnight (14.2 mg/dl at 0500 h; $P < 0.05$) and noon (17.3 mg/dl at 1700 h; $P < 0.05$). Rumen $\text{NH}_3\text{-N}$ concentration steadily increased in the pTMR treatment during the morning (6.2 mg/dl at 0500 h, 7.1 mg/dl at 0900 h, and 9.7 mg/dl at 1300 h). A very important increase in rumen $\text{NH}_3\text{-N}$ concentration was detected in the pTMR from 1700 h to 2100 h due to the ingestion of TMR when cows were kept indoors overnight (9.3 vs. 20.8 mg/dl; $P < 0.05$), with a latter reduction at 0100 h indicating that cows stopped eating TMR (20.8 vs. 11.3 mg/dl; $P < 0.05$). Although the mean rumen $\text{NH}_3\text{-N}$ concentration was similar between the pTMR and the TMR treatments, the pattern differed during the 24-h period. The TMR treatment had a peak of rumen $\text{NH}_3\text{-N}$ at 0900 h (13.2 mg/dl) and then remained relatively constant (10.8 mg/dl at 1300 h and 12.6 mg/dl at 1700 h; $P > 0.05$). After 1700 h, rumen $\text{NH}_3\text{-N}$ concentration decreased reaching the lowest values at 0100 h (6.1 mg/dl) and 0500 h (5.4 mg/dl). The cows fed pTMR exhibited the greatest variations in rumen $\text{NH}_3\text{-N}$ over the 24 h period (minimum 6.2 mg/dl of at 0500 h, maximum of 20.8 mg/dl at 2100 h), while the cows fed TMR treatment had the most constant pattern of rumen $\text{NH}_3\text{-N}$ concentration (minimum 5.4 mg/dl of at 0500 h, maximum of 12.6 mg/dl at 1700 h).

A significant period and treatment by period interaction ($P < 0.05$) was found for rumen $\text{NH}_3\text{-N}$ concentration, which indicates that this parameter changed differently between periods among treatments (*Table 45*). Comparing across periods and within treatments, cows on the PC treatment had lowest $\text{NH}_3\text{-N}$ during period 1, intermediate during periods 2 and 4, and a remarkably high peak in period 3 (*Figure 27*). The highest rumen $\text{NH}_3\text{-N}$ concentration in the PC treatment during period 3 corresponded to the highest CP content in the grazed pasture (29.5%; *Table 42*). In contrast, both the pTMR and the TMR treatments showed an increase in $\text{NH}_3\text{-N}$

concentration during periods 2, 3, and 4 compared to period 1, and then it remained constant (*Table 45; Figure 27*). Comparing within periods and across treatments, $\text{NH}_3\text{-N}$ concentration was always higher on the PC treatment compared to both the pTMR and the TMR treatments, as shown in *Figure 27*.

6.4.3 In Situ Rumen Digestion of Concentrate and TMR

In situ rumen digestion of the concentrate supplemented to cows on both the PC and the pTMR treatments, and of the TMR fed to cows on both the pTMR and the TMR treatments are shown in *Table 46*. This information is provided to characterize the feedstuffs.

In situ degradation data of the concentrate were obtained from two cows in the PC treatment and two cows on the pTMR treatment. None of the in situ DM, or CP kinetics parameters were affected by treatment ($P > 0.05$), thus the overall mean of the four cows are presented in *Table 46*. The concentrate averaged 27.8 %, 62.3 %, and 7.4 %/h for the soluble fraction, insoluble potentially degradable fraction, and degradation rate of DM. Similar values for those fractions (28.4 %, 70.2 %, and 6.4 %/h for OM digestion) were reported for a corn-based concentrate (Kolver et al., 1998). The soluble fraction, insoluble degradable fraction, and degradation rate of CP averaged 24.8 %, 57.7 %, and 5.2 %/h. Kolver et al. (1998) reported similar values for the soluble fraction and rate of degradation (28.2 % and 4 %/h) but higher values for the insoluble degradable fraction (73.2%) of CP for a corn-based concentrate with 14.4% CP.

In situ DM, CP, and NDF degradation of TMR was determined in two cows fed the TMR treatment. This resulted in substantial variation between cows (i.e. large SEM). The TMR

averaged 39.3%, 43% and 4.2 %/h for the soluble, insoluble potentially degradable, and degradation rate of DM, respectively; and 42.0%, 45.5%, and 3.0 %/h for the soluble, insoluble potentially degradable, and degradation rate of CP, respectively. A 5.6 h lag time was observed for the in situ NDF digestion of the TMR. The potentially digestion fraction of NDF averaged 70.5%, and the degradation rate of NDF averaged 3.3 %/h.

6.4.4 In Situ Rumen Digestion of Pasture

The in situ DM, CP, and NDF digestion of pasture grazed by cows on the PC and the pTMR treatments is presented in *Table 47*. The main goal of the in situ rumen digestion data of pasture was to determine the effects of feed interactions in the rumen of the cows on the pTMR treatment.

6.4.4.1 In situ DM digestion

The soluble fraction (A) of DM was not affected by treatment ($P > 0.05$) and averaged 7.75% (*Table 47*). The low soluble fraction value resulted because of a low DM disappearance in the 0-h bags, which were not incubated in the rumen and soaked for 15 min in 39°C water. For bromegrass and orchardgrass (incubated dried and ground), Hoffman et al. (1993) reported a soluble fraction of DM of 36.3 and 39.0%, respectively at second node maturity stage. The soluble fraction of DM decreased to 32.7 and 34.0% for bromegrass and orchardgrass when maturity stage advanced to boot stage (Hoffman et al., 1993). Differences between Hoffman et al. (1993) and this study are likely related to sample processing (ground vs. 1-cm cut, respectively) and soaking time (30 vs. 15 min, respectively). Soluble fraction of DM for fresh

bromegrass at tillering averaged 24.3%, and decreased to 18.6% at stem elongation (Elizalde et al., 1999); no information about sample processing was provided in that study. When a predominantly orchardgrass pasture was incubated in situ using the same procedure (fresh forage cut to 1-cm) as used in this study, a soluble fraction of OM of 13.0% was found (Kolver et al., 1998). However, when an orchardgrass-bromegrass pasture was freeze-dried and ground to 4-mm (Hongerholt and Muller, 1998), the soluble fraction of DM was higher and averaged 24.4%.

Neither the insoluble potentially degradable fraction (B) (76.2%), nor the degradation rate (kd) (5.9 %/h) of DM were affected by inclusion of TMR in a pasture-based diet. Kolver et al. (1998) reported a degradation rate of OM of orchardgrass pasture of 7.3 %/h, and Hongerholt and Muller (1998) reported a degradation rate of DM of orchardgrass-bromegrass pasture of 7.6 %/h. Elizalde et al. (1999) found a significant reduction in degradation rate of DM of bromegrass from 11.5 to 6.6 %/h as maturity stage advanced from tillering to stem elongation. Hoffman et al. (1993) reported degradation rate of DM of bromegrass between 6 to 5 %/h and of orchardgrass between 10 to 8 %/h for second node to boot stage of maturity.

The total potentially degradable fraction (TD) of DM of pasture was significantly reduced (85.5 vs. 82.3%; $P < 0.05$) with the inclusion of TMR in the diet, indicating a negative rumen associative effect for this treatment. The actual value of this fraction depends on the length of in situ rumen incubation. Studies where pasture was incubated in situ for 48 h (Hongerholt and Muller, 1998; Kolver et al., 1998) reported that this fraction ranged from 82.2 to 84.7%. Other studies using a final incubation time of 72 h (Elizalde et al., 1999; Hoffman et al., 1993) reported total potentially degradable fraction of DM of orchardgrass and bromegrass forage between 85.3 to 88.3%. Although it has been recently proposed that forages should be incubated in situ for 72

h (NRC, 2001), the difference in total potentially degradable fraction (TD) of DM for this type of pasture between 48 h or 72 h incubations was not large.

6.4.4.2 In situ CP digestion

Neither the soluble (A), nor the insoluble potentially degradable (B), nor the total potentially degradable (TD) fractions of pasture CP were affected by treatments ($P > 0.05$; *Table 47*). Similar to results found for pasture DM digestion, pasture grazed in this study had very low soluble fraction of CP (6.3 as % of DM), which was likely related to the physical form of the pasture samples. In situ data from Kolver et al. (1998), using the same procedures as used in this study, reported for an orchardgrass-based pasture a soluble fraction of CP of 11.4%. Higher values were reported by Elizalde et al. (1999) for bromegrass (27.1 to 32.3%), by Hoffman et al. (1993) for orchardgrass (46.1 to 51.5%) and bromegrass (45.1 to 46.1%), and by Honerholt and Muller (1998) for orchardgrass-bromegrass pastures (19.0%). All these studies, however, used different sample processing (e.g. grinding as in Honerholt and Muller, 1998) and procedures (e.g. soaking the 0-h bags for a longer period of time as in Hoffman et al., 1993) in comparison to this study. The total potentially degradable fraction (TD) of pasture CP averaged 88.2%, which agrees with previously reported for orchardgrass (90.1%, Kolver et al., 1998) and orchardgrass-bromegrass (94.3%, Honerholt and Muller, 1998) pastures. Others studies (Elizalde et al., 1999; Hoffman et al., 1993) reported greater values for this fraction (92.2 to 96.1%), which could be attributed to longer incubation time (72-h). Degradation rate of CP tended to be slightly greater in the pTMR treatment (8.2 to 7.8%/h; $P < 0.06$). Honerholt and Muller (1998) reported a degradation rate of 7.6%/h, while Kolver et al. (1998) found a

degradation rate of 10.9%. Others also reported greater degradation rate of CP of bromegrass (11.8 %/h; Elizalde et al., 1999) and orchardgrass (15.0 %/h; Hoffman et al., 1993) than in this study.

6.4.4.3 In situ NDF digestion

In situ pasture NDF digestion was lower on the TMR treatment than on the PC treatment (*Table 47*). The inclusion of TMR in the pTMR treatment tended to increase the lag time (5.4 vs. 6.5 h; $P < 0.08$) and significantly reduced the potentially digested fraction (82.1 vs. 74.9%) of pasture NDF ($P < 0.05$). The reduction in NDF digestion of pasture is in agreement with the lower total potentially degradable fraction of DM discussed above. This also indicates the presence of negative associative effects in the rumen of cows on the pTMR treatment, which may be related with the higher rumen pH daily variations (*Figure 24*) and lower $\text{NH}_3\text{-N}$ concentrations at 0500, 0900, 1300 and 1700 h (*Figure 26*) compared to the PC treatment. Degradation rate of NDF did not differ between treatments and averaged 7.2 %/h.

None of the previous in situ studies that used similar procedures (Kolver et al., 1998) or similar forages (Elizalde et al., 1993; Hongerholt and Muller, 1998) to the present study reported data on NDF digestion. Hoffman et al. (1993) studied in situ NDF degradation of orchardgrass and bromegrass forages at different stages of maturity. Orchardgrass averaged 3.2, 73.1, and 23.7%, and bromegrass averaged 1.9, 77.4, and 20.7% for the soluble fraction, the potentially digested fraction, and the undigested fraction at 72-h, respectively (Hoffman et al., 1993). These values are similar to those found in our study. Also in agreement with our study, Hoffman et al. (1993) reported a degradation rate of NDF for orchardgrass from 7 to 8 %/h and for bromegrass

from 5 to 6 %/h. Although in that study data were fitted to a model including lag time (Mertens and Lofton, 1980), values of lag time were not reported (Hoffman et al., 1993).

6.5 CONCLUSIONS

The three different feeding systems combining pasture and TMR had similar rumen pH (5.87). Neither total VFA concentration (137.5 mmol/L), nor the molar proportion of the most important individual VFA were affected by feeding system. Acetate, propionate and butyrate molar proportions averaged 64.4, 20.6, and 12.0 mol/100 mol. Rumen $\text{NH}_3\text{-N}$ concentration was significantly higher for the PC treatment (19.9 mg/dl) than for both the pTMR and the TMR treatments (10.2 mg/dl). The inclusion of TMR in the pTMR treatment reduced the total potentially degradable fraction of DM (85.5 vs. 82.3 %) and the potentially digested fraction (82.1 vs. 74.9%) of NDF of pasture.

Dietary nitrogen utilization was improved when combining pasture and TMR compared to pasture plus concentrate. Both feeding systems including TMR resulted in lower rumen $\text{NH}_3\text{-N}$ concentrations than with only pasture plus concentrate. Rumen pH was not affected with the inclusion of TMR for grazing cows. Combining pasture with TMR resulted in negative associative rumen effects resulting in reduced potential digestion of pasture DM and NDF, and increased lag time of NDF pasture digestion.

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Table 42: Chemical composition of hand-plucked pasture samples during the four intake measurement periods.

	May	June	August	September	X	SEM
DM, %	19.9 ^a	17.0 ^{ab}	15.7 ^b	17.1 ^{ab}	17.4	1.0
% DM						
OM, %	93.2 ^a	92.9 ^a	90.9 ^b	91.1 ^b	92.1	0.2
CP, %	25.5 ^a	26.2 ^a	29.5 ^b	23.9 ^a	26.3	0.9
Soluble CP, %	6.4	6.3	7.2	6.2	6.5	0.5
TNC, %	13.0 ^a	14.1 ^{ab}	13.5 ^{ab}	14.9 ^b	13.9	0.6
NDF, %	53.5 ^a	52.1 ^a	46.5 ^b	47.5 ^b	49.9	1.0
ADF, %	25.9 ^{ab}	27.4 ^a	25.0 ^b	26.1 ^{ab}	26.1	0.6
IVDMD, %	57.6	57.5	58.3	58.5	58.0	1.2

^{a, b, c}Least square means in the same row with different superscripts differ ($P < 0.05$).

Table 43: Ingredient and chemical composition (mean $\pm$ SD) of the concentrate and the TMR.

	Concentrate	TMR
Ingredient composition		
Dry corn	59.6	14.9
Wheat midds	20.7	...
Molasses	4.9	...
Animal protein blend ¹	4.6	...
Animal fat	2.3	...
Canola meal	1.5	3.4
Limestone	1.4	...
Dicalcium phosphate	1.3	...
Salt	1.3	...
Corn gluten meal	1.0	...
Dynamate 22S	0.57	...
Magnesium oxide	0.52	...
PS TM premix	0.16	...
Selenium premix	0.09	...
Vitamins A, D, E	0.06	...
Sodium bicarbonate	...	0.7
Bentonite	...	0.4
Commercial concentrate ²	...	18.8
Cookie by-products	...	8.8
Roasted soybean	...	2.6
Corn silage	...	27.1
Alfalfa haylage	...	13.4
Alfalfa hay	...	9.9
Chemical composition		
DM, %	89.4 $\pm$ 4.4	54.5 $\pm$ 1.9
% DM		
OM, %	91.4 $\pm$ 0.7	92.8 $\pm$ 0.2
CP, %	15.2 $\pm$ 0.4	16.9 $\pm$ 0.3
Soluble CP, %	2.8 $\pm$ 0.5	5.1 $\pm$ 0.4
TNC, %	53.7 $\pm$ 1.4	35.1 $\pm$ 1.1
NDF, %	15.7 $\pm$ 0.4	30.1 $\pm$ 1.5
ADF, %	6.5 $\pm$ 0.2	19.4 $\pm$ 1.2
IVDMD, %	72.2 $\pm$ 3.2	64.3 $\pm$ 1.4
NE _L , Mcal/kg ³	1.89	1.64

¹One-third of each: fish meal, feather meal, and blood meal.

²Pellet composed by 44.2% distillers grain, 17.8% wheat midds, 17.8% soyhulls, 12.3% animal protein blend, 1.5% salt, 1.1% tallow, and 5.3% minerals and vitamins. The pellet had 27.7% CP, 1.85 Mcal of NE_L/kg, 1.92% Ca, 0.74% P, 1.16% Mg, 1.10% K, 0.41% S, 166.6 ppm Mn, 58.2 ppm Cu, 215.1 ppm Zn, 427.8 ppm Fe, 2.01 ppm Se, 2.57 ppm I, 1.03 ppm Co, 26795 UI/kg vitamin A, 6589 UI/kg vitamin D, and 176 UI/kg vitamin E.

³Estimated using values of NRC (2001).

Table 44: Ruminal fermentation parameters of dairy cows fed three different feeding systems.

	Treatments ¹			SEM	Effects, ² <i>P</i> <			
	PC	pTMR	TMR		T	P	T x P	T x H
pH	5.89	5.88	5.83	0.04	0.55	0.05	0.17	<0.01
NH ₃ -N, mg/dl	19.96 ^a	10.75 ^b	9.74 ^b	0.48	<0.01	<0.01	<0.01	<0.01
Total VFA, mmol/L	140.92	130.05	141.56	3.96	0.22	0.01	0.55	0.03
VFA, mol/100 mol								
Acetate (A)	64.88	63.59	60.71	1.42	0.25	0.06	<0.01	<0.01
Propionate (P)	19.42	19.74	22.49	1.23	0.29	0.13	0.04	0.42
Butyrate (B)	11.56	12.19	12.37	0.92	0.82	0.01	0.01	0.04
Iso-Butyrate	0.97 ^a	0.87 ^{ab}	0.78 ^b	0.04	0.09	<0.01	<0.01	<0.01
Valerate	1.68	2.22	2.19	0.23	0.31	0.02	<0.01	0.02
Iso-Valerate	1.49	1.38	1.44	0.10	0.71	<0.01	0.01	0.01
A/P	3.35	3.25	2.74	0.22	0.25	0.48	0.01	0.06
(A+B)/P	3.95	3.87	3.30	0.25	0.28	0.13	0.04	0.31
Rumen content								
Wet fill, kg	78.3	76.1	89.2	7.9	0.53	0.59	0.82	...
Dry fill, kg DM	13.6	12.6	13.7	0.3	0.12	0.46	0.53	...
DM content, %	17.4	17.0	15.3	1.5	0.60	0.90	0.91	...

¹PC = pasture plus concentrate (PC), pTMR = partial total mixed ration, TMR = total mixed ration.

²T = treatment, P = period, T x P = treatment x period interaction, T x H = treatment x hour.

^{a, b, c}Least square means in the same row with different superscripts differ (*P* < 0.05).

Table 45: Period effects on ruminal fermentation parameters of dairy cows fed three different feeding systems.

	Treatments ¹			SEM ³	Effects, ² $P <$	
	PC	pTMR	TMR		P	T x P
pH						
Period 1 (May)	6.07 ^{A, a}	5.88 ^{ab}	5.81 ^b	0.10	0.05	0.17
Period 2 (June)	5.70 ^{B, a}	5.87 ^{ab}	5.69 ^a	0.10		
Period 3 (August)	5.88 ^{AB}	5.85	5.91	0.10		
Period 4 (September)	5.91 ^A	5.90	5.91	0.10		
NH ₃ -N, mg/dl						
Period 1 (May)	10.34 ^{A, a}	6.34 ^{A, b}	6.94 ^{A, b}	1.52	<0.01	<0.01
Period 2 (June)	19.19 ^{B, a}	12.74 ^{B, b}	8.99 ^{AB, c}	1.52		
Period 3 (August)	28.75 ^{C, a}	11.98 ^{B, b}	10.52 ^{B, b}	1.52		
Period 4 (September)	21.56 ^{B, a}	11.94 ^{B, b}	12.53 ^{B, b}	1.52		

¹PC = pasture plus concentrate (PC), pTMR = partial total mixed ration, TMR = total mixed ration.

²P = period, T x P = treatment x period interaction.

³Standard error of the differences of Least Squares Means.

^{A, B, C}Least square means in the same column with different superscripts differ ($P < 0.05$).

^{a, b, c}Least square means in the same row with different superscripts differ ($P < 0.05$).

Table 46: In situ DM, CP, and NDF degradation kinetics (mean $\pm$ SEM) of the concentrate and the TMR.

	Concentrate	TMR
DM degradation ¹		
A, %	27.83 $\pm$ 1.23	39.27 $\pm$ 0.76
B, %	62.31 $\pm$ 0.84	43.00 $\pm$ 2.05
TD, %	90.13 $\pm$ 0.46	82.27 $\pm$ 1.96
kd, %/h	7.41 $\pm$ 0.31	4.15 $\pm$ 0.42
CP degradation ¹		
A, %	24.78 $\pm$ 0.96	41.99 $\pm$ 4.49
B, %	57.69 $\pm$ 3.01	45.52 $\pm$ 2.58
TD, %	82.47 $\pm$ 2.98	87.51 $\pm$ 6.21
kd, %/h	5.16 $\pm$ 0.95	3.03 $\pm$ 1.01
NDF degradation ²		
L, h	...	5.58 $\pm$ 1.23
S, %	...	2.22 $\pm$ 0.75
P, %	...	70.54 $\pm$ 14.18
U, %	...	27.24 $\pm$ 14.21
kd, %/h	...	3.32 $\pm$ 1.83

¹A = soluble fraction, B = insoluble potentially degradable fraction, TD = total potentially degradable fraction (A + B), kd = fractional degradation rate (Ørskov and MacDonald, 1979).

²L = lag time, S = soluble fraction (100 – P – U), P = potentially digested fraction, U = undigested fraction at 48 h, kd = fractional degradation rate (Mertens and Loftén, 1980).

Table 47: In situ DM, CP, and NDF degradation kinetics of pasture grazed by dairy cows fed two different feeding systems.

	Treatments ¹		SEM	Effects, ² <i>P</i> <		
	PC	pTMR		T	P	T x P
DM degradation ³						
A, %	8.71	6.79	1.57	0.48	<0.01	0.57
B, %	76.81	75.54	1.58	0.63	0.01	0.53
TD, %	85.53	82.33	0.38	0.03	0.07	0.12
kd, %/h	5.68	6.03	0.44	0.63	<0.01	0.14
CP degradation ³						
A, %	6.83	5.81	1.05	0.55	<0.01	0.15
B, %	82.99	81.14	2.38	0.64	<0.01	0.16
TD, %	89.82	86.48	1.82	0.31	0.16	0.52
kd, %/h	7.77	8.23	0.08	0.06	0.08	0.60
NDF degradation ⁴						
L, h	5.41	6.48	0.23	0.08	0.52	0.81
S, %	1.67	0.89	0.49	0.37	0.05	0.63
P, %	82.05	74.95	0.66	0.02	0.02	0.33
U, %	16.28	24.16	0.66	0.01	0.02	0.33
kd, %/h	6.51	7.86	0.68	0.29	0.08	0.68

¹PC = pasture plus concentrate (PC), pTMR = partial total mixed ration, TMR = total mixed ration.

²T = treatment, P = period, T x P = treatment x period interaction.

³A = soluble fraction, B = insoluble potentially degradable fraction, TD = total potentially degradable fraction (A + B), kd = fractional degradation rate (Ørskov and MacDonald, 1979).

⁴L = lag time, S = soluble fraction (100 – P – U), P = potentially digested fraction, U = undigested fraction at 48 h, kd = fractional degradation rate (Mertens and Loftén (1980).

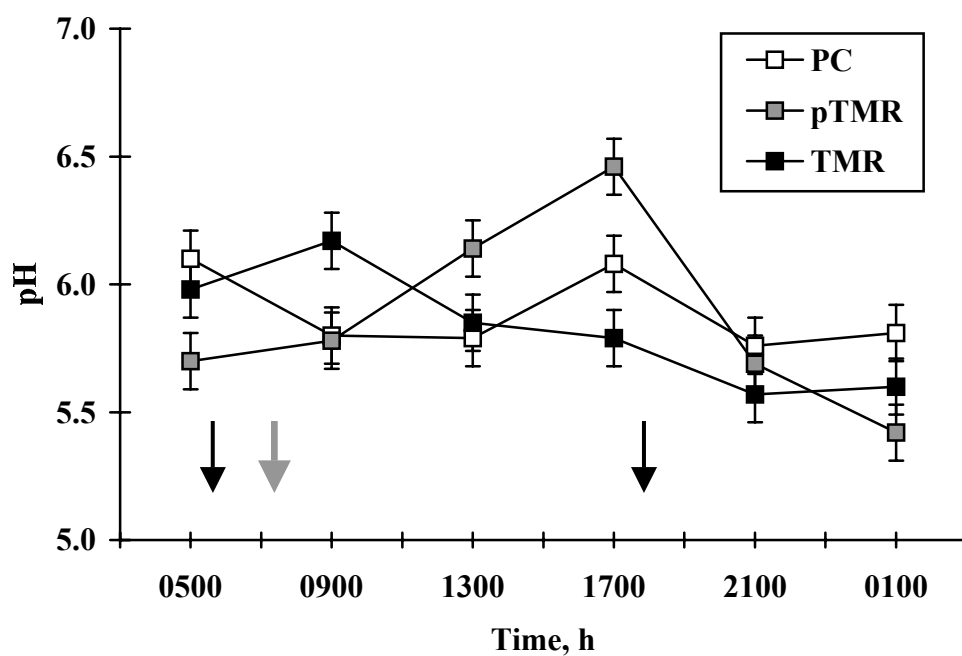


Figure 24: Daily rumen pH variations of dairy cows with three different feeding systems: pasture plus concentrate (PC), partial total mixed ration (pTMR) or total mixed ration (TMR). The two black arrows indicate the time concentrate was supplemented to the cows of the PC treatment (0600 and 1800 h), and the gray arrow the time TMR was fed (0730 h). Overall SEM = 0.11.

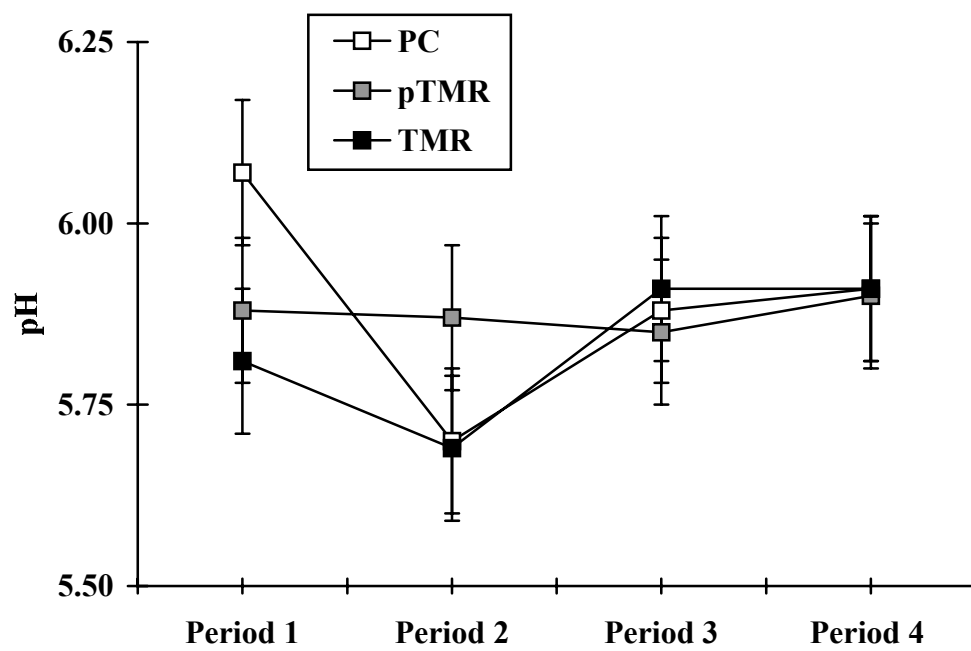


Figure 25: Rumen pH variations across periods of dairy cows with three different feeding systems: pasture plus concentrate (PC), partial total mixed ration (pTMR) or total mixed ration (TMR). Overall SEM = 0.10.

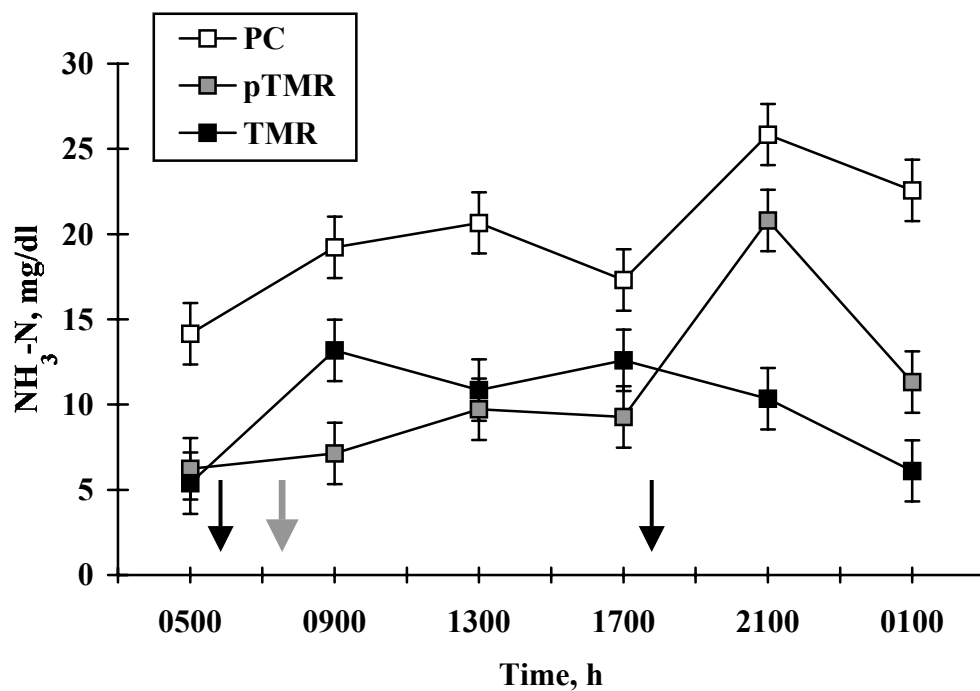


Figure 26: Daily rumen $\text{NH}_3\text{-N}$ variations of dairy cows with three different feeding systems: pasture plus concentrate (PC), partial total mixed ration (pTMR) or total mixed ration (TMR). The two black arrows indicate the time concentrate was supplemented to the cows of the PC treatment (0600 and 1800 h), and the gray arrow the time TMR was fed (0730 h). Overall SEM = 1.8.

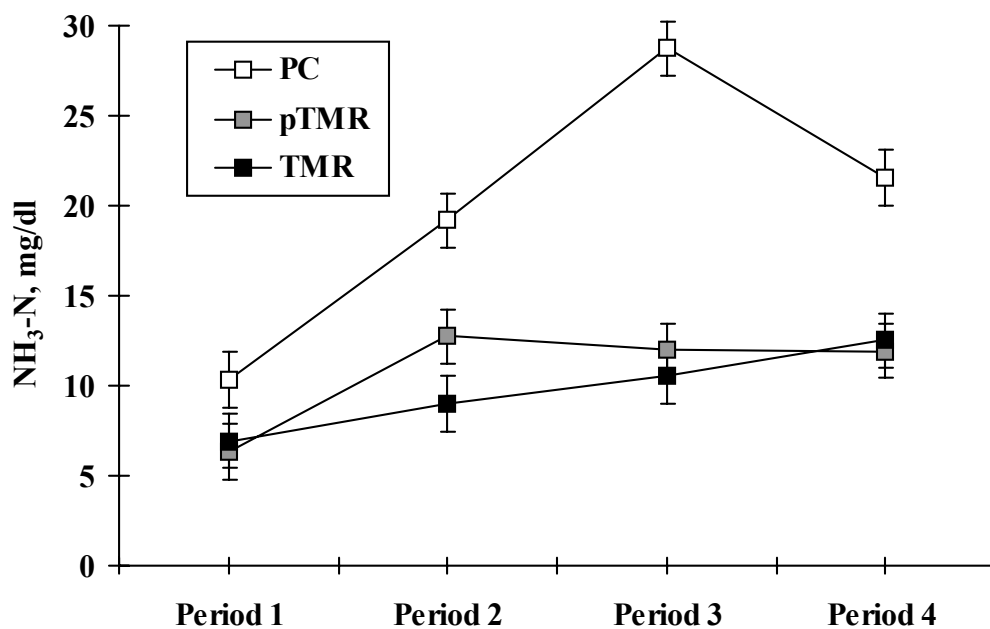


Figure 27: Rumen $\text{NH}_3\text{-N}$ variations across periods of dairy cows with three different feeding systems: pasture plus concentrate (PC), partial total mixed ration (pTMR) or total mixed ration (TMR). Overall SEM = 1.52.

Chapter 7

Conclusions

This thesis investigated two feeding strategies for high producing Holstein cows on pasture: 1) management of the amount of pasture offered on a daily basis, known as pasture allowance, and its interaction with concentrate supplementation, and 2) the use of feeding systems combining pasture and total mixed rations (TMR).

In the first study, the effect of pasture allowance (low: 25 vs. high: 40 kg DM/cow/d) and concentrate supplementation (zero vs. 1 kg concentrate/4 kg milk) on substitution rate and milk response was evaluated. Concentrate supplementation reduced pasture DMI, but the reduction in pasture DMI was greater when cows grazed at the high pasture allowance; i.e. substitution rate was greater at the high pasture allowance (0.55 vs. 0.26 kg pasture/kg concentrate). Milk production was increased by concentrate supplementation, but the milk response was greater at low pasture allowance (1.36 vs. 0.96 kg milk/kg concentrate). Regardless of pasture allowance, concentrate supplementation decreased milk fat percentage but increased total protein percentage. At both pasture allowances, concentrate supplementation reduced concentrations of ammonia N in the rumen and urea N in blood and milk, and increased the allantoin/creatinine ratio, indicating a higher efficiency of dietary nitrogen utilization and microbial protein synthesis. Concentrate supplementation reduced rumen pH, in situ rate of degradation of pasture, and NDF apparent digestibility at both pasture allowances. Concentrate supplementation also reduced grazing time by 75 min/d at the low pasture allowance and by 104 min/d at the high pasture allowance with concentrate supplementation. These results show that the reduction in

pasture DMI by concentrate supplementation or substitution rate is related to both negative associative effects in the rumen and reduction in grazing time.

In the second study, grazing behavior and rumen digestion of unsupplemented or supplemented dairy cows grazing at low (25 kg DM/cow/d) or high (40 kg DM/cow/d) pasture allowance was investigated. Pasture allowance affected performance, grazing behavior and rumen digestion of unsupplemented cows. Pasture DMI and milk production of unsupplemented cows was higher at high pasture allowance, which was related to a higher daily grazing time (638 vs. 461 min/d) and number of bites (36663 vs. 24353 bites/d) than at low pasture allowance. Rumen pH was lower and total VFA concentration was higher at high pasture allowance, while $\text{NH}_3\text{-N}$ was not affected. In contrast, pasture allowance had no major effects on performance, rumen digestion and grazing behavior of supplemented dairy cows. Total DMI, milk production, milk composition and rumen fermentation parameters were similar in supplemented cows regardless of pasture allowance. None of the grazing behavior variables (grazing time, biting rate, and bite mass) of supplemented cows were affected by pasture allowance. The pattern of grazing time of both unsupplemented and supplemented cows explained the daily variations in rumen pH and $\text{NH}_3\text{-N}$ concentration. For both the unsupplemented and supplemented cows, lower rumen pH were associated with higher grazing time per hour, while similar rumen $\text{NH}_3\text{-N}$ concentrations were associated with similar grazing time per hour.

The third study compared animal performance during the entire grazing season of high producing dairy cows on three different feeding systems combining pasture and TMR: 1) pasture plus concentrate (PC), 2) pasture plus partial TMR (pTMR), and 3) TMR (non pasture). The TMR feeding system maximized total DMI and milk production. The TMR treatment had the highest total DMI, the PC treatment the lowest, and the pTMR treatment intermediate. The TMR

treatment produced 19% more milk than the pTMR treatment, and 33% more than the PC treatment. Both the pTMR and the TMR treatments had higher milk fat percentage. True protein percentage in milk was higher in the TMR treatment than in the PC treatment. The cows on the three treatments gained BW after 21 wk of study, however, the cows on the TMR treatment had a higher increase in BW in comparison with both the PC and the pTMR treatments. The cows on the PC treatment lost, the pTMR treatment maintained, and the TMR treatment gained body condition. Compared to pasture as the only forage plus concentrate, the combination of pasture and TMR resulted in higher milk production, milk fat and protein percentage, maintenance in body condition score, and lower NEFA concentrations.

The fourth study investigated rumen digestion and associative effects in the rumen of high producing dairy cows on pasture plus concentrate (PC), pasture plus partial TMR (pTMR), and 3) TMR (non pasture) feeding systems. The three treatments had a similar rumen pH (5.87). Neither total VFA concentration (137.5 mmol/L) nor the molar proportion of the most important individual VFA were affected by feeding system. Rumen ammonia N concentration was significantly higher for the PC treatment (19.9 mg/dl) than for both the pTMR and the TMR treatments (10.2 mg/dl). In situ pasture digestion data showed that the inclusion of TMR in the pTMR treatment reduced the total potentially degradable fraction of DM (85.5 vs. 82.3 %), and increased lag time (5.4 vs. 6.5 h) and reduced the potentially digested fraction (82.1 vs. 74.9%) of NDF. Compared to the PC treatment, including TMR in the diet reduced rumen ammonia N concentration, while ruminal pH was not affected. Combining pasture with TMR resulted in associative rumen effects reducing the potential digestion of pasture DM and NDF.

VITA

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