University of Alberta

# Evaluation of planting date on fibre digestibility of barley silage and its effects on performance of lactating dairy cows

by

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## ABSTRACT

The effect of barley differing in planting date on in vitro fibre digestibility (IVFD), and its effects on the productivity of lactating dairy cows were evaluated. Whole crop barley planted in June (BJ) had greater IVFD compared to that planted in May (BM). However, there was no effect of treatment on dry matter intake and milk yield. Total tract nutrient digestibility was greater and body weight gain tended to be greater for cows fed BJ compared to those fed BM. Multiparous cows fed BJ increased body condition score gain. Forages can improve the energy balance of animals by increasing the digestibility, but may not improve milk production unless physical fill limits their feed intake. In another study, the efficacy of two digestion markers was compared, and it was found that the method using in situ indigestible neutral detergent fibre as an internal maker provided repeatable measurements.

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| | |
|---|---|
| ADF | Acid detergent fibre |
| BCS | Body condition score |
| BJ | Barley silage planted in June |
| BM | Barley silage planted in May |
| BMR | Brown midrib |
| BMRR | Brown midrib corn silage fed to approximate ad libitum DMI of isogenic ration |
| BW | Body weight |
| CCK | Cholecystokinin |
| CAD | Cinnamyl alcohol dehydrogenase |
| COMT | Caffeic acid O-methyl transferase |
| CP | Crude protein |
| DIM | Days in milk |
| DM | Dry matter |
| DMI | Dry matter intake |
| FCM | Fat corrected milk |
| HCW | High cell wall content and high fibre digestibility |
| HCWN | High cell wall content and digestibility diet substituted on a NDF basis |
| ISIDF | In situ indigestible NDF |
| ISO | Isogenic |
| IVFD | In vitro fibre digestibility |
| LCW | Low cell wall content and low fibre digestibility |
| MUN | Milk urea nitrogen |
| NDF | Neutral detergent fibre |
| NEL | Net energy of lactation |

| | |
|---|---|
| OM | Organic matter |
| peNDF | Physically effective fibre |
| PSPS | Penn State Particle Separator |
| RIB | Rumen inert bulk |
| SD | Standard deviation |
| TMR | Total mixed ration |
| VDMI | Voluntary dry matter intake |
| VFA | Volatile fatty acid |
| Yb | Ytterbium |

## 1.0. LITERATURE REVIEW

### 1.1. Introduction

The study of factors affecting voluntary dry matter intake **(VDMI)** has been of significant importance to the dairy cattle industry. Increasing the VDMI of high-producing dairy cows increases milk production and profitability of dairy operations (Holcomb et al., 2001). Greater VDMI can also improve animal health as dairy cattle are at an increased risk for metabolic diseases that are associated with high energy demands and a reduction of VDMI (Grummer et al., 1995). Sovani et al. (2000) reported that nearly 80% of dairy cows experience negative energy balance during the peripartum period. This negative energy balance results in the animal mobilizing body fat for energy, which increases the concentrations of serum non-esterified fatty acids, ß-hydroxybutyrate, glucagon, growth hormone, and liver triglycerides. Concurrently, levels of serum glucose and insulin are decreased (Petersson-Wolfe et al., 2007). This imbalance of metabolites and hormones increases the risk of metabolic and health problems including ketosis, milk fever, retained placenta, displaced abomasum, and reduced reproductive performance. Maximizing VDMI is also important during the peak lactation; insufficient VDMI can decrease milk yield by 5 to 10 kg/d, totalling 1000 to 2000 kg of lost milk for the total lactation cycle (Holcomb et al., 2001, Petersson-Wolfe et al., 2007).

The regulation of VDMI is complex and influenced by numerous factors. However, these factors can be divided into two categories including short-term and long-term control. Long-term control includes metabolic signals that affect hunger and satiety thresholds over a lactation cycle, including leptin, gut peptides, insulin, cytokines, neuropeptides (i.e. neuropeptide Y, corticotrophin-releasing factor, and

galanin), and stress and reproductive hormones (Ingvartsen and Andersen, 2000). The VDMI of cows in the final 3 wk of gestation has been found to be mainly affected by parity, body condition score **(BCS)**, gestation day, and dietary concentrations of ether extract and neutral detergent fibre **(NDF)** (Hayirli et al. 2002, 2003). This review will focus on dietary factors that regulate the short-term control of VDMI of lactating dairy cows, including physical limitations via reticulo-rumen distension, forage length and quality, and effects of starch, fat, and protein digestion. Forages with enhanced in vitro fibre digestibility **(IVFD)** have been found to increase VDMI and milk yield. The effects of enhanced IVFD on animal performance and agronomic factors affecting IVFD of forages will also be discussed in this review.

## 1.2. Factors Affecting Voluntary Dry Matter Intake

### 1.2.1. Physical limitations of the rumen

Distension of the reticulo-rumen can limit VDMI as shown via the addition of rumen inert bulk **(RIB)** into cows (Dado and Allen, 1995, Johnson and Combs, 1991, Tjardes et al., 2002). Johnson and Combs (1991) found that the addition of water-filled bladders depressed VDMI in prepartum (70 d) and postpartum (28 d) cows by 99 and 130 g/L of RIB, respectively. However, no decrease in VDMI was observed when RIB was added to cows in later lactation (229 d) (Johnson and Combs, 1992). The lack of RIB effects on VDMI may be due to lower digesta volume in the rumen before the addition of RIB, or increased digesta disappearance from the rumen. Similarly, Dado and Allen (1995) found that the addition of water-filled plastic containers into the rumen of early-lactation cows (17 d postpartum) decreased VDMI by 95 g/L RIB if fed a high NDF diet (35% dietary NDF); however, no change in VDMI

was observed for cows fed a low NDF diet (25% dietary NDF). They concluded that VDMI of cows fed a low NDF diet was less likely to be limited by physical fill or ruminal distension as compared to cows fed a high NDF diet. Tjardes et al. (2002) also found that the addition of plastic-coated tennis balls filled with sand decreased VDMI by an average of 65.5 g/L of RIB when Holstein steers were fed either a 33.8 or 50.8% NDF diet. No interaction was found between dietary fibre concentration and RIB effects, suggesting that VDMI was limited by physical fill at both levels of dietary fibre. The reticulo-rumen is the primary site of control of VDMI by physical fill. Tension receptors are located primarily in the reticulum and cranial rumen sac (Allen, 1996). Distension of the reticulum and rumen, as caused by volume and weight of digesta, activates these receptors. The frequency of neuron discharge in the ventromedial hypothalamus is increased while those in the lateral hypothalamus are inhibited, signalling satiety and for the animal to stop eating (Allen, 1996, Forbes, 1996). Thus, the effect of RIB on VDMI seems to be dependent primarily on the filling effect of the diet and the energy requirements of the animal as indicated by the stage of lactation or physiological stage.

### 1.2.2. Neutral detergent fibre characteristics

The amount of NDF in a diet plays an important role in filling the rumen since fibre ferments slower than non-forage components and occupies space within the rumen for a longer time (Oba and Allen, 1999b). Previous studies have shown that increasing the dietary NDF content reduces VDMI (Allen, 2000, Beauchemin et al., 1994b, Beauchemin and Rode, 1997, Dado and Allen, 1995, Llamas-Lamas and Combs, 1991, Ruiz et al., 1995, Tomlinson et al., 1991, Wang et al., 2001, West et

al., 1997, West et al., 1998). As discussed earlier, the extent to which VDMI is regulated by distension from dietary fibre depends on the animal's energy requirement and the filling effect of the diet (Allen, 2000). This distension is caused by the slower rate of digesta removal from the reticulo-rumen via digestion and passage (Allen, 2000). Additional factors affecting the rate of passage include size and density of digesta particles and reticulo-rumen motility. Density of digesta particles in the reticulo-rumen is determined partly by the amount of fermentable fibre and rate of fibre fermentation. An enhanced fermentation increases production of carbon dioxide and methane by microbes, making the fibre particles more buoyant (Jung and Allen, 1995 Jung, 1997). Lechner-Doll et al. (1991) reported that the retention time of digesta in the reticulorumen decreased from 91 to 19 h as inert particle density increased from 0.9 to 1.5 g/mL. Particle density and retention time are negatively related, because small and dense particles descend to the ventral rumen where they move cranially to the reticulum, and are easily expelled at the second reticular contraction through the reticulo-omasal orifice. In contrast, less dense particles become entrapped in the raft or fibre mat, being pushed further away from the reticulo-omasal orifice when the reticulum contracts.

The size of digesta particles is determined by forage chop length and rate and duration of chewing during eating and rumination. When VDMI is limited by physical fill, a reduction in forage particle size can increase VDMI if density of particles ingested increases. Chewing both decreases particle size and increases surface area, increasing microbial fermentation rate. The higher particle density arises from less gas production due to the reduction of fermentable substrate and gas retention due to the breakdown of cell walls (Allen, 1996). Beauchemin et al. (1994a) found that VDMI was decreased by approximately 3 kg/d when dietary allocation of long

alfalfa silage (10 mm) was increased from 35 to 65% dietary dry matter **(DM)**. However, VDMI was decreased by less than 0.5 kg/d when diets contained shorter alfalfa silage (5 mm). Similarly, Tafaj et al. (2001) found that VDMI of sheep was increased by 13% when hay particle size was decreased from 28.7 to 9.2 mm when sheep were fed a low concentrate diet (13% DM). However, there was no effect on VDMI when sheep were fed a high concentrate diet (40% DM).

Many studies have found no significant effect of forage particle length on VDMI. Bal et al. (2000) found that varying the length of corn silage (0.95, 1.45 or 1.90 cm) had no effect on VDMI. Similarly, Clark and Armentano (1999) evaluated the effect of chop length for corn silage (7.6 vs. 3.4 mm), and Yang et al. (2001) evaluated the effect of the chop length (6.08 vs. 7.59 mm) for alfalfa silage, barley silage, and alfalfa hay, and observed no treatment effects on VDMI. It is difficult to compare these various experiments as they use different chop lengths, forages, and cows at different stages of lactation. Distension in the reticulo-rumen in these studies may not have been limiting VDMI, and thus decreasing forage particle size had no effect on VDMI.

### 1.2.3. Effects of starch, fat, and protein

Additional short-term factors regulating VDMI include site of starch digestion, concentration of dietary fat, and the amount of protein degradation in the rumen (Allen, 2000). The site of starch digestion depends on grain type and processing method (i.e. rolling, steam flaking or grinding) (Knowlton et al., 1998). Starch in wheat, barley, and oats is more degradable than starch in corn or sorghum. Processing breaks the kernel outer coat which increases the surface area for micro-

organism and enzyme digestion (Knowlton et al., 1998). Increasing ruminal starch degradation can decrease VDMI due to the greater osmotic pressure in the rumen or absorbed propionate in the liver (Allen, 2000). Oba and Allen (2003) showed that increasing intraruminal infusion of propionate into lactating dairy cows decreased VDMI via tendencies to decrease meal size ($P < 0.09$) and increase intermeal interval ($P < 0.07$).

Fat supplementation is used to increase energy intake to sustain a high milk production. However, high levels of fat interfere with fibre digestion in the rumen (Eastridge, 2006). Choi and Palmquist (1996) found that the inclusion of greater than 30 g/kg calcium salts of long-chain fatty acids of total diet DM depressed VDMI and increased cholecystokinin **(CCK)** concentration in the plasma. The CCK stimulates satiety by acting on CCK receptors in the brain. In addition, activation of gut CCK receptors results in the inhibition of gastric emptying, increased distension of the reticulo-rumen, and stimulation of hepatic satiety mechanisms via increased pancreatic enzyme secretion and gallbladder contraction (Reidelberger, 1994). Thus, total dietary fatty acids should be below 6%, and dietary unsaturated fatty acids should be below 4% (Eastridge, 2006).

Increasing dietary crude protein **(CP)** content has been found to increase VDMI mainly due to the increased digestibility of the ration due to an increase in ruminally degradable protein concentration (Oldham, 1984). Also, Allen (2000) noted that experiments substituting protein for starch could decrease ruminal propionate production, reducing the hypophagic effects of propionate on VDMI. However, when the diet consists of >15% CP, the % unit increase in dietary CP has less incremental benefits in increasing DM digestibility (Oldham, 1984). Roffler et al. (1986) found that a 1-unit increase in dietary CP resulted in approximately 0.9 kg/d increase in VDMI at

12% dietary CP, but only a 0.04 kg/d increase in VDMI at 18% dietary CP. The increase of dietary amino acid content or supplementation with ruminally protected methionine or lysine, which may be limiting for milk production, have minimal effects on VDMI, because the majority of amino acids absorbed are derived from microbial protein (Robinson et al., 1995). Thus, increasing the CP content of diets can increase VDMI if the dietary CP is low, but typically this would not be a significant factor with the high level of CP commonly fed to high producing cows (Allen, 2000).

## 1.3. Significance of Dietary Forage

Dairy cows require adequate fibre in their diets to maintain healthy rumen function. Feeding insufficient fibre in dairy diets results in an increased risk of ruminal acidosis, lower acetate-to-propionate ratio, milk fat depression, and reductions in chewing, rumination, saliva secretion, and fibre digestion (Beauchemin and Rode, 1997). Mertens (1987) created a model to predict VDMI using the amount of dietary NDF since there is a positive relationship between NDF and the bulk density of the ration. However, dietary NDF content is insufficient as the sole predictor of VDMI because its filling effects also depend on particle fragility and NDF digestibility. Whereas NDF is a measurement of the forage chemical characteristics, physically effective fibre **(peNDF)** also measures forage particle size and quantifies the value of the fibre to rumen function (Zebeli et al., 2006). The requirement of peNDF may be greater if the diet contains rapidly digested starch sources such as barley grain (Beauchemin and Rode, 1997). Decreasing the dietary forage-to-concentrate ratio decreases rumen pH and changes the ruminal microbial profile; the number of fibre digesting bacteria decrease as pH decreases (pH <6.0), whereas starch digesting

bacteria increase as they can tolerate a more acidic environment (pH <5.0) (Nocek, 1997).

Lammers et al. (1996) proposed that peNDF be determined by measuring the proportion of DM retained by the 19 and 8 mm Penn State Particle Separator **(PSPS)** screens, multiplied by dietary NDF content (peNDF>8). However, Mertens (1997) proposed that peNDF should be measured as the proportion of DM retained by a 1.18 mm screen multiplied by dietary NDF (peNDF>1.18) with a dry-sieving technique. Similarly, Kononoff et al. (2003) modified the PSPS to have the additional 1.18 mm screen as it has been found that an average of 57.7% of total mixed ration **(TMR)** sample passes through the 8 and 19 mm screens. Zebeli et al. (2006) quantitatively analyzed data from recent research studies, and found that peNDF>1.18 explained 67% of ruminal pH variation and 56% NDF digestibility, but was poorly correlated to chewing ($r^2$=0.17) and rumination ($r^2$=0.24). The peNDF>8 only explained 27% of ruminal pH and was also poorly correlated to chewing ($r^2$=0.13) and rumination ($r^2$=0.27). An assumption of the peNDF system is that all particles measured on the 1.18 mm screen are equally effective at stimulating rumination activity. However, it has been found that particles > 19 mm are twice as effective for stimulating rumination vs. those which are between 8 and 19 mm. Also, particles <8 mm are one-fifth as effective in stimulating rumination as compared to particles which are between 8 and 19 mm (Zebeli et al., 2006).

Grant et al. (1990) suggested that diets containing less than 7% long particles retained on the top screen of the PSPS would increase the risk of sub-acute ruminal acidosis. However, inclusion of long forage particles does not always decrease the risk of acidosis, because diets containing longer particles are easily sorted by animals (Zebeli et al., 2006). Also, the estimation of peNDF in TMR is higher when the 1.18

mm screen is used because measurements with the 8 mm screen do not consider particles less than 8 mm or steam-rolled concentrates, which can make up 30-50 % of the TMR (Zebeli et al., 2006). Furthermore, estimating peNDF with only the 8 mm screen does not necessarily reflect the peNDF of diets actually consumed by animals, because cows sort against coarse particles of TMR. Thus, estimating peNDF based on particles retained on the 1.18 mm screen seems to be better for the TMR consumed by dairy cows (Zebeli et al., 2006). Currently, there are no standard methods to measure peNDF, and thus requirements have not been determined by the NRC (NRC, 2001).

## 1.4. Effects of Enhanced In Vitro Fibre Digestibility on Animal Performance

### 1.4.1. Overview of relationship between IVFD and animal performance

Fibre ferments slowly and is retained longer than non-fibre fractions, contributing to ruminal fill (Oba and Allen, 1999a). Neutral detergent fibre varies in degradability in the rumen, ranging from less than 35% to over 75% for different forage types (Nocek and Russell, 1988), and also varies within fibre sources (Llamas-Lamas and Combs, 1990, Robinson and McQueen, 1992). Through a statistical analysis of 13 sets of forage comparisons, Oba and Allen (1999b) found that for every 1-unit increase in NDF digestibility, the VDMI and 4.0% fat-corrected milk yield **(FCM)** increased by 0.17 kg/d and 0.25 kg/d, respectively. Similar increases in VDMI and/or milk yield have also been found for the following forages: corn silage (Ballard et al., 2001, Ivan et al., 2005, Oba and Allen, 1999a, 2000a, Qiu et al., 2003, Thomas et al., 2001, Tine et al., 2001), sorghum (Aydin et al., 1999, Grant et al., 1995), alfalfa hay (Dado and Allen, 1996), and wheat straw (Kendall and Combs,

2004). The majority of research has focused on brown midrib **(BMR)** mutants which have decreased the lignin content and increased in vitro NDF digestibility with minimal effects on NDF and CP compared to their isogenic controls (Oba and Allen, 2000a). Comparison of BMR forages with their isogenic control isolates specific effects of fibre digestibility on animal performance with minimal confounding effects of different dietary NDF concentration, protein concentration, and forage-to-concentrate ratio (Oba and Allen, 1999a).

### 1.4.2. Voluntary dry matter intake and feeding behavior

Oba and Allen (2000a) found that cows fed BMR corn silage had higher VDMI than cows fed normal corn silage, regardless of dietary NDF content. They also found that the dietary NDF level influenced meal patterns; cows fed BMR corn silage in a high NDF diet had greater meal sizes (2.2 vs. 2.0 kg) but greater intervals between meals (98.8 vs. 87.8 min) compared to those fed control corn silage, suggesting that the greater VDMI was due to the greater meal size. In contrast, cows fed BMR corn silage in a low NDF diet had smaller meal sizes (2.2 vs. 2.3 kg) and shorter intervals between meals (90.4 min vs. 98.2 min) compared to those fed control corn silage, suggesting that physical fill was not limiting VDMI for cows fed low NDF diets. This speculation was also supported by the lower volume of ruminal digesta for cows fed the low NDF diets, demonstrating that there was additional physical capacity in the rumen. The authors suggested that another factor which could have influenced VDMI for cows fed the low NDF diet was the lower ruminal pH for the BMR corn silage treatment; a lower pH increases the absorption of fermentation acids, resulting in the cows feeling hunger sooner due to metabolite

uptake by peripheral tissues (Oba and Allen, 2000a). In another experiment, Oba and Allen (1999a) also found an increased VDMI (25.6 vs. 23.5 kg/d) when cows were fed BMR corn silage as compared to normal corn silage even though cows consuming the BMR corn silage were consuming a greater amount of NDF (7.7 vs. 7.2 kg/d).

Similarly, Qiu et al. (2003) compared BMR corn silage to normal corn silage at two dietary NDF contents. They found that cows fed the BMR corn silage had increased VDMI for both levels of dietary NDF. An interaction between corn silage hybrid and forage NDF level showed that increasing the amount of normal corn silage in the diet decreased VDMI. However, increasing dietary BMR corn silage did not decrease VDMI due to the greater NDF digestibility or higher passage rate. Ivan et al. (2005) found that a 1% unit increase in 30-h in vitro NDF digestibility of corn silage resulted in a 0.29 kg/d increase in VDMI. This increase in VDMI was also significant when converted into % of BW, showing that the results were not due to larger cows eating more as a result of a higher rumen capacity. Instead, the increase in VDMI was due to an increase in the turnover of organic matter **(OM)** and NDF in the rumen for cows fed more digestible corn silage. Tine et al. (2001) fed cows diets containing either BMR or isogenic normal corn silage ad libitum **(ISO)**. Cows fed BMR corn silage had an increase of VDMI by 2.6 kg/d as compared to ISO.

Feeding forages with enhanced fibre digestibility have been shown to have no effect on chewing or rumination time (Aydin et al., 1999, Grant et al., 1995, Oba and Allen, 2000b). Grant et al. (1999) compared normal and BMR sorghum, and found no treatment effects on rumination (min/d or min/kg of NDF intake) or total chewing (min/d), but found that cows fed the normal sorghum had greater chewing per kg of NDF intake (70.0 vs. 56.3 min/kg of NDF intake). Similarly, Aydin et al. (1999) and

Oba and Allen (2000b) found no treatment effects between cows fed normal and BMR sorghum or normal and BMR corn silage on rumination (min/d) or total chewing time (min/d).

Other studies have shown that forage digestibility has no effect on VDMI (Aydin et al., 1999, Robinson and McQueen, 1992, Weiss and Wyatt, 2002). Weiss and Wyatt (2002) fed diets differing in corn silage hybrid that differed in digestibility and NDF content. Although they did not observe positive effects of enhanced digestibility on VDMI, this may have been due to that the hybrid with greater fibre digestibility (40.1 vs. 35.4 %) had greater NDF content (49.0 vs. 42.4%). In addition, diets contained a low amount of NDF (28.9 vs. 31.9%) and cows used in the experiment were in late lactation (174 ± 20 DIM; mean ± SD). Thus, VDMI of cows may not have been limited by physical fill. Similarly, Robinson and McQueen (1992) found no effect of good vs. poor quality timothy hay on VDMI. However, cows used in this trial were not in early lactation and VDMI may not have been limited by physical fill. In addition, the treatment effects might have been confounded by the different levels of a second forage source, alfalfa silage, and different sources of concentrate.

### 1.4.3. Milk yield and milk composition

Numerous studies have found that increasing forage NDF digestibility increases milk yield (Aydin et al., 1999, Ballard et al., 2001, Dado and Allen, 1996, Grant et al., 1995, Ivan et al., 2005, Oba and Allen, 1999b, 2000a, Thomas et al., 2001). It has been suggested that this increase in milk yield is related to the increase in VDMI when cows are fed more digestible forages (Ivan et al., 2005, Tine et al.,

2001). Oba and Allen (1999b) showed that, within their data set, an increase in VDMI of 0.17 kg is equivalent to 0.29 Mcal of net energy of lactation **(NEL)**, from which 70% of the energy would be required for a 0.25 kg increase in 4% fat-corrected milk. Any additional energy could contribute to body weight **(BW)** gain. However, it was also suggested that enhancing the digestibility of forages of low NDF digestibility (i.e. from 30 to 31%) would have a greater impact on animal performance than enhancing the digestibility of forages that are already at a high NDF digestibility (from 60 to 61%; Oba and Allen,1999b).

Tine et al. (2001) did not see an increase in milk yield when cows were fed BMR corn silage vs. normal corn silage. This could have been due to the mid-lactation cows (155 ± 23 days in milk; **DIM**) used in the experiment; physical fill may not have limited VDMI and milk production. Similarly, Weiss and Wyatt (2002) used cows in late-lactation (174 ± 20 DIM) and saw no treatment effects. Qiu et al. (2003) observed an increase in VDMI when cows were fed BMR corn silage as compared to normal corn silage, but saw no increase in milk yield. They suggested that the increased VDMI resulted in an increase in digesta passage rate; this increased passage rate decreased NDF total tract digestibility, resulting in similar energy levels and no increase in milk production. Further, milk production may not have been limited by energy intake. Robinson and McQueen (1992) also did not see any effects on milk yield when good versus poor quality timothy hay was compared. However, the diets had inadequate protein levels which could have limited milk production.

Milk composition was not affected by forage NDF digestibility in many previous studies (Aydin et al., 1999, Ballard et al., 2001, Ivan et al., 2005, Oba and Allen, 1999b, Qiu et al., 2003, Robinson and McQueen, 1992, Thomas et al., 2001, Tine et al., 2001). However, some studies have found differences in fat composition

(Grant et al., 1995, Oba and Allen, 2000a). Grant et al. (1995) found that cows fed BMR sorghum had greater milk yield and fat content (% and kg/d). However, Oba and Allen (2000a) found that cows fed BMR corn silage had a lower milk fat concentration than cows fed normal corn silage. The BMR treatment, compared to control, did not decrease milk fat concentration as much for cows fed high NDF diets (3.86 vs. 3.90%) compared to those fed low NDF diets (3.28 vs. 3.67%). It was suggested that the apparent decrease in milk fat concentration may have been caused by the increased milk fluid yield as compared to the milk fat yield, which did not differ between treatments. Other studies have found differences in lactose percentage (Grant et al., 1995, Oba and Allen, 1999a, 2000a). Oba and Allen (1999a, 2000a) found that feeding BMR corn silage increased milk lactose content as compared to feeding normal corn silage. They speculated that either the mineral content (i.e. Na+ and K+) in the milk decreased or that both the blood and milk osmolarities increased. However, since these parameters were not measured, the mechanism for this increase in lactose concentration was not clear.

### 1.4.4. Body weight and body condition score

Results have been inconsistent regarding changes in BW and BCS of animals fed diets with enhanced fibre digestibility. Some studies have found that forage digestibility had no significant effect on BW and BCS (Aydin et al., 1999, Ballard et al., 2001, Oba and Allen, 2000c, Thomas et al., 2001, Weiss and Wyatt, 2002). Oba and Allen (2000a) observed that cows fed BMR corn silage utilized additional energy for milk production compared to those fed normal corn silage, and thus observed no BW or BCS gain. However, cows fed a low NDF diet had increased NEL intake and

BW gain compared to those fed a high NDF diet, but did not increase milk yield (Oba and Allen, 2000a).

One of the factors affecting the partition of energy is the stage of lactation; cows at a later stage of lactation decrease milk yield as they pass their peak stage of lactation, while VDMI remains constant, resulting in animals depositing more body fat (Kuehn et al., 1999). Tine et al. (2001) found that feeding BMR corn silage to ad libitum dry matter intake **(DMI)** resulted in greater BW as compared to cows fed the ISO diet (616 kg vs. 598 kg). However, cows fed the BMR diet had greater BW than cows fed BMR fed to ad libitum DMI of isogenic diet **(BMRR)** (621kg vs. 598 kg). This suggests that the greater BW was due to an increase in VDMI (25.2 vs. 22.3 kg/d for BMR vs. BMRR cows, respectively) and energy availability.

### 1.4.4. Ruminal Kinetics and total tract digestibility

Oba and Allen (1999a) found that cows fed BMR corn silage as compared to normal corn silage had slightly higher NDF and acid detergent fiber **(ADF)** total tract digestibility. In contrast, there were no differences in apparent total tract digestibility of DM, OM, CP, and starch. Although in vitro digestibility of the BMR corn silage was 9.7 units higher than the normal silage, the BMR treatment only increased the in vivo total tract digestibility of NDF by 2.2 units. Oba and Allen (1999b) stated the importance of measuring in vitro or in situ NDF digestibility as opposed to in vivo digestibility as a predictor of the potential of forages to increase VDMI. In vivo digestibility of NDF is confounded by varying retention times in the rumen, and is decreased by an increase in VDMI which increases the passage rate, decreasing the time for microbial degradation. Furthermore, fermentation in the large intestine may

decrease the differences in digestibility seen in the rumen. Ivan et al. (2005) found that cows fed corn silage with greater in vitro NDF digestibility had greater total tract NDF digestibility but no changes in total tract OM digestibility. They also found that the ruminal turnover of OM and NDF was increased for the diet with enhanced forage NDF digestibility, allowing for a greater VDMI. Also, the numerically lower wet digesta weight and ruminal volume suggested that the higher NDF digestibility decreased ruminal fill in their study. Contrarily, Aydin et al. (1999) found no significant change in passage rate of BMR as compared to normal sorghum silage as VDMI was similar for all treatments. This resulted in the total tract digestibility of ADF for BMR sorghum being significantly greater, and NDF digestibility tended to be greater for cows fed the BMR sorghum silage, allowing for greater milk production.

However, other studies have found no effect of increased in vitro forage NDF digestibility on NDF digestibility in vivo. Oba and Allen (2000c) found that cows fed BMR corn silage had no increase in NDF digestibility in the rumen, postruminally, or total tract. Since BMR corn silage has greater in vitro NDF digestibility, the rate of particle size reduction in the rumen is expected to be greater, resulting in the faster rate of passage, causing the decreased in vivo NDF digestibility. Qiu et al. (2003) also found that an increased passage rate led to no treatment effects of enhanced in vitro digestibility on total tract digestibility of NDF or OM. Grant et al. (1995) observed a higher ruminal forage passage rate for animals fed BMR compared to those fed normal sorghum silage (0.055 vs. 0.041/h). However, they also found that cows fed the BMR sorghum silage had greater NDF total tract digestibility as compared to cows fed the normal sorghum silage (46.7 vs. 44.8%). Since ruminal NDF disappearance was similar, they suggested that hindgut fermentation compensated for the more rapid passage rate, resulting in the higher NDF total tract digestibility for

the BMR treatment. Dado and Allen (1996) found that rumen digesta and weight of NDF were similar when cows were fed alfalfa silages differing in NDF digestibility. Also, rumen turnover time was not significantly affected, but DMI was greater for cows fed the diet with greater fibre digestibility. Since the alfalfa with the greater fibre digestibility also had lower NDF content, the greater DMI without changes in rumen pool size was most likely attributed to the difference in NDF content for that study.

### 1.4.5. Ruminal pH and volatile fatty acid concentrations

Previous studies have found that ruminal pH is not affected by forage NDF digestibility (Aydin et al., 1999, Grant et al., 1995, Ivan et al., 2005, Qiu et al., 2003). In contrast, Oba and Allen (2000a) found that daily mean ruminal pH was lower for cows fed the BMR corn silage treatment, which had greater fibre digestibility as compared to the control corn silage treatment. Differences in ruminal pH could have been due to different starch or forage sources between treatments, but the aforementioned studies were not confounded by these differences. Also, forages with enhanced digestibility can increase VDMI, which could further decrease ruminal pH by increasing the total amount of fermentable organic matter consumed. A concern with the potential of forages with enhanced fibre digestibility to reduce the ruminal pH is that the activity of fibrolytic bacteria could be disrupted, decreasing fibre digestion.

Many studies also reported no treatment effects of forage fibre digestibility on ruminal volatile fatty acid **(VFA)** concentrations (Ballard et al., 2001, Dado and Allen, 1996, Grant et al., 1995, Weiss and Wyatt, 2002). However, Ivan et al. (2005) found that diets containing forages of lower in vitro rumen NDF digestibility resulted in a

greater concentration of propionate and tended to have a decreased acetate-to-propionate ratio. This difference could have been caused by the lower NDF and higher starch contents as compared to the other diet. Similarly, Qiu et al. (2003) found that cows fed BMR corn silage had a lower acetate-to-propionate ratio, and that increasing the dietary NDF content resulted in an increase in the acetate-to-propionate ratio. An increase in ruminal propionate has been found to have hypophagic effects on cows (Oba and Allen, 2003). However, BMR treatments as compared to the control for both studies resulted in an increase in VDMI as physical fill may have been the more dominant factor in determining VDMI (Ivan et al., 2005, Qiu et al., 2003).

### 1.4.6. Relationship of milk response to pre-trial milk yield

Previous studies in the literature have shown that high yielding cows respond to a greater extent to forages with enhanced NDF digestibility with higher VDMI and milk yield compared to low yield cows. Oba and Allen (1999a) plotted milk yield and VDMI against pre-trial milk yield, and found that high yielding cows had greater increases in VDMI ($p < 0.06$) and milk yield ($p < 0.03$) when fed BMR corn silage. They suggested that it is more difficult for high yielding cows to meet their energy requirements, and VDMI is limited to a greater extent by physical fill of the rumen. Thus, increased digestibility of the BMR corn silage resulted in an increase in rumen turnover, allowing for increased VDMI and milk production. However, Ivan et al. (2005) found no relationship between pre-trial milk yield and milk response when cows were fed corn silage with either high cell wall content with high fibre digestibility **(HCW)** or low cell wall content with low fibre digestibility **(LCW)**. They suggested that

other factors such as parity, days in milk, or BW could affect pre-trial milk yield. In another trial, Ivan et al. (2005) compared the effects of feeding a LCW diet with a diet containing the aforementioned HCW at a concentration that would result in the 2 diets containing equal NDF content **(HCWN)**. They found that high yielding cows fed the HCWN had a greater milk yield response; a 1% unit increase in pre-trial milk resulted in a 0.15 kg increase in milk yield when fed the HCWN diet as compared to the LCW diet. It was also found that cows at later DIM had a lower pre-trial milk yield, reflecting the lower range of milk production for the second study. Cows in the later stages of lactation partition less energy toward milk production, and thus cannot benefit as much as cows at peak lactation from forages with enhanced fibre digestibility (Tine et al. 2001).

## 1.5. Agronomic Factors Affecting In Vitro Fibre Digestibility of Forages

### 1.5.1. Genetics

Genetic and chemical approaches have been used to increase NDF digestibility by decreasing the amount of lignin or the amount of lignin cross-linking with cell wall carbohydrates (Oliver et al., 2005). Neutral detergent fibre primarily consists of hemicellulose, cellulose and lignin. Lignin decreases cell wall digestibility by blocking the polysaccharides from enzymatic hydrolysis (Jung and Allen, 1995). The BMR mutation, when present in the homozygous recessive state, reduces lignification resulting in increased IVFD (Casler et al., 2003). Though the BMR genes increase IVFD, they have minimal effects on NDF and CP content (Oba and Allen, 2000a, Oliver et al., 2005). Lignin concentration of BMR plants has been decreased by 5 to 50% as compared to the isogenic control, where a 10 g/kg reduction in lignin

resulted in a 40 g/kg increase in digestibility (Cherney et al., 1991). However, a disadvantage of growing BMR plants is the decrease in yield associated with the phenotype including a reduction of 10-17% for stover, 20% for grain, and 16% for fodder yields (Casler et al., 2003).

The BMR mutation has been seen in corn, sorghum, and millet, which all belong to the Panicoideae subfamily, but have not been found in barley. The first BMR corn plant was a self-pollinated line observed in 1924, and the gene was named bm-1; presently, there are three other genes which induce the BMR phenotype in corn including bm-2, bm-3 and bm-4, all of which are from natural mutations (Barrièrea et al., 2003, Pedersen et al., 2006). Considerable research has focused on the mechanism of lignin reduction by bm-3 and bm-1; the bm-3 mutation reduces caffeic acid O-methyl transferase **(COMT)** while the bm-1 mutation reduces cinnamyl alcohol dehydrogenase **(CAD)** activity (Jung and Allen, 1995). In addition, the BMR mutation has been found to shift the lignin composition to increase guaiacyl-type units vs. syringyl-type units, which decreases cell wall digestibility further (Jung and Allen, 1995). In sorghum, BMR mutations were induced in 1975 by soaking sorghum seeds in diethyl sulphate, creating 19 BMR mutant lines. Currently, three of these lines are being developed including BMR-6, BMR-12, and BMR-18; BMR-12 and BMR-18 are allelic genes (Oliver et al., 2005). BMR-6 decreases CAD activity while BMR-12 and BMR-18 genes decrease COMT activity (Oliver et al., 2005). Brown midrib mutations in pearl millet were induced chemically by ethyl methyl sulfonate (Cherney et al., 1988) and also through naturally occurring mutations (Degenhart et al., 1995).

1.5.2. Physiological stage of maturity

As forages mature, the CP as a proportion of total DM decreases, concentration of cell wall increases, and cell wall composition changes, decreasing IVFD (Jung and Casler, 2006). After cellulose and hemicellulose are incorporated into the cell wall, lignin is deposited. As lignification commences, hydroxycinnamic acids cross-link cell wall polysaccharides to monolignols. The extent of lignificaton depends on tissue type, with stems lignified to a greater extent than leaves (Morrison et al., 1998). Thus, an increase in maturity is positively correlated with lignin concentration in stems and negatively correlated to the leaf-to-stem ratio (Jung and Allen, 1995). Lignin decreases the ability of microbes to attack and degrade the cell wall (Morrison et al., 1998). Jung and Engels (2002) showed that the primary cause for reduction in cell wall digestion of alfalfa stems was that lignin is deposited in xylem tissue via cambial activity after internode elongation. Also, alfalfa harvested at the late flowering stage has a higher stem portion than leaves due to advanced maturity (Sheaffer et al., 2000). Lignin composition changes from guaiacyl-type units to syringyl-type units with maturation of cell walls, also decreasing digestibility (Jung and Allen, 1995). Though lignin is thought to be the primary cause of decreasing degradation of cell walls in the rumen, it has also been suggested that ferulic acid plays a major role in cross-linking of lignin to polysaccharides, decreasing digestibility (Jacquet et al., 1995). Bal et al. (1997) found that the maturation of corn silage from early dent to black layer decreased lignin (% DM) and NDF (% DM) concentrations. However, this paradox was due to the increasing proportion of grain in the whole-plant crop, and NDF digestibility was still reduced. Although less mature forages have a greater fibre digestibility, harvesting too early decreases yields, and may

increase seepage losses of nutrients from the silo, and decrease energy concentration due to poor starch development in the kernel (Neylon and Kung, 2003).

Cherney and Marten (1982a) evaluated the effects of maturation on small grain crop forages including wheat, oats, triticale, and barley. Six maturity stages were evaluated including: (1) flag leaf, (2) inflorescence emergence, (3) 7 days, (4) 14 days, (5) 21 days, and (6) 28 days after stage 2. They showed that plant maturity is associated with increased cell wall constituents and ADF concentrations in the early stages, but reached the plateau at approximately stage 3. Acid detergent lignin concentration increased linearly with advanced maturation stage, and was highly negatively correlated with in vitro digestible dry matter concentration at all maturity stages. In contrast, cell wall constituents and ADF were only highly negatively correlated with in vitro digestible dry matter at maturity stages 1 to 3, but decreased during the later stages. In further studies, Cherney and Marten (1982b) found that the decreased digestibility of mature plants was mainly caused by the increased lignin concentration in the stem. Based on the morphological analyses, they also found that at maturity stage 1, the forages consisted of over 80% DM of leaf blade and sheath. With increasing maturity, the proportion of leaves to the total forage decreased, while concentration of in vitro digestible dry matter decreased in the sheath, leaf blade, and stem. Digestibility of stems was 18% units less than that of leaves. Leaf sheaths had similar digestibility to stems in the early maturity stages, but leaf sheaths were more digestible than stems in the latter stages.

### 1.5.3. Environmental factors

#### 1.5.3.1. Temperature and forage quality

Temperature affects biochemical processes via its effect on the kinetic energy of molecules (Buxton and Fales, 1994). Generally, higher temperatures result in an increase in enzyme-catalyzed reactions in the range of temperature where the enzymes are stable. Thus, enzymes involved in mediating the composition and amount of cell wall constituents are affected by changes in temperature (Buxton and Fales, 1994). At higher temperatures, fibre digestibility is decreased since there is an increase in the conversion of photosynthates to structural cell wall components, and lignin synthesis is preferentially increased. Not only do higher temperatures increase enzyme activity, but they also affect enzyme concentrations and rate of active transport of glucose across cell membranes, reducing fibre digestibility further (Fahey and Hussein, 1999, Fales and Fritz, 2007). In addition to the increase in lignin synthesis, high temperatures reduce the leaf-to-stem ratio which decreases fibre digestibility further as stems are generally less digestible than leaves (Fales and Fritz, 2007). It has been found that the digestibility of timothy is decreased by 0.060 ± 0.008 % units for each 1°C increase in temperature (Thorvaldsson, 1992). Atkin et al. (1987) evaluated the effect of altering temperature conditions on tall fescue plants at two diurnal temperature treatments of 20°C/18°C or 30°C/27°C. They found that lower temperatures increased the growth of leaf tissue (including mesophyll, epidermis, parenchyma sheath, and pholem) which was completely digested during in vitro digestion. However, plants grown under higher temperatures had leaves that were only partially digestible.

Forage digestibility has been found to be increased by cooler growing conditions as a result of an increased proportion of readily fermentable non-structural carbohydrates to less digestible cell wall components (Fahey and Hussein, 1999, Maloney et al., 1999). Livingston and Premakumar (2002) found that at suboptimal temperatures, oat crown accumulated fructans due to reduced photosynthesis. Fructan accumulation at low temperature is related to the increase in sucrose:sucrose fructosyl transferase and sucrose synthase activities and overall sucrose biosynthesis (Savitch et al., 2000). Savitch et al. (2000) observed that cold stress resulted in an increase in the sucrose-to-starch ratio by 5-10 fold and neutral invertase activity increased by 2- to 2.5 fold in wheat leaves. Invertases are sucrose hydrolyzing enzymes associated with plant tissues acting as physiological sinks (Rehill and Schultz, 2003). Similarly, Mayland et al. (2000) harvested tall fescue cultivars at 4 different harvest times (May, June, August, and September), and found that the cooler temperatures of May, June, and September as compared to August resulted in decreased aging of cellular tissue and increased total non-structural carbohydrates.

Regional temperatures determine the distribution of C3 and C4 plant species, with the C4 species being more prevalent in warm climates (Fales and Fritz, 2007). Designation of C3 as compared to C4 species is determined by the first stable photosynthetic products with 3-carbon compounds for temperate C3 plants as compared to 4-carbon compounds for tropical C4 plants (Van Soest, 1988). Generally, C4 plants are less digestible and have higher concentrations of structural polysaccharides such as cellulose and hemicellulose (Buxton and Fales, 1994). Deinum and Dirven (1975) found that warm-season grasses have lower digestibility due to a lower leaf-to-stem ratio and a greater decrease in stem digestibility than leaf

digestibility with increasing temperature. Warm-season grasses also have more efficient photosynthetic pathways and have fewer mesophyll cells, which makes them less digestible since mesophyll cells are unlignified in temperate grasses (Van Soest, 1988). Barley belongs to the C3 family while corn silage belongs to the C4 family, which includes forages with lower fibre digestibility. However, due to extensive research, corn with the BMR gene has increased fibre digestibility as compared to its isogenic control (Oba and Allen, 2000a).

1.5.3.2. Light and forage quality

The duration of light exposure, light wavelength, and light intensity affect forage digestibility (Briggs, 1978). The duration of light exposure determines the upper limit of energy for plant use (Van Soest, 1988). Longer photoperiods increase photosynthetic activity which results in the accumulation of soluble carbohydrates, metabolism of nitrogen, and the conversion of nitrate to ammonia and amino acids. This accumulation of carbohydrates was also found by Burns et al. (2005) when they harvested alfalfa at sunset as compared to sunrise. Thus, the proportion of cell wall components is decreased with increasing light, making plants more digestible (Van Soest, 1988). Light wavelength is affected by canopy structure with light in the blue and red range decreasing with depth of canopy (Fales and Fritz, 2007). Guerra et al. (1985) found that ultraviolet and blue radiation stimulated lignin synthesis in wheat by decreasing phenylalanine ammonia-lyase activities.

Latitude affects the amount of solar radiation received at the earth's surface with higher latitudes having longer day lengths but lower flux density of solar radiation. The time of year also affects forage growth with cool-season forages

generally flowering during the long days of late spring (Fales and Fritz, 2007). Van Soest (1988) noted that light and temperature associate with each other to affect forage digestibility. For example, at mid-temperature latitudes, total light decreases, which would result in lower fibre digestibility, but the simultaneous decrease in temperature overrides these negative effects.

1.5.3.3. Water stress and forage quality

Water stress due to an excess or deficit of moisture has a greater effect on forage growth as compared to forage digestibility (Fales and Fritz, 2007). An excess of soil moisture can result in anoxia for forage roots, reducing forage growth and yield. Water deficits delay maturity and negatively affect forage yield. It results in stomata closure, and reductions of transpiration rates and tiller production, while accelerating leaf senescence (Fales and Fritz, 2007). Forage digestibility is affected by water deficits via a decrease in NDF concentration while increasing soluble carbohydrate concentrations in grasses (Crasta et al., 1997). Similarly, Halim et al. (1989) found that moisture deficits for alfalfa increased hemicellulose concentration, the more digestible fraction of the cell wall, as compared to the decrease in cellulose concentration. Sheaffer et al. (1992) found that moisture deficits resulted in shorter grass internodes, higher leaf-to-stem ratio, decreased NDF and ADF contents, and increased CP for timothy and orchardgrass.

## 1.6. Barley Silage

### 1.6.1. Barley growth, ensiling process, and nutrient composition

Barley silage is one of the main forage components used in dairy rations in western Canada (Khorasani et al., 1993). Optimal ambient temperatures for barley vegetative growth and at heading are 15°C and 17-18 °C, respectively. Optimal soil temperature is approximately 15 °C (Briggs, 1978).

The Zadoks two-digit code system includes 9 principal stages (from 0 to 9) for forage growth including: germination, seeding development, tillering, stem elongation, boot, head emergence, flowering (not readily visible in barley), milk development in kernel, dough development in kernel (includes early, soft, and hard stages), and ripening (Zadoks et al., 1974). Barley silage is commonly harvested at the dough stage and ensiled at approximately 30 to 40% DM (Baron et al., 2000). Baron et al. (1992) found that the visual inspection of the appearance of the dough stage was an imprecise indicator of whole plant percent DM to indicate an appropriate harvesting time. Instead, a more precise indicator was the use of cumulative growing degree days greater than 5 °C from heading.

However, as forages mature, fibre content increases while fibre digestibility and CP content decrease (Jung and Casler, 2006). Acosta et al. (1991) found that soft dough stage barley silage had greater concentrations of NDF, ADF, lignin, and lower CP content than boot stage barley silage. Apparent digestibility of DM, OM, CP, NDF, and ADF were greater for the boot stage silage. However, DMI and milk yield did not differ between cows fed barley silage at the soft dough and at the boot stage. Furthermore, cows fed the soft dough barley silage had an increase in BW whereas the cows fed the boot stage barley silage decreased BW. Although starch

content was not reported, starch content of soft dough barley silage is expected to be greater than boot stage barley, providing the additional energy for BW gain.

For an optimal ensiling environment, lactic acid production dominates and butyric acid fermentation is suppressed. Under proper conditions, silage is well-sealed and additives are not required. However, aerobic stability after exposure of silage to the air is often a concern. Taylor et al. (2002) found that the addition of *Lactobacillus buchneri* to barley silage resulted in less yeast and mold, and took it longer to heat and to be spoiled than untreated silage. However, DMI and milk production did not differ between cows fed the treated and untreated silage. Kung and Ranjit (2001) also found that the addition of *Lactobacillus buchneri* and enzymes to barley silage resulted in lower pH, and reduced yeast growth. In addition, treated silage also had a lower NDF content. Both studies found that the use of *Lactobacillus buchneri* effectively improved the aerobic stability of the barley silage.

### 1.6.2. Barley silage and animal production studies

Previous barley silage research with dairy cows in the literature includes the comparison of barley silage with other forages, effects of particle length and peNDF, and the effects of processing barley silage on DMI and milk yield.

Barley silage contains a relatively high concentration of NDF, at approximately 52 to 58%, compared to alfalfa which usually contains less than 46% NDF (Khorasani et al. 1993). Other whole crop grain silages such as oats and triticale can contain even greater NDF concentrations than barley silage (Khorasani et al. 1993). Khorasani et al. (1993, 1996) compared the effects of substituting barley, oats, or triticale silages for alfalfa silage in the diet of lactating dairy cows. They found that cows fed alfalfa and barley silage diets had the greatest DMI as compared to cows

fed the oats and triticale silage diets. Since the latter two diets contained greater NDF contents (37.9 and 36.5% for oat and tricticale, respectively compared to 32.2 and 35.4% for alfalfa and barley, respectively), DMI of cows may have been limited by ruminal fill. Using regression analyses, it was found that for each % increase in dietary NDF concentration, DMI was reduced by 0.95 and 0.38 kg/d for early lactation and mid-lactation cows, respectively (Khorasani et al. 1993). Whole tract digestibility of DM, OM, CP, and ADF was greatest for cows fed the alfalfa diet and lowest for cows fed the oat and triticale diets. Cows fed alfalfa and barley silage had the greatest BW gain while cows fed the oat silage had the lowest BW gain. However, milk production was not affected by treatments, suggesting that the additional energy supplied from increased DMI and nutrient digestion was partitioned to BW gain instead of milk production (Khorasani et al. 1993).

Ahvenjarvi et al. (2006) compared the effects of feeding grass silage with barley silage on animal performance. Barley silage had a higher concentration of non-fibre carbohydrates, but a lower ruminal and total tract NDF digestibility than the grass silage. There were no treatment effects on DMI, but milk production was decreased when cows were fed barley silage. Decreased milk production was not only attributed to reductions in diet digestibility but also to reduced non-ammonia nitrogen flow into the omasal canal.

Several studies evaluated the effects of particle length and peNDF of barley silage on DMI and milk production. Einarson et al. (2004) found that decreasing the chop length of barley silage from 19 to 10 mm had no effect on milk yield and composition, rumen pH, or total volatile fatty acids. However, cows fed the shorter chopped silage had increased DMI and propionate concentration in rumen fluid, and decreased rumen acetate-to-propionate ratio and rumen ammonia concentration. In

agreement to these results, Kononoff et al. (2000) reduced barley silage chop length from 9.0 to 4.8 mm and found that shorter silage resulted in an increase in DMI but no change in milk yield or milk composition. Yang and Beauchemin (2006) found that decreasing the chop length of barley silage form 9.5 to 4.8 mm decreased rumination time. However, they also found that chop length had no effect on ruminal pH and did not decrease the incidence of subclinical acidosis.

In addition to forage chop length, the amount of forage in the diet is also important in determining DMI and milk production. Beauchemin and Rode (1997) found that increasing the amount of barley silage in diets from 30.77 to 64.96% (with an increase of dietary NDF from 36.6 to 49.3%) decreased DMI (17.0 vs. 19.7 kg/d for high vs. low barley silage diets, respectively). Similarly, milk yield was decreased when cows were fed the high barley silage diet (23.1 vs. 27.5 kg/d).

Eun et al. (2004) mechanically processed whole crop barley before ensiling to improve nutrient utilization by dairy cattle. They found that mechanically processing barley silage had no effect on the digestibility of the silage, DMI or milk yield. They speculated that the mechanical processing did not sufficiently damage stem tissue to increase fibre digestibility since the equipment used for their study was specialized for corn silage processing. Since the grain kernel of corn silage has a different structure and size, the rollers should be modified for the processing of barley silage.

## 1.7. Conclusion

The VDMI of lactating dairy cows is affected by numerous factors. However, a major limitation of VDMI is the filling effect of NDF in the rumen. Animal production studies have shown that forages with enhanced IVFD can increase VDMI and milk production. Positive effects of more digestible forage fibre were observed more clearly with high producing cows in early lactation as their VDMI is limited to a greater

extent by physical fill compared to low producing cows or cows in late lactation. Fibre digestibility of forages is affected by numerous factors including genetics, physiological stage of maturity, and the growing environment. The physiological stage of maturity and ambient temperature are the two most significant variables; reductions in fibre digestibility are caused by an advanced stage of plant maturity or higher temperature.

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## 2.0. EVALUATION OF PLANTING DATE ON FIBRE DIGESTIBILITY OF BARLEY SILAGE AND ITS EFFECTS ON PERFORMANCE OF LACTATING DAIRY COWS

### 2.1. Introduction

Maximizing DMI has been of significant importance to the dairy cattle industry. Greater DMI can increase milk yield and reproductive performance and reduce the risk of metabolic diseases due to the negative energy balance caused by high energy demands during early lactation (Grummer et al., 1995). Dairy cows require adequate fibre to maintain healthy rumen function and maximize milk yield. The amount of NDF largely determines the amount of chewing activity and hence salivary buffer secretion. Reducing dietary fibre decreases chewing and salivary production which increases the risk of ruminal acidosis and milk fat depression, and reduces fibre digestion (Beauchemin and Rode, 1997). However, fibre ferments slowly and is retained in the rumen longer than non-fibre fractions, which distends the reticulo-rumen and limits DMI (Dado and Allen, 1995, Johnson and Combs, 1991, Tjardes et al., 2002).

It has been suggested that DMI be predicted based on the dietary NDF content due to the negative relationship between DMI and NDF content (Mertens, 1987). However, NDF varies in degradability in the rumen, ranging from less than 35% to over 75% for different forage types (Nocek and Russell, 1988), and also varies within fibre sources (Llamas-Lamas and Combs, 1990, Robinson and McQueen, 1992). Enhanced IVFD can increase DMI due to the greater rate of NDF clearance from the rumen, creating additional space for further intake (Dado and Allen, 1995). Through a statistical analysis of 13 sets of forage comparisons, Oba and Allen (1999a) found that for every 1-unit increase in in vitro or in situ NDF digestibility, DMI and 4.0% fat-corrected milk yield increased by 0.17 and 0.25 kg/d,

respectively. Similar increases in DMI and/or milk yield have also been found for corn silage (Ballard et al., 2001, Ivan et al., 2005, Oba and Allen, 1999a, 2000a, Qiu et al., 2003, Thomas et al., 2001, Tine et al., 2001), sorghum (Aydin et al., 1999, Grant et al., 1995), alfalfa hay (Dado and Allen, 1996), and wheat straw (Kendall and Combs, 2004).

The IVFD of forages is affected by numerous factors including genetics, physiological maturity of the forage, and growing environment (Fahey and Hussein, 1999, Jung and Casler, 2006, Oliver et al., 2005, Van Soest, 1988). Physiological maturity is an important aspect, because as forages mature, CP decreases, the concentration of cell wall increases, and cell wall composition changes, resulting in decreased IVFD (Jung and Casler, 2006). The increase in maturity is positively correlated with lignin concentration, which decreases the cell wall degradation by microbes (Jung and Allen, 1995, Morrison et al., 1998). An increase in plant maturity also results in an increase of starch content (Bal et al. 1997). Higher temperatures decrease fibre digestibility mainly by increasing indigestible fibre since lignin synthesis is increased (Fahey and Hussein, 1999, Van Soest, 1988).

Barley silage is the primary forage used by dairy producers in western Canada as it makes up over 85% of the annual cereal silage production (Alberta Government, 2005). However, to this date, the effects of IVFD as a quality parameter of barley silage have not been evaluated. Specific objectives of this study were to 1) evaluate the effect of planting date on IVFD of whole crop barley and 2) evaluate the effect of barley silage differing in planting date on DMI and milk production. It was hypothesized that 1) barley planted in June would have lower IVFD compared to that in May, if harvested at the late dough stage, due to the higher environmental

temperature, and that 2) barley silage with enhanced IVFD would reduce the physical fill of the rumen allowing for greater DMI and milk production.

## 2.2. Material and Methods

### 2.2.1. Agronomic study

Two cultivars of barley (*Hordeum vulgare*) were planted on either May 5, 2005 **(BM)** or June 7, 2005 **(BJ)** in 12.4 ha fields at the Edmonton Research Station, University of Alberta. The two cultivars were AC Lacombe and Vivar. Seeding rate was at 112.4 kg/ha. In the fall prior to planting, 78.6 kg nitrogen/ha was applied in a band 24 cm apart and 10 cm in the ground to all fields. At planting, a mixture of fertilizers (16.8 kg Nitrogen/ha, 22.5 kg phosphorous/ha, 7.9 kg sulphur/ha, and 7.9 kg potassium/ha) was placed in the seed rows with the seeds. DyVel® herbicide, which included dicamba as dimethylamine salt and 4-chloro-*o*-tolyloxyacetic acid as potassium salt, was applied once at 1.2 L/ha with the appearance of 4 leaves on the barley plants for broadleaf weed control.

Temperature and precipitation data for the months of May to August 1987 to 2006 were collected from the University of Alberta Metabolic Centre (Environmental Canada, 2006). Four samples of whole plants were collected weekly after the plant reached the heading stage until they were harvested at the late dough stage. Samples were collected from a row of 30 to 60 cm, and stands were cut at 1 cm above the ground. Samples were collected on July 8, 15, and 22 for BM and July 26, August 2, 9, 16, and 23 for BJ. Harvest dates were July 26 and August 25 for the BM and BJ barley, respectively.

Samples were dried in a 55 °C forced-air oven for 72 h and analyzed for DM concentration. Dried samples were ground through a 1-mm screen with a Wiley mill (Thomas-Wiley, Philadelphia, PA), and sent to Cumberland Valley Analytical Services (Hagerstown, MD) for chemical analysis of NDF content (Van Soest et al., 1991) and 30-h IVFD (Tilley and Terry, 1963).

### 2.2.2 Animal production study

#### 2.2.2.1 Vivar barley silage

Both Vivar and AC Lacombe were packed into Agbags, and allowed to ferment for 79 or 49 d, for the BM and BJ silages, respectively. Vivar barley was used for the animal production study due to the lower IVFD standard deviations between samples as compared to AC Lacombe (0.91 and 1.27 % for Vivar BM and BJ, respectively vs. 6.54 and 4.82 % for AC Lacombe BM and BJ, respectively). Concentrations of NDF and starch were 43.7 and 25.3% for BM, and 38.0 and 25.2% for BJ, respectively. The 30-h IVFD was 50.3% and 45.5% for BJ and BM, respectively (Table 2. 1).

#### 2.2.2.2 Cows and treatments

Sixteen multiparous (6 ruminally-cannulated) and 14 primiparous Holstein cows in mid- to late-lactation (183 ± 71.7 days in milk; mean ± SD) were used at the University of Alberta Dairy Research and Technology Centre. Cows were randomly assigned to one of two diets after they were blocked by parity within a crossover design. At the beginning of the experiment, cows averaged 591 ± 68 kg and BCS

was 2.98 ± 0.31 (mean ± SD). Parity of cows averaged 1.70 ± 084. Treatment periods were 19 d, with the final 5 d used as a collection period. Cows were housed in tie-stalls throughout the experiment except for 2 h of daily exercise. Cows were cared for according to the guidelines of the Canadian Council on Animal Care (Institutional Animal Use Approval Number: Exp. 2005-12-CO).

Experimental diets contained either the BM or BJ (58.5% dietary DM) silage, dry rolled barley grain, corn gluten meal, and a premix of minerals and vitamins, and were fed as TMR. Since the BM had lower CP, the BM diet also included canola meal and urea in place of beet pulp in the BJ diet so that the diets would be isonitrogenous. The nutrient composition of the diets were 19.7% CP and 30.6% NDF for BM, and 20.0 % CP and 28.8% NDF for BJ (Table 2.2). All diets were formulated to meet all the nutrient requirements according to the Dairy NRC (NRC, 2001).

2.2.2.3 Data and sample collection

Cows were fed once daily (1100 h) at 110% of expected intake. The amount of feed offered and orts were weighed and recorded daily during the collection period. Samples of dietary ingredients (0.5 kg) and orts (10%) were collected daily during the collection period and pooled into one sample per cow per period. The DM content of the barley silage was determined weekly to adjust dietary allocation of forages to ensure a consistent forage-to-concentrate ratio. Cows were milked twice per day in the tie stalls at 0600 and 1600 h. Milk yield was measured daily during the collection period and averaged over the 5-d collection period. Milk was sampled from both milkings on d 15, 17, and 19. Body weight was recorded on 2 consecutive days

immediately prior to the start of the first period and on the last 2 d of each period. Body condition score, on a scale of 1 to 5 (1=thin and 5=fat; Wildman et al., 1982), was determined by 2 trained investigators 1 day prior to the first period, and on the last day of each period. Fecal and blood samples were collected from each cow every 16 h for 4 d (n=4), representing every 6 h of a 24 h period to account for diurnal variation. Fecal samples (150 g) were collected from the rectum, frozen at -20°C, and composited into one sample per cow per period immediately prior to drying in a forced-air oven at 55 °C. Blood was collected from the coccygeal vessels using Vacutainer tubes containing sodium heparin (Becton Dickinson, Franklin Lakes NJ). Blood samples were immediately placed on ice and centrifuged within 1 h at 4 °C for 30 min at 3,000 x g. Plasma was collected and stored at -20 °C for further analysis.

Rumen fluid samples were collected every 2 h, starting at 0900 h on d 17 and continued for 24 h of each period to determine rumen pH, VFA, and ammonia concentration. Samples (100 g) were taken from 5-6 different sites in the rumen, strained through cheesecloth, and frozen at -20°C for further analysis. Contents from the rumen were evacuated manually through the rumen fistula at 1400 h (3 h after feeding) on d 18 and at 0800 h (3 h before feeding) on d 19 of each period. During evacuation of ruminal contents, a 10% aliquot of digesta was separated for the ease of sub-sampling. This aliquot was squeezed through cheesecloth to separate the sample into primarily solid and liquid phases. Samples were taken from both phases to obtain representative samples accounting for proportions of liquid and solid phases more accurately. These samples were analyzed for nutrient composition to calculate ruminal pool size of nutrients and ruminal turnover rates.

2.2.2.4. Sample analyses

Diet ingredients, orts, feces, and solid ruminal digesta were dried in a 55 °C forced-air oven for 72 h and analyzed for DM concentration. Liquid ruminal digesta samples were freeze-dried because they would not be completely dried in a 55 °C forced-air oven. Dried samples were ground through a 1-mm screen with a Wiley mill (Thomas-Wiley, Philadelphia, PA). Samples were analyzed for concentrations of DM, NDF, indigestible NDF, ash, CP, ether extract, and starch. DM concentration was determined by drying samples at 105 °C for 2 h (AOAC, 2002; method 930.15). Ash concentration was determined after 5 h in a 550 °C furnace (AOAC 2002; method 942.05). Crude protein concentration was determined by flash combustion with gas chromatography and thermal conductivity detection (Carlo Erba Instruments, Milan, Italy; (Rhee, 2005). Neutral detergent fibre concentration was determined using sodium sulphite and amylase (Van Soest et al., 1991). Indigestible NDF was estimated as NDF residue after 120 h in the rumen (Cochran et al., 1986). Samples measured for starch were gelatinized with sodium hydroxide and then measured with an enzymatic method (Karkalas, 1985), and glucose concentration was measured using a glucose oxidase/peroxidise enzyme (No. P7119, Sigma, St. Louis, MO) and dianisidine dihydrochloride (No. F5803, Sigma). A plate reader was used to determine absorbance (SpectraMax 190, Molecular Devices Corp., Sunnyvale, CA). Ether extract concentration was determined using a Goldfisch extraction apparatus (Labconco, Kansas City, MO; AOAC, 1980). Particle size distribution for the TMR and orts samples was determined using a Penn State Forage Particle Separator (19.0, and 8.0 mm sieves; Nasco, Fort Atkinson, WI; Lammers et al., 1996).

Milk samples were analyzed for fat, protein, lactose, and somatic cell count concentrations with infrared spectroscopy by Edmonton-Alberta DHIA (MilkOScan

605, Foss Electric, Hillerød, Denmark; AOAC, 1996). Milk urea nitrogen **(MUN)** was determined with an automated infrared Fossomatic 400 milk analyzer (Foss North America, Brampton, Ontario, Canada). Milk fatty acids were extracted, and after esterification, fatty acid profiles were determined using gas chromatography (Khorasani et al., 1991). Plasma insulin concentrations were determined using commercial kits (Coat-A-Count, Diagnostic Products Corporation, Los Angeles, CA). Plasma glucose concentration was determined using a glucose oxidase/peroxidise enzyme and dianisidine dihydrochloride as described above.

Ruminal pool size (kg) of DM, NDF, indigestible NDF, OM and starch were determined by multiplying the concentration of each component by the ruminal digesta DM weight (kg) except for DM. Rumen turnover rate was calculated for each component by the equation below:

Turnover rate in the rumen (%/h) =

(Intake of component/Ruminal pool of component)/24 x 100

Rumen fluid samples were centrifuged at 26,000 x g for 15 min and supernatants were collected. The centrifuged supernatant was analyzed for VFA by gas chromatography (Khorasani et al., 1996), and ammonia concentration was also determined (Fawcett and Scott, 1960).

2.2.2.5. Statistical analysis for agronomic study

Data were analyzed using ANOVA on JMP® (version 5.1, SAS® Inc., Cary, NC) with the fixed effects of planting date (BM vs. BJ; cultivars, AC Lacombe and Vivar, were used as replications; n=2 for each planting date).

2.2.2.6 Statistical analysis for animal production study

Data were analyzed using the fit model procedure of JMP® (version 5.1, SAS® Inc., Cary, NC) according to the below model:

$Y_{ijkl} = \mu + P_i + C_j(P_i) + E_k + T_l + PT_{il} + e_{ijkl}$,

Where,

$\mu$ = overall mean,

$P_i$ = the parity (i = 1 to 2; primiparous vs. multiparous)

$C_j(P_i)$ = random effect of cows nested in parity (j=1 to 30)

$E_k$= fixed period effect (k = 1 to 2)

$T_l$ = fixed effect of treatment (l = 1 to 2)

$PT_{il}$ = effect of interaction between parity and treatment, and

$e_{ijkl}$ = residual, assumed to be normally distributed.

## 2.3. Results

### 2.3.1. Agronomic Data and IVFD

The BJ took 12 days less to reach the heading stage from planting than BM (50 vs. 62 d) (Table 2.3). Average daily mean temperature and average precipitation over the period from planting to heading were greater for BJ compared to BM (15.1 vs. 13.3 °C and 2.3 vs. 1.6 mm/d). However, BJ took 9 d longer to reach the late dough stage for harvest from heading (30 vs. 21 d). From heading to harvest, average daily mean temperature was greater for BM (15.9 vs. 14.3 °C), and average precipitation was greater for BJ (3.4 vs. 2.5 mm/d). The BJ had greater IVFD than BM for samples collected closest to heading (72.4 vs. 57.5%; Fig. 2.1). BJ had no reduction of IVFD for the last 2 sampling dates. Harvested samples of BJ had greater 30-h IVFD and CP than BM ($P < 0.01$ and $P < 0.01$) (Table 2.4).

Average daily mean temperatures recorded for the 2005 growing season were similar to average daily mean temperatures recorded within the last 20 years. For BM, standard deviations were 0.06, 1.04, and 0.33 for the following time periods respectively: planting to heading, heading to harvest, and planting to harvest. For BJ, standard deviations were 0.75, 1.67, and 1.10 for the same aforementioned time periods, respectively. Average daily mean temperatures for the 20 year average were significantly greater for BJ as compared to BM from planting to heading and from planting to harvest, but were not different from heading to harvest ($P < 0.01$, $P > 0.01$, and $P < 0.01$).

2.3.2. Animal production study

2.3.2.1. DMI, milk yield and composition, energy utilization, and plasma metabolites

There were no treatment effects on DMI (Table 2.5). DMI was 20.4 and 19.9 kg/d for BM and BJ cows, respectively. However, intake of OM, NDF and indigestible NDF were significantly greater for cows fed the BM diet ($P = 0.03$, $P < 0.01$, and $P < 0.01$).

Milk yield was not affected by treatment with average milk yield being 27.2 and 27.1 kg/d for BM and BJ cows, respectively. No differences were found for milk fat, protein, or lactose %. Milk fatty acid composition was affected by diet (2.6). Concentrations of C13:0, C15:0, C17:0, and C19:0 were greater ($P = 0.01$, $P = 0.01$, $P < 0.01$, and $P = 0.04$, respectively) for cows fed BJ. However, concentrations of C6:0 were greater ($P = 0.03$) for cows fed BM. Although some individual fatty acids were significantly different between treatments, the total short- and mid-chain fatty acid concentration (C:4-C:14) was not different. Somatic cell counts were significantly greater for cows fed BJ, but the count was below 200,000 cells/ml for both treatments (195,000 vs. 153,000 cells/ml; $P=0.03$).

Cows fed the BJ diet had a tendency for an increase in BW ($P = 0.06$). For BCS, there was a significant interaction between parity and treatment ($P = 0.04$). Primiparous cows were not affected by treatment, but multiparous cows had greater BCS when fed the June silage ($P = 0.04$). There was no significant increase in plasma glucose or insulin for cows fed BJ as compared to the BM diet.

2.3.2.2. Ruminal kinetics and fermentation characteristics and nutrient digestibility

Ruminally cannulated cows fed the BM diet had significantly greater NDF and indigestible NDF intakes ($P = 0.01$ and $P < 0.01$) (Table 2.7). Cows fed the BM diet also had significantly greater ruminal pool size for DM ($P = 0.05$), and a tendency for a greater NDF pool size ($p = 0.06$). However, the turnover rates of NDF and indigestible NDF were not affected by treatment. The turnover rate for DM of BJ cows tended to be greater than BM cows ($P = 0.08$).

Ammonia concentration in rumen fluid was not affected by source of silage (Table 2.8). The ruminal pH was also not different between BM and BJ diets (6.07 and 5.97, respectively). There were no significant treatment effects on total VFA or composition of VFA.

Total tract nutrient digestibility was significantly greater for cows fed the BJ diet as compared to the BM diet for DM, OM, and CP ($P < 0.01$, $P < 0.01$, and $P = 0.01$, respectively), but was lower for starch ($P = 0.02$) (Table 2.9). The NDF digestibility for BJ tended to be greater than BM ($P = 0.10$).

2.4. Discussion

2.4.1. Effect of planting date on barley IVFD

To harvest at the late dough stage, the growing season was staggered by one month as barley planted in May was harvested in July, and barley planted in June was harvested in August. Mean daily temperatures averaged over growth periods were greater for BJ as compared to BM for planting to harvest for both the weather data collected within our data set and for the 20 year average. Due to these higher

temperatures, it was expected that BJ would have lower IVFD, because as temperatures increase, lignin synthesis is increased and IVFD is decreased (Fahey and Hussein, 1999, Fales and Fritz, 2007). However, we found that BJ had greater IVFD than BM. Although average daily mean temperature was greater for BJ from planting to heading, the average daily mean temperature was lower for BJ from heading to harvest. Greater IVFD of BJ could have been due to the average daily mean temperature from heading to harvest having a more dominant effect on IVFD than average daily mean temperature from planting to heading.

When mean temperatures averaged weekly were evaluated within our data set, it was found that there were three main periods of different temperature levels from heading to harvest for BM and BJ. From heading to 2 weeks after heading for BJ, BJ was exposed to the highest weekly temperatures, and also had the greatest reduction of IVFD (13.6 units) within that period. However, from 2 weeks after heading to harvest for BJ, BJ was exposed to the lowest weekly temperatures, and also had no reduction in IVFD. From heading to harvest for BM, BM was exposed to temperatures intermediate to the aforementioned two periods, and experienced a 4.7 unit decrease in IVFD. The association between higher weekly temperatures and the greater reductions in IVFD suggests that higher temperatures resulted in greater cell wall lignification. However, we only have 1 year of IVFD data which is not sufficient to validate these statements. Thus, additional data is required from the repetition of the agronomy study.

For the Vivar fresh whole crop barley, BJ had an 8.8 unit greater IVFD than BM. However, when samples were ensiled, BJ and BM silages had a 10.2 and 6.2 unit reductions in IVFD, respectively. Thus, the difference in IVFD was reduced to 4.8 units between BJ and BM. The decrease in IVFD was caused by the reduction of

the more soluble fraction of the cell wall; ensiled samples decreased in NDF but there was little change in ADF, CP, and starch content. Since ADF consists primarily of lignin and cellulose while NDF consist of lignin, cellulose, and hemicellulose (Jung and Allen, 1995), the decrease in IVFD suggests that hemicelluose, the more soluble fraction, disappeared via non-enzymatic hydrolysis. Similarly, Dado and Allen (1996) found that ensiling alfalfa decreased NDF content and NDF digestibility (average of 1.4 and 2.9 % unit decrease for NDF and neutral detergent fibre digestibility-24 h, respectively for two alfalfa silages). Also, alfalfa with greater fibre digestibility prior to ensiling resulted in a greater decrease in NDF and fibre digestibility.

### 2.4.2. DMI and milk production

It was expected that feeding BJ would increase DMI and milk production due to its greater IVFD and lower NDF content as compared to BM. However, no treatment effects were found on DMI or milk yield. In this experiment, cows were at a late stage of lactation (183 ± 71.7 DIM; Mean ± SD), and diets had originally been formulated based on data from the fresh whole crop barley to supply a high dietary NDF content, approximately 37 and 39% of dietary DM for BM and BJ diets, respectively. This high forage and high dietary NDF was intended to cause ruminal distension and challenge cows by physical fill in regulation of VDMI (Dado and Allen, 1995, Johnson and Combs, 1991, Tjardes et al., 2002). However, rations fed to cows had a lower than expected dietary NDF due to the reductions in silage NDF, which decreased NDF content of diets to 30.6 and 28.8% DM for BM and BJ diets, respectively. Thus, the DMI of cows was not likely limited by physical fill, which was supported by the ruminal pool size and turnover rate data. Cows fed the BM diet had

significantly greater NDF and indigestible NDF intakes, and also had significantly greater ruminal pool size for DM and a tendency for a greater NDF pool size. However, the turnover rates of NDF and indigestible NDF were not significantly different. If physical fill had limited maximum DMI of animals, a greater NDF intake would have been associated with no change in rumen pool size of NDF and a greater turnover rate, but this was not observed.

Milk yield was not affected in this study (27.2 vs. 27.1 kg/d for BM vs. BJ), which disagreed with the finding that increasing forage digestibility increases milk yield (Aydin et al., 1999, Ballard et al., 2001, Dado and Allen, 1996, Grant et al., 1995, Ivan et al., 2005, Oba and Allen, 1999b, 2000a, Thomas et al., 2001). However, all but one of these studies also found that DMI was increased when cows were fed a diet with higher fibre digestibility. Oba and Allen (1999b) found that within their data set of 13 forage comparisons, an increase in DMI of 0.17 kg was equivalent to 0.29 Mcal of NEL; 70% of the energy would be required for a 0.25 kg increase in 4% fat-corrected milk. In our study, we did not find the increase in DMI, and thus the additional energy for increased milk production was not provided. Also, the late stage of lactation for the cows used limited maximal milk production. Similarly, Tine et al. (2001) did not see an increase in milk yield when cows were fed the more digestible BMR corn silage vs. normal corn silage; this could have been due to the late lactation cows (155 ± 23 DIM) used in the experiment; milk production may not be limited by DMI. Weiss and Wyatt (2002) also used cows in later lactation (174 ± 20 DIM), and saw no treatment effects with two corn silage hybrids (35.4 vs. 40.1% 30-h IVFD)

2.4.3. Energy utilization and rumen fermentation

Total tract nutrient digestibility was significantly greater for cows fed the BJ diet compared to those fed the BM for DM, OM, and CP, and tended to be greater for NDF. The greater total tract digestibility of BJ may have provided additional energy for the tendency for an increase in BW for cows fed BJ. For BCS, there was a significant interaction between parity and treatment. Primiparous cows were not affected by treatment, but multiparous cows had greater BCS when fed the BJ. This suggested that the increased total tract nutrient digestibility of the BJ diet provided additional energy; BW may have been deposited as fat for multiparous cows, but since primiparous cows did not change in BCS, the additional BW may have been used for lean tissue gain. According to the NRC (2001), cows reach a mature BW at the beginning of the third lactation, suggesting that primiparous cows still gain lean tissue mass. However, nitrogen excretion in urine was not determined in this study. Thus, it cannot be concluded that cows fed BJ retained more nitrogen than those fed BM.

There has been inconsistent results regarding BW and BCS changes of animals fed diets with enhanced fibre digestibility. Some studies have found that forage digestibility has no significant effect on BW and BCS (Aydin et al., 1999, Ballard et al., 2001, Oba and Allen, 2000b, Thomas et al., 2001, Weiss and Wyatt, 2002). Oba and Allen (2000a) observed that cows fed BMR corn silage vs. normal corn silage utilized additional energy for milk production, and thus saw no BW or BCS gain. Another factor in the partition of energy is the stage of lactation; cows at a late stage of lactation decrease milk yield as they pass their peak stage of lactation, while DMI remains constant, resulting in animals depositing more body fat (Kuehn et al., 1999). Tine et al. (2001) evaluated the effects of feeding BMR corn silage to

approximate ad libitum DMI of the isogenic control as compared to the isogenic control. They found that cows fed the isogenic diet had a greater BW (616 kg vs. 598 kg), which was not consistent with the results found in this study. However, other studies have found that the extra energy from bm3 corn silage was partitioned to BW gain instead of increasing milk production (Block et al., 1981, Sommerfeldt et al., 1979).

There were no significant effects of treatment on plasma glucose and insulin concentrations. It was expected that greater BCS gain for multiparous cows fed BJ would have been associated with an increase in plasma glucose and insulin concentrations (Gaynor et al. 1995, Rao et al. 1973). It has been found that an increase in plasma insulin and glucose is associated with an increase in lipoprotein lipase activity in the adipose tissue and a decrease in lipoprotein lipase in activity in mammary tissue. This increase in lipoprotein lipase activity increases the uptake of fatty acids into adipose tissue, promoting the deposition of fat (Gaynor et al., 1995, Rao et al. 1973). However, no increase of glucose or insulin was observed for multiparous cows, suggesting that we need to be cautious about the interpretation of BCS data, and that 19-d for the experimental periods may have been too short of a time interval to evaluate BCS changes.

There were no significant effects of treatment on ruminal pH, which is in agreement with many previous studies evaluating forage fibre digestibility (Aydin et al., 1999, Grant et al., 1995, Ivan et al., 2005, Qui et al., 2003). Concentration of the fatty acid, $C18:1_{trans\ 11}$, in milk fat was also not significantly different between the treatments. If rumen pH had decreased, $C18:1_{trans\ 11}$ would have been expected to increase (Oba and Allen, 2000a, Kalscheur et al, 1997). It has been found that a decrease in rumen pH inhibits the conversion of trans-C18:1 fatty acid to stearic acid,

decreasing milk fat (Kalscheur et al, 1997). The VFA concentrations between treatments were similar in the present study, which was consistent with the lack of change in ruminal pH. Thus, data from the current study suggested that rumen fermentation was not affected by treatments.

## 2.5. Conclusion

Delaying the planting date of barley changed its growing environment and affected nutrient composition and IVFD of whole crop barley. The BJ had low NDF content and enhanced IVFD compared to BM, but cows fed BJ did not increase DMI or milk production. The lack of responses could have been attributed to the lower milk production or late stage of lactation of cows used in this experiment. Ruminal physical fill might not have limited DMI. Cows fed BJ increased nutrient digestibility, and the additional energy may have been partitioned to body weight gain. Further research is needed to repeat the agronomy trial to obtain additional data on the effects of planting date on IVFD. Also, the effects of IVFD of barley silage on DMI and milk production need to be evaluated using high producing cows, in which physical fill is more dominant in regulation of feed intake.

Table 2.1. Nutrient composition and particle size of fresh whole crop and ensiled Vivar barley used in experimental diets

| | BM[1] | | BJ[2] | |
|---|---|---|---|---|
| | Mean | SE | Mean | SE |
| Fresh samples[3] | | | | |
| DM, % | 37.6 | 0.54 | 39.1 | 0.47 |
| NDF, % of DM | 50.4 | 0.54 | 52.6 | 0.46 |
| ADF, % of DM | 26.1 | 1.1 | 21.8 | 1.0 |
| 30-h IVFD, % of NDF | 51.7 | 0.65 | 60.5 | 0.57 |
| CP, % of DM | 8.6 | 0.14 | 12.4 | 0.12 |
| Starch, % of DM | 26.3 | 0.17 | 24.6 | 0.14 |
| Silage[4] | | | | |
| DM, % | 38.0 | 0.35 | 38.5 | 0.35 |
| NDF, % of DM | 43.7 | 0.38 | 38.0 | 0.38 |
| ADF, % of DM | 26.1 | 1.52 | 23.4 | 1.52 |
| 30-h IVFD, % of NDF | 45.5 | 1.47 | 50.3 | 1.47 |
| CP, % of DM | 8.6 | 0.21 | 13.5 | 0.21 |
| starch, % of DM | 25.3 | 0.15 | 25.2 | 0.15 |
| Particle size[5] (% of DM) | | | | |
| Top (>19 mm) | 9.6 | 2.53 | 13.3 | 2.53 |
| Middle (8-19 mm) | 55.8 | 2.98 | 52.2 | 2.98 |
| Bottom (<8 mm) | 34.7 | 0.75 | 34.6 | 0.75 |

[1]BM = barley silage planted on May 5
[2]BJ = barley silage planted on June 7
[3]Samples were taken from each load at harvest (n=4 for BJ and n=3 for BM)
[4]Samples were taken from both period 1 and period 2 of the experiment (n=2 for each treatment; samples collected daily during the collection period of the animal study were composited for each period)
[5]Determined with PSPS from samples taken during both periods of experiment (Lammers et al., 1996).

Table 2.2. Ingredients and nutrient composition of experimental diets (% of dietary DM)

| | Treatment[1] | |
|---|---|---|
| | BM | BJ |
| Ingredients | | |
| Vivar barley silage planted in May | 58.5 | - |
| Vivar barley silage planted in June | - | 58.5 |
| Dry rolled barley grain | 25.0 | 25.0 |
| Corn gluten meal | 10.0 | 9.3 |
| Canola meal | 4.1 | - |
| Urea | 0.2 | - |
| Beet pulp | - | 5.0 |
| Minerals and vitamins[2] | 2.3 | 2.3 |
| Nutrient composition | | |
| DM | 47.2 | 49.1 |
| Forage NDF | 23.0 | 20.9 |
| NDF | 30.6 | 28.8 |
| CP | 19.7 | 20.0 |
| Starch | 28.1 | 28.2 |
| Ether extract | 1.8 | 1.9 |
| Forage:concentrate ratio | 59:41 | 59:41 |

[1] BM = barley silage planted on May 5, BJ = barley silage planted on June 7
[2]Premix of minerals and vitamins contained 56% trace minerals and vitamins (<11.5% Na, 17.8% Cl, 0.7% K, 0.1% Ca, 0.6% P, 0.3% Mg, 0.23% S, 25 mg/kg Se, 6.2 mg/kg Co, 80 mg/kg I, 1170 mg/kg Cu, 3100 mg/kg Mn, 5000 mg/kg Zn, 1265 KIU/kg Vitamin A, 142 KIU/kg vitamin D, and 3800 IU/kg vitamin E), 22% limestone, 11% dicalcium phosphate, and 11% magnesium oxide (DM basis)

Table 2.3. Climatic conditions during the 2005 growing season and during a 20 year average[1]

| | BM | | | BJ | | |
|---|---|---|---|---|---|---|
| | 2005 | Avg.[2] | SD[3] | 2005 | Mean | SD |
| Planting date | May 5 | | | June 7 | | |
| Heading date | July 5 | | | July 26 | | |
| Harvest date | July 26 | | | August 25 | | |
| Planting to heading | | | | | | |
| # days | 62 | | | 50 | | |
| Average daily mean temperature (°C)[a] | 13.3 | 13.4 | 0.06 | 15.1 | 16.2 | 0.75 |
| Average precipitation/d (mm) | 1.6 | 2.5 | 0.60 | 2.3 | 3.1 | 0.48 |
| Heading to Harvest | | | | | | |
| # days | 21 | | | 30 | | |
| Average daily mean temperature (°C) | 15.9 | 17.4 | 1.04 | 14.3 | 16.6 | 1.67 |
| Average precipitation/d (mm) | 2.5 | 2.8 | 0.02 | 3.4 | 2.6 | 0.60 |
| Planting to harvest | | | | | | |
| # days | 83 | | | 80 | | |
| Average daily mean temperature (°C)[a] | 14.0 | 14.5 | 0.33 | 14.8 | 16.4 | 1.1 |
| Average precipitation/d (mm) | 1.9 | 2.6 | 0.47 | 2.7 | 2.9 | 0.07 |

[1]Data collected from University of Alberta Metabolic Centre .
[2]Average of 20 yr of data (1987-2006).
[3]The standard deviation between 2005 climatic data and 20 year average climatic data
[a]BM Mean vs. BJ Mean ($P < 0.01$)

Table 2.4. Effects of planting date on nutrient composition of fresh whole crop barley[1]

| | Treatment[2] | | | |
|---|---|---|---|---|
| | BM | BJ | SE | P-value |
| NDF, % DM | 51.3 | 52.5 | 0.66 | 0.34 |
| 30-h IVFD, % of NDF | 51.9 | 61.2 | 0.52 | <0.01 |
| CP, % DM | 8.7 | 12.4 | 0.08 | <0.01 |
| Starch, % DM | 25.9 | 23.0 | 1.11 | 0.21 |

[1]Approximately 8-10 samples were collected during harvesting and pooled (cultivars, AC Lacombe and Vivar, were as replications; n=2 for each planting date)

[2]May = fresh whole crop barley planted on May 5, June = fresh whole crop barley planted on June 7

Table 2.5. Effects of BM and BJ silage on DMI, milk production, BW, BCS, and plasma metabolites

| | Treatment[1] | | | |
|---|---|---|---|---|
| | BM | BJ | SE | P-value |
| Intake (kg/d) | | | | |
| DM | 20.4 | 19.9 | 0.55 | 0.17 |
| OM | 17.0 | 16.3 | 0.46 | 0.03 |
| CP | 3.8 | 3.7 | 0.10 | 0.19 |
| Starch | 5.5 | 5.3 | 0.14 | 0.06 |
| NDF | 5.9 | 5.3 | 0.15 | <0.01 |
| Indigestible NDF | 1.8 | 1.3 | 0.04 | <0.01 |
| Ether extract | 0.3 | 0.4 | 0.009 | 0.43 |
| Yield (kg/d) | | | | |
| Milk | 27.2 | 27.1 | 1.11 | 0.84 |
| Fat-corrected milk (4.0%)[2] | 26.1 | 25.7 | 0.99 | 0.46 |
| Somatic cell count (x1000) | 153 | 195 | 35.0 | 0.03 |
| Milk fat | 1.01 | 0.99 | 0.04 | 0.41 |
| Milk protein | 0.93 | 0.93 | 0.03 | 0.95 |
| Milk lactose | 1.26 | 1.26 | 0.06 | 0.92 |
| Milk Composition | | | | |
| Fat,% | 3.77 | 3.72 | 0.11 | 0.50 |
| Crude protein, % | 3.45 | 3.48 | 0.06 | 0.19 |
| Lactose, % | 4.58 | 4.60 | 0.04 | 0.65 |
| MUN, mg/dl | 12.0 | 11.6 | 0.29 | 0.19 |
| BW change, g/d | 504 | 864 | 129 | 0.06 |
| BCS change, /19 d | | | | |
| Parity x treatment interaction | | | | 0.04 |
| Primiparous cows | 0.00 | -0.05 | 0.04 | 0.41 |
| Multiparous cows | -0.02 | 0.13 | 0.05 | 0.04 |
| Plasma glucose (mg/dl) | 56.2 | 57.1 | 0.70 | 0.37 |
| Plasma insulin (µIU/ml) | 11.4 | 11.7 | 0.74 | 0.81 |

[1]BM = barley silage planted on May 5, BJ = barley silage planted on June 7
[2]4% Fat-corrected milk = ((0.4 x milk yield (kg)) + ((15 x milk fat yield (kg))

TABLE 2.6. Effects of BM and BJ silage on composition of milk fatty acids

| | Treatment[1] | | | |
|---|---|---|---|---|
| Fatty Acid | BM | BJ | SE | P-value |
| % of total fatty acids | | | | |
| $C_{4:0}$ | 1.37 | 1.34 | 0.03 | 0.46 |
| $C_{6:0}$ | 1.99 | 1.92 | 0.03 | 0.03 |
| $C_{8:0}$ | 1.51 | 1.48 | 0.03 | 0.39 |
| $C_{10:0}$ | 3.91 | 3.91 | 0.11 | 0.99 |
| $C_{11:0}$ | 0.18 | 0.21 | 0.02 | 0.06 |
| $C_{12:0}$ | 4.88 | 5.00 | 0.15 | 0.30 |
| $C_{13:0}$ | 0.23 | 0.27 | 0.02 | 0.01 |
| $C_{14:0}$ | 13.4 | 13.3 | 0.23 | 0.75 |
| $C_{14:1}$ | 1.48 | 1.58 | 0.05 | 0.06 |
| $C_{15:0}$ | 1.70 | 1.95 | 0.10 | 0.01 |
| $C_{16:0}$ | 34.6 | 34.6 | 0.56 | 0.87 |
| $C_{16:1}$ | 2.21 | 2.27 | 0.08 | 0.33 |
| $C_{17:0}$ | 0.66 | 0.71 | 0.02 | <0.01 |
| $C_{17:1}$ | 0.38 | 0.39 | 0.02 | 0.81 |
| $C_{18:0}$ | 7.73 | 7.44 | 0.18 | 0.09 |
| $C_{18:1}$ *trans* | 1.79 | 2.06 | 0.15 | 0.10 |
| $C_{18:1}$ *cis* -9 | 19.0 | 18.6 | 0.62 | 0.22 |
| $C_{18:2}$ | 2.69 | 2.58 | 0.14 | 0.54 |
| $C_{18:3}$ ω6 | 0.10 | 0.09 | 0.02 | 0.85 |
| $C_{18:3}$ ω 3 | 0.14 | 0.16 | 0.03 | 0.70 |
| $C_{19:0}$ | 0.09 | 0.16 | 0.02 | 0.04 |
| % fatty acids from $C_{4:0}$ –$C_{14}$ | 28.9 | 29.0 | 0.52 | 0.79 |

[1]BM = barley silage planted on May 5, BJ = barley silage planted on June 7

Table 2.7. Effects of BM and BJ silage on intake, nutrient mass in rumen, and ruminal turnover rate on ruminally cannulated cows[1]

| | Treatment[2] | | | |
|---|---|---|---|---|
| | BM | BJ | SE | P-value |
| Intake (kg/d) | | | | |
| DM | 19.7 | 19.7 | 0.61 | 0.84 |
| OM | 17.4 | 17.2 | 0.54 | 0.59 |
| NDF | 6.0 | 5.7 | 0.07 | 0.01 |
| Indigestible NDF | 1.8 | 1.4 | 0.05 | <0.01 |
| Starch | 5.6 | 5.6 | 0.18 | 0.66 |
| Nutrient mass in rumen (kg) | | | | |
| Wet digesta | 81.5 | 76.0 | 2.51 | 0.16 |
| DM | 16.7 | 14.8 | 0.58 | 0.05 |
| OM | 13.8 | 12.5 | 0.50 | 0.10 |
| NDF | 8.7 | 7.8 | 0.36 | 0.06 |
| Indigestible NDF | 4.5 | 3.5 | 0.39 | 0.13 |
| Starch | 0.8 | 0.7 | 0.11 | 0.75 |
| Ruminal turnover rate (%/h) | | | | |
| DM | 4.95 | 5.60 | 0.24 | 0.08 |
| OM | 5.28 | 5.80 | 0.26 | 0.16 |
| NDF | 2.89 | 3.10 | 0.17 | 0.34 |
| Indigestible NDF | 1.84 | 1.85 | 0.25 | 0.99 |
| Starch | 30.2 | 32.3 | 4.29 | 0.77 |

[1] Measurements taken from ruminally cannulated cows (n=6)

[2]BM = barley silage planted on May 5, BJ = barley silage planted on June 7

Table 2.8. Effects of BM and BJ silage on ruminal pH and rumen fermentation on ruminally cannulated cows[1]

| | Treatment[2] | | | |
|---|---|---|---|---|
| | BM | BJ | SE | P-value |
| Ruminal pH | 6.07 | 5.97 | 0.04 | 0.16 |
| $NH_3$ (mM) | 9.3 | 11.3 | 0.87 | 0.19 |
| Total VFA (µmol/ml) | 144 | 151 | 5.01 | 0.43 |
| VFA (mol/100 mol) | | | | |
| Acetic | 58.5 | 56.5 | 1.25 | 0.17 |
| Propionic | 20.1 | 20.6 | 1.54 | 0.78 |
| Butyric | 13.1 | 14.1 | 0.66 | 0.33 |
| Branched-chain VFA[3] | 2.3 | 2.4 | 0.13 | 0.48 |
| Acetic:Propionic ratio | 3:1 | 2.8:1 | 0.23 | 0.34 |

[1] Measurements taken from ruminally cannulated cows (n=6)
[2]BM = barley silage planted on May 5, BJ = barley silage planted on June 7
[3]Branched chain VFA: isobutyric and iso-valeric

Table 2.9. Effects of BM and BJ silage on total tract nutrient digestibility (%)

| | Treatment[1] | | | |
|---|---|---|---|---|
| | BM | BJ | SE | P-value |
| DM | 66.1 | 68.9 | 0.56 | <0.01 |
| OM | 69.0 | 70.9 | 0.52 | <0.01 |
| CP | 69.8 | 72.0 | 0.79 | 0.01 |
| NDF | 42.1 | 44.1 | 1.06 | 0.10 |
| Starch | 97.5 | 97.0 | 0.18 | 0.02 |
| Ether extract | 65.7 | 63.6 | 1.78 | 0.27 |

[1]BM = barley silage planted on May 5, BJ = barley silage planted on June 7

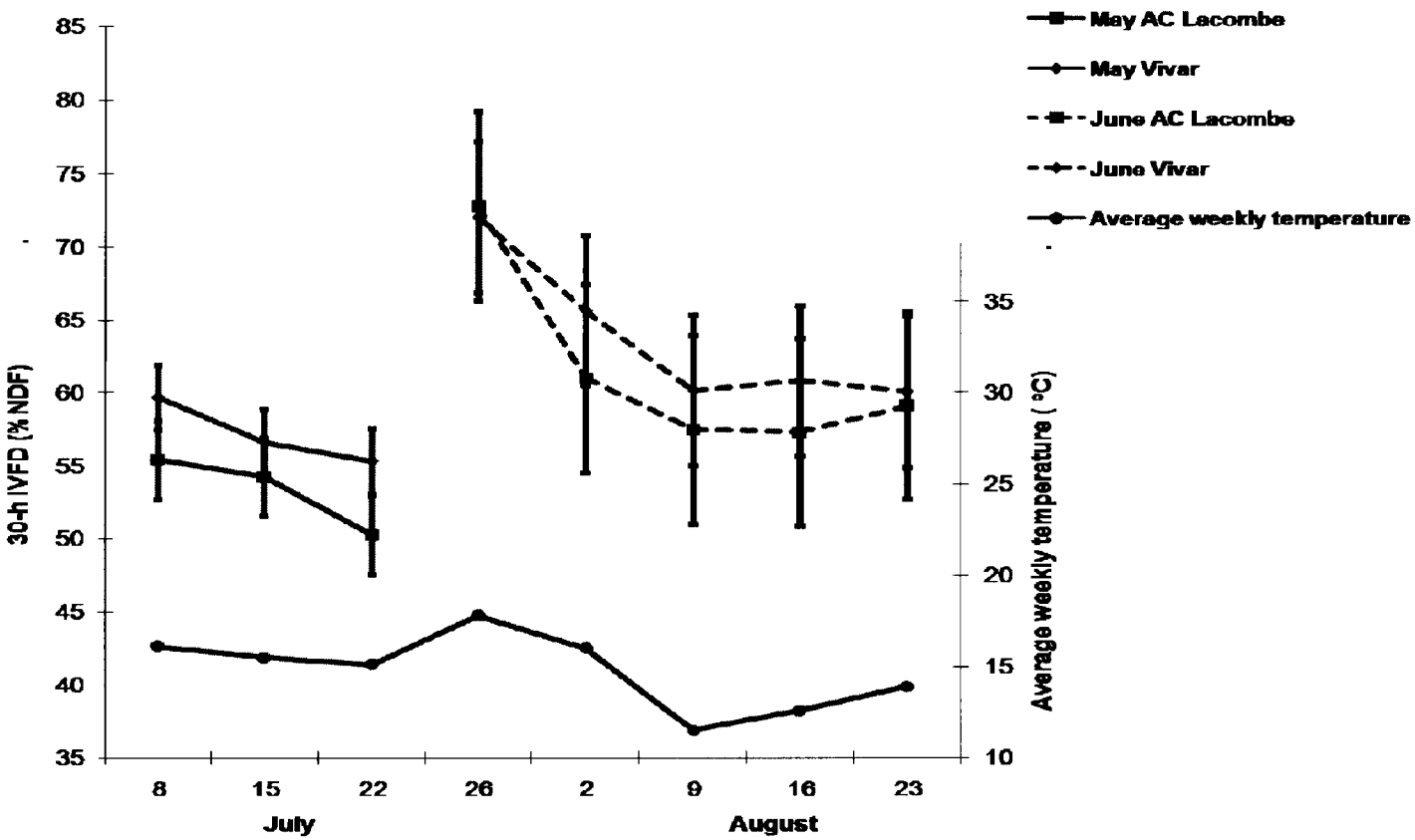


Figure 2.1. Mean daily temperatures, averaged weekly, and effects of planting date (May 5 vs. June 7) on 30-h IVFD of AC Lacombe and Vivar barley

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## 3.0 EVALUATION OF IN SITU INDIGESTIBLE NEUTRAL DETERGENT FIBRE AS AN INTERNAL MARKER TO DETERMINE DIGESTIBILITY OF NUTRIENTS

### 3.1. Introduction

Digestibility of nutrients is an important response variable in nutrition research. External markers such as chromium or ytterbium **(Yb)** have been used extensively to estimate the flow of duodenal digesta or feces, but they require labor for the frequent dosing of markers. Alternatively, internal markers used to determine nutrient digestibility include indigestible NDF, indigestible ADF, acid insoluble ash, acid detergent lignin concentrations (Cochran et al., 1986, Sunvold and Cochran, 1991), and alkanes (Ohajuruka and Palmquist, 1991). Indigestible NDF can be determined relatively easily and inexpensively by in situ methods if ruminally cannulated animals are available (Stern et al., 1997). The 30-h or 48-h NDF digestibility determined by in situ methods may be variable as the fermentation environment is not always optimized for fibrolytic bacteria which can affect measurements. The rumen environment is variable depending on the diet and feeding behaviour of the animals, and diurnal variation in the rumen greatly affects lag time for microbial attachment and rate of digestion (Michalet-Doreau and Ould-bah, 1992). However, the determination of in situ indigestible NDF **(ISIDF)** is an end-point measurement, which should be affected little by lag time or rate of digestion given that samples should be incubated in the rumen for a sufficient time to be digested completely. It is hypothesized that in situ methods provide repeatable indigestible NDF measurements and that ISIDF can be used as a reliable internal marker to estimate nutrient digestibility. Specific objectives of this study were 1) to determine the intra- and inter-assay CV for ISIDF measurements for various types of samples (concentrate mix, silage, TMR, and fecal samples) in dry and lactating cows

and 2) to compare the apparent total tract digestibility of nutrients determined by the use of ISIDF vs. Yb.

## 3.2. Materials and Methods

### 3.2.1. Study 1

Three lactating cows (51, 61, and 62 days in milk) and three dry cows (45, 56, and 129 days prior to calving) were used to determine the ISIDF concentrations of concentrate mix, silage, TMR, and fecal samples. Diets consisted of 60 and 85% forage for lactating and dry cows, respectively (Table 3.1). Two-g of each sample was placed in a 5 x 10 cm in situ bag with a pore size of 50 μm (R510; ANKOM Technology, Macedon, NY), and incubated for 120 h in the rumen of the cows. Samples were replicated three times per cow per sample type; thus a total of 72 in situ bags were prepared (6 cows, 4 types of sample, 3 replications for each sample type). The ISIDF was defined as the NDF residue after 120-h ruminal incubation. Immediately after the 120-h incubation in the rumen, samples were rinsed in a bucket with cold running water for approximately 20 min until effluent water became visibly clear. In situ bags containing the fermentation residues were then placed in boiling NDF solution with sodium sulfite for 1 h (Van Soest et al., 1991), and dried in a forced air oven at 105°C for 24 h prior to the determination of NDF residues. Intra-assay CV was defined as: standard deviations of each triplicate sample within a cow / means of each triplicate sample within a cow × 100, and averaged for each sample type. Inter-assay CV was defined as: standard deviations of each sample across cows / means of each sample across cows × 100, and averaged for each sample type. Data were analyzed with JMP® (version 5.1, SAS® Inc., Cary, NC) using ANOVA to

determine the effect of cow type (dry vs. lactating cows) for ISIDF of the different sample types.

### 3.2.2. Study 2

Eight ruminally cannulated cows were used in a duplicated 4 × 4 Latin square design with a 2 × 2 factorial arrangement of treatments. Treatments included two levels of dietary starch concentrations (30 vs. 23% dietary DM) and two lots of steam-rolled barley cultivars (Dillon vs. Xena) differing in expected ruminal starch degradation. Xena had a greater starch concentration (58.7 vs. 50.0%) and higher in vitro 6-h starch digestibility (78.0 vs. 73.5%) compared to Dillon. Treatment periods were 16 d with the final 6 d used as a collection period. Animals, diets, and sample collection procedures were described in details elsewhere (Silveira et al., 2007). Briefly, during d 7 to 16 of each period, 1.5 g/d of $YbCl_3$ (GFS Chemicals, Inc., Powell, OH) dissolved in 550 mL water was continuously infused into the rumen of each cow via the ruminal cannula using an automatic pump. Fecal samples (100 g) were collected from the rectum twelve times per period on d 13 to 16 so that each sample represented every 2-h of a 24-h period to account for diurnal variation (i.e. 0600, 1200, 1800, and 2400 on d 13; 0800, 1400, and 2000 h on d 14; 0200, 0900, 1600, and 2100 on d 15; and at 0400 h on d 16). Fecal samples were pooled by cow within each period, dried at 55°C in a forced air oven, and ground through a 1 mm screen (Thomas-Wiley, Philadelphia, PA). The fecal samples were analyzed for Yb concentration using inductively coupled plasma optical emission spectroscopy according to the AOAC method (1990) with no KCl used during the sample digestion.

Feed ingredients, orts, and fecal samples were also analyzed for indigestible NDF concentration using three lactating dairy cows. Apparent total tract digestibility of nutrients was estimated either using indigestible NDF as an internal marker (Cochran et al., 1986) or Yb as an external marker. Digestibility data were analyzed using the fit model procedure of JMP® (version 5.1, SAS® Inc., Cary, NC) according to the model:

$$Y_{ijkl} = \mu + S_i + C(S)_{ij} + P_k + T_l + e_{ijklm}$$

Where,

$\mu$ = overall mean

$S_i$ = fixed effect of square (i = 1 to 2)

$C(S)_{ij}$ = random effect of cow nested in squares (j = 1 to 8)

$P_k$ = fixed effect of period (k = 1 to 4)

$T_l$ = fixed effect of treatment (l = 1 to 4)

$e_{ijklm}$ = residual, assumed to be normally distributed.

Orthogonal contrasts were made to evaluate effects of dietary starch concentration, barley grain cultivars, and their interactions.

## 3.3. Results and discussion

### 3.3.1. Study 1

Overall intra- and inter-assay CV for the ISIDF measurements were 2.9 and 6.9%, respectively (Table 3.2). Both intra- and inter-assay CV were greater for concentrate mix samples compared with silage, TMR, and fecal samples. This difference was likely attributed to the low concentration of indigestible NDF for concentrate mix samples, which mathematically inflated the standard deviation expressed as a percent of mean. Because the intra-assay CV was reasonably low, it is recommended that all samples from a study be incubated together for determination of indigestible NDF in nutrition studies. If this is not possible, it is recommended that samples are grouped by blocks or periods that contain samples from all treatments so that each group of samples can be incubated together in the same cow. The effort to minimize inter-assay variation will increase the chance to detect significant treatment effects on digestibility measurements.

Inter-assay CV was lower for samples incubated in the rumen of dry cows compared with lactating cows. In this study, the dry cows were fed a diet containing 85% forage while the lactating cows were fed a diet containing 60% forage (Table 3.1). In situ degradation of forage is decreased in animals fed a concentrate-based diet vs. a roughage-based diet (Vanzant et al., 1998). It is speculated that less diurnal variation in ruminal fermentation for the dry cows provided a more consistent environment for fibrolytic bacteria, resulting in less inter-assay CV for the dry cows. Although the measurements of 120-h indigestible NDF concentration are not greatly affected by lag time or rate of digestion, the use of dry cows rather than lactating dairy cows may also help increase the precision of the measurements.

The ISIDF of fecal samples was greater when lactating vs. dry cows were used (Table 3.3). The indigestible NDF content of feces was greater compared to that of silage, TMR, and concentrate mix. The fecal ISIDF measurements were lower when samples were incubated in dry cows, suggesting that the rumen environment of dry cows was more optimal for fibrolytic bacteria compared to that of lactating cows. A decrease in dietary forage-to-concentrate ratio increases fermentation acid production, and decreases chewing activity and salivary buffer secretion, leading to a decrease in rumen pH (Beauchemin and Rode, 1997). The number of fibre digesting bacteria decrease as pH decreases (pH <6.0), whereas starch digesting bacteria increase as they can tolerate a more acidic environment (pH <5.0) (Mertens, 1997, Nocek, 1997, Sarwar et al., 1991). Thus, to avoid the overestimation of indigestible NDF, the incubation time for fecal samples in the rumen may have to be increased to ensure complete digestion of samples.

### 3.3.2. Study 2

The statistical significance of treatment mean differences was affected by the marker used to estimate nutrient digestibility (Table 3.4). Treatment effects of barley grain cultivar were significant for total tract digestibility of DM, OM, and starch when indigestible NDF was used as the digestion marker ($p < 0.01$, $<0.01$, and $p < 0.001$). However, treatment effects of barley cultivar were significant for starch and NDF ($p < 0.05$ and $p < 0.05$) when Yb was used as the digestion marker. Standard error of means for DM digestibility were smaller when indigestible NDF was used as a digestion marker compared with Yb, indicating that fecal DM flow was estimated more precisely by the use of 120-h ISIDF as an internal marker.

The apparent total tract digestibility of nutrients estimated by Yb was greater than that estimated by ISIDF, indicating that nutrient digestibility was overestimated by the Yb method, underestimated by the ISIDF method, or both. However, for this study, it is not known which method estimated nutrient digestibility more accurately since a total fecal collection was not performed (Owens and Hanson, 1992). If the use of Yb overestimated digestibility, the concentration of the marker must have been overestimated to underestimate fecal output. Problems with marker recovery could include inconsistent mixing of the marker and unrepresentative fecal samples (Titgemeyer, 1997). Brandyberry et al. (1991) reported that fecal recovery of Yb was 104 ± 4% of marker infused, and Siddons et al. (1985) found that Yb recovery was 103.9%. An underestimation of nutrient digestibility by ISIDF would result from an underestimation of indigestible NDF to overestimate fecal output. Vanzant et al. (1998) found that rinsing procedures between laboratories were variable ranging from hand-rinsing to machine washing. Also, subjective endpoints for the determination of water clarity affected the removal of contaminating ruminal residue; though increasing the number of washes decreases ruminal contamination, excessive washing could cause loss of small particles. Accordingly, dry matter digestibility has been found to be overestimated when Dacron bags were used. It was found that mechanical stress caused pore size of Dacron bags to be variable, allowing particles to pass through (Marinucci et al., 1992). Olubobokun and Craig (1990) found that even after multiple washes and physical agitation of Dacron bags, microbes remained firmly attached to feed particles. Failure to correct for contamination by microbes resulted in an underestimation of feed digestibility values since weight of in situ bags would be overestimated, giving an overestimation of indigestible NDF weight.

## 3.4. Conclusion

The in situ method provided repeatable ISIDF measurements, and was a reliable marker to estimate nutrient digestibility. Due to the variation of digestibility results depending on the marker used, it may not be appropriate to determine absolute nutrient digestibility, but it is still useful to determine relative differences among treatments. With the numerous array of markers, the choice of marker may depend on the availability of methods and objectives of the study. The use of in situ method for the determination of indigestible NDF should be evaluated further to ensure adequate incubation time in the rumen for the complete digestion of samples.

Table 3.1. Ingredients and nutrient composition of diets fed to dry and lactating cows (% of dietary DM)

| | Cows | |
|---|---|---|
| | Dry | Lactating |
| Ingredient | | |
| Alfalfa hay | 15.0 | 10.0 |
| Barley silage | 70.0 | 28.0 |
| Alfalfa silage | - | 22.0 |
| Concentrate mix | 15.0 | 40.0 |
| Rolled barley grain | 10.7 | 19.7 |
| Canola meal | 2.3 | 1.0 |
| Dairy pre-mix[1] | 1.1 | 0.6 |
| Molasses | 0.2 | 0.4 |
| Canola oil | 0.7 | 1.8 |
| Ground corn | - | 4.4 |
| Gluten meal 60 | - | 6.8 |
| Fish meal | - | 1.1 |
| Megalac | - | 1.8 |
| Limestone | - | 0.6 |
| Biofos | - | 0.4 |
| Magnesium oxide | - | 0.4 |
| Sodium bicarbonate | - | 0.7 |
| Vitamin E (5000 IU/kg) | - | 0.1 |
| Vitamin $D_3$ (500,000 IU/kg) | - | 0.2 |
| Nutrient composition | | |
| DM | 40.9 | 53.83 |
| Forage NDF | 32.6 | 22.17 |
| CP | 12.8 | 18.4 |
| Forage-to-concentrate ratio | 85:15 | 60:40 |

[1]Dairy pre-mix included: <11.5% Na, 17.8% Cl, 0.7% K, 0.1% Ca, 0.6% P, 0.3% Mg, 0.23% S, 25 mg/kg Se, 6.2 mg/kg Co, 80 mg/kg I, 1170 mg/kg Cu, 3100 mg/kg Mn, 5000 mg/kg Zn, 1265 KIU/kg Vitamin A, 142 KIU/kg vitamin D, and 3800 IU/kg vitamin E.

Table 3.2. Mean, intra- and inter-assay CV (%) of 120-h in situ indigestible NDF concentration of various types of samples

| | Mean, %DM | Intra-assay CV, % | Inter-assay CV, % | | |
|---|---|---|---|---|---|
| | | | Total[1] | Dry cows[2] | Lactating cows[3] |
| Concentrate Mix[4] | 9.7 | 5.9 | 12.3 | 7.0 | 15.9 |
| Silage | 27.6 | 3.5 | 5.0 | 2.6 | 6.8 |
| TMR | 18.2 | 0.9 | 4.5 | 3.8 | 3.9 |
| Feces | 32.6 | 1.0 | 5.9 | 0.5 | 2.2 |
| Overall | 21.9 | 2.9 | 6.9 | 3.5 | 7.2 |

[1] Inter-assay CV of indigestible NDF for samples incubated in the rumen of 6 cows (3 lactating cows and 3 dry cows)
[2] Inter-assay CV of indigestible NDF for samples incubated in the rumen of 3 dry cows
[3] Inter-assay CV of indigestible NDF for samples incubated in the rumen of 3 lactating cows
[4] Concentrate mix sample contained 60.6% dry rolled barley, 24.3% corn gluten meal, 9.3% canola meal, 5.4% premix of minerals and vitamins, 0.3% urea

Table 3.3. Effects of using lactating vs. dry cows for 120-h in situ indigestible NDF analysis of various types of samples

| | Cows | | | |
|---|---|---|---|---|
| | Dry | Lactating | SE | P-value |
| TMR | 17.7 | 18.6 | 0.42 | 0.19 |
| Silage | 28.1 | 27.1 | 0.81 | 0.43 |
| Concentrate | 10.2 | 9.13 | 0.66 | 0.30 |
| Feces | 30.9 | 34.3 | 0.32 | 0.001 |

Table 3.4. Effects of dietary starch concentration (low vs. high) and barley grain cultivars (Dillon vs. Xena) on apparent total tract digestibility of nutrients estimated by either 120-h in situ indigestible NDF or Yb

| | Low starch | | High starch | | SE | *P* value | | |
|---|---|---|---|---|---|---|---|---|
| | Dillon | Xena | Dillon | Xena | | Starch[1] | BG[2] | INT[3] |
| Total tract digestibility estimated by use of 120-h in situ indigestible NDF as an internal marker, % | | | | | | | | |
| DM | 61.1 | 63.7 | 61.9 | 64.5 | 0.9 | 0.31 | < 0.01 | 0.97 |
| OM | 63.2 | 66.0 | 64.2 | 66.7 | 0.9 | 0.26 | < 0.01 | 0.85 |
| Starch | 90.9 | 93.7 | 93.0 | 94.2 | 0.9 | 0.02 | < 0.001 | 0.12 |
| EE | 67.2 | 69.0 | 71.2 | 72.1 | 2.2 | 0.13 | 0.55 | 0.82 |
| NDF | 50.2 | 51.1 | 50.8 | 49.2 | 2.5 | 0.72 | 0.86 | 0.50 |
| CP | 67.6 | 69.2 | 67.1 | 68.9 | 1.9 | 0.77 | 0.16 | 0.93 |
| Total tract digestibility estimated by use of Yb as an external marker, % | | | | | | | | |
| DM | 66.1 | 66.3 | 68.6 | 65.9 | 1.3 | 0.38 | 0.31 | 0.24 |
| OM | 67.9 | 68.3 | 70.5 | 67.9 | 1.3 | 0.37 | 0.39 | 0.23 |
| Starch | 91.8 | 94.2 | 94.1 | 94.4 | 0.9 | 0.05 | 0.04 | 0.11 |
| EE | 71.1 | 71.4 | 76.1 | 73.1 | 2.0 | 0.12 | 0.52 | 0.43 |
| NDF | 56.8 | 54.5 | 59.0 | 50.9 | 2.9 | 0.77 | 0.04 | 0.02 |
| CP | 71.5 | 71.4 | 73.1 | 70.2 | 1.8 | 0.85 | 0.28 | 0.31 |

[1] Effect of dietary starch concentration (low vs. high; 23 vs. 30% dietary DM)
[2] Effect of barley grain cultivar (Dillon vs. Xena)
[3] Effect of interaction between dietary starch and barley grain cultivars

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## 4.0. GENERAL DISCUSSION AND IMPLICATIONS

### 4.1. Environmental Factors Affecting IVFD

Numerous factors affect the IVFD of forages including the environment, genetics, and physiological parameters. Chap. 2 examined the effects of altered growing environment on IVFD of forages by delaying the planting date by one month (May vs. June), and harvesting at the same physiological maturity. In this study, barley planted in June had greater fibre digestibility compared to that in May. Environmental factors affecting forages quality include temperature, water stress, soil nutrient availability, and photoperiod. A later planting date usually increases photoperiod per day, generally resulting in greater plant growth. It is also known that higher temperatures increase the rate of plant growth, decrease the leaf-to-stem ratio, and increase lignin concentration, resulting in decreased digestibility. Also, high temperatures increase the respiration rate, decreasing the soluble sugar concentration. Diurnal variation affects the quality of forages with harvesting in the afternoon being most favourable due to the plant having the greatest digestibility, and leaf sugar concentration. Whereas temperature and photoperiod affect forage yield the effects of maturity usually play a more important role in forage quality. An increase in maturity reduces leaf-to-stem ratio, and increases lignin concentration with a proportional decrease of cell solubles. The variability of the environment from year to year warrants further study on IVFD of barley silage with the goal of enhancing animal performance.

## 4.2. Silage

Ensiled barley had a drastic decrease in NDF concentration and 30-h IVFD compared to the fresh whole crop barley (Chap. 2). Also, the greater decrease in digestibility for barley silage with higher fresh crop fibre digestibility could decrease the benefits of harvesting forages with higher digestibility. Successful ensiling should theoretically preserve nutrients. However, ensiling may not completely preserve crop quality, and respiration and effluent losses may affect the silage quality; plant and microbial respiration may oxidize the most digestible portions of the forages. To ensure the optimum ensiling process, forages should be harvested at the optimal physiological maturity, plant enzyme and microbial degradation should be minimized, and the lactic acid bacteria population should be maximized. The use of silage additives including bacterial inoculants, enzymes, acids, urea and anhydrous ammonia help to improve aerobic stability and improve the quality of silage. Further research should investigate the effects of the ensiling process on barley nutrient composition since many dairy nutritionists send fresh whole crop barley samples to be analyzed for the formulation of animal rations.

## 4.3. Relationship between IVFD and Animal Performance

The relationship between IVFD and animal performance has been extensively studied for corn silage due to the discovery of the brown midrib mutant, which decreases lignin content and increases IVFD with minimal effects on NDF and CP. Research has also been done on sorghum, alfalfa hay, and wheat straw. However, the current study in Chap. 2 was the first to evaluate the effects of IVFD of barley silage on animal performance. The IVFD measurement is a useful tool to identify

forages with higher quality and a greater potential to increase DMI and milk production. It can also be used to aid in trouble-shooting when switching to a new silage. Past studies have found that forages with enhanced IVFD can increase DMI but may also increase passage rate, decreasing digestibility so that the in vitro digestibility does not necessarily represent the in vivo digestibility. In Chap. 2, barley silage with enhanced digestibility did not have an effect on DMI and milk yield, but it tended to increase cow BW and BCS. Forages with enhanced IVFD can improve the energy balance of animals, but may not improve milk production unless physical fill limits their voluntary feed intake. The stage of lactation also plays an important role in energy partitioning, with early lactation cows partitioning more resources for maximal milk yield. Utilization of IVFD data for barley silage will refine nutritional management to optimize animal performance. It will also enhance the profitability of the dairy industry, especially for Western Canada where the majority of producers use barley silage for dairy rations

### 4.4. In Situ Indigestible Neutral Detergent Fibre as a Digestion Marker

The ISIDF was found to give consistent results for nutrient digestibility, but differed from Yb measurements (Chap. 3). Future improvements to marker development must ensure that measurements are both accurate and precise by comparing values to those found from total fecal collections. Standardization of procedures is vital to reduce inter- and intra- laboratory variations. Currently, variations with measurements from the in situ method can be caused by differences in feeding behaviour of animals, pore size of bags, sample weight to bag surface area ratio, sample particle size, amount of bacteria contamination, and bag

placement in the rumen. Since ISIDF is an end-point measurement, variable rumen fermentation should have little effect on values. However, in Chap. 3, it was found that the use of dry vs. lactating cows did have an effect on ISIDF values, with samples incubated in dry cows having lower inter-assay CV. Also, the incubation of fecal samples in lactating cows resulted in higher ISIDF values compared to dry cows, suggesting incomplete digestion of samples for lactating cows. Although, after 120 h in the rumen, digestion should be complete, limitations with using lactating cows should be considered.

## 4.5. Conclusion

In summary, whole crop barley planted in June had greater 30-h IVFD, and the silage tended to increase BW gain of dairy cows, and increased BCS gain for multiparous cows. However, barley silage planted in June did not affect DMI and milk yield and composition. In conclusion, planting date of barley may affect IVFD of whole crop barley possibly due to the differences in the growing environment. Forages with enhanced IVFD can improve the energy balance of animals, by increasing the digestibility but may not improve milk production unless physical fill limits their voluntary feed intake

## APPENDIX

### Appendix 1: Neutral detergent fibre determination

1. Hot weigh dry, clean crucible (105°C)
2. Weigh 0.5 g dry, ground sample into a 600 ml beaker
3. Add 50 ml NDF solution and 75 µl amylase (Sigma A3306)
4. Turn on the water for the condensing unit, and turn on the switch for the burners
5. Place beakers on refluxing apparatus
6. At the onset of boiling add 0.5 g sodium sulfite to the beaker
7. Reflux for 1 hour
8. Slowly pour hot sample from the beaker into the crucible and filter solution using minimal vacuum
9. Rinse crucible with hot water
10. Fill crucible half full with hot water and 75 µl amylase
11. Let sit for 1 minute
12. Evacuate crucible and fill crucible with hot water
13. Let sit for 1-3 minutes
14. Evacuate and then fill crucible with hot water again
15. Let sit 3-5 minutes, repeat
16. Evacuate and then fill the crucible ¼ full with acetone and let sit for 3-5 minutes, repeat
17. Let residue and crucible dry for 60 minutes and place in oven at 105°C for at least 8 hours
18. Calculation:

    %NDF = [(wt. cruc + residue) – (wt. cruc)] / (wt. sample) x 100

**Appendix 2: Starch determination**

1. Prepare acetate buffer 2 M, pH 4.9
    a. Dissolve 120 ml glacial acetic acid and 164 g sodium acetate abhydrous in 300-500 ml of d water in a 1000 ml volumetric flask. Dilute to volume
2. Determine free glucose in sample
    a. Weigh 0.50 g sample into 125 ml Erlenmeyer flask
    b. Add 50 ml dH2O to flask and swirl
    c. Allow the sample to hydrate 15 min
    d. Mix well and transfer ~5 ml sample to a polycarbonate centrifuge tube
    e. Spin at 26,000 x g for 10 minutes
    f. Decant 1.5 ml to micro-centrifuge tubes for analysis by plate reader
3. Starch gelatination and analysis
    a. Weigh sample into 125 ml Erlenmeyer flask. Be sure to include 100% starch standard (and known lab standard in each run)

<u>Following sample weight suggestions according to predicted starch %</u>:

| | |
|---|---|
| 0-10 % | 0.50g |
| 11-60% | 0.25g |
| 61-80% | 0.20g |
| 81-100% | 0.10g |

    b. Add 20 ml $dH_2O$ to flask and swirl

c. Allow the sample to hydrate 15 min

d. While swirling flask, add 0.5 ml 50% w/water NaOH and let sample gelatinize for 15 minutes. Consistently shake samples so that they do not concentrate in the middle of the flask

e. Add 10 ml 2 M acetate buffer to flask and rinse the sides of the flask with 15 ml $dH_2O$ and swirl.

f. Add 0.8 ml concentrated HCl (12N) in a fume hood

g. Add 250 µl amylase (Crystalzyme 40L Valley Research, Inc., South Bend, IN) to each flask, seal each flask with aluminum foil and set in a 55°C water bath for 16 hours.

h. Transfer to a 200 ml volumetric flask and dilute with $dH_2O$ to 200 ml.

i. Mix well and transfer ~5 ml sample to a polycarbonate centrifuge tube

j. Spin at 26,000 x g for 10 minutes.

k. Decant 1.5 ml to micro-centrifuge tubes for analysis by plate reader.

4. Glucose determination is the same as Appendix 6 except for different set of standards for free glucose determination (0, 2, 4, 6, 8, 10 mg Glucose/100 ml)

5. Calculation of results:

$$\text{Starch}(\%\ DM)=(0.9 \times [Glc]/1000 \times 2/W_s \times 100)-\text{Sugar}\ \%$$

$$\text{Sugar}\ (\%\ DM)=0.9 \times [Glc]/1000 \times 0.5/\ W_s \times 100$$

Where:

$W_S$ = sample dry weight (mg)

[Glc] = concentration of glucose as read from the standard curve (mg/dl)

**Appendix 3: In situ indigestible neutral detergent fibre determination**

1. Label ANKOM bags (Part #R510) with appropriate ID, use black permanent marker
2. Place bags in oven for 8 hours (105 °C)
3. Hot weigh bags
4. Weigh 0.5 g sample, and put into bag
5. Heat seal bags twice
6. Place bags into mesh bags
7. Place bags in cannulated cow for 120 hours
8. Remove mesh bags, place on ice, and wash mesh bags with cold water for 20 min (or until water is clear)
9. Weigh 2 g sodium sulfite into 600 ml beakers add 400 ml of NDF solution and place 15 ANKOM bags in the beaker
10. Turn on the water for the condensing unit, and turn on the switch for the burners
11. Reflux for 1 hour from onset of boiling
12. Rinse bags with water (~15 minutes)
13. Dry bags with sample for 8 h in 105 °C oven
14. Hot weigh bags
15. Calculate indigestible NDF

INDF% = [(wt. bag + residue) – (wt. bag)] / (wt. sample) x 100

**Appendix 4: Ether extract determination**

1. Weigh in duplicate 2 g dry, ground sample into a cone of an appropriate grade filter paper for the sample, then cover the material with small amount of glass wool
2. Weigh clean labelled extraction beakers
3. In a fume hood, add 40 ml petroleum ether (Fisher Scientific, Pittsburgh, PA, USA) into each beaker
4. Run at least one blank with 40 ml petroleum ether to determine residue after evaporation
5. Put samples in sample holders and attach to clamps at the condenser unit. Attach extraction beaker by tightening the beaker ring clamp
6. Turn on water condenser
7. Turn Goldfisch (Labconco, Kansas City, MO, USA) unit on and raise heater unit to near but not touching the beakers.
8. Once vigorous boiling has started check that there is no leakage of solvent
9. Extract fat for 4-6 hours with the heat set on high
10. Once extraction is completed lower the heaters and let the beaker contents cool
11. Remove each beaker so that the sample holder can be replaced with a glass solvent collector tube, then reattach the beaker
12. Heat until only a small amount of solvent is in the beaker
13. Lower heat unit again and turn off main power
14. Allow beakers to cool, remove them and transfer to a fume hood
15. Remove solvent collect and pour solvent into a waste ether bottle

16. Once all petroleum ether has evaporated place in a 110°C oven for 30 minutes
17. Cool beaker to run temperature in a desiccator and weigh
18. Calculation:

    % Fat = 100 x [(wt. beaker + wt. extract – wt. blank residue) – (wt. beaker)] / wt. sample

**Appendix 5: Glucose assay**

1. Dissolve the contents of 2 capsules of peroxidase-glucose oxidase (P7119, Sigma Chemical Co., St. Louis, MO) into 100 ml of d $H_2O$ in an amber flask (solution A)
2. Using a squirt bottle, add 20 $\pm$ 0.01 g of d $H_2O$ into the vial of dianisidine dihydrochloride (F5803, Sigma Chemical Co., St. Louis, MO; solution B)
3. Add 3.2 ml of solution B to 100 ml of solution A (solution AB). Solution can be stored at 4°C for 3 weeks
4. Prepare a standard glucose curve into test tubes using the stock solution in the glucose kit:

| mg Glucose/100 ml: | *0* | *20* | *40* | *60* | *80* | *100* |
|---|---|---|---|---|---|---|
| Stock solution (μL); | 0 | 200 | 400 | 600 | 800 | 1000 |
| D $H_2O$ (μL); | 1000 | 800 | 600 | 400 | 200 | 0 |

1. Assay each sample in duplicate
2. Add 10 μL of sample, standard or blank (no sample) to wells in UV flat bottom 96-well plate (Corning Incorporated, Corning, NY, USA).
3. Add 300 μL solution AB to each well with a multi-channel micropipette, turn the plate reader on and shake the plate with the plate reader for at least 10 sec. Allow to sit at room temperature for 45 minutes covered with tin foil.
4. Read absorbance of samples at 450 nm

## Appendix 6: Plasma insulin radioimmunoassay (Coat-A-Count ® Insulin)

1. Instructions are the same as provided in the kit, only difference is Calibrator B should also be diluted in half (B/2), for a total of 16 standards

## Appendix 7: Ammonia-N determination

1. **Reagents:**

   **a) Sodium phenate**

   12.5 g phenol + 6.2 g NaOH in 500 ml volumetric flask (dissolved and made to volume with de-ionized water)

   **b) Sodium nitroprusside**

   Stock solution (1%) – 1 g/100 ml $H_20$

   Working solution (0.01%) – 5 ml stock diluted to 500 ml with de-ionized water

   Note: working solution should be prepared fresh daily

   b) **Sodium hypochlorite**

   0.02 N; 15 NaOCL (4-6%) diluted to 500 ml with de-ionized water

   NOTE: the pH should be adjusted to 12.0 with 50% NaOH

   -All solutions should be stored in the fridge with the tin foil wrapped around the containers. The solutions should be brought to room temperature before use.

2. **Standard solution: 100 µg $NH_3$- N/ml**

0.4716 g ammonium sulphate (dried at 60 °C for 2 h)/L de-ionized water

The pH should be close to that of the samples

3. **Color development**

   a) Prepare a standard curve by pipetting 0, 10, 30, 40, 50, 60 µl from standard solution (in duplicate) into 16 x 100 mm glass tubes. Standards must be run with every set (approximately 40 tubes) (for the "0 standard", pipette 20 µl distilled water). A control sample should be run in duplicate with each standard curve.

   b) Add reagents to the standards and samples in the following order:

   -2 ml phenate solution and vortex

   -3 ml nitrprusside solution and vortex

   -3 ml hypochlorite, cover tube with parafilm and invert several times

   c) Develop color at room temperature in the dark for 1 h

   d) Read absorbance on dipping probe at 600 nm using distilled water to zero spectrophotometer (mix sample by inversion before reading absorbance)

   NOTE: Cover sample with parafilm at all times as ammonia is absorbed form the air.

**Calculations:**

1. Run regression analysis using 0, 1, 2, 3, 4, 5, and 6 as x and absorbances as y.
2. µg/volume added to tube = $\frac{\text{Absorbance} - \text{intercept}}{\text{slope}}$

Dry sample = DM at 105°C x DM at 60 °C x 0.001

Conc. mgN = average µg/tube x (1000/volume added) x 0.001

Total N = conc. mgN x (1000-dry sample)

NH3 (g) / sample (kg) = total N/dry sample

NOTE: [(maximum – minimum)/average) x 100] < 10 % is acceptable

**Appendix 8: Ytterbium determination**

1. Prepare 2% v/v nitric acid solution
    a. Add 20 ml concentrated $HNO_3$ to 980 ml double distilled water
    b. Dissolve 1.91 g $KCl_2$ into solution to act as an ionization buffer
2. Hot weigh empty, clean 100 ml beakers
3. Weigh 0.50 g of dried ground sample into hot weighed beakers in duplicate
4. Include two beakers as blanks
5. Dry samples in the oven at 135°C for 2 hours
6. Turn oven down to 105°C, wait 15 minutes and hot weigh beakers to obtain DM
7. Ash the samples in the beakers at 550°C for 5 hours
8. When samples are cooled to room temperature add 40 ml nitric acid solution to each beaker (to extract the Yb)
9. Place beaker on a shaker for 2 hours (100 rpm)
10. Transfer the solution in each beaker to a 50 ml centrifuge tube and centrifuge at 10,000 rpm for 10 minutes
11. Decant supernatant into labelled scintillation vial and analyze sample
12. Calculation

    Yb (μg/g DM) = (reading ppm x dilution factor) / (sample weight x DM)

**Appendix 9: Rumen VFA assay**

1. Sample preparation
    a. Thaw sample and pipette 5 ml into a centrifuge tube
    b. Centrifuge sample for 15 minutes at 3000 rpm
    c. Pipette 1 ml of centrifuged sample into a GC vial
    d. Add 200 μL of 25% phosphoric acid and 200 μL isocaproic acid solution (as an internal standard) and mix well
    e. Allow solution to stand for 30 minutes (if solution is not clear, centrifuge again at 3000 rpm for 15 minutes and analyze as soon as possible)
2.
    a. Standard:

| | g/100 ml water | Approx. RF | Approx. mg/ml rumen fluid |
|---|---|---|---|
| Acetic | 0.30 | 2.60 | 2-5 |
| Propionic | 0.20 | 1.60 | 0.5-2 |
| Isobutyric | 0.05 | 1.20 | 0.1 |
| Butyric | 0.10 | 1.20 | 0.5-1 |
| Isovaleric | 0.05 | 1.10 | 0.1 |
| Valeric | 0.05 | 1.10 | 0.1 |
| Caproic | 0.05 | 1.00 | 0.05 |

3. GC condition:
    a. Column: Stabilwax-DA 30 m x 0.25 mm I.D.
    b. Temperature: 120°C to 170°C at 10 °C per minute
4. Calculations:

a. RF= response factor

b. RF (acetic, A) = mg 'A' x area IS / mg IS x area 'A'

c. mg 'A' / ml RuFl = ((( mg IS / ml RuFl) x area 'A') / area IS) x RF 'A'