Towards Understanding Feeding Motivation and Management Factors Affecting Feeding Behaviour in Limit-Fed Dairy Heifers

by

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ABSTRACT

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The objective of this dissertation was to determine the feeding motivation of dairy heifers and to determine management factors that contribute to satiety in limit-fed dairy heifers. The first study of this dissertation determined the difference in behavioural patterns between limit-fed and ad libitum-fed dairy heifers so that we may understand how limit feeding diverges from normal behaviour. Limit-fed heifers were found to decrease time spent feeding, consume less DM, and increase their feeding rate across the day in comparison to ad libitum-fed heifers. Additionally, the diurnal patterns of ad libitum-fed heifers were much closer to normal behaviour as they consisted of several short, small meals distributed throughout the day. The next three studies examined the management factors of frequency of feed provision and feed bunk space and how these may impact the behaviour of heifers. Increased frequency of feed provision did result in more distribution of feeding activity throughout the day but also imposed feeding amounts that were likely insufficient to achieve satiety. Heifers fed once daily likely experienced satiety in the short term and did spend the most time feeding throughout the day but the diurnal patterns of these heifers did not allow for expression of normal foraging behaviour. Providing increased feed bunk space did not allow limit-fed heifers to spend more time feeding or impact competition within a pen whereas providing straw alongside of a nutrient-dense TMR did result in more normal diurnal patterns, albeit with an increase in competitive behaviour. There was no interaction found between feed bunk space and frequency of feed provision, indicating that limit-fed heifers must be provided with sufficient bunk space to feed simultaneously. Heifers provided with un-restricted bunk space did gain more and were more feed efficient and less variable in feeding time than heifers given restricted bunk space. Feeding once daily resulted in an increase in competition but also enabled heifers to gain well and to spend more time feeding each day. The remaining studies sought to quantify whether a preference for supplementary feed exists in limit-fed heifers and whether heifers will work, and to what extent, for this extra feed. The results indicated that heifers will consume similar amounts of supplementary long or short straw if provided to them alongside of a limit-fed TMR. The limit-fed heifers do, however, show a clear preference for long straw when offered the choice, suggesting that they find long straw to be more satisfactory for achieving rumen fill and/or meeting their behavioural foraging needs. Heifers were also found to work harder (push more weight), spend less time feeding and ruminating, and consume feed faster when provided a high-concentrate, limit-fed ration than when provided a high-forage, ad libitum-fed ration, suggesting that these animals are experiencing feelings of hunger and may not be physically or behaviourally satisfied. These findings indicate that there are clear behavioural differences between limit-fed and ad libitum-fed dairy heifers.
Management factors, such as frequency of feed provision and feed bunk space, may be altered in such a way as to provide some benefit to the animals but are limited in their ability to normalize feeding behaviour and diurnal patterns. Provision of supplemental, long particle low-nutritive roughage (i.e. straw) aids much more in allowing limit-fed heifers to achieve satiety and is considered a desirable resource by the animals, as evidenced through their willingness to work for this supplemental feed.
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LIST OF ABBREVIATIONS

TMR – total mixed ration
DM – dry matter
NDF – neutral detergent fibre
ADF – acid detergent fibre
CP – crude protein
NFC – non-fibre carbohydrates
TDN – total digestible nutrients
ME – metabolizable energy
NEₘ – net energy for maintenance
NE₉ – net energy for gain
DMI – dry matter intake
ADG – average daily gain
ADGV – variation in ADG within a pen
SARA – sub-acute ruminal acidosis
HCR+S – high concentrate ration with ad libitum wheat straw
LCR – low concentrate ration
TMR-0.34 – provision of 0.34 m/heifer feed bunk space
TMR-0.68 – provision of 0.68 m/heifer feed bunk space
TMR-S – provision of 0.34 m/heifer feed bunk space with straw
LS – long straw
SS – short straw
C – control treatment providing ad libitum access to high forage ration
LF – provision of high concentrate ration in limited amount
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CHAPTER 1: GENERAL INTRODUCTION

1.1 Factors Affecting Feed Intake

Feed intake and feeding behaviour are closely integrated aspects of cattle life. There are a number of metabolic and physiological factors that affect feed intake including sensory cues, gastrointestinal signaling, and hormones (Baile and Della Fera, 1981; Allen, 2000). Our increased knowledge of these factors has aided greatly in the improved management, health, and care of these animals.

When choosing which foods to consume, cattle primarily use their senses of sight, smell, and taste. Through post-ingestive feedback of consumed nutrients (or toxins), the animal will continue to feed or will cease feeding on a particular food (Provenza, 1995). For example, it is known that high energy foods are typically associated with a sweet flavour. Thus, when a sweet flavour is added to a feed, dairy cattle will increase consumption of that feed (Murphy et al., 1997), indicating that cattle are able to associate the high energy feed with the sensory cue of “sweet” flavour.

Gastrointestinal signalling may occur through hormones, such as cholecystokinin (CCK), opioid peptides, and volatile fatty acids (VFAs), or through rumen fill. CCK is known to control satiety whereas opioid peptides are key hormones in the control of hunger (Forbes, 2007). Injections of CCK have been shown to decrease feed intake in sheep (Della-Fera and Baile, 1980) whereas opioid peptides have been shown to stimulate feeding in sheep (Forbes, 2007). Neuropeptide Y (NPY) is a pancreatic polypeptide involved in stimulation of feeding. Miner (1992) reviewed studies finding that a bolus of NPY into the cerebral ventricles immediately stimulated feeding in sheep while continuous infusion slowly increased feeding. Overall, NPY can overcome the intake-reducing effects of ruminal distension and VFA infusion (Miner, 1992). Ghrelin, a peptide
produced predominantly in the abomasum, has been reported to stimulate feeding in ruminants through stimulation of NPY in the hypothalamus (Wertz-Lutz et al., 2006). Leptin, a polypeptide product primarily produced by adipose tissue, plays a key role in regulating energy intake and energy expenditure. Physiologically large amounts of leptin have been reported to decrease voluntary feed intake and there are indications of seasonal dependency (Roche et al., 2008). Intracerebral injections of leptin in sheep induced a 30% decrease in DMI in autumn, but not in the spring, suggesting an interaction of leptin with photoperiod (Miller et al., 2002, Adam et al., 2003).

Chemical receptors also play a role in signalling the central nervous system to cease feeding. Although the mechanisms by which VFAs influence feeding are not completely understood, it does appear that propionate plays a larger role than acetate or butyrate (Leuvenink et al., 1997; Sheperd and Combs, 1998). Insulin (initiates feeding) and glucagon (depresses feeding) secretion are stimulated by VFAs (Mineo et al., 1990) and by diet type and frequency of feeding (Sutton et al., 1988).

Within the GI tract, mechanoreceptors send signals to the central nervous system to initiate or terminate feeding (Leek, 1986) depending on distension in the rumen and/or physical fill in other areas of the GI tract (Allen, 1996).

Feed intake is ultimately controlled by the behaviour of the cow, which is further influenced by availability of feed, social environment, and internal state (i.e. nutrient requirements in the short- and long-term).

1.2 Feeding Behaviour of Dairy Cattle

Feeding behaviour of ruminants includes both ingestive and ruminating behaviour (Baumont et al., 2006). The observed feeding patterns of cattle throughout the day provide insight into how best to manage these animals and, perhaps, even what the
animals themselves desire. In this way we may be able to maximize production potential without compromising the welfare of the animals. Feeding behaviour is flexible in that different rations, management factors, and social environments can have an impact on the feeding patterns of the animals.

1.2.1 Diurnal Feeding Patterns

Under more natural conditions (i.e. pasture or rangeland), cattle exhibit a very distinct diurnal feeding rhythm. Periods of feeding and ruminating are interspersed with periods of resting and these three categories of behaviour comprise approximately 90-95% of the animal’s time (Kilgour, 2012). Gregorini (2012) describes the grazing pattern of ruminants as being composed of three to five grazing events. These events may merge as a result of an increase in duration of a particular feeding event on short days or, during long and warm days, events may become longer and fewer as well as become concentrated in the cooler parts of the day. Regardless, major grazing events occur early in the morning and late in the afternoon/early evening. There may be shorter and less intense grazing events occurring at night. Cattle spend the longest period of time feeding during the evening grazing event and this is when the majority of feed is consumed (Gregorini, 2012).

Kilgour (2012) breaks this diurnal pattern down further, describing how long cattle spend grazing, ruminating, and resting. During daylight hours, cattle may spend 4-9 h grazing but this period of time increases to 6-13 h over a 24 h period. Time spent ruminating ranges from 4.7-10.2 h over a 24 h period with the majority occurring during the night and when lying down. Finally, the average time spent resting ranges from 3.6-10.3 h daily. Feeding and ruminating events roughly alternate with each other throughout the day. This type of diurnal feeding pattern and feeding behaviour occurring in this
rhythm would be considered “normal” and reflects the needs and motivation of ruminants evolving over millennia to become efficient at gathering and digesting feed.

The above diurnal patterns are still observed, albeit to a lesser extent, in cattle that are intensively housed. The feeding patterns of these animals are influenced by many different management factors, including feed delivery and milking times (in lactating cattle). Individually housed cows in tie stalls tended to consume the majority of their feed during the day, with peak feeding periods occurring immediately after milking and feed delivery (Haley et al., 2000). This pattern has also been demonstrated in dairy cattle housed in free stall facilities (DeVries et al., 2003; Wagner-Storch and Palmer, 2003). Time of day does not have as great an influence on the diurnal patterns of intensively housed animals, although peaks in feeding activity are still observed around dawn and dusk in both young (Greter et al., 2008) and adult cattle (DeVries et al., 2003). Additionally, ad libitum-fed, intensively housed cattle only spend 3-5 h feeding each day, typically spread between 6-10 meals (Tolkamp et al., 2000; DeVries et al., 2003). This decrease in time spent feeding is likely due to the fact that intensively raised cattle that are not grazed on pasture do not require the same amount of time to harvest and manipulate their feed as this feed is usually mixed and/or processed for the animals.

1.2.2 Feed Delivery Methods

The most common method of feeding for dairy cattle in North America over 6 months of age (DeVries and von Keyserlingk, 2009) is to provide a total mixed ration (TMR), which is a homogeneous mixture of several different feed components (both grain concentrates and forages). The goal of a TMR is to minimize the selective consumption of particular feed types, thereby helping to promote intake of adequate fibre levels and nutrients to ensure proper rumen function (Coppock et al., 1981). Cattle are, however, adept at sorting more desirable dietary components out of a mixture, typically
selecting for short, highly fermentable particles and against long, fibrous particles (Leonardi and Armentano, 2003; DeVries et al., 2007). This sorting behaviour results in consequences on the health and productivity of the animals, regardless of age. If cows are consuming a ration that is higher in fermentable carbohydrates than was intended, they increase the risk of developing sub-acute ruminal acidosis (SARA; DeVries et al., 2008). In group-fed cattle this problem may be exacerbated by differing social status within the herd. Dominant animals, with access to the feed bunk immediately following feed delivery, may consume a ration that is higher in nutrients than intended while subordinate animals are left with a ration that is reduced in nutritive value (DeVries et al., 2005). Thus dominant animals increase their risk of developing metabolic disorders (Hosseinkhani et al., 2008) while subordinate animals may not be able to maintain adequate nutrient intake to meet their energy and nutrient requirements (Krause and Oetzel, 2006).

Young cattle are often fed a diet consisting of roughage (hay or silage) and a form of grain concentrate provided as a TMR, separate components, or concentrate top-dressed on roughage (Hutjens, 2004; Murphy, 2004). When young dairy heifers are fed separate components or a top-dressed ration, they will rapidly consume the grain portion of their ration in a few, large meals prior to consuming the forage portion of the ration (slug feeding; DeVries and von Keyserlingk, 2009; Greter et al., 2010). This slug feeding behaviour may result in significant post-prandial drops in rumen pH, leaving the animals susceptible to the development of SARA or problems with rumen fermentation (Quigley, 1992).

1.2.3 Social Factors

Most domesticated species of ruminant animals are kept in some form of social setting, whether in a barn, on pasture, or on rangeland. The behaviour of individual
animals affects the behaviour of conspecifics in both positive and negative ways. For example, older or more experienced individuals may pass nutritional wisdom (ability to select a balanced diet from various foods or situations) along to younger or naïve animals (Provenza and Balph, 1987). This wisdom can be highly beneficial for the development of feeding behaviour or patterns in that it may teach an animal what foods to consume and what foods to avoid as well as the best methods to achieve optimal feed intake. Animals kept in groups may, however, also engage in negative interactions with others through competition for feed and other resources. These agonistic interactions may result in injury to the animals involved or, with specific regard to feeding behaviour, may prevent an animal from consuming a sufficient amount of a particular nutrient or an otherwise unbalanced diet.

Social facilitation has been defined as an ‘increase in the frequency or intensity of responses, or the initiation of a particular response, when shown in the presence of others engaged in the same behaviour at the same time’ (Ralphs and Provenza, 1999). Foraging with experienced social partners decreases neophobia to particular foods and facilitates acceptance of novel food by naïve animals (Ralphs et al., 1994). Furthermore, cattle can learn to avoid harmful foods by observing conspecifics avoiding this food. For example, lambs generally avoided elm leaves if their mothers avoided them and consumed elm leaves if their mothers did (Provenza et al., 1993). Social facilitation is, in fact, one of the most powerful influences on feeding behaviour and can easily extinguish a previously learned aversion. Galef (1986) concluded that a rat ‘will abandon, to a greater or lesser extent, reliance on information it personally has collected concerning the value of a food, in favour of information it acquired from others’. This appears to be true with cattle as well. Through conditioning with lithium chloride (LiCl), heifers were trained to avoid eating larkspur and retained this aversion for two years. However, when
pastured with heifers that had not been conditioned, the conditioned heifers began sampling larkspur and the aversion was quickly extinguished (Lane et al., 1990).

Another social influence on feeding behaviour is the effect of the social hierarchy and/or competition on individual feeding bouts and intake. The establishment of a hierarchy among cattle takes little time and, in most cases, does not involve much physical contact (Bouissou et al., 2001). Furthermore, dominance relationships among cattle are extremely stable and may persist for several years (Bouissou et al., 2001). It is important to note, however, that dominance is an adaptive mechanism which can become maladaptive in certain circumstances, such as when the social structure of the group is frequently changed (i.e. typical practice in an intensively raised dairy herd). In these situations, dominant animals gain the advantage when feed availability is reduced (Manson and Appleby, 1990). Metz and Mekking (1978) found that even when food is permanently available and cows had access to food during the night, low ranking individuals consumed less feed and did not gain as well as more dominant animals. In lactating dairy cattle, competition can lead to rapid intake of the diet (Proudfoot et al., 2009), possibly resulting in the development of metabolic disorders (i.e. SARA) or problems with rumen fermentation (Stone, 2004). As previously discussed, competition at the feed bunk also causes problems with sorting behaviour, creating disparity in the nutritive value of the feed consumed by dominant or subordinate animals. These examples show that competitive interactions in intensive settings can be highly detrimental to both dominant and subordinate animals.

Competition for feed has also been observed in several studies with young cattle, both ad libitum- and limit-fed animals. DeVries and von Keyserlingk (2009) found that ad libitum-fed dairy heifers, when housed under conditions of high feed bunk competition, spend 10% less time feeding throughout the day than heifers with no feed bunk.
competition. Keys et al. (1978) also found that increasing stocking density within a pen, and consequently reducing feed bunk space, resulted in a linear decrease in time spent feeding. Longenbach et al. (1999) found a similar result when reducing feed bunk space from as much as 47 cm to 15 cm per heifer in a pen. In these situations, the heifers rarely decrease their DMI. Instead, they compensate by increasing the rate at which they eat, particularly during periods of peak feeding activity (i.e. immediately following feed delivery). This competition may also increase the day-to-day variation in meal patterns (meal duration and size), resulting in inconsistent patterns. Additionally, increased competition and resulting variation in feeding patterns may contribute to an increase in variability between individuals in weight gain and growth (Longenbach et al., 1999; Gonzalez et al., 2008).

It is necessary to develop management strategies that take advantage of the positive effects of nutritional wisdom and learning while minimizing or eliminating the negative consequences of social groups. A recent study by De Paula Vieira et al. (2010) found that pair-housed calves reduced their response to weaning (less vocalization, consumed more solid feed more quickly, etc.) and improved performance (greater weight gain) in comparison to calves housed singly. In a similar study, unweaned calves housed with a weaned older calf consumed more hay during the pre-weaning period, consumed more starter during the weaning and post-weaning periods and gained more weight throughout the study than groups comprised of only unweaned calves (De Paula Vieira et al., 2011). Finally, studies with sheep have shown that lambs that observed their mothers consuming grain readily consumed this grain 18 months later while naive lambs were much less likely to consume this grain immediately (Lynch and Bell, 1987). These studies indicate that housing young animals with one another and/or with an older, experienced animal (mother or conspecific) can have beneficial effects on feed
intake and acceptance of foods, thereby positively affecting performance. It is also
important for management strategies to target minimization of negative consequences of
social housing. When feed is spatially distributed or feeding barriers (Huzzey et al.,
2006) are designed so that high-ranking individuals cannot prevent low-ranking ones
from feeding, competitive interactions are minimized (Phillips, 2002). Cattle generally
prefer concentrates to forage and aggression may result as dominant cows attempt to
control a concentrate feeder (Phillips, 2002). Thus, the feeding of a TMR rather than
separate components of concentrate and roughage may be preferable. However, this
must be done only in situations where cattle may all feed simultaneously to avoid effects
of sorting and to minimize competition (Milinkski and Parker, 1991). Competitive
interactions for feed do not typically occur in grazing situations once a social hierarchy is
established, making management of pastured or extensively raised animals somewhat
tsimpler in this respect (Mendl and Newberry, 1997).

1.3 Feeding Behaviour and Health

Most animals will eventually go off feed when experiencing illness or disease and
this is often considered one of the clinical signs of illness. Abnormal feeding and drinking
behaviour are also considered to be indicative of more general malaise in animals and
may alert caregivers to potential problems (von Keyserlink and Weary, 2010). There
have been several recent studies demonstrating that changes in feeding behaviour may
help to identify which animals are at greatest risk of becoming ill in the future (Weary et
al., 2009). As feeding behaviour can be measured quite easily and accessibly with
commercially developed equipment (providing information on feeding frequency,
duration, and intake), it may prove to be an excellent means to identify animals at risk of
illness or disease in a commercial setting.
Sowell et al. (1998) found that healthy feedlot steers spent considerably more time at the feed bunk than ill steers and that a greater percentage of the healthy animals were present at the feed bunk immediately following feed delivery. Furthermore, these healthy steers spend more time at the feed bunk and also had more feeding bouts than sick animals (Sowell et al., 1999). Quimby et al. (2001) was able to detect morbidity in calves approximately 4 d earlier than clinical evaluations by monitoring feeding behaviour electronically at the feed bunk. And finally, Huzzey et al. (2007) used electronic monitoring of dairy cattle feeding behaviour to determine that cows spending less time feeding were more likely to develop metritis after calving. In that study, the differences in feeding behaviour between healthy individuals and those that ultimately developed disease were able to be detected up to 2 weeks before calving and before any clinical signs of infection.

It is clear that there is much potential for the use of feeding behaviour measures in the early detection of illness and disease. Further research is needed into the use of these measures as indicators of metabolic disease, such as ruminal acidosis or ketosis.

1.3.1 Feeding Behaviour and Metabolic Disorders

Feeding management practices that cause cattle to eat fewer and larger meals more quickly may be associated with an increased incidence of SARA (Krause and Oetzel, 2006). The ruminal pH declines following meals and the rate of decline increases as meal size increases and as dietary effective fibre concentration decreases (Allen, 1997). Competition at the feed bunk may further exacerbate this decline in rumen pH due to rapid intake of the ration (slug feeding; Proudfoot et al., 2009; Greter et al., 2010). Rapid consumption of feed often results in a decrease in salivary secretion due to decreased chewing, thereby diminishing the buffering capacity of the rumen (Beachemin et al., 2008). Large postprandial drops in rumen pH, resulting from this style of feeding
behaviour, increase the risk of development of SARA or the creation of problems with rumen fermentation (Stone, 2004). These problems may have a serious impact on the production potential, growth and development, and welfare of the cattle. Understanding the role of feeding management and its influence on feeding behaviour, as well as recognizing any early changes in behaviour, will aid greatly in the development of new management strategies aimed at preventing these problems from occurring.

1.4 Feed Restriction and Feeding Behaviour

Access to feed is restricted in many domesticated species to avoid obesity, to decrease feed costs and wastage, to improve feed and growth efficiency, and to ensure that body weights are conducive to good health and reproductive efficiency (D’Eath et al., 2009). Mammalian farm animals that are commonly restricted include: growing and gestating sows in the swine industry; backgrounding and finishing cattle in the beef industry; and lactating cows at dry-off and dairy heifers in the dairy industry. The method of feed restriction, quantitative or qualitative, will have different effects on the animals, both behaviourally and with respect to growth and performance. Perhaps the most important welfare (behavioural) issue arising from this feeding practice is the development of oral stereotypies, which are believed to reflect continued feeding motivation, lack of satiety, and may therefore indicate hunger, frustration, and/or boredom.

1.4.1 Quantitative Restriction

In general, quantitative restriction refers to feeding a nutritious food in a limited amount or providing ad libitum access to a food for a limited period of time. This is done for the reasons stated above and does not take into account the behavioural effects on the animals.
Gestating sows are typically feed restricted by offering a nutrient-dense ration in an amount that is approximately 60% of their ad libitum intake (Kyriazakis and Savory, 1997). Restricted-fed sows have been shown to increase feeding rate and decrease time spent feeding relative to ad libitum-fed animals (Terlouw et al., 1991; Bergeron et al., 2000). Furthermore, quantitatively feed restricted sows increase general activity, foraging-related oral behaviour, drinker use, and respond to operant conditioning tests regardless of time since last meal, all suggesting that these animals have not achieved satiety due to either metabolic hunger, physical hunger, or both (Lawrence and Illius, 1989; Terlouw et al., 1991; Robert et al., 1997; Bergeron et al., 2000). There are also situations where feed allowance appears to be ad libitum but where a nutrient is quantitatively restricted, accidentally or purposefully. For example, an ad libitum diet deficient in protein fed to growing pigs resulted in an increase in general activity, walking, and rooting of straw. These appetitive behaviours were interpreted as an indicator of unsatisfied hunger due to gut fill limiting sufficient intake of the deficient diet to meet protein requirements (Jensen et al., 1993).

Beef cattle are typically feed restricted at some stage of their growth, particularly when starting cattle on feed, backgrounding, and during the finishing period. This is often done to increase feed efficiency and to decrease feed wastage, feed costs, and nutrient excretion (Murphy and Loerch, 1994; Galyean, 1999). The concept of compensatory growth, wherein animals are given ad libitum access to feed following a period of feed restriction, is often utilized in growing beef cattle and may result in more efficient deposition of muscle mass (Galyean, 1999). Sainz et al. (1995) found that calves fed a restricted diet during the growing period were more efficient during the subsequent finishing phase than calves fed a high-forage diet ad libitum and also showed greater compensatory growth. Although feed restricting beef cattle is not a new concept, there is
very little information available on the behavioural differences associated with this practice relative to feeding a high-forage ration. However, Schwartzkopf-Genswein et al. (2002) found that in an industrial feedlot setting, beef cattle fed either ad libitum or restricted diets differed in their feeding behaviour with ad libitum-fed cattle attending the feed bunk for a longer period of time and visiting more frequently than restricted-fed cattle. Additionally, although ad libitum-fed cattle consumed more DM overall, restricted-fed cattle consumed more feed in the 3 h period immediately following feed delivery, a result further reflected in increased feeding rates of restricted-fed cattle (Schwartzkopf-Genswein et al., 2002). Overall, these researchers concluded that the restricted-fed cattle were likely to be more hungry and, thus, more anxious to consume a large amount of food quickly, leaving them vulnerable to development of metabolic disorders (Stone, 2004). Much more research is needed on the effects of quantitative restriction on the feeding behaviour of beef cattle as this practice likely has major welfare implications.

Quantitative restriction also occurs in the dairy industry, particularly with calves and growing heifers. Calves are rarely fed ad libitum in commercial production, with most receiving only 10-15% of their body weight, an amount that has been shown to be insufficient to satisfy hunger and ensure optimal growth and development (Jasper and Weary, 2002). Restricted-fed calves make many more visits to the milk feeder than ad libitum-fed calves, suggesting that they are still hungry and are clearly still motivated to feed (De Paula Vieira et al., 2008).

Limit feeding of dairy heifers is discussed in detail in section 1.5.2 below.

1.4.2 Qualitative Restriction

The term ‘qualitative restriction’ implies that a food is reduced in quality but is offered ad libitum (D’Eath et al., 2009). In this situation, we still consider the feeding
strategy as restricted feeding because the animal is consuming less energy and/or nutrients from a food that is of lower quality. The ration (and nutrient content of the ration) is usually diluted through addition of fibrous or bulky feedstuffs.

There is evidence to suggest that qualitative restriction does not reduce performance in comparison to ad libitum-fed animals. For example, Whittaker et al. (2000) compared the behaviour of gestating sows fed a restricted amount of a nutrient-dense pregnancy ration vs. a diet diluted with sugar beet pulp and fed ad libitum. Feeding regime did not affect backfat thickness, reproductive performance, or litter performance but the diet diluted with beet pulp did increase the costs for producers. Additionally, qualitative restriction has been shown to provide many behavioural benefits to the animals in that it allows them to increase or ‘normalize’ performance of feeding-related behaviours such as feeding rate, meal patterns, feeding time, and general activity (D’Eath et al., 2009). Robert et al. (1997) examined the feeding behaviour and feeding motivation of gilts when fed different high-fibre or high-concentrate diets. They found that gilts fed the bulky diets spent more time feeding, exhibited reduced heart rate responses to feeding, and had a reduced response to operant conditioning tests where animals could receive food rewards. Despite the strength of these findings, this feeding strategy must be used with caution. Providing the animals with a bulky diet may aid in increasing their feeding behaviour, but if gut-filling properties of the bulky food limit nutrient intake in such a way as to prevent satisfaction of nutrient requirements, then the animal may still experience ‘metabolic hunger’ (Kyriazakis and Emmans, 1995).

As the addition of bulky ingredients to a ration decreases the feed efficiency of cattle (Kitts et al., 2011), qualitative feed restriction is rarely employed with finishing beef cattle. However, cattle are often inadvertently faced with qualitative restriction through provision of poor quality roughage during the growing phase (Kyriazakis and Savory,
1997). Beef cattle grazing in upland, hilly areas, or rangeland, despite spending most of their active time feeding, are often unable to meet their nutrient requirements and may even move into a state of negative energy balance due to effort related to locomotion and grazing and the quantity and quality of herbage available (Oldham et al., 1993; Bailey, 2005). There is very little information available on the feeding behaviour of qualitatively restricted beef cattle due to lack of this practice in feedlots and the difficulty of observation in the field.

There has been little work on qualitative restriction with lactating dairy cattle since maximizing feed and nutrient intake, and subsequently maximizing milk production, is a major goal in the dairy industry. However, there has been some work with these cattle around the dry-off period. In an effort to decrease milk production, Valizaheh et al. (2008) altered the ration of lactating cows during the dry-off period by providing either grass hay or oat hay ad libitum. Dry matter intake declined when cows were fed either type of hay, but with a greater reduction when fed grass hay. This feeding strategy resulted in more frequent vocalizations, especially in cows fed oat hay, suggesting that these cows may be experiencing some distress, possibly due to hunger from this feedstuff not meeting their nutrient requirements (Valizaheh et al., 2008). Dairy cows grazed on pasture may experience seasonal fluctuation in quantity and quality of herbage available, thereby impacting the animals' feeding motivation. In this situation, although still fed ad libitum, cows have been shown to work for extra, nutritious food. Schütz et al. (2006) measured cows' motivation to walk for food at different levels of feed deprivation and found that feeding motivation increases within a few hours of feed deprivation, indicating that pasture conditions preventing the satisfaction of nutrient requirements likely result in hunger in these animals. Finally, there has been some work with qualitative restriction of growing dairy heifers. Greter et al. (2008) found that feeding
growing dairy heifers a ration containing either 10 or 20% straw altered their feeding behaviour in comparison to a control ration. Specifically, DMI, feeding rate, meal size, and meal frequency linearly decreased, while feeding time and meal duration linearly increased with increased straw in the diet. Additionally, requirements for growth of 0.9 kg/d were met even when the ration contained 20% straw (Greter et al., 2008). Kitts et al. (2011) also found that heifers fed a nutrient-dense ration with straw mixed into it (qualitative-style feed restriction) spent more time feeding, more time ruminating, less time unrewarded at the feed bunk, and less time inactively standing. Thus, incorporating straw into a nutritious ration for growing dairy cattle can result in a return to more natural feeding behaviours and patterns, particularly in comparison to quantitatively restricted heifers.

1.5 Heifer Management

As the future potential of any dairy operation lies in the efficient raising of dairy replacement heifers, the area of heifer management is gaining in popularity. In the past, heifers were largely overlooked on most farms as the rearing process provides little benefit to producers until animals reach lactating age, wherein they begin producing calves and milk for the farm (Hutjens, 2004). The costs associated with replacement dairy heifer rearing are second only to the feed costs of lactating cows, representing 15-20% of total farm expenses (Heinrichs, 1993). Thus, improper management from an early age may cause problems with growth and production in both the short- and long-term, thereby increasing the overall costs to the producer (Quigley, 1997). Feed costs for replacement animals represent over 50% of overall costs associated with raising these animals (Cady and Smith, 1996) and, therefore, improvements in feeding management and new feeding strategies will greatly aid in reducing rearing costs.
1.5.1 Feeding Strategies – Ad Libitum Feeding

Replacement dairy heifers have traditionally been provided with a high-forage, low-energy ration that may or may not be formulated to meet the nutrient requirements of growing animals, depending on individual farm management (MidWest Plan Service, 2003). This strategy has the potential to target ADG by controlling caloric intake and is usually provided to the animals ad libitum. However, as previously discussed, cattle fed ad libitum are capable of sorting through a TMR (Hoffman et al., 2006; Greter et al., 2008). As a result of sorting behaviour, the heifers may end up consuming a ration that is not balanced for their needs. Subsequently, it may be difficult to target actual growth rate, decrease feed efficiency, and increase the risk of metabolic disorders (DeVries, 2010).

Another strategy of ad libitum feeding involves formulating a nutrient-dense diet and then limiting the nutrient density of the feed (Hoffman et al., 1996) by diluting it with a low-nutritive feedstuff (i.e. straw). Additionally, a low-nutritive feedstuff will decrease passage rate and increase rumination time, thereby increasing saliva production and rumen buffering. Greter et al. (2008) found that adding straw to a TMR fed ad libitum resulted in a decrease in daily DMI, feeding rate, meal size, and meal frequency (as straw level increased) while feeding time and meal duration increased. This indicates that a diluted ration fed ad libitum helps to provide a foraging substrate for the heifers, thereby allowing them to meet their natural foraging needs. Heifers in this study were also able to achieve ADG of 0.9 or 1.0 kg/d (on 20 and 10% straw dilution, respectively), suggesting that, when the ration is balanced properly, this feeding strategy may enable producers to effectively target growth while reducing DMI and allowing heifers to engage in natural behaviours.
1.5.2 Feeding Strategies – Limit Feeding

In contrast to feeding an ad libitum diet, limit feeding consists of providing a nutrient-dense ration in a limited amount (discussed in detail in section 1.4.1). This ration contains high levels of grain concentrates and/or high quality forages that enable the animals to gain efficiently at a reduced DMI, thus decreasing the overall feed costs associated with heifer rearing (Hoffman et al., 2007; Zanton and Heinrichs, 2007). As a result of increased feed efficiency, limit-fed cattle also produce less manure and, consequently, less nitrogen is excreted; a public concern as excess nitrogen from intensive feeding operations leads to environmental contamination.

Zanton and Heinrichs (2007) found that, when fed a high concentrate diet (HC) versus a high forage diet (HF), Holstein heifers decreased their DMI by 0.64 kg/d. A follow-up study by this same research group found similar results, with heifers fed a HC diet consuming 0.18 kg/d less than heifers fed a HF diet (Lascano et al., 2009). When considering the number of heifers fed on a typical dairy operation in North America, the reduction in feed costs from feeding a high concentrate, limit-fed ration has the potential to be quite dramatic.

In addition to the decrease in DMI, average daily gain (ADG) may also be maintained. Indeed, ADG may be more easily controlled and growth rates more accurately targeted under a limit feeding regime. Zanton and Heinrichs (2007) found that there were no differences in ADG between heifers fed a HC or HF diet. Hoffman et al. (2007) also found no differences in ADG, body weight, hip height, heart girth, and body condition score in limit-fed heifers compared to ad libitum-fed heifers. Kitts et al. (2011) found that heifers fed a high concentrate TMR or a high concentrate TMR with free choice straw offered alongside of the TMR had increased ADG over heifers fed the
TMR with straw mixed into the ration (0.9 vs 0.8 kg/d). It is clear that a limit-fed diet can successfully control ADG, perhaps much more effectively than ad libitum feeding.

Since DMI decreases and ADG is maintained when heifers are provided a limit-fed ration, it follows that an increase in feed efficiency is observed in animals managed under this regime. This has long been established in beef cattle (Hicks et al., 1990; Loerch, 1990) with improvements in feed efficiency of over 15% observed in cattle fed a high-grain ration; an improvement that is primarily due to the increased digestibility of grains in comparison to forages (Klinger et al., 2007). Studies with limit-fed dairy heifers have also found improvements in feed efficiency. Hoffman et al. (2007) found an improvement of 28.9% in efficiency when comparing limit-fed and ad libitum-fed heifers; Kitts et al. (2011) found a feed efficiency of 6.3 (DMI/ADG) in limit-fed heifers versus 9.9 in heifers fed a TMR mixed with straw. Similar improvements in efficiency were also observed by Lascano et al. (2009) and Zanton and Heinrichs (2007). Alongside these results of improved feed efficiency, many researchers have found a decrease in total manure output. Lascano et al. (2009) reported a decreased total manure output in HC-fed heifers vs. HF-fed heifers. Similar results have also been found with beef cattle managed under a typical limit feeding program (Dreidger and Loerch, 1999). This decrease in fecal output translates to a decrease in nitrogen output as ruminant animals fed a low-forage diet have been shown to have improved nitrogen retention and efficiency (Driedger and Loerch, 1999; Moody et al., 2007, Zanton and Heinrichs, 2009).

Despite all the positive benefits to limit feeding dairy heifers, there are a number of behavioural and health concerns. Intensively housed and fed dairy heifers typically spend 3-5 h feeding each day (Greter et al., 2008; DeVries and von Keyserlingk, 2009) whereas limit-fed heifers often finished their ration within 1-2 h of feed delivery (Hoffman et al., 2007; Kitts et al., 2011). As natural foraging conditions allow for a further increase
in feeding time (4-9 h; Hafez and Bouissou, 1975), it seems that limit feeding poses a stark contrast to the feeding behaviour of cattle in more natural settings (“normal” behaviour). Provided that the ration is formulated properly, the animals are receiving sufficient nutrients to meet all metabolic needs and are considered metabolically satiated. However, they may not be experiencing sufficient rumen fill or allowed enough time or DM for feed manipulation (foraging needs) to be physically or mentally satisfied. Indeed, Lindstrom and Redbo (2000) found that, whether the rumen was full or not, lactating cows were motivated to spend time manipulating their feed, suggesting that cattle may have a ‘behavioural need’ to forage.

Researchers have also noted an increase in inactive standing time (standing without eating; Hoffman et al., 2007; Kitts et al., 2011). One study found an increase in vocalization levels in the heifers as well as an increase in aggressive ‘reaching’ (stretching of the neck and front legs) to acquire feed (Hoffman et al., 2007). These behaviours may be interpreted as indicators of hunger or frustration as a result of lack of satiety (Watts and Stookey, 2000; Valizaheh et al., 2008). There have also been a number of studies examining oral stereotypies, including tongue rolling, tongue playing, head nodding, and bar biting, associated with limit feeding (Redbo et al., 1996; Redbo and Nordblad, 1997; Lindstrom and Redbo, 2000), providing further evidence of frustration and hunger (Savory et al., 1993).

Potential health effects associated with limit feeding include increased potential for lameness or hoof pathologies and increased risk of metabolic disorders. As limit-fed heifers often stand for more time than ad libitum-fed heifers (Hoffman et al., 2007; Kitts et al., 2011), they are at increased risk of hoof pathologies, particularly when housed in barns with hard flooring surfaces (Cook et al, 2004; Vanegas et al., 2006). There have been no studies to examine the relationship between inactive standing resulting from
limit feeding and the development of lameness and further research is needed on this issue.

Sub-acute ruminal acidosis is a metabolic disorder affecting ruminants, particularly those fed a low-forage, high-energy diet in a situation where the rumen environment has not adapted sufficiently to the feed type (Kleen et al., 2003). This situation results in high acid production, causing the rumen pH to decrease to a range of 5.6 to 5.2, which is considered sub-acute (Plaizier et al., 2008). As limit-fed rations consist of rapidly fermentable carbohydrates and/or highly digestible forages as well as a low forage:concentrate ratio, these animals may be more susceptible to development of this disorder. When limit-fed heifers were fed a HC diet, there was a significant decrease in rumen pH levels conducive to the development of SARA (Moody et al., 2007). Heifers in that study were clearly experiencing larger within-day bouts of SARA when fed a HC diet in comparison to heifers fed a HF diet. Along with the short-term detrimental effect on health and production, SARA has the potential to cause long-term problems, particularly when experienced early in life. Ruminal acidosis may cause shedding of the stratum corneum (the protective barrier between the rumen environment and portal circulation) (Steele et al., 2009) which can cause rumen microbes to leak into the body's circulation, leading to liver abscesses and other immunological problems (Nocek, 1997).

A further detrimental effect of long-term high-grain feeding is that this feeding regime may cause excessive keratinization of the epithelium which can lead to parakeratosis; thereby limiting the long-term absorptive capacity of the rumen (Steele et al., 2009). It is clear that the long-term effects of limit feeding on rumen health must be investigated in an effort to ensure proper health and welfare of these animals.
1.6 Preferences and Motivation

Designing appropriate environments and feeding management strategies for domesticated animals is greatly aided by understanding what the animals themselves prefer or desire.

1.6.1 Preference Tests

Herbivores in free-ranging situations are able to successfully select a diet from available choices (Dumont and Gordon, 2003). Intensified livestock, however, are often provided with either a few feedstuffs combined into a homogeneous mixture (TMR) or with a field or feed bunk providing a few different types of feed that the animal may choose from (Rutter, 2006). Understanding the preferences that an animal has for a particular food can be helpful in considering the dietary needs of our domesticated animals. The term ‘preference’ indicates a difference between the strength of motivation to obtain or avoid one resource and the strength of motivation to obtain or avoid another (Kirkden and Pajor, 2006).

Preference tests, with reference to feeding behaviour, determine the proportions of 2 or more different foods that are consumed by the subject (D'Eath et al., 2009). Many believe this technique to be a direct way of asking what it is that animals want to consume (Rushen et al., 2008). Additionally, it has been suggested that animals will select an appropriate diet from different food sources by learning to associate the sensory properties of the food with the post-ingestive consequences of that food (Provenza, 1995). In this way, they can develop a preference or aversion for a particular food over another due to the food inducing either satiety or malaise. For example, when given preloads of energy or nitrogen, lambs prefer flavours previously paired with nitrogen or energy, respectively, during subsequent meals (Villalba and Provenza, 1999). Lambs in this experiment seem to show preference for the feedstuff that will help
to balance their metabolic state. Preference tests may be used with much success but certain limitations must be kept in mind. Although one food source may be preferred over another, the choices are limited to those provided or available to the animals (Kirkden and Pajor, 2006). Tests reflect the rank that animals give certain options but do not tell us anything about the strength of those preferences. We may try to quantify this through total intake of a food source but, as different foods vary in nutrient content, this may not provide the full story of the preference. The choices that an animal makes may be related to previous experience. Herbivorous animals are notoriously neophobic and, therefore, will select a food that they have encountered in the past rather than a novel food, regardless of whether the novel food is higher in nutritive properties (Provenza, 1995). Preferences may also vary depending on the circumstances surrounding the animal’s behaviour; for example, a hen may prefer different environments depending on whether she is foraging, laying, or resting (Fraser, 2008). Finally, social facilitation can affect the preferences of animals, particularly herd species that operate within a social hierarchy and/or with a leader-type animal (Ralphs and Provenza, 1999). Chapple et al. (1987) found that sheep unfamiliar with wheat consumed wheat much more readily when exposed to the feedstuff with experienced sheep, suggesting that the preference was developed at least partially due to social facilitation. Preference tests may still tell us much about the options that animals would choose for themselves, but we must recognize these caveats when interpreting results from a preference test.

1.6.2 Feeding Motivation

Motivation is defined as ‘the process within the brain controlling which behaviours and physiological changes occur and when’ (Fraser and Broom, 1990). Kirkden and Pajor (2006) further suggest that motivation reflects subjective experiences. For example, a motivation to feed is said to be accompanied by the subjective experience of
hunger. Motivations can vary in strength and, if the motivated behaviour is thwarted, may have a negative impact on the animal's welfare. Motivation testing is often deemed to be more powerful than preference testing because it tells us the strength of a preference (Kirkden and Pajor, 2006).

Specific to feeding behaviour, we may measure feeding motivation with feeding rate, compensatory feeding tests, and the aforementioned operant responding tests for food (D'Eath et al., 2009). Feeding rate generally increases with increasing food deprivation; thus, an alteration of feeding behaviour (increase in feeding rate in feed deprived animals) may indicate a high motivation to feed and may suggest negative affect in the form of hunger. However, lower quality foods cannot be ingested as quickly as high quality foods so this measure of feeding motivation must be used with caution. Compensatory feeding tests involve a situation where previously restricted animals are allowed ad libitum access to food and their total intake during a compensatory feeding period is used as a measure of the hunger that resulted from the restriction period, reflecting the feeding motivation of the animal (D'Eath et al., 2009). Here, animals may be limited by gut fill and this type of test must also be used with caution.

Perhaps the simplest approach to discovering how motivated an animal is to obtain a resource is to train the animal to perform an instrumental task (i.e. pushing a lever, running down a runway, pushing a door) in order to gain the resource. If testing elicits a response from the animal, then a motivation is said to exist. We can further quantify the strength of the motivation by measuring the number of responses or by increasing the amount of work that an animal must do (increase the amount of effort required or alter the fixed ratio schedule) to gain the reward and measuring this work until the animal no longer performs (Kirkden and Pajor, 2006).
Dawkins (1990) has proposed an approach to motivation testing that is described in the language of microeconomics. Consumers will continue to purchase certain commodities despite price increases (inelastic demand) whereas they will forego the purchase of other commodities as price increases (elastic demand). Elasticity of demand may be able to show us how important different environments or commodities are to animals (Dawkins, 1990). With respect to motivational analysis, a commodity, such as food, may be provided in response to some work ('price') that an animal performs. This price can then be varied experimentally to determine the price elasticity of the demand for food. Alternatively, the animal can be given a limited amount of time ('income') in which to access different resources. This amount of time can be varied experimentally to determine the income elasticity of the demand. In general, commodities that are important to the animal should show little price or income elasticity. More simply, an animal’s willingness to pay for a resource can be measured by determining the highest price that the animal will pay for a unit of the resource (reservation price) (Fraser, 2008). For example, Verbeek et al. (2011) found that feed restricted ewes worked for a higher number of rewards, walked a greater total distance, and approached the feeding station faster than ewes fed ad libitum, suggesting increased feeding motivation reflected in increased work performed by the restricted animals.

### 1.6.3 Frustrated Motivation and Stereotypies

Restricted feeding and feeding motivation cannot be discussed without the mention of oral stereotypies. Briefly, stereotypies are behaviours that are regularly repeated in a very similar way and lacking an obvious function (Kyriazakis and Savory, 1997). Pigs will express a range of stereotypic activities including bar-biting, chain-manipulation, excessive drinking, and sham chewing (Fraser, 1975; Terlouw et al., 1991). Cattle express many different stereotypies as well, including head nodding, bar-
biting, cross-sucking, and tongue rolling or playing (Rushen et al., 2008). It is widely believed that these oral-type stereotypies represent the expression of foraging behaviour, modified by physical constraints of the environment (Lawrence et al., 1993). Furthermore, they likely arise as a result of chronic frustration (Wiepkema et al., 1987), lack of stimulation inducing boredom (Wemelsfelder, 1990), or lack of satiety due to metabolic or physical hunger (Lindstrom and Redbo, 2000; Rushen et al., 2008). These types of behaviours are substantially reduced if suitable substrates are provided to which foraging behaviour of feed restricted animals can be directed (Spoolder et al., 1995). Thus, they are more commonly seen in quantitatively restricted animals. Indeed, feeding motivation has been demonstrated as having a major role in the development of stereotypies in farm animals as these behaviours are typically preceded or succeeded by feed-searching behaviours (Redbo, 1990, 1992; Bergeron et al., 2006). Adding long straw to the ration of dairy heifers has been shown to decrease tongue rolling, possibly due to satisfaction of their foraging needs (Redbo and Nordblad, 1997). Similarly, providing straw on the pen floor of housed sows appears to serve as a rooting and foraging substrate, consequently decreasing oral stereotypies in sows (Fraser, 1975).

Although the presence of oral stereotypies may themselves indicate poor animal welfare, it is possible that animals expressing these behaviours may be self-soothing or coping in some way (Rushen et al., 2008). For example, veal calves that developed tongue rolling stereotypies did not have abomasal ulcers or erosions whereas all calves that did not develop these behaviours had ulcers or scars (Wiepkema et al., 1987). Clearly this is an area requiring future research as the link between oral stereotypies and gastrointestinal health is not well established.
1.7 Thesis Objectives

This review has explained what is known regarding the feeding behaviour of dairy cattle, the practice of restricted feeding, management strategies for replacement dairy heifers, and the measurement of preference and motivation. There is a need to understand the relationship between restricted feeding strategies, hunger, and feeding motivation in limit-fed dairy heifers. Thus, the overall objective of this dissertation was to determine the feeding motivation of dairy heifers and to determine management factors that contribute to feeding behaviour in limit-fed dairy heifers. This objective was addressed using six experiments, with individual objectives as follows:

1) To describe the diurnal feeding patterns of dairy heifers fed ad libitum and compare these to those of heifers fed a limited amount.

2) To determine the effect that frequency of feed provision, fed in a limited amount, has on the feeding behaviour patterns.

3) To examine the feeding and competitive behaviour of limit-fed dairy heifers when provided with an increase in feed bunk space and to determine if the effects of provision of extra bunk space would be comparable to those seen when limit-fed heifers are provided a low nutritive feedstuff.

4) To determine the interactive effect that feed bunk space and frequency of feed provision, fed in a limited amount, has on the feeding behaviour patterns and growth rates of growing dairy heifers.

5) To determine whether limit-fed heifers are motivated to consume large particles of a low-nutritive feedstuff (i.e. straw) in order to alleviate health concerns and foraging needs.

6) To determine how motivated limit-fed heifers are, in comparison to ad libitum-fed heifers, to work for extra feed (straw) using operant conditioning.
CHAPTER 2: RATION COMPOSITION AFFECTS SHORT-TERM DIURNAL FEEDING PATTERNS OF DAIRY HEIFERS

2.1 Introduction

Replacement dairy heifers are normally fed a high-forage, moderate-energy diet to meet their nutrient requirements and control their average daily gain (ADG; DeVries, 2010; MidWest Plan Service, 2003). As a result of rising feed costs and the need for increased efficiency, there has been interest in feeding heifers a limited amount of a nutrient dense diet (often referred to as limit feeding) resulting in similar total intake of nutrients but consumed in a much smaller daily amount and shorter period of time (Hoffman et al., 2007). A number of researchers have shown that this strategy can be used to effectively target ADG in replacement heifers, while reducing feed costs, decreasing fecal excretion, and increasing feed efficiency (Hoffman et al., 2007; Kitts et al., 2011; Lascano et al., 2009).

Despite the perceived benefits of limit feeding, this feeding management regime poses a number of behavioural concerns. Researchers have recently shown that limit feeding reduces feeding and lying time, resulting in animals spending more time standing while not eating, or inactively standing (Hoffman et al., 2007; Kitts et al., 2011). It has been suggested that inactive standing may increase the potential for development of hoof pathologies, possibly resulting in lameness (Cook et al., 2004; Vanegas et al., 2006). Hoffman et al. (2007) also found that limit feeding increased vocalization levels in heifers. Furthermore, limit feeding dairy cattle has also been associated with increased levels of oral stereotypies, including tongue rolling, head nodding, and bar biting (Lindstrom and Redbo, 2000; Redbo et al., 1996). Changes in behaviour associated with limit feeding may be attributed to frustration or hunger due to a lack of satiety resulting
from feed only being available for a short duration and in a limited amount (Lindstrom and Redbo, 2000; Savory et al., 1993).

When dairy heifers are limit-fed (at 2.05% of body weight or less), their daily allotment of feed is typically consumed within 2 h after feed delivery (Chapter 4; Hoffman et al., 2007; Kitts et al., 2011). This raises concern as this feeding pattern differs greatly from more natural grazing conditions, where cattle will engage in foraging behaviour for 4 - 9 h per day, distributing their feeding time across the day, resulting in a distinct diurnal feeding pattern involving several small, short meals (Albright, 1993; Hafez and Bouissou, 1975). It is clear that such limit feeding strategies fail to allow the animals to spend an adequate amount of time engaging in foraging behaviour. Alternatively, researchers have recently shown that providing a low-nutritive feedstuff, such as straw, alongside a limit-fed diet offers the potential to help minimize these negative effects (Kitts et al., 2011). Specifically, providing dairy heifers straw in addition to a high-concentrate ration fed in a limited amount increased feeding time and decreased inactive standing, resulting in a feeding pattern that more closely resembles natural grazing behaviour (Chapter 4; Kitts et al., 2011). Kitts et al. (2011) utilized 28-d periods in that experiment, providing ample time for heifers to acclimate to treatment rations, both behaviourally and ruminally. To date there have been no shorter-term, direct comparisons of the diurnal feeding patterns of intensively-housed and -fed growing dairy heifers provided rations of varied composition and fed in different amounts. Thus, the objective of this study was to describe the short-term (first week of adaptation to a new ration) behavioural feeding patterns (including diurnal feeding patterns; feeding behaviours such as feeding time, feeding rate, DMI, and meal patterns; and nutrient intakes) of dairy heifers when provided ad libitum access to a low-concentrate, high-forage ration and compare these to when heifers are provided a high-concentrate ration.
in a limited amount, followed by ad libitum access to chopped wheat straw. We hypothesized that heifers fed a low-concentrate, high-forage ration would, contrary to heifers provided a high-concentrate ration in a limited amount followed by ad libitum access to chopped wheat straw, exhibit diurnal feeding patterns similar to those observed for grazing heifers, distributing their feeding time and intake throughout the day by consuming more frequent, smaller, and shorter meals.

2.2 Materials and Methods

2.2.1 Animals and Housing

Twelve Holstein dairy heifers that were 203.9 ± 15.2 (mean ± SD) d old, had a BW of 225.1 ± 16.8 kg, and measured 118.6 ± 3.2 cm high at the rump at the beginning of the experimental period were used in this study. Heifers had a BW of 233.9 ± 16.7 kg and measured 119.8 ± 3.6 cm high at the rump at the end of the experiment. Heifers were housed in the heifer research barn at the University of British Columbia Dairy Education and Research Centre (Agassiz, BC, Canada) and were managed according to the guidelines outlined by the Canadian Council on Animal Care (2009). Use of animals was approved by the University of British Columbia’s Animal Care Committee (#A06-1562). The experiment was conducted between March and April 2010. Heifers were housed in a pen with a sawdust bedded-pack area (9.2 x 9.0 m; width x depth) and a standing alley (9.2 x 3.05 m) that divided the pack from the feeding area. Bedding material was replenished as needed. Feed was provided using six roughage intake control feed bins (Insentec B.V., Marknesse, the Netherlands). Each heifer was assigned and trained to eat exclusively from one feed bin. Water was available ad libitum in two water bowls located in the pen.
2.2.2 Experimental Design and Diets

The number of replicates required per treatment was determined through power analysis (Morris, 1999) for the primary response variables, including DMI and feeding time. Estimates of variation for these variables were based on previously reported values ( DeVries and von Keyserlingk, 2009; Kertz and Chester-Jones, 2004). Heifers were divided into two groups of six and assigned, in two sequential replicates, to each of two dietary treatments using a crossover design. Four days before exposure to the treatment diets, the first group of six heifers were housed together and trained to access their assigned feed bin. During this training period all heifers were fed a high-forage ration ad libitum (Table 2.1), as per the research centre standard replacement heifer feeding protocol. Heifers were then exposed to each of two dietary treatments (Table 2.1), three heifers per treatment, in a random order over two successive 7-d treatment periods. This included a 2-d adaptation period and a 5-d data collection period. Following completion of the first replicate, a second group of six heifers entered the experiment following the same procedure as described for the first replicate. Treatment diets were: 1) low-concentrate total mixed ration (TMR) fed ad libitum (LCR), and 2) high-concentrate TMR fed in a limited amount (2.0% of BW) followed by ad libitum access to wheat straw after the TMR was consumed (HCR+S). These rations were formulated according to the National Research Council’s (2001) nutrient requirement recommendations for a 225 kg nonbred Holstein heifer targeted to grow at 0.9 kg/d. To ensure that heifers on the HCR+S treatment were able to achieve satiety, they were provided ad libitum access to chopped wheat straw after they had finished consuming the HCR+S ( Kitts et al., 2011). The ingredient and chemical composition, as well as particle size distribution, of the treatment rations and the forages are provided in Tables 2.1 and 2.2, respectively. Orts were removed at 07:30 h and heifers were fed at 08:00 h daily with bins containing the
HCR+S checked at half-hour intervals to determine complete consumption of the TMR, at which time straw was added to the bin.

2.2.3 Experimental Measurements

Insentec feed bins (Insentec B.V., Marknesse, the Netherlands), previously validated by Chapinal et al. (2007), continuously measured the feeding behaviour and individual intakes throughout the course of the study. From the recorded data, the duration of each visit to the feed bin, the amount of feed consumed (start weight-end weight) during each visit, and the rate of consumption for each visit were determined. These data were then summarized to calculate daily DMI (kg/d), daily time spent feeding (min/d), and average feeding rate (kg/min). Visits to the feed intake system were separated into meals using an individual meal criterion (minimum time interval that a heifer is away from the feed bin to be considered a meal). Meal criteria were determined for TMR and straw consumption using a software package (MIX 3.1.3; MacDonald and Green, 1988) that fitted a mixture of normal distributions to the distributions of log$_{10}$-transformed time intervals between the recorded periods of time spent feeding. A bimodal normal distribution was fitted to the distribution of intervals for each calf in each week. As described by (DeVries et al., 2003), the meal criterion was determined from these modeled distributions as the time point at which the distribution curve of inter-meal intervals intersects the distribution curve of intra-meal intervals. The meal criteria were calculated to be 33.3 min for TMR consumption on both treatments and 132.4 min for straw consumption. Animals consume TMR and straw differently due to the different physical and chemical properties of each feed type (Beauchemin et al., 2008) and, as a result, the meal criteria may differ, as found, between these feeds. Using these criteria, meal frequency, duration, and size were calculated.
Standing and lying behaviour data of individual heifers were obtained via electronic data loggers (HOBO Pendant G Data Logger, Onset Computer Corporation, Pocasset, MA, USA), previously validated by Ledgerwood et al. (2010). The loggers were attached to the hind leg of each heifer using veterinary bandaging (Vetrap Bandaging Tape, 3M, St. Paul, MN, USA) and measured the orientation of the leg at 1-min intervals. These data were summarized to calculate the average daily lying time and inactive standing time (i.e. daily standing time – feeding time) for each heifer.

2.2.4 Feed Sampling and Analysis

Representative samples of the treatment rations, straw, and orts (if any) were taken daily throughout the experiment for dry matter (DM) and chemical analysis. Duplicate samples of the treatment rations, straw, and orts (if any) were taken on d 2, 4, and 6 of each treatment period for particle size separation. Samples of the dietary components were also taken once a week for DM and chemical analysis, with duplicate samples of forage components being taken at that time for particle size separation. All samples were immediately frozen at -20°C until time of analyses.

Samples for particle size separation (TMRs, forage components, straw, and orts) were separated using the three-screen (19, 8, 1.18 mm) Penn State Particle Separator (PSPS; Nasco, Fort Atkinson, WI, USA; Kononoff et al., 2003). Samples were separated into four fractions: long (>19 mm), medium (<19, >8 mm), short (<8, >1.18 mm), and fine (<1.18 mm) particles. After separation, the DM of each separated fraction was determined by oven drying at 55 °C for 48 h. Dry matter content of samples taken for chemical analysis was determined by drying samples in a forced-air oven at 55 °C for 48 h. These samples were then ground to pass through a 1-mm screen (Brinkmann mill, Brinkmann Instruments Co., Westbury, NY, USA). All ground samples were then

2.2.5 Calculations and Statistical Analysis

Preliminary inspection of the data revealed that all dependent variables were normally distributed. A preliminary analysis of the effect of day within treatment period was conducted for the DMI, feeding behaviour, and lying behaviour data. For this analysis, data were analyzed by period using the MIXED procedure of SAS (SAS Institute, 2003). The model included the fixed effect of treatment, day, and treatment by day interaction, the random effects of replicate and heifer within replicate, and the residual error. This analysis revealed a day effect; visual assessment of the data suggested that variability within treatments was greatest in the first 2 d of each treatment period, suggesting heifers were acclimating to their treatment rations. Due to this variability, the first 2 d of data from each period were excluded; further analysis of the last 5 d of each treatment period revealed no day effect. Thus, only data from the last 5 d of each treatment period were considered in further analyses. These data were averaged by treatment period for each heifer. To test for the effect of treatment, data were analyzed using the MIXED procedure of SAS (SAS Institute, 2003). The model included the fixed effect of treatment, period, and treatment by period interaction, the random effects of replicate and heifer within replicate, and the residual error.
Data for DMI, feeding time, and feeding rate were also summarized on an hourly basis for each animal on each treatment. Differences among treatments in the distribution of these variables over a 24-h period were analyzed using the MIXED procedure of SAS treating hour as a repeated measure. The model included the fixed effects of hour, treatment, and hour by treatment interaction, the random effect of replicate, and the residual error. Heifer within replicate was included in the model as the subject of the repeated statement. Compound symmetry was selected as the covariance structure on the basis of best fit according to Schwarz’s Bayesian information criterion. All values reported are least squares means. Significance was declared a $P \leq 0.05$, and a trend was reported if $0.05 < P \leq 0.10$.

2.3 Results

Daily DMI was greater when heifers consumed the LCR than when they consumed the HCR+S (Table 2.3) and this translated into greater intakes of crude protein (CP), acid detergent fibre (ADF), neutral detergent fibre (NDF), non-fibre carbohydrates (NFC), total digestible nutrients (TDN), metabolizable energy (ME), net energy of maintenance ($NE_M$), and net energy of gain ($NE_G$). Although feeding rates were similar between the two treatments, heifers fed the LCR spent more time feeding per day (Table 2.4). When fed the HCR+S, heifers altered their feeding behaviour depending on the type of feed provided (TMR or straw). On average, heifers spent more time feeding, consuming the DM at a higher rate, when eating the TMR than when consuming the straw (Tables 2.5 and 2.6), resulting in greater DMI of the TMR than the straw. Heifers spent more time lying down and less time standing inactively when consuming the LCR compared to the HCR+S (Table 2.4).
Analysis of the diurnal pattern of feeding behaviour showed a treatment by hour interaction in DMI (SE = 0.01, \(P < 0.001\); Figure 2.1A), indicating that treatment differences in hourly DMI were affected by time of day. Of note is the peak in DMI by heifers on the HCR+S immediately following feed delivery and the consistently greater DMI of heifers fed the LCR in the hours following the initial peak after feed delivery. Similarly, we observed a treatment by hour interaction for hourly feeding time (SE = 0.4, \(P < 0.001\); Figure 2.1B), indicating that treatment differences in hourly time spent feeding were affected by the time of day. A treatment by hour interaction for feeding rate was also noted (SE = 0.002, \(P < 0.001\); Figure 2.1C). Of particular interest, was that the heifers on the HCR+S had a greater feeding rate when consuming the TMR compared to when the heifers were fed the LCR. Feeding rate declined when heifers fed the HCR+S had finished the TMR portion of the ration and began consuming the straw, but remained relatively consistent over the course of the day when they were provided with the LCR.

When fed the LCR, heifers consumed a greater number of meals per day, albeit of smaller size and shorter duration, which translated into an overall reduction in total daily meal time compared to when they were fed the HCR+S (Table 2.4). Although the duration of the first meal was similar between the two treatment groups, heifers fed the HCR+S consumed more feed during the first meal than heifers fed the LCR (Table 2.4). There was a period by treatment interaction for both first meal length (SE = 10.2; \(P = 0.02\)) and first meal size (SE = 0.18; \(P = 0.05\)), suggesting that order of treatment had an effect on meal patterns. Heifers initially fed the HCR+S had shorter first meal lengths than heifers fed the HCR+S after being on the LCR (102.6 vs. 125.3 min/meal), whereas heifers initially fed the LCR had longer meals than heifers fed the LCR after initially being fed the HCR+S (129.5 vs. 85.6 min/meal). Heifers initially fed the HCR+S had smaller first meals than heifers fed the HRC+S after being on the LCR (4.0 vs. 4.4 kg/meal),
whereas heifers initially fed the LCR had larger meals than heifers fed the LCR after initially being fed the HCR+S (2.5 vs. 2.1 kg/meal). The differences in meal patterns between the two treatments may be better understood when considering the TMR and straw portions of the HCR+S treatment separately (Table 2.6). Overall the number of meals per day was similar between the TMR and straw components of the HCR+S treatment, but the TMR meals were larger in size in comparison to the straw meals. Additionally, meals were shorter in duration when heifers were consuming the TMR portion of the HCR+S and, consequently, the proportion of total daily meal time spent consuming the TMR portion was less than that on the straw.

2.4 Discussion

There is an increasing body of knowledge regarding the diurnal feeding patterns and feeding behaviour of adult dairy cattle (DeVries et al., 2003; Nikkhah et al., 2011). However, the behaviour patterns of young dairy cattle are not yet well understood. This study was conducted to better understand the effect of ration composition on the behavioural patterns of growing dairy heifers. In support of our hypothesis, heifers fed the LCR distributed their feeding activity throughout the day and exhibited diurnal feeding patterns that were more similar to those observed for grazing heifers compared to when heifers were fed the HCR+S. Ad libitum-fed dairy heifers typically show a peak in feeding activity immediately after feed delivery followed by additional feeding activity throughout the day, resulting in approximately 3 to 5 h per day spent feeding (DeVries and von Keyserlingk, 2009; Greter et al., 2010), similar to that observed under natural (‘grazing’) conditions (Hafez and Bouissou, 1975). In contrast, heifers provided the HCR+S showed a dramatic peak in feeding activity immediately following delivery of the TMR, followed by a dramatic decline in feeding activity for the remainder of the day. This feeding pattern is consistent, and not surprising, given previous work with limit-fed dairy
heifers (Chapter 4; Kitts et al., 2011). Limit-fed heifers appear to be highly motivated to consume the TMR portion of the ration compared to straw (Chapter 4; Kitts et al., 2011). This pattern results in a very large TMR meal being consumed immediately following feed delivery. Consumption of straw is considerably lower in comparison, likely due to the fibrous nature of the feedstuff and also, possibly, to lower palatability of straw (Baumont, 1996; Beauchemin et al., 2008). In contrast, heifers fed the LCR had the same ration available throughout the day, and were therefore less motivated to consume the entire ration immediately following feed delivery. This was reflected in a more consistent rate of intake, as well as consumption of smaller and shorter meals throughout the day.

Despite having ad libitum access to straw after consuming the HCR+S, heifers consumed less DM per day when fed the HCR+S compared to when provided the LCR. The high fibre content of the straw likely contributed to rumen fill (distension of the rumen), which may have decreased the motivation to continue feeding (Allen, 1996; Campling and Balch, 1961). Additionally, straw may be an unpalatable feedstuff for cattle (Baumont, 1996), further reducing the motivation to consume this feed. Thus, heifers on the HCR+S spent less overall time feeding throughout the day. Some insight into why this was observed can be obtained when the feeding patterns for the two components of the HCR+S (i.e. TMR or straw) are looked at independently. When provided the HCR+S, heifers consumed the TMR portion of the ration at a higher rate of intake compared to when they consumed the straw, suggesting that they were more motivated to consume the TMR. Similarly, Kitts et al. (2011) reported that when heifers were offered straw alongside of a limit-fed TMR, they consistently consumed all of the TMR before beginning to consume straw. In the present study, the heifers on the HCR+S slowed their rate of intake once they transitioned from consuming the TMR to
the straw. Not surprisingly, meals were smaller and longer when heifers fed on the straw portion of the ration, likely due to the fibrous nature of the feedstuff requiring the animals to spend more time chewing than when consuming the TMR (Yang and Beauchemin, 2006).

Heifers on the LCR consumed more daily DM and, consequently, more CP, ADF, NDF, NFC, TDN, ME, NE\textsubscript{M}, and NE\textsubscript{G}. Unfortunately, this feeding strategy resulted in the heifers exceeding the daily requirements for dairy heifers averaging 225 kg in weight with a target growth rate of 0.9 kg/d (NRC, 2001). In contrast, heifers on the HCR+S were well within the range of acceptable nutrient intake for heifers of this size and targeted growth rate. This experiment consisted of 7-d treatment periods, which were too short to provide reliable growth measurements for the heifers on the treatment rations. However, given that the heifers on an ad libitum LCR would be predicted to grow at a faster rate than heifers on a HCR+S, it could be speculated that it would be more difficult to target a specific growth rate in heifers fed this type of ration. There are feeding strategies that can help to better target growth rates in high-forage fed heifers. For example, diluting the high-forage ration with a low-nutritive feedstuff (i.e. straw) can decrease nutrient intake at a level sufficient to prevent over-conditioning of animals while still maintaining necessary nutrient requirements (Greter et al., 2008). Further, high-forage rations may be control-fed in such a way as to limit excess growth (Zanton and Heinrichs, 2007), although this feeding strategy may be accompanied by negative consequences on the feeding behaviour and welfare of the animals, albeit to a lesser extent than limit feeding of a high-concentrate ration.

Heifers on the LCR maintained consistent meal patterning throughout the day, consuming a greater number of smaller, shorter meals in comparison to when they were fed the HCR+S. Despite heifers on both treatments spending a similar amount of time
consuming their first meal after feed delivery, heifers on the HCR+S consumed more DM during this meal, providing further evidence that they were highly motivated to consume the TMR portion of the ration. In fact, the entire TMR portion of the HCR+S was rapidly consumed in one or two large meals immediately following feed delivery. Consumption patterns of this nature have the potential for negative rumen health effects. Rapid consumption of such a ration may result in large post-prandial drops in rumen pH immediately following TMR consumption (Quigley, 1992), potentially causing some temporary discomfort in these animals (Paton et al., 2006; Webster, 1986). The consumption of straw following the TMR portion of the daily ration may have mitigated any significant reduction in pH by stimulating an increase in salivary secretion (Beauchemin et al., 2008) and rumination (Beauchemin and Buchanan-Smith, 1990). Unfortunately, we were unable to measure any of these potential rumen health effects, and thus encourage future research in this area to validate these hypotheses.

Of note, provision of straw alongside of a limit-fed TMR has been shown to be an economical alternative to feeding a low-concentrate, high-forage ration ad libitum. Kitts et al. (2011) found that, using local prices, which may vary by region, feeding a limit-fed TMR alone would cost $1.17/kg of gain. Those researchers reported that adding straw would increase this cost by $0.17/kg of gain. However, they also reported that feeding a traditional low-concentrate, high-forage ration ad libitum would increase the cost to $1.50/kg of gain. Thus, in addition to improving the welfare and potentially improving the health of limit-fed animals through provision of straw, this feeding strategy may also help to decrease feed costs on farm.

A period by treatment interaction for first meal length and size suggests that the order of exposure to treatments affected heifer meal patterns. Heifers initially exposed to the HCR+S decreased the length of their first meal after feed delivery when switched to
the LCR treatment whereas heifers initially exposed to the LCR maintained a similar first meal length when switched to the HCR+S. As all of the heifers were housed in the same pen, it is possible that heifers initially fed the LCR synchronized their feeding behaviour with the heifers fed the HCR+S. Additionally, heifers initially exposed to the LCR had smaller first meals, translating into persistence of smaller first meals when switched to the HCR+S. In contrast, heifers initially exposed to the HCR+S continued to have larger first meals when switched to the LCR, suggesting that the animals may have habituated to a particular feeding pattern and simply maintained that behaviour across the treatment change. Recent research has shown that dairy heifers may develop feeding patterns and behaviours and maintain those feeding patterns across a ration change (Greter et al., 2010). It is important to consider the potential effect of behavioural habituation and development on response to treatment when designing future experiments involving growing dairy heifers.

In addition to behavioural habituation and response development, the long-term effect of the treatments should be considered. In this experiment, we were only able to demonstrate short-term (7 d) treatment differences. Post-ingestive consequences of feed intake may alter behavioural response in the long-term (i.e. weeks to months) due to both metabolic and physical factors (Gregorini et al., 2006). A short-term supply of excess nutrients or an imbalanced diet may result in satiety, but this satiety may be a form of metabolic discomfort due to the ratio of nutrients absorbed from the gastrointestinal tract resulting in metabolic imbalance (Forbes, 2007). Conversely, hunger is dependent on both short- and long-term nutrition (Forbes and Provenza, 2000). Heifers on the HCR+S may have experienced satiety in the short-term (perhaps for a period of several hours following feed consumption) due to high intake of a nutrient-dense ration, but may have experienced metabolic hunger in the long-term (over a period of a few
days or weeks) due to gut fill as a result of ingestion of straw preventing ingestion and absorption of sufficient nutrients to induce metabolic satiety (D'Eath et al., 2009). As straw contributed high levels of forage to the HCR+S, both treatment rations likely contained sufficient levels of fibre to allow rumen microflora populations to remain relatively stable across treatment changes. However, a longer-term study would be helpful in assessing whether the behavioural differences observed in our study would persist through any further ruminal adaptation occurring in these animals.

Interestingly, when fed the LCR, heifers spent more time lying down and less time standing inactively (standing without feeding) than when fed the HCR+S. Previous research has shown that heifers fed a limit-fed TMR spend more time standing inactively than heifers given ad libitum access to feed (Hoffman et al., 2007; Kitts et al., 2011). Traditionally, cattle spend a large portion of their day grazing (Hafez and Bouissou, 1975), so it could be argued that the increased time spent standing while not feeding may be a result of animals attempting to engage in grazing behaviour without a desirable, available food source. Additionally, cattle tend to ruminate while lying down (Albright, 1993). As heifers fed the HCR+S consumed less feed, they likely also spent less time ruminating and, therefore, may have spent more time engaged in inactive standing due to boredom (Albright, 1993) or discomfort (Paton et al., 2006; Webster, 1986) from subacute acidotic conditions resulting from a lack of salivary buffering (due to lack of chewing activity; Beauchemin et al., 2008). Recently, it has been speculated that inactive standing may be redirected feed searching behaviour due to hunger (D'Eath et al., 2009). In the present study, the heifers fed the HCR+S were allowed ad libitum access to straw in an effort to prevent hunger. However, straw may not have been a desirable feedstuff and, thus, the heifers may have continued to search for alternative feed sources. Unfortunately, the use of the data loggers does not give an indication of
where and what the heifers were doing while inactively standing and, therefore, further investigation into the oral behaviour of these animals is encouraged. Increased standing time may increase the susceptibility to claw horn lesions, which have the potential to cause pain and discomfort, leading to reduced mobility and lameness (Chapinal et al., 2009).

This experiment was designed to investigate the behavioural effects of ration composition utilizing short treatment periods and, therefore, we encourage future work to investigate feeding these diets for longer periods of time to assess whether there are any detrimental effects on health, both rumen function and lameness.

2.5 Conclusions

This study utilized short treatment periods and, thus, results must be interpreted in the short-term with recognition that behaviour may differ in a longer-term study. In the short-term, providing heifers a HCR+S followed by ad libitum access to wheat straw allowed for better targeting of nutrient intakes relative to growth requirements, but the LCR fed ad libitum promoted more natural diurnal feeding and meal patterns (similar to a grazing situation), increased lying times and decreased inactive standing times. However, long-term consequences of consuming large amounts of a LCR may result in over-conditioning of animals, negatively impacting growth and development of young dairy heifers. Additionally, behavioural patterns may differ in the long-term due to post-ingestive consequences of the different rations and ruminal microflora changes impacting digestibility and absorption of nutrients.

2.6 Acknowledgements
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Table 2.1. Ingredient, chemical composition, and particle size (mean ± SD) of the high concentrate ration (HCR) and low concentrate ration (LCR).

<table>
<thead>
<tr>
<th>Composition</th>
<th>HCR</th>
<th>LCR</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ingredient, %DM</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Heifer Mash$^{1,2}$</td>
<td>60.7</td>
<td>22.3</td>
</tr>
<tr>
<td>Alfalfa Hay</td>
<td>27.0</td>
<td>53.3</td>
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<tr>
<td>Grass Silage</td>
<td>3.6</td>
<td>7.2</td>
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<tr>
<td>Corn Silage</td>
<td>8.7</td>
<td>17.2</td>
</tr>
<tr>
<td>Chemical composition$^3$</td>
<td></td>
<td></td>
</tr>
<tr>
<td>DM, %</td>
<td>74.0 ± 2.9</td>
<td>67.1 ± 5.5</td>
</tr>
<tr>
<td>OM, % of DM</td>
<td>91.9 ± 0.3</td>
<td>91.2 ± 0.4</td>
</tr>
<tr>
<td>CP, % of DM</td>
<td>18.0 ± 0.4</td>
<td>18.4 ± 0.5</td>
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<tr>
<td>ADF, % of DM</td>
<td>21.7 ± 2.0</td>
<td>25.8 ± 1.6</td>
</tr>
<tr>
<td>NDF, % of DM</td>
<td>36.8 ± 2.4</td>
<td>40.6 ± 3.0</td>
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<tr>
<td>NFC, % of DM</td>
<td>34.9 ± 2.3</td>
<td>30.1 ± 2.7</td>
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<tr>
<td>Calculated Nutrients</td>
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<td></td>
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<tr>
<td>TDN, % of DM</td>
<td>70.3 ± 1.2</td>
<td>67.7 ± 1.4</td>
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<tr>
<td>ME, MJ/kg</td>
<td>10.63 ± 0.17</td>
<td>10.26 ± 0.21</td>
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<td>NE$_{G}$, MJ/kg</td>
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<td>4.02 ± 0.17</td>
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<td>NE$_{M}$, MJ/kg</td>
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<td>6.53 ± 0.17</td>
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<td>Particle Size$^4$, %</td>
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<tr>
<td>Long</td>
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<tr>
<td>Medium</td>
<td>18.9 ± 2.0</td>
<td>20.8 ± 2.5</td>
</tr>
<tr>
<td>Short</td>
<td>36.6 ± 7.5</td>
<td>27.1 ± 2.8</td>
</tr>
<tr>
<td>Fine</td>
<td>17.8 ± 3.9</td>
<td>14.5 ± 2.4</td>
</tr>
</tbody>
</table>

$^1$ Chemical composition of heifer mash (DM basis) was 17.9 ± 1.4% CP, 12.3 ± 0.9% ADF, and 29.2 ± 0.8% NDF
$^2$ Supplied by Unifeed Ltd. (Chilliwack, BC, Canada), containing (on as-is basis): 57.4% dairy concentrate pellet (Unifeed Ltd., Chilliwack, BC, Canada), 14.1% flatted barley,
13.0% steamed flatted oats, 10.0% steamrolled corn, 3.5% molasses spray, 2.0% PFI spray dairy.

3 Values were obtained from chemical analysis of TMR samples. NFC = 100 – (%CP + %NDF + %fat + %ash).

4 Particle size determined by Penn State Particle Separator which has a 19 mm screen (long), 8 mm screen (medium), 1.18 mm screen (short) and a pan (fine).
Table 2.2. Chemical composition and particle size distribution of the forages (mean ± SD; DM basis).

<table>
<thead>
<tr>
<th>Item</th>
<th>Alfalfa Hay</th>
<th>Grass Silage</th>
<th>Corn silage</th>
<th>Straw</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Chemical Composition</strong>&lt;sup&gt;1&lt;/sup&gt;</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>DM, %</td>
<td>92.6 ± 1.1</td>
<td>32.7 ± 9.3</td>
<td>32.7 ± 2.7</td>
<td>89.8 ± 1.8</td>
</tr>
<tr>
<td>CP, %</td>
<td>17.5 ± 3.0</td>
<td>22.2 ± 0.1</td>
<td>9.1 ± 0.6</td>
<td>5.5 ± 0.8</td>
</tr>
<tr>
<td>ADF, %</td>
<td>40.8 ± 3.0</td>
<td>28.4 ± 1.1</td>
<td>23.9 ± 0.6</td>
<td>50.3 ± 1.5</td>
</tr>
<tr>
<td>NDF, %</td>
<td>48.1 ± 4.1</td>
<td>45.8 ± 6.4</td>
<td>39.8 ± 0.6</td>
<td>73.8 ± 2.5</td>
</tr>
<tr>
<td>NFC&lt;sup&gt;2&lt;/sup&gt;, %</td>
<td>25.4 ± 0.4</td>
<td>18.0 ± 6.2</td>
<td>43.7 ± 0.8</td>
<td>14.9 ± 1.5</td>
</tr>
<tr>
<td>OM, %</td>
<td>91.6 ± 0.5</td>
<td>88.7 ± 0.2</td>
<td>96.0 ± 0.2</td>
<td>94.0 ± 0.5</td>
</tr>
<tr>
<td><strong>Calculated Nutrients</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>TDN, % of DM</td>
<td>56.4 ± 2.6</td>
<td>65.6 ± 2.3</td>
<td>73.7 ± 1.3</td>
<td>54.3 ± 0.9</td>
</tr>
<tr>
<td>ME, MJ/kg</td>
<td>8.54 ± 0.38</td>
<td>9.92 ± 0.33</td>
<td>11.14 ± 0.21</td>
<td>8.21 ± 0.13</td>
</tr>
<tr>
<td>NE&lt;sub&gt;G&lt;/sub&gt;, MJ/kg</td>
<td>2.64 ± 0.33</td>
<td>3.77 ± 0.25</td>
<td>4.69 ± 0.13</td>
<td>2.34 ± 0.13</td>
</tr>
<tr>
<td>NE&lt;sub&gt;M&lt;/sub&gt;, MJ/kg</td>
<td>4.98 ± 0.38</td>
<td>6.24 ± 0.33</td>
<td>7.33 ± 0.21</td>
<td>4.65 ± 0.17</td>
</tr>
<tr>
<td><strong>Particle Size</strong>&lt;sup&gt;3&lt;/sup&gt;, %</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Long</td>
<td>61.8 ± 2.6</td>
<td>71.2 ± 17.8</td>
<td>9.2 ± 1.6</td>
<td>49.9 ± 10.0</td>
</tr>
<tr>
<td>Medium</td>
<td>13.6 ± 2.2</td>
<td>19.3 ± 9.9</td>
<td>69.8 ± 4.1</td>
<td>31.0 ± 6.1</td>
</tr>
<tr>
<td>Short</td>
<td>17.0 ± 1.9</td>
<td>7.4 ± 5.4</td>
<td>20.1 ± 2.5</td>
<td>15.6 ± 4.2</td>
</tr>
<tr>
<td>Fine</td>
<td>7.6 ± 1.1</td>
<td>2.2 ± 2.7</td>
<td>0.9 ± 0.2</td>
<td>3.5 ± 1.8</td>
</tr>
</tbody>
</table>

<sup>1</sup>Values were obtained from chemical analysis of feed component samples.

<sup>2</sup>NFC = 100 – (% NDF + % CP + % ether extract + % ash).

<sup>3</sup>Particle size determined by Penn State Particle Separator which has a 19 mm screen (long), 8 mm screen (medium), 1.18 mm screen (short) and a pan (fine).
Table 2.3. Nutrient and energy intake of growing dairy heifers fed a high concentrate ration (HCR) and low concentrate ration (LCR; \( n = 12 \) HCR+S heifers, \( n = 12 \) LCR heifers). ¹

<table>
<thead>
<tr>
<th>Item</th>
<th>Dietary Treatment²</th>
<th>SE</th>
<th>( P )-value</th>
<th>( F )-value</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>HCR+S</td>
<td>LCR</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Nutrient Intake</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>DM, kg/d</td>
<td>5.3</td>
<td>7.3</td>
<td>0.2</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>CP, kg/d</td>
<td>0.85</td>
<td>1.34</td>
<td>0.04</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>ADF, kg/d</td>
<td>1.40</td>
<td>1.88</td>
<td>0.07</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>NDF, kg/d</td>
<td>2.28</td>
<td>2.96</td>
<td>0.11</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>NFC, kg/d</td>
<td>1.69</td>
<td>2.19</td>
<td>0.07</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Energy Intake</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>TDN, kg/d</td>
<td>3.61</td>
<td>4.93</td>
<td>0.16</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>ME, MJ/d</td>
<td>54.64</td>
<td>74.69</td>
<td>2.43</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>NE₁, MJ/d</td>
<td>34.71</td>
<td>47.48</td>
<td>1.55</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>NE₀, MJ/d</td>
<td>21.27</td>
<td>29.31</td>
<td>0.96</td>
<td>&lt;0.001</td>
</tr>
</tbody>
</table>

¹ Data are averaged across 5 d for 12 heifers on each treatment.
² Treatments were 1) a high concentrate ration fed in a limited amount with ad libitum wheat straw provided following consumption of TMR (HCR+S), and 2) a low concentrate ration provided ad libitum (LCR).
Table 2.4. Feeding and lying behavior data from growing dairy heifers fed a high concentrate ration (HCR) and low concentrate ration (LCR; \( n = 12 \) HCR+S heifers, \( n = 12 \) LCR heifers). ¹

<table>
<thead>
<tr>
<th>Item</th>
<th>Dietary Treatment⁴</th>
<th>SE</th>
<th>( P )-value</th>
<th>( F )-value</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>HCR+S</td>
<td>LCR</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Feeding time, min/d³</td>
<td>119.9</td>
<td>195.4</td>
<td>8.3</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Feeding rate, kg/min</td>
<td>0.05</td>
<td>0.05</td>
<td>0.004</td>
<td>0.9</td>
</tr>
<tr>
<td>Meal frequency, meals/d</td>
<td>4.8</td>
<td>7.0</td>
<td>0.8</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Meal duration, min/meal</td>
<td>134.4</td>
<td>59.6</td>
<td>22.9</td>
<td>0.03</td>
</tr>
<tr>
<td>Meal size, kg/meal</td>
<td>1.4</td>
<td>1.1</td>
<td>0.1</td>
<td>0.01</td>
</tr>
<tr>
<td>First meal length, min/meal⁴</td>
<td>114.0</td>
<td>107.6</td>
<td>7.2</td>
<td>0.4</td>
</tr>
<tr>
<td>First meal size, kg/meal⁴</td>
<td>4.2</td>
<td>2.3</td>
<td>0.13</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Total daily meal time, min/d³</td>
<td>455.8</td>
<td>381.4</td>
<td>41.7</td>
<td>0.1</td>
</tr>
<tr>
<td>Lying time, min/d</td>
<td>813.8</td>
<td>851.1</td>
<td>13.4</td>
<td>0.007</td>
</tr>
<tr>
<td>Inactive standing time, min/d</td>
<td>506.3</td>
<td>393.5</td>
<td>14.9</td>
<td>&lt;0.001</td>
</tr>
</tbody>
</table>

¹ Data are averaged across 5 d for 12 heifers on each treatment.
² Treatments were 1) a high concentrate ration fed in a limited amount with ad libitum wheat straw provided following consumption of TMR (HCR+S), and 2) a low concentrate ration provided ad libitum (LCR).
³ Feeding time is defined as the time each heifer spent at the feed bin consuming feed. Total daily meal time is the time spent at the feed bin + the time spent away from the bin within each meal (i.e. non-feeding intervals up to the length of the meal criterion).
⁴ First meal consumed after delivery of fresh feed.
Table 2.5. Nutrient and energy intake of growing dairy heifers on the high concentrate ration with straw offered ad libitum, separated by feed type ($n = 12$ heifers).

<table>
<thead>
<tr>
<th>Item</th>
<th>Dietary Treatment</th>
<th>SE</th>
<th>P-value</th>
<th>F-value</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>TMR</td>
<td>Straw</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Nutrient Intake</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>DM, kg/d</td>
<td>4.5</td>
<td>0.9</td>
<td>0.1</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>CP, kg/d</td>
<td>0.81</td>
<td>0.05</td>
<td>0.02</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>ADF, kg/d</td>
<td>0.97</td>
<td>0.43</td>
<td>0.04</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>NDF, kg/d</td>
<td>1.65</td>
<td>0.63</td>
<td>0.07</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>NFC, kg/d</td>
<td>1.56</td>
<td>0.13</td>
<td>0.03</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Energy Intake</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>TDN, kg/d</td>
<td>3.14</td>
<td>0.46</td>
<td>0.08</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>ME, MJ/d</td>
<td>47.60</td>
<td>7.03</td>
<td>1.17</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>NE$_{m}$, MJ/d</td>
<td>30.73</td>
<td>3.98</td>
<td>0.75</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>NE$_{G}$, MJ/d</td>
<td>19.26</td>
<td>2.01</td>
<td>0.46</td>
<td>&lt;0.001</td>
</tr>
</tbody>
</table>

1Data are averaged across 5 d for 12 heifers.
Table 2.6. Feeding behavior measures from growing dairy heifers on the high concentrate ration with straw offered ad libitum, separated by feed type (n = 12 heifers).¹

<table>
<thead>
<tr>
<th>Item</th>
<th>Feed Type</th>
<th>SE</th>
<th>P-value</th>
<th>F-value</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>TMR</td>
<td>Straw</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Feeding time, min/d²</td>
<td>79.9</td>
<td>40.0</td>
<td>5.5</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Feeding rate, kg/min</td>
<td>0.06</td>
<td>0.04</td>
<td>0.005</td>
<td>0.002</td>
</tr>
<tr>
<td>Meal frequency, meals/d</td>
<td>2.2</td>
<td>2.6</td>
<td>0.5</td>
<td>0.3</td>
</tr>
<tr>
<td>Meal duration, min/meal</td>
<td>70.3</td>
<td>202.6</td>
<td>43.8</td>
<td>0.05</td>
</tr>
<tr>
<td>Meal size, kg/meal</td>
<td>2.5</td>
<td>0.4</td>
<td>0.2</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Total daily meal time, min/d²</td>
<td>136.7</td>
<td>319.1</td>
<td>36.4</td>
<td>0.005</td>
</tr>
</tbody>
</table>

¹ Data are averaged across 5 d for 12 heifers.
² Feeding time is defined as the time each heifer spent at the feed bin consuming feed. Total daily meal time is the time spent at the feed bin + the time spent away from the bin within each meal (i.e. non-feeding intervals up to the length of the meal criterion).
**Figure 2.1.** Hourly averages for A) DMI (kg), B) feeding time (min), and C) feeding rate (kg/min) for growing dairy heifers fed 1) a high concentrate ration in a limited amount (2.0% of BW) with ad libitum wheat straw provided following consumption of TMR, and 2) a low concentrate ration provided ad libitum. Data are averaged over 5 d for 12 animals on each treatment.
CHAPTER 3: EFFECT OF FREQUENCY OF FEED DELIVERY ON THE FEEDING
BEHAVIOUR, GROWTH, AND RUMEN TEMPERATURE OF LIMIT-FED DAIRY
HEIFERS

3.1 Introduction

The practice of feeding a nutrient-dense ration in a controlled amount (limit feeding) has received recent consideration as producers are experiencing increased pressure due to rising feed costs and public concerns regarding farm waste excretion and the environment. Recent research has shown that this feeding practice may provide benefits to producers in the form of targeted ADG, decreased feed costs and fecal excretion and increased feed efficiency (Hoffman et al., 2007; Lascano et al., 2009; Kitts et al., 2011).

Despite these apparent benefits, this feeding strategy poses certain behavioral concerns. Dairy heifers that are limit-fed (at 2.05% of BW or less) a nutrient-dense ration have been shown to spend less time feeding throughout the day, often consuming feed within 1.5 h after feed delivery, and spending more time at the feed bunk without feed present (unrewarded visits) (Hoffman et al., 2007; Kitts et al., 2011, Chapter 4). This rapid intake of a nutrient-dense ration may increase the risk for development of sub-acute ruminal acidosis (SARA) due to post-prandial drops in rumen pH (Moody et al., 2007). Additionally, limit feeding results in a decrease in time spent lying, thereby increasing the time heifers spend standing without feeding (Kitts et al., 2011; Greter et al., submitted). These abnormal behaviors suggest that the animals may be engaging in redirected searching behavior due to hunger (D'Eath et al., 2009). An increase in time spent standing without eating may also contribute to the development of hoof pathologies due to increased contact time of claws with hard, often wet, concrete (Cook
et al, 2004; Vanegas et al., 2006). Furthermore, limit-fed heifers have been shown to vocalize more than ad libitum-fed animals (Hoffman et al., 2007), another familiar sign of frustration and hunger. Finally, limit-fed cattle often display oral stereotypies such as head nodding, tongue rolling, or bar-biting (Redbo et al., 1996; Lindstrom and Redbo, 2000), all behaviors that are associated with frustration and/or hunger, and may be indicative of high feeding motivation.

Frequency of feed delivery has been shown to modulate the feeding patterns of lactating dairy cattle with cows fed more frequently spending more time feeding and distributing feeding behavior throughout the day (DeVries et al., 2005). Intensively raised and fed cattle, young and mature, fed ad libitum, will spend 3-5 hours feeding throughout the day and will also consume their feed in small meals occurring throughout the day (DeVries et al., 2003; Greter et al., 2008). We hypothesized that frequent delivery of a limit-fed ration would encourage more feeding activity throughout the day, rather than just in the short time period after feed delivery when fed 1x/d (Hoffman et al., 2007; Kitts et al, 2011; Chapter 4); and this may increase satiety and reduce behavioral indicators of hunger and frustration. Additionally, we hypothesized that heifers fed more frequently would grow at a more consistent rate and with less variability between individuals. Thus, the objective of this experiment was to determine the effect that frequency of feed provision, offered in a restricted amount, had on the feeding behavior patterns, growth, and rumen temperature of growing dairy heifers.

3.2 Materials and Methods

3.2.1 Animals and Housing

Twenty-four Holstein dairy heifers were used in this study and were acquired, on loan, from a local commercial dairy operation. Upon arrival, heifers were given a broad-
spectrum antibiotic (Draxxin, tulathromycin, Pfizer Animal Health, Kirkland, Quebec, Canada) to prevent potential sickness due to transport and mixing stresses (Stanton et al., 2010). Heifers were given a 14-d adaptation period prior to exposure to experimental treatments. Heifers were 178.2 ± 9.3 (mean ± SD) d of age, weighed 200.4 ± 19.4 kg and measured 117.6 ± 4.3 cm tall at the rump at the beginning of the study. Heifers weighed 272.2 ± 26.9 kg and were 127.5 ± 4.3 cm tall at the rump at the end of the study. Heifers were housed in groups of 4 in 6 pens. Pens were located in a naturally-ventilated barn at the University of Guelph, Kemptville Campus (Kemptville, Ontario, Canada) and were managed according to the guidelines set by the Canadian Council on Animal Care (2009). Use of heifers was approved by the University of Guelph’s Animal Care Committee (AUP#09R022). The study was conducted between May and August of 2010. Pens consisted of an indoor sand-bedded pack area (3.6 m x 10.9 m; width x depth) and an outdoor concrete run (3.6 m x 16.4 m). Sand bedding was cleaned out and replenished once weekly throughout the experiment. Feed bunks were located along the front of each indoor pack area and measured 1.60 m in length, allowing a total of 0.40 m of bunk space/heifer. Water was available ad libitum to the heifers through a water bowl in each pen. Heifers were given ad libitum access to trace mineral salt blocks (Windsor TM Stock Salt, The Canadian Salt Company Limited, Pointe-Claire, Quebec, Canada).

3.2.2 Experimental Design and Diets

The number of animals required per treatment was determined through power analysis (Morris, 1999) for primary response variables, including DMI, feeding behavior and ADG. Estimates of variation for these variables were based on previously reported values (Kertz and Chester-Jones, 2004; Greter et al., 2008; Kitts et al., 2011). Heifers were divided into groups of 4 that were balanced for age and weight. Heifers had
previously been fed a TMR. Upon arrival, heifers were fed a high-concentrate TMR (Table 3.1) that was formulated, and fed at a restricted level (target = 2.05% of BW; actual across study = 2.03 ± 0.06% of BW), to meet the nutrient requirements for a non-bred Holstein heifer growing at 0.8 kg/d (NRC, 2001). Over the 14-d adaptation period, heifers were fed straw (initially offered at 8.0 kg/pen as-fed) offered alongside the TMR. The amount of straw offered was gradually reduced by 1.0 kg/pen/d until heifers were only provided with the high concentrate, limit-fed TMR.

Following the adaptation period, groups of heifers were exposed to each of 3 treatments, in 28-d periods, using a replicated 3x3 Latin square design. The treatments were delivery of daily allotment of TMR (Table 3.1): 1) once per day (1x; 0800 h), 2) twice per day (2x; 0800 and 1600 h), and 3) four times per day (4x; 0800, 1200, 1600, and 2000 h). Heifers in adjacent pens were on different treatments. Each 28-d treatment period consisted of a 21-d adaptation period (to allow heifers to acclimate to the treatment), followed by a 7-d data collection period. Once daily, dietary components were mixed in a TMR mixer wagon (Jaylor 4425, Jaylor Fabricating, Orton, ON, Canada). The appropriate amount of TMR was manually weighed out for each pen. Feed remaining for subsequent feed delivery was kept in an air conditioned room set at 12 °C. The total amount of feed offered to each pen was adjusted weekly, according to total pen BW. The ration DM used to calculate feed amounts on a pen basis was adjusted every 2 wk.

3.2.3 Experimental Measurements

Group intakes were recorded daily throughout the study by weighing the amount of feed offered and amount of feed refused (if any) using a calibrated floor scale precise to the nearest 0.2 kg (Model 31-0851-T17430, Toledo Scale Company of Canada Ltd.,
Windsor, ON, Canada). This data was used to calculate daily DMI (kg/d) on a pen basis. Heifers were weighed and measured for the same 2 consecutive days each week and these weekly weights were averaged for each heifer and used to calculate ADG. Feed efficiency was calculated, on a pen basis, as total pen DMI per week/total pen BW per week. Scale errors resulting in inaccurate BW for some heifers within a pen resulted in inaccurate total pen BW values. Thus, feed efficiency values for 4 pens over various weeks during the course of the experiment were excluded from further analysis.

Feeding and competitive behavior were monitored, using time-lapse video, continuously for the last 7-d of each treatment period. Heifers were recorded using 1 video camera (Panasonic WV-BP330; Osaka, Japan) per pen, a time-lapse video cassette recorder (Panasonic AG-6740) and a video multiplexer (Panasonic WJ-FS 616). Video cameras were mounted 3.2 m above each of the 6 feed bunks. Red lights (100W), mounted adjacent to the cameras, were used to facilitate recording at night. Digital photographs were taken showing a dorsal view of the back of each animal. These photos were used during video analysis to identify individuals within each pen.

The amount of time spent feeding during the 7-d recording period was scored for individual heifers using instantaneous scan sampling every 1 min. For each scan, feeding was defined when a heifer had her head completely past the feed rail and over the feed. Total time spent feeding was then calculated for each heifer for each day of the 7-d recording period. To identify changes in diurnal feeding patterns between treatments, these scans were used to calculate the percentage of heifers feeding at the feed bunk over a 24-h period. Unrewarded time at the feed bunk (presence at the feed bunk when no feed was present) was also measured using 1 min scans. For each scan, unrewarded time at the feed bunk was defined when a heifer had her head completely
past the feed rail and over an empty feed bunk. Total daily unrewarded time at the feed bunk was calculated for each day of the 7-d recording periods.

Feeding competition, recorded as displacements from the feed bunk while feeding, was measured on d 23, 25 and 27 of each treatment period. A displacement was marked when a butt or a push from the actor (instigator) resulted in the complete withdrawal of the reactor’s head from beneath the feed rail (DeVries et al., 2004). These observations were used to calculate the number of times each heifer was displaced from the feed bunk per minute of feeding time.

Ruminal temperature was selected as an indicator of rumen health, as opposed to rumen pH, due to the lower level of invasiveness associated with administration of rumen temperature boluses versus cannulation of the rumen for the placement of rumen pH probes that require regular calibration. The association between rumen temperature and rumen pH has been validated and discussed elsewhere (AlZahal et al., 2008; 2009). Ruminal temperature was recorded for the last 7 d of each treatment period using a telemetric acquisition system (SmartStock LLC, Pawnee, OK, USA), which was composed of the following: a telemetric ruminal bolus (3 cm in diameter and 8.5 cm in height, 120 g in weight), an antenna, a barn receiver unit, a base receiver unit, and a personal computer equipped with a software program for data logging, as validated by AlZahal et al. (2009). Ruminal temperature measurements were broadcasted through a radio frequency (0.3-3.0 GHz) from the bolus to the barn receiver unit through the antenna that was within 100 m of the heifers. The signal was then transmitted (0.9 GHz) from the barn receiver unit to the base receiver unit (located within 10 m), which was connected via a cable to the personal computer. The bolus was administered, using a bolus gun, to each heifer prior to the start of the study. The bolus was customized to transmit every minute and each transmission included 12 recordings.
Lying behavior was recorded during the last 7-d of each treatment period. Daily lying times of individual heifers were collected using electronic data loggers (HOBO Pendant G Data Logger, Onset Computer Corporation, Pocasset, MA), previously validated by Ledgerwood et al. (2010). These devices were placed on the hind leg of each heifer using veterinary bandaging (Vetrap Bandaging Tape, 3M, St. Paul, MN) and measured the orientation of the leg at 1-min intervals. This data was summarized to calculate the average daily standing/lying time (min/d) as well as time spent standing without eating. This latter measurement included all time that heifers were standing without eating, including unrewarded visits to the feed bunk.

3.2.4 Feed Sampling and Analysis

Representative grab samples of the TMR were taken for DM and chemical analysis during the last 7 d (recording weeks) of each treatment period. Samples of the dietary components were also taken for DM and chemical analysis once during each of these recording weeks. Duplicate samples of forage components were taken, once a week during the last 7 d of each treatment period, for particle size separation. Grab samples of the TMR were also collected for particle size separation at the time of feed delivery twice weekly during the last 7 d of each treatment period. All samples were immediately frozen at -20°C until they were analyzed.

Samples for particle size separation were thawed at a later date and separated using a 3-screen (19, 8, 1.18 mm) Penn State Particle Separator (PSPS; Kononoff et al., 2003). This separated the samples into 4 fractions: long (>19mm), medium (<19, >8mm), short (<8,>1.18mm) and fine (<1.18mm) particles. After separation, DM of each separated fraction was determined by forced air drying at 55°C for 48 h.
Samples taken for DM and chemical analysis were oven dried at 55°C for 48 h and then ground to pass through a 1mm screen (Wiley Mill, Arthur H. Thomas Co., Philadelphia, PA). These samples were then sent to Cumberland Valley Analytical Services Inc. (Maugansville, MD, USA) for analysis of DM (135°C; AOAC, 2000: method 930.15), ADF (AOAC, 2000: 973.18), NDF with heat-stable α-amylase and sodium sulfite (Van Soest et al., 1991) and CP (N x 6.25) (AOAC 2000: method 990.03; Leco FP-528 Nitrogen Analyzer, Leco, St. Joseph, MI).

3.2.5 Calculations and Statistical Analysis

For analyses of treatment effects, the pen was considered the experimental unit. Data for intakes, growth (ADG), feed efficiency, feeding time, unrewarded time, lying/standing behavior, and competitive behavior were averaged for each pen by treatment period. Pen variance in ADG (ADGv) was calculated by averaging, per pen, the absolute difference between individual heifer ADG and pen mean ADG. Data for feed bunk attendance (percentage of heifers feeding) was summarized by hour for each pen for each treatment period.

Preliminary inspection of the data revealed that all dependent variables were normally distributed. To test for differences among treatments, DMI, ADG, feed efficiency, feeding and unrewarded time, displacements, lying time, and time spent standing without eating were analyzed using the MIXED procedure of SAS (2008). The model included the fixed effects of period, square, and treatment, the random effect of pen within square and the residual error. Interactions of the period and square with treatment were tested in the model and were not significant; therefore, these are not further reported. Linear and quadratic orthogonal contrasts were tested using the CONTRAST statement of SAS. To test for the effect of treatment for diurnal feeding
patterns, the data were analyzed using the MIXED procedure of SAS (2003) treating hour as a repeated measure. The model included the fixed effects of period, square, treatment, hour, and the random effect of pen within square. The covariance structure was autoregressive, according to the best-fit Schwarz’s Bayesian information criterion.

Heifers with less than 800 temperature recordings/d were removed from further analysis. Mean, minimum, and maximum daily ruminal temperatures were calculated from all available observations using PROC MEANS of SAS. The duration (min/d) that ruminal temperature was elevated above a given threshold (i.e. 38.0 °C, 38.2 °C, 38.4 °C, etc.) was computed to describe the magnitude of elevation in temperature in the rumen (Alzahal et al., 2008). The durations that ruminal temperatures were above a given temperature threshold were calculated for each day of recording using PROC MEANS of SAS. Daily average ruminal temperature data (mean, maximum, minimum, and time (min/d) above a specific cut-off point) were analyzed using the MIXED procedure of SAS. The model included the fixed effects of period, square, and treatment, the random effect of pen within square and the residual error. Interactions of the period and square with treatment were tested in the model and were not significant; therefore, these are not further reported. Linear and quadratic orthogonal contrasts were tested using the CONTRAST statement of SAS. All values reported are least squares means. Significance was declared as $P \leq 0.05$ and trends were reported if $0.05 < P \leq 0.10$.

3.3 Results

There was a decrease in both feeding time and unrewarded time at the feed bunk with increasing frequency of feed delivery (Table 3.3), with this decrease being greater for heifers fed 2x compared to 4x (Table 3.3). The differences in feeding time are seen in Figure 3.1, with a clear difference in the percentage of heifers present at the
feed bunk over the course of the day ($SE = 1.3$, $P_{linear} = 0.03$; $P_{quadratic} = 0.01$). A treatment × hour interaction ($P < 0.001$) was detected in the analysis of diurnal feed bunk attendance, reflecting the different percentages of heifers present at the feed bunk across the day, depending on treatment. Despite the difference in feeding time, the daily frequency of displacements and the number of displacements per unit of feeding time were similar between treatments (Table 3.3). Although daily lying time was similar between treatments, there was a linear increase in the amount of time spent standing without eating as frequency of feed delivery increased (Table 3.3).

Heifers completely consumed all offered feed each day. Even though feed was provided at the same rate to each pen, DMI was greatest when heifers were fed 1x (Table 3.3). Despite the difference in DMI, the ADG was similar across treatments. There was, however, a tendency for within-pen variation of ADG to be greater with increasing frequency of feed delivery. Feed efficiency was similar between treatments.

Heifers maintained similar mean, minimum, and maximum rumen temperature across treatments (Table 3.4). The amount of time that rumen temperature was elevated over 38.0°C decreased linearly with increasing frequency of feed delivery, while the amount of time that rumen temperature was elevated over 38.8°C increased linearly with increasing frequency of feed delivery (Table 3.4).

3.4 Discussion

To date, little is known about what modulates the feeding behavior of growing dairy heifers, particularly when managed according to a limit feeding regime. Previous research has shown that the diurnal feeding patterns of lactating dairy cattle are governed, in part, by the frequency of feed delivery. Thus, it is possible that increasing
the frequency of feed provision may result in more natural feeding patterns and behavior of limit-fed dairy heifers.

In support of our hypothesis we found that heifers distributed their feeding time throughout the day to a greater degree when provided with feed more frequently. Specifically, heifers fed 4x consumed their feed in 4 smaller, shorter bouts than heifers fed 1x or 2x, demonstrating a pattern more comparable to natural grazing, wherein cattle consume several small, short meals throughout the day (Albright, 1993; Hafez and Bouissou, 1975). However, heifers fed 1x spent the greatest amount of time feeding, consuming their ration in just over 1 h, albeit in one large feeding bout immediately following feed delivery. Even though this increase in feeding time allows the animals to express feeding behavior for a larger portion of the day, heifers fed 1x also spent the most time in unrewarded visits to the feed bunk in comparison to heifers fed 2x or 4x. Restricted-fed calves have been shown to perform more unrewarded visits to the milk feeder than ad libitum-fed calves (De Paula Vieira et al., 2008), suggesting that more frequent unrewarded visits were indicative of increased hunger in restricted animals. Additionally, Redbo et al. (1996) found that restricted-fed dairy cattle spent more time in feeding-related behaviors (i.e. sniffing or licking around the empty feed trough); those researchers suggested that these appetitive behaviors were triggered by a motivation for food-searching or feeding. Redbo et al. (1996) concluded that these behaviors indicated increased feeding motivation due to restrictive feeding. In our study, heifers fed 1x may have shown an increase in unrewarded time at the feed bunk due to increased feeding motivation. However, as cattle are highly social animals, there were periods of time when heifers fed 1x were visiting the feed bunk, despite the lack of feed, while heifers on the other two treatments were being fed (Figure 3.1b). The pens utilized in the current study were not solid-sided and, therefore, heifers were able to view the behavior of
heifers on other treatments in the barn, possibly contributing to this increase in unrewarded time. Thus, this result must be interpreted with caution. Indeed, Robles et al. (2007) found that heifers fed less frequently spent more time observing other heifers when the latter were fed. Heifers fed 1x may not be demonstrating increased feelings of frustration or hunger but, rather, may be showing motivation to engage in characteristic social behaviors (i.e. feeding as a herd). It is important to note that both ingestion and rumination reflect the overall feeding behavior of cattle (Baumont et al., 2006). We were unable to measure rumination in the present study and, therefore, further research in this area is encouraged.

Contrary to our hypothesis, feeding frequency did not affect competition between treatments, either throughout the day or per unit of time spent feeding. Other studies have also found competitive interactions between limit-fed heifers to be reduced in comparison to values reported for ad libitum-fed animals (Kitts et al., 2011; Chapter 4). Furthermore, heifers in this study were allowed 0.4 m/heifer of feed bunk space, which was sufficient space for all heifers to feed simultaneously. Longenbach et al. (1999) found that variability in growth and increased competition only occur when limit-fed heifers of this age category are provided with feed bunk space that is less than 0.31 m/heifer. This consistent lack of competition between limit-fed heifers is interesting, as it provides further support to our hypothesis that feeding motivation in these animals is substantial (as they will forego competition for the “best” feed in an effort to maximize intake of any feed) and that they may be experiencing hunger throughout the day (Kitts et al., 2011; Chapter 4). Stocking density within the pen must also be considered as increased competition has been observed in heifers that were given increasingly limited access to a concentrate feeder via an increase in stocking density (Gonzalez et al., 2008). Similarly, competition for feed increases in lactating cattle that are overstocked,
with subordinate individuals being most affected (Huzzey et al., 2006). In these situations, the animals did not have sufficient space to feed simultaneously and thus gained access to the resource only by displacing others. It follows that heifers in a situation of increased stocking density may be more competitive than heifers kept in smaller groups with adequate bunk space. The group size in this study consisted of only 4 heifers per pen; it is possible that competition at the feed bunk would increase with increased group size as the social hierarchy within each pen may be altered. However, other researchers have found that increased group size did not alter competition at the feed bunk in lactating dairy cattle (Telezhenko et al., 2012) or ewes (Jorgensen et al., 2009). As the animals in those studies were not feed restricted, group size may prove to have more of an impact on limit-fed heifers. Further research on group size and the effect on the feeding behavior of dairy heifers is encouraged.

Heifers spent a similar amount of time lying down each day across treatments. This is supported by results from previous studies on feeding frequency with cattle. Robles et al. (2007) found that increasing feeding frequency from 1x to 4x daily did not affect the percentage of time that Holstein heifers spent resting throughout the day. Similarly, DeVries et al. (2005) found no difference in lying time between lactating dairy cattle fed 1x, 2x, or 4x daily. These researchers speculated that cows were able to increase the time spent feeding and reduce the amount of time that they spend waiting for feed or waiting to access the feed bunk without altering lying behavior.

Although, in the current study, heifers did not differ in lying time between treatments, they did spend different amounts of time standing without eating. Indeed, heifers fed 4x spent the most time, whereas heifer fed 1x spent the least amount of time standing without eating. This result may be attributed to the feeding regime, as there is evidence to suggest an increase in appetitive behavior surrounding the feeding time in
restricted-fed species (D’Eath et al., 2009). It is likely that as the frequency of feeding increased, the animals also became stimulated more frequently. As feed ingestion in itself may intensify feeding motivation and encourage animals to continue eating (Terlouw et al., 1993), heifers may spend more time in appetitive-type behavior following the feeding event, despite the absence of feed. These behaviors may be directed towards other parts of the pen that were not captured by video, such as outdoor feed bunks (empty throughout this experiment) and water bowls. Indeed, several researchers examining the behavior of restricted-fed pigs have found that the animals will increase their drinker use and water intake as a result of quantitative feed restriction of diets high in concentrate (Rushen, 1984; Terlouw et al., 1991; Robert et al., 1993). This is a welfare concern as this increase in time spent standing without eating (resulting from a decrease in feeding time) suggests that the heifers may be continuing to perform appetitive behaviors following a feeding event as a result of lack of satiety (Terlouw et al., 1993; Toscano et al., 2007). Although heifers fed 4x did not spend as much time unrewarded at the feed bunk, it is possible that these animals were directing their behavior towards other areas of the pen, as previously suggested, particularly considering that they were likely aware that their feed bunk was empty, having recently consumed any available feed. Complete observation of the time budgets of limit-fed heifers may shed light on this result.

Heifers fed 1x consumed 0.1 kg/d more feed than heifers fed 2x or 4x. This difference in the amount of feed consumed was very small, and was likely not biologically relevant. As expected in a study with controlled feeding levels, the SE of DMI was extremely small (0.02 kg/d). The equipment used to weigh pen feed amounts was not sensitive enough to measure to the nearest tenth and, therefore, this significance must be interpreted with caution. Indeed, the irrelevance of the difference in DMI is
further reflected in the fact that heifers did not differ in their ADG or feed efficiency between treatments. This may not be surprising as the heifers were consuming the same ration in very similar quantities. Additionally, the ration was formulated to provide an ADG of 0.8 kg/d, which was achieved throughout this study.

There was a tendency for heifers fed 1x to have less variation in ADG between different individuals within a pen. This is likely a result of variability in intake between heifers within a pen. As we were unable to measure individual intake in this study, we encourage further research on limit feeding and the effect on individual animals’ intake and growth.

Although heifers fed 1x spent more time feeding daily, the distribution of this feeding time in one large meal may increase the potential for the development of metabolic disorders, such as SARA, due to rapid intake of a large quantity of rapidly fermentable carbohydrates (Quigley, 1992; Moody et al., 2007). Researchers have shown that consuming feed more frequently throughout the day may decrease the risk of acidosis (Kaufmann, 1976) and minimize ruminal pH fluctuations (French and Kennelly, 1990). Rumen temperature (heat of fermentation) may be measured as an indicator of rumen health, as rumen temperature and rumen pH are inversely correlated (AlZahal et al., 2009). In our study, heifers fed 1x spent the greatest amount of time with rumen temperature elevated over 38.0°C, suggesting that a single, large meal may cause a large postprandial drop in rumen pH lasting for a longer period of time than when heifers consume their feed in smaller meals more frequently. Alternatively, heifers spending any period of time with rumen temperature elevated over 38.8°C may have experienced a spike in postprandial rumen pH, albeit for a short period of time throughout the day. Similarly, Sutton et al. (1986) and Yang and Varga (1989) found that increasing the frequency of feeding tended to decrease the average ruminal pH, but also minimized pH
fluctuation in dairy cattle. Overall, mean, maximum, and minimum ruminal temperature did not differ between treatments, indicating that there may be considerable variation between individuals. It should be noted that AlZahal et al. (2008) found that the correlation between ruminal temperature and ruminal pH for dairy cows receiving a diet formulated to induce SARA was greatest for time above ruminal temperature 39.2°C. Heifers in this study were not under the same treatment conditions as the adult cattle in the study by AlZahal et al. (2008) and, as such, it is difficult to estimate acidotic conditions without sufficient pH validation. Based on rumen temperature, however, it is likely that heifers in this study experienced some depression in pH and thus may have encountered issues with rumen health and function. Further research is needed on the effect of feeding frequency on rumen dynamics of limit-fed heifers.

### 3.5 Conclusions

Frequency of feed delivery affects the feeding behavior and may have an impact on the rumen health of growing limit-fed dairy heifers. Limit-fed heifers provided their daily allotment of feed 1x spent more time feeding throughout the day, but also spent more unrewarded time at the feed bunk (present at the bunk with no feed available). Additionally, heifers fed 1x spent less time standing without eating. Heifers fed 1x consumed their daily allotment of feed within 1.5 h after feed delivery. It is likely that these heifers experienced satiety, at least in the short-term, following that large meal, whereas heifers fed smaller meals 2x or 4x spent less time feeding and may never have achieved sufficient rumen fill to reach satiety. Further research to consider impact on animal welfare and behavior with respect to oral stereotypies, hunger, and frustration is warranted to ensure that optimal conditions are provided for animals managed with this feeding strategy.
3.6 Acknowledgements

We thank the staff and students at the University of Guelph, Kemptville Campus Dairy Education and Research Centre, particularly Mikayla Baxter, Megan Bruce, Julie Fish, and Bas Westerveld for their technical help and support. Angela Greter was supported by a Natural Sciences and Engineering Research Council of Canada (NSERC) Alexander Graham Bell Canadian Graduate Scholarship. This project was funded through an Ontario Ministry of Agriculture, Food and Rural Affairs (OMAFRA)/University of Guelph Production Systems research grant. This project was also supported through contributions from the Canadian Foundation for Innovation (CFI) and the Ontario Research Fund.
Table 3.1. Ingredient, chemical composition, calculated nutrients, and particle size (mean ± SD) of the diet.

<table>
<thead>
<tr>
<th>Composition</th>
<th>Ingredient, %DM</th>
<th>Composition 2 DM, %</th>
<th>OM, % of DM</th>
<th>CP, % of DM</th>
<th>ADF, % of DM</th>
<th>NDF, % of DM</th>
<th>NFC, % of DM</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ingredient</td>
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<tr>
<td>Corn silage</td>
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<tr>
<td>Grass/alfalfa haylage</td>
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<tr>
<td>High moisture corn</td>
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<tr>
<td>Protein supplement$^1$</td>
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<tr>
<td>Chemical composition$^2$</td>
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<tr>
<td>DM, %</td>
<td></td>
<td>63.7 ± 3.3</td>
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<tr>
<td>OM, % of DM</td>
<td></td>
<td>94.2 ± 0.6</td>
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<td>CP, % of DM</td>
<td></td>
<td>15.6 ± 1.0</td>
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<td>ADF, % of DM</td>
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<td>14.8 ± 1.3</td>
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<tr>
<td>NDF, % of DM</td>
<td></td>
<td>24.3 ± 2.0</td>
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<td>NFC, % of DM</td>
<td></td>
<td>51.6 ± 2.5</td>
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<tr>
<td>Calculated nutrients$^3$</td>
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<tr>
<td>TDN$^4$, % of DM</td>
<td></td>
<td>76.7 ± 1.3</td>
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<tr>
<td>ME, Mcal/kg</td>
<td></td>
<td>2.77 ± 0.05</td>
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<tr>
<td>NE$_G$, Mcal/kg</td>
<td></td>
<td>1.21 ± 0.04</td>
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<tr>
<td>NE$_M$, Mcal/kg</td>
<td></td>
<td>1.84 ± 0.04</td>
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<tr>
<td>Particle size$^5$, %</td>
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<tr>
<td>Long</td>
<td></td>
<td>4.1 ± 2.5</td>
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<td></td>
</tr>
<tr>
<td>Medium</td>
<td></td>
<td>48.2 ± 3.7</td>
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<td></td>
</tr>
<tr>
<td>Short</td>
<td></td>
<td>35.1 ± 3.5</td>
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<td></td>
</tr>
<tr>
<td>Fine</td>
<td></td>
<td>12.6 ± 1.7</td>
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</tr>
</tbody>
</table>

$^1$Supplied by Dundas Feed and Seed (Winchester, ON, Canada), containing (on as-is basis): 25.6% corn gluten meal, 24.4% Tri-Pro Gold (Tri-County Protein Corp., Winchester, ON, Canada), 24.4% soybean meal, 10.0% canola meal, 4.8% ground limestone, 4.5% trace mineral/vitamin premix, 4.4% sodium bicarbonate, 1.9% cobaltized-iodized salt.
Values were obtained from chemical analysis of TMR samples. NFC = 100 – (%CP + %NDF + %fat + %ash).

3 Calculated according to NRC (2001) equations.

4 Total digestible nutrients (calculated from ingredients).

5 Particle size determined by Penn State Particle Separator which has a 19 mm screen (long), 8 mm screen (medium), 1.18 mm screen (short) and a pan (fine).
Table 3.2. Chemical composition, calculated nutrients, and particle size distribution of the forages (mean ± SD; DM basis)

<table>
<thead>
<tr>
<th>Item</th>
<th>Grass/Alfalfa Haylage</th>
<th>Corn Silage</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Chemical composition</strong>(^1)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>DM, %</td>
<td>59.8 ± 4.4</td>
<td>43.0 ± 5.9</td>
</tr>
<tr>
<td>CP, %</td>
<td>16.8 ± 1.2</td>
<td>7.9 ± 0.3</td>
</tr>
<tr>
<td>ADF, %</td>
<td>32.4 ± 0.4</td>
<td>21.2 ± 1.9</td>
</tr>
<tr>
<td>NDF, %</td>
<td>45.4 ± 0.7</td>
<td>45.4 ± 0.7</td>
</tr>
<tr>
<td>NFC(^2), %</td>
<td>28.0 ± 1.3</td>
<td>51.9 ± 4.1</td>
</tr>
<tr>
<td>OM, %</td>
<td>91.7 ± 0.8</td>
<td>97.5 ± 0.3</td>
</tr>
<tr>
<td><strong>Calculated nutrients</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>TDN, % of DM</td>
<td>63.4 ± 0.8</td>
<td>76.3 ± 1.7</td>
</tr>
<tr>
<td>ME, Mcal/kg</td>
<td>2.29 ± 0.03</td>
<td>2.76 ± 0.06</td>
</tr>
<tr>
<td>NE(_G), Mcal/kg</td>
<td>0.84 ± 0.02</td>
<td>1.20 ± 0.05</td>
</tr>
<tr>
<td>NE(_M), Mcal/kg</td>
<td>1.41 ± 0.03</td>
<td>1.83 ± 0.06</td>
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<tr>
<td><strong>Particle size</strong>(^3), %</td>
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<tr>
<td>Long</td>
<td>23.0 ± 9.1</td>
<td>13.6 ± 8.4</td>
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<tr>
<td>Medium</td>
<td>38.2 ± 5.0</td>
<td>54.6 ± 6.1</td>
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<tr>
<td>Short</td>
<td>28.7 ± 5.1</td>
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<tr>
<td>Fine</td>
<td>10.0 ± 2.9</td>
<td>4.8 ± 1.1</td>
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</tbody>
</table>

\(^1\)Values were obtained from chemical analysis of feed component samples.

\(^2\)NFC = 100 – (% NDF + % CP + % ether extract + % ash).
Particle size determined by Penn State Particle Separator which has a 19 mm screen (long), 8 mm screen (medium), 1.18 mm screen (short) and a pan (fine).
Table 3.3. Growth and behavioral data from growing dairy heifers fed at various frequencies.\textsuperscript{1}

<table>
<thead>
<tr>
<th>Item</th>
<th>Treatment\textsuperscript{2}</th>
<th>SE</th>
<th>( P )-value</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>1x</td>
<td>2x</td>
<td>4x</td>
</tr>
<tr>
<td>DMI, kg/d</td>
<td>5.0</td>
<td>4.9</td>
<td>4.9</td>
</tr>
<tr>
<td>ADG, kg/d</td>
<td>0.7</td>
<td>0.8</td>
<td>0.8</td>
</tr>
<tr>
<td>ADGv, kg/d</td>
<td>0.2</td>
<td>0.3</td>
<td>0.3</td>
</tr>
<tr>
<td>Feed efficiency (DMI/ADG)</td>
<td>5.7</td>
<td>5.8</td>
<td>5.7</td>
</tr>
<tr>
<td>Feeding time, min/d</td>
<td>63.1</td>
<td>44.2</td>
<td>51.5</td>
</tr>
<tr>
<td>Unrewarded time, min/d</td>
<td>49.5</td>
<td>27.7</td>
<td>33.1</td>
</tr>
<tr>
<td>Displacements, #/d</td>
<td>4.5</td>
<td>1.9</td>
<td>2.8</td>
</tr>
<tr>
<td>Displacements, #/min feeding time</td>
<td>0.07</td>
<td>0.04</td>
<td>0.06</td>
</tr>
<tr>
<td>Lying time, min/d</td>
<td>818.0</td>
<td>805.8</td>
<td>783.8</td>
</tr>
<tr>
<td>Standing without eating, min/d</td>
<td>558.9</td>
<td>590.0</td>
<td>604.7</td>
</tr>
</tbody>
</table>
Data for DMI, ADG, ADGv, feed efficiency, feeding time, unrewarded time at the feed bunk, lying time, and time spent standing without eating are averaged for 6 pens (4 heifers/pen) over 7 d on each treatment period. Data for displacements are averaged for 6 pens (4 heifers/pen) for d 23, 25, and 27 of each treatment period.

Treatments were delivery of daily allotment of TMR in: 1) one feeding per day (1x; 0800 h), 2) two feedings per day (2x; 0800 and 1600 h), and 3) four feedings per day (4x; 0800, 1200, 1600, and 2000 h).
Table 3.4. Least squares means for ruminal temperature characteristics obtained by a telemetric monitoring system for growing dairy heifers fed at various frequencies.  

<table>
<thead>
<tr>
<th>Item</th>
<th>Treatment$^2$</th>
<th>SE</th>
<th>$P$-value</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>1x</td>
<td>2x</td>
<td>4x</td>
</tr>
<tr>
<td>Mean, °C</td>
<td>37.8</td>
<td>37.8</td>
<td>37.7</td>
</tr>
<tr>
<td>Maximum, °C</td>
<td>38.6</td>
<td>38.6</td>
<td>38.6</td>
</tr>
<tr>
<td>Minimum, °C</td>
<td>35.5</td>
<td>35.2</td>
<td>35.3</td>
</tr>
<tr>
<td>Duration, min/d</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>&gt;38.0°C</td>
<td>613.7</td>
<td>578.3</td>
<td>556.2</td>
</tr>
<tr>
<td>&gt;38.4°C</td>
<td>185.6</td>
<td>188.2</td>
<td>160.2</td>
</tr>
<tr>
<td>&gt;38.8°C</td>
<td>12.2</td>
<td>26.8</td>
<td>28.2</td>
</tr>
</tbody>
</table>

$^1$ Data are averaged over 7 d on each treatment.

$^2$ Treatments were delivery of daily allotment of TMR in: 1) one feeding per day (1x; 0800 h), 2) two feedings per day (2x; 0800 and 1600 h), and 3) four feedings per day (4x; 0800, 1200, 1600, and 2000 h).
Figure 3.1. Percentage of heifers feeding at the feed bunk over a 24-h period (percentage for each 1-min interval during the day) for 3 treatments when: a) feed was available, and b) when no feed was available (unrewarded visits). Treatments were delivery of daily allotment of TMR in: 1) one feeding per day (1x; 0800 h), 2) two feedings per day (2x; 0800 and 1600 h), and 3) four feedings per day (4x; 0800, 1200, 1600, and 2000 h). Data are averaged across 7 d for 6 pens (4 heifers/pen) on each treatment.
CHAPTER 4: LIMIT FEEDING DAIRY HEIFERS: EFFECT OF FEED BUNK SPACE AND PROVISION OF A LOW-NUTRITIVE FEEDSTUFF

4.1 Introduction

Limit feeding of replacement dairy heifers is a feeding management strategy that is gaining popularity with dairy producers. The use of limit feeding in dairy heifers allows for controlled growth rates, improved feed efficiency, reduced cost of feed per kg of gain, and a decrease in fecal excretion (Hoffman et al., 2007; Moody et al., 2007; Kitts et al., 2011). However, there are some behavioral concerns associated with this practice, including increased vocalizations (Hoffman et al., 2007) and oral stereotypies (Redbo et al., 1996), which may be indicative of hunger and frustration, ultimately suggesting a reduction in welfare. There are also potential health concerns associated with limit feeding, including increased standing time without eating (Hoffman et al., 2007; Kitts et al., 2011) and increased potential for the development of sub-acute ruminal acidosis (SARA; Moody et al., 2007).

Limit-fed dairy heifers require varied amounts of feed bunk space, depending on size and age, to achieve adequate ADG to meet target growth and calving age goals (Longenbach et al., 1999). Failure to provide adequate space results in an increase in attempts to feed, shorter duration of meals, less time spent feeding, and, ultimately, a decrease in overall ADG, as well as an increase in variability of ADG between individuals (Longenbach et al., 1999). At low levels of feed bunk space it appears that limit-fed dairy heifers are highly motivated to compete for feed access. It could be hypothesized that providing additional space at the feed bunk may provide ample opportunity for all animals to feed simultaneously and, thus, aid in reducing this competition. Therefore, the objective of this study was to examine the behavior of limit-fed dairy heifers when provided with an increase in feed bunk space in comparison to
recommended feed bunk allowance. In addition, we also set out to determine if the effects of provision of extra bunk space would be comparable to those seen when limit-fed heifers are provided a low-nutritive feedstuff, which has been previously demonstrated to increase feeding time, decrease inactive standing time, and maintain a targeted ADG (Kitts et al., 2011). The hypothesis was that both the addition of straw and the provision of additional feed bunk space would allow heifers to satisfy their foraging needs while minimizing competition.

4.2 Materials and Methods

4.2.1 Animals and Housing

Twelve Holstein dairy heifers were used in this study, which was conducted between November and December of 2009. Animals were 381.1 ± 44.8 (mean ± SD) d of age, and weighed 417.3 ± 47.9 kg at the beginning of the study. Over the course of the experiment, the animals gained 0.84 kg/d. Heifers were housed in 3 pens in the heifer research barn at the University of Guelph, Kemptville Campus (Kemptville, ON, Canada) and were managed according to the guidelines set by the Canadian Council on Animal Care (2009). The pens, located in a naturally-ventilated cold barn, consisted of an indoor sand-bedded pack area (3.6 x 10.9 m; width x depth) and an outdoor concrete run (3.6 x 16.4 m). Bedding material (fine-grain, washed sand) was replenished as needed. Feed bunks were located along the front of each pack area within each pen.

Prior to the beginning of the study, heifers were gradually acclimated to a reduced amount of a nutrient-dense TMR (Table 4.1) during a 10-d adaptation period. At the beginning of the adaptation period, heifers were fed the TMR ad libitum. Over the 10 d, the amount of feed offered was incrementally decreased until heifers were fed the ration at a restricted level of 1.93% of BW. At this feeding rate, the ration, fed once daily
at 1130 h, was formulated to meet the nutrient requirements of a dairy heifer growing at 0.9 kg/d (NRC, 2001). Heifers were given ad libitum access to trace mineral salt blocks (Windsor TM Stock Salt, The Canadian Salt Company Limited, Pointe-Claire, Quebec). Water was available ad libitum through a water bowl in each pen.

4.2.2 Experimental Design

The number of replicates required per treatment was determined through power analysis (Morris, 1999) for the primary response variables, including feeding behavior and DMI. Estimates of variation for these variables were based on previously reported values (Kertz and Chester-Jones, 2004; Hoffman et al., 2007; Kitts et al., 2011). Heifers were divided into 3 groups of 4, which were balanced for weight and age. Following the adaptation period, heifers were subjected, using a 3x3 Latin square design, to each of 3 treatments: 1) 0.68 m of feed bunk space/heifer, 2) 0.34 m of feed bunk space/heifer and 3) 0.34 m of feed bunk space/heifer with an additional 0.34 m of feed bunk space available for free-choice straw (offered at 2.0 kg as-fed/heifer/d). Heifers were kept on each treatment for a total of 7 d, which included a 3-d adaptation period to the treatment, followed by a 4-d data collection period.

4.2.3 Experimental Measurements

Group intakes were recorded daily by weighing the amount of feed offered and amount of feed refused (if any). These data were used to calculate daily DMI (kg/d) on a pen basis. Heifers were weighed on the same day each week to track ADG. Feeding and competitive behavior were monitored using time-lapse video, recorded continuously for the last 4 d of each treatment period. Heifers were recorded using 1 video camera (Panasonic WV-BP330; Osaka, Japan) per pen, a time-lapse cassette recorder (Panasonic AG-6740) and a video multiplexer (Panasonic WJ-FS 616). Video cameras
were mounted 3.2 m above each feed bunk. Red lights (100W), mounted adjacent to the cameras, were used to facilitate recording at night. Individual heifers were identified within each pen by their unique patterns. Digital photographs were taken of each animal’s head and another showing a dorsal view of the back. These photos were used during video analysis to identify individuals within each pen. The amount of time spent feeding during the 4-d recording period was scored for individual heifers using instantaneous scan sampling every 10 min. For each scan, a heifer was recorded as feeding when its head was completely past the feed rail and over the feed. If all feed had been consumed, the visit was recorded as an unrewarded bout. As validated by Kitts et al. (2011), total time spent feeding and total time spent in unrewarded visits were then calculated by multiplying the number of scans by 10. Unrewarded time and time spent feeding were then calculated for each heifer for each day of the recording periods (min/d). Additionally, to detect changes in the diurnal pattern of feed bunk attendance, these scans were used to calculate the percentage of heifers at the feed bunk over the course of each 24-h period. Based on these diurnal patterns, we calculated feeding time for each heifer during the 1.5 h period (period of peak feeding activity) following the delivery of feed, during which all of the TMR was consumed.

Feed bunk competition, recorded as displacements from the feed bunk while feeding, was measured for the last 4-d of each treatment period. A displacement was recorded when a butt or a push from the actor (instigator) resulted in the complete withdrawal of the reactor’s head from beneath the feed rail (DeVries et al., 2004). These observations were used to calculate the number of times each heifer was displaced from the feed bunk each day. Further, these data were used to calculate, according to DeVries et al. (2004), an index of success in agonistic interactions for each individual heifer. This index of success was calculated as follows: number of individuals
subdominant to focal heifer / number of individuals dominant to focal heifer + number of individuals subdominant to focal heifer x 100%.

4.2.4 Feed Sampling and Analysis

Representative grab samples of the TMR and straw were collected daily, for DM and chemical analysis, at the time of feed delivery during the recording days. Orts straw samples were also collected, when available, for DM analysis. Samples of the TMR components were taken weekly for DM and chemical analysis. Additionally, duplicate samples of the TMR, straw, and forage components were taken weekly for particle size analysis. All samples were immediately frozen at -20°C until they were analyzed. Samples for particle size separation were separated using a 3-screen (19, 8, 1.18 mm) Penn State Particle Separator (PSPS; Kononoff et al., 2003). Samples were separated into 4 fractions: long (>19mm), medium (<19, >8mm), short (<8, >1.18mm) and fine (<1.18mm) particles. After separation, the DM of each separated fraction was determined by forced air drying at 55°C for 48 h. Dry matter content of samples taken for chemical analysis was determined by drying samples in a forced air oven at 55°C for 48 h. These samples were then ground to pass through a 1-mm screen (Wiley Mill, Arthur H. Thomas Co., Philadelphia, PA). The ground samples were then sent to Cumberland Valley Analytical Services Inc. (Maugansville, MD, USA) for analysis of DM (135°C; AOAC, 2000: method 930.15), ash (535°C; AOAC, 2000: method 942.05), ADF (AOAC, 2000: 973.18), NDF with heat-stable α - amylase and sodium sulfite (Van Soest et al., 1991), NFC, and CP (N x 6.25) (AOAC 2000: method 990.03; Leco FP-528 Nitrogen Analyzer, Leco, St. Joseph, MI).

4.2.5 Calculations and Statistical Analysis
Preliminary inspection of the data revealed that all dependent variables were normally distributed. For analyses of treatment effects, the pen was considered the experimental unit. To ensure that the 3-d adaptation period allowed sufficient time for the animals to adapt to the treatments, a preliminary analysis of the effect of day within treatment period was conducted on the DMI, feeding, and competitive behavior data collected in the last 4 d of each treatment period. This analysis revealed no effect of day or day x treatment interaction. Therefore, DMI, feeding, and competitive behavior data were averaged for each pen across the 4 d of each treatment period. To test for differences among treatments, data were analyzed using the MIXED procedure of SAS (2003). The model included the fixed effect of treatment, the random effects of pen and period, and the residual error. All values reported are least square means. The Tukey-Kramer method was used to adjust the probability differences between least square means. Data for feed bunk attendance (% of heifers feeding) were summarized by hour for each pen for each treatment period. To test for effect of treatment on feed bunk attendance patterns, these data were analyzed using the MIXED procedure of SAS (2003) treating hour as a repeated measure. The model included the fixed effects of treatment and hour and the random effects of pen and period. The variance-covariance structure was first-order autoregressive, according to best fit with Schwarz’s Bayesian information criterion. Overall, treatment responses were tested using the pen as the experimental unit; however, we had a specific prediction that certain heifers would respond differently in their peak feeding activity based on their index of success. Thus, the effect of treatment on peak feeding activity was also evaluated using heifer as the observational unit and the heifers’ index of success as a covariate. These data were analyzed using the MIXED procedure of SAS (2003); the model included the index of success as a covariate, the fixed effect of treatment, the random effects of heifer within
pen and period, and the residual error. Significance was declared as $P \leq 0.05$ and trends were reported if $0.05 < P \leq 0.10$.

4.3 Results and Discussion

The amount of TMR provided was restricted across treatment groups and, as a result, DMI was similar for the TMR-0.68 and TMR-0.34 treatments. However, DMI was over 20% higher for the TMR-S treatment due to consumption of straw (Table 4.2). This is consistent with data from recent research wherein limit-fed heifers were offered straw alongside or mixed into their TMR (Kitts et al., 2011). The consumption of straw in addition to the limit-fed TMR indicates that restricting intake of TMR to 1.93% of BW or less is not sufficient to allow dairy heifers to become satiated.

Analysis of the diurnal feed bunk attendance of heifers revealed a treatment by hour interaction (SE = 0.5, $P < 0.001$; Figure 4.1), indicating the different feeding pattern exhibited by heifers while on the TMR-S treatment. A peak in feeding activity was seen immediately following feed delivery, regardless of treatment, which is consistent with the normal diurnal pattern of feeding behavior seen in young and adult cattle (DeVries and von Keyserlingk, 2005; DeVries and von Keyserlingk, 2009). Across treatments, there was a similar percentage of heifers feeding at the bunk during the period of peak feeding activity following feed delivery, resulting in similar feeding times by heifers across treatments during this time period (Table 4.2). This is not surprising as it was during this period of peak feeding activity that all heifers, regardless of treatment, consumed the entire allotment of TMR. Similarly, other researchers measuring feeding time of limit-fed heifers found that the TMR was completely consumed in just over 1 h following feed delivery (Hoffman et al., 2007; Kitts et al., 2011).
During this period of peak feeding activity, the frequency of displacements from the feed bunk was also similar between treatments. Kitts et al. (2011) found a comparable level of competition between heifers when provided a limit-fed TMR alone or with straw alongside. Our prediction was that with increased bunk space, limit-fed heifers would compete less for feed than heifers given less bunk space. However, all treatments allowed for sufficient space, well within suggested guidelines for feed bunk allowance (Longenbach et al., 1999), for all heifers to access feed simultaneously (Figure 4.1). The similar frequency of displacements when the heifers were consuming the TMR suggests that they were highly motivated to simply consume the feed, rather than engaging in competitive behaviour (Kitts et al., 2011). There was, however, an effect of index of success on peak feeding time \( (P < 0.001) \) with more dominant heifers spending more time feeding than those less dominant (peak feeding time = \([0.34 \times \text{index of success}] + 49.7; R^2 = 0.46\)). There was no index of success × treatment interaction \( (P = 0.9) \), indicating that this effect of dominance on peak feeding time was consistent across treatments. Longenbach et al. (1999) suggested that when feed bunk space for 11.5-15.5 mo old limit-fed heifers was less than 0.31 m/heifer, increased competition may have contributed to the increased variation in growth they observed. Based on those results, and that of the current study, it can be hypothesized that in situations of increased competition for a limit-fed ration, such as when feed bunk space is limited, domination of the feed bunk by certain animals could restrict DMI in subordinate heifers, leading to increased variability in ADG between heifers of differing social status. Further work is encouraged to address this hypothesis.

While on the TMR-0.34 and TMR-0.68 treatments, heifers consumed all their feed in just over 1 h. There is evidence from previous studies suggesting that ad libitum-fed dairy heifers will spend 3-5 hours feeding throughout the day, in an intensively-raised
setting, when given the opportunity (Greter et al., 2008; DeVries and von Keyserlingk, 2009), suggesting that heifers in this study were not meeting their diurnal feeding requirements. In fact, it has been demonstrated that lactating dairy cattle are motivated to orally manipulate (consume) feed whether the rumen is full or not, suggesting that the act of feeding itself (length of time spent feeding) has some impact on an animal's ability to feel satiated (Lindström and Redbo, 1990). In our study, heifers fed the limit-fed ration alone were not only limited in the time spent feeding but were also unlikely to achieve sufficient rumen fill, possibly resulting in frustration due to a lack of satiety and/or a lack of substrate to manipulate. Once all the TMR was consumed, feeding activity was only possible on the TMR-S treatment for the rest of the day (Figure 4.1). As a result of the consumption of the straw, feeding time was over twice as long on the TMR-S treatment, as compared with the other treatments (Table 4.2). It is likely, therefore, that the consumption of straw alongside the limit-fed TMR contributed to greater satiety due to the consumption of sufficient bulk over a longer period of time throughout the day, as seen also in the Kitts et al. (2011) study.

The amount of unrewarded time at the feed bunk was similar across treatments (Table 4.2). A recent study with calves showed that visits to the milk feeder doubled when calves were limit-fed compared with ad libitum-fed calves (De Paula Vieira et al., 2008). Those researchers concluded that unrewarded visits to the feeder could be used as an indicator of hunger. In our study, then, it could be suggested that some heifers on all treatments were expressing hunger behavior at some point during the day. Hunger is an indicator of negative welfare (von Keyserlingk et al., 2009) and, as such, limit feeding may pose a welfare concern. Interestingly, heifers on the TMR-S treatment spent a similar amount of unrewarded time at the feed bunk, despite having access to a low-nutritive feedstuff throughout the day, suggesting that heifers on this treatment were
highly motivated to consume the nutrient-dense TMR rather than the straw. Further research on the motivation of limit-fed heifers to achieve satiety is needed to determine the importance of feeding behavior to these animals.

4.4 Conclusions

Overall, the provision of feeding space in excess of that which allows heifers to feed simultaneously had no impact on the feeding or competitive behavior of limit-fed dairy heifers. Alternatively, the provision of straw alongside of a limit-fed TMR did have a positive impact on dairy heifers fed a TMR in a limited amount as it resulted in greater DMI, which would contribute to further rumen fill, and an increase in time spent feeding throughout the day.

4.5 Acknowledgements

We thank the staff and students at the University of Guelph, Kemptville Campus Dairy Education and Research Centre. In particular, we thank Megan Bruce, Albert Koekkoek, and Kerri Stevenson for their technical assistance with this project. Angela Greter was supported by a Natural Sciences and Engineering Research Council of Canada (NSERC) Canadian Graduate Scholarship. This project was funded through an Ontario Ministry of Agriculture, Food and Rural Affairs (OMAFRA)/University of Guelph Production Systems research grant. This project was also supported through contributions from the Canadian Foundation for Innovation (CFI) and the Ontario Research Fund.
Table 4.1. Ingredient, chemical composition, and particle size (mean ± SD) of the treatment diet.

<table>
<thead>
<tr>
<th>Composition</th>
<th>Ingredient, %DM</th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Corn silage$^1$</td>
<td>20.1</td>
<td></td>
<td></td>
<td></td>
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</tr>
<tr>
<td></td>
<td>Grass/alfalfa haylage$^2$</td>
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<td></td>
<td></td>
<td></td>
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</tr>
<tr>
<td></td>
<td>High moisture corn$^3$</td>
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<td></td>
<td></td>
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<tr>
<td></td>
<td>Protein supplement$^4,5$</td>
<td>10.4</td>
<td></td>
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</tr>
<tr>
<td>Chemical composition$^6$</td>
<td>DM, %</td>
<td>56.7 ± 1.5</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>OM, % of DM</td>
<td>94.0 ± 0.1</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>CP, % of DM</td>
<td>14.1 ± 0.3</td>
<td></td>
<td></td>
<td></td>
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<tr>
<td></td>
<td>ADF, % of DM</td>
<td>17.0 ± 1.1</td>
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<td></td>
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<tr>
<td></td>
<td>NDF, % of DM</td>
<td>25.9 ± 1.4</td>
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<td></td>
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<tr>
<td></td>
<td>NFC, % of DM</td>
<td>50.8 ± 1.8</td>
<td></td>
<td></td>
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<tr>
<td>Particle Size$^7$, %</td>
<td>Long</td>
<td>2.4 ± 1.6</td>
<td></td>
<td></td>
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<tr>
<td></td>
<td>Medium</td>
<td>46.7 ± 2.5</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Short</td>
<td>40.4 ± 3.4</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Fine</td>
<td>10.5 ± 0.6</td>
<td></td>
<td></td>
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<td></td>
</tr>
</tbody>
</table>

$^1$Chemical composition of corn silage (DM basis) was 8.1 ± 0.2% CP, 19.8 ± 0.8% ADF, and 34.1 ± 0.7% NDF.

$^2$Chemical composition of grass/alfalfa haylage (DM basis) was 17.5 ± 0.3% CP, 36.6 ± 1.0% ADF, and 45.3 ± 0.8% NDF.
3Chemical composition of high moisture corn (DM basis) was 8.3 ± 0.4% CP, 4.7 ± 0.7% ADF, and 12.6 ± 1.1% NDF.

4Chemical composition of the protein supplement (DM basis) was 39.4 ± 0.6% CP, 11.0 ± 1.4% ADF, and 16.1 ± 1.6% NDF.

5Supplied by Ritchie Feed & Seed Inc. (Ottawa, ON, Canada), containing (on as-is basis): 30.3% soybean meal, 24.2% Tri-Pro Gold (Tri-County Protein Corp., Winchester, ON, Canada), 16.5% corn gluten meal, 11.0% canola meal, 5.9% ground limestone, 5.8% trace mineral/vitamin premix, 4.1% sodium bicarbonate, 2.2% cobaltized-iodized salt.

6Values were obtained from chemical analysis of TMR samples. NFC = 100 – (%CP + %NDF + %fat + %ash).

7Particle size determined by Penn State Particle Separator which has a 19 mm screen (long), 8 mm screen (medium), 1.18 mm screen (short) and a pan (fine).
Table 4.2. Intake and behavior measures from growing dairy heifers on experimental treatments\textsuperscript{1}.

<table>
<thead>
<tr>
<th>Item</th>
<th>Treatment\textsuperscript{2}</th>
<th></th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>TMR-0.68</td>
<td>TMR-0.34</td>
<td>TMR-S</td>
<td>SE</td>
<td>(P)-value</td>
</tr>
<tr>
<td>DMI, kg/d</td>
<td>7.8</td>
<td>7.8</td>
<td>9.4</td>
<td>0.1</td>
<td>0.001</td>
</tr>
<tr>
<td>Feeding time, min/d</td>
<td>64.6</td>
<td>64.4</td>
<td>147.7</td>
<td>6.6</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Peak\textsuperscript{3} feeding time, min/d</td>
<td>65.2</td>
<td>64.2</td>
<td>70.8</td>
<td>4.9</td>
<td>0.9</td>
</tr>
<tr>
<td>Unrewarded time, min/d</td>
<td>39.6</td>
<td>36.0</td>
<td>24.6</td>
<td>6.9</td>
<td>0.4</td>
</tr>
<tr>
<td>Displacements, no./d</td>
<td>13.0</td>
<td>13.2</td>
<td>23.8</td>
<td>2.9</td>
<td>0.08</td>
</tr>
<tr>
<td>Peak\textsuperscript{3} displacements, no./d</td>
<td>13.0</td>
<td>13.2</td>
<td>15.1</td>
<td>2.4</td>
<td>0.5</td>
</tr>
</tbody>
</table>

\textsuperscript{1}Data are averaged across 3 pens (4 heifers/pen) for 4 d on each treatment.

\textsuperscript{2}Heifers were allowed either: 1) 0.68 m of bunk space, 2) 0.34 m of bunk space, or 3) 0.34 m of bunk space for a limit-fed TMR and an additional 0.34 m of bunk space for straw.

\textsuperscript{3}Peak feeding activity period = 1.5-h period immediately following feed delivery.
Figure 4.1. Percentage of heifers present at the feed bunk over a 24-h period (percentage for each 10-min interval during the day) for 3 treatments: 1) 0.68 m of bunk space/heifer, 2) 0.34 m of bunk space/heifer, and 3) 0.34 m of bunk space/heifer for TMR and an additional 0.34 m of bunk space/heifer for straw. Data were averaged across 4 d for 3 pens (4 heifers/pen) on each treatment.
5.1 Introduction

Recent interest in limit feeding strategies has driven an increase in research relating to heifer feeding management. Limit feeding involves feeding a nutrient-dense ration, composed of high levels of concentrate and/or high-quality forages, in a restricted quantity. There are many benefits to this feeding strategy, including a decrease in fecal excretion (and subsequent nitrogen excretion), reduced feed costs, increased feed efficiency, and effective control of ADG (Hoffman et al., 2007; Lascano et al., 2009; Kitts et al., 2011).

Despite the many demonstrated benefits to limit feeding, there are a number of behavioral, health, and welfare concerns associated with this practice. These include decreased feeding and lying time, and increased unrewarded time at the feed bunk, vocalizations, and inactive standing time (Hoffman et al., 2007; Greter et al., submitted; 2011; Kitts et al., 2011). Additionally, rations high in fermentable carbohydrates, when rapidly consumed, leave replacement heifers susceptible to sub-clinical depressions in rumen pH (Quigley, 1992; Moody et al., 2007). Researchers have recently attempted to identify why these concerns arise, and what factors may help diminish or eliminate them. When offered straw alongside a limit-fed TMR, heifers will immediately begin consuming the straw upon completion of the TMR, suggesting that they are still hungry and continue to be motivated to feed (Chapter 4; Kitts et al., 2011). In a more recent study we demonstrated that increased frequency of feed delivery of a limit-fed TMR did not result in a return to more natural feeding patterns as heifers fed more frequently (2x or 4x/d) spent less time feeding throughout the day (less than 1 h) and more time standing without eating than heifers fed 1x/d (Greter et al., submitted). We have speculated that
this is due to the high feeding motivation in these animals. Additionally, in that study, variability in ADG between individuals within a pen was greater when heifers were fed more frequently.

One of the essential management aspects associated with limit feeding concerns the amount of feeding space needed for heifers to feed simultaneously. When limit-fed heifers lack adequate space, the level of competition at the feed bunk increases, time spent feeding decreases, and variability in heifer growth between animals increases (Keys et al., 1978; Longenbach et al., 1999). Alternatively, providing more than adequate space (0.68 vs. 0.34 m/heifer) has been shown to have no benefit on the behavior of limit-fed heifers (Chapter 4). Zanton and Heinrichs (2008) recently suggested that, when feed bunk space is insufficient for limit-fed heifers to feed simultaneously, producers should consider feeding 2x/d at close intervals (i.e. 2 h apart). The untested hypothesis of these researchers was that this provides more opportunity for larger, dominant animals to feed at will after the first feed delivery and the more timid animals to feed after the second feed delivery. Unfortunately, given that limit-fed heifers consume their feed so quickly and are still motivated to feed following complete consumption of the TMR present in the bunk (Kitts et al., 2011), it could be hypothesized that the dominant heifers may return and dominate the feed bunk after the second feed delivery as well.

Therefore, the objective of this experiment was to determine the interaction between feed bunk space and frequency of feed provision on the feeding behavior patterns and growth of growing dairy heifers fed in a limited amount. We hypothesized that delivering a limit-fed ration twice daily (2x/d) in two equal amounts (2 h apart) to heifers with restricted feed bunk space will result in less time feeding due to increased competition at the feed bunk in comparison to heifers given adequate feed bunk space and fed once per day (1x/d).
5.2 Materials and Methods

5.2.1 Animals and Housing

Sixteen dairy heifers were used in this study, 8 of which were owned by the University of Guelph, Kemptville Campus and the remaining 8 acquired, on loan, from a local commercial dairy operation. Upon arrival, all heifers were given a broad-spectrum antibiotic (Draxxin, tulathromycin, Pfizer Animal Health, Kirkland, Quebec, Canada) to prevent potential sickness due to transport and mixing stresses (Stanton et al., 2010) and were given a 14-d adaptation period to acclimate to groups and their environment. Heifers were 183.4 ± 9.1 (mean ± SD) d of age and weighed 223.3 ± 20.5 kg at the beginning of the study. Heifers weighed 312.6 ± 29.1 kg at the end of the study. Heifers were housed in pens of 4 that were balanced for age and weight. Pens were located in a naturally-ventilated barn at the University of Guelph, Kemptville Campus (Kemptville, Ontario, Canada) and were managed according to the guidelines set by the Canadian Council on Animal Care (2009). Use of heifers was approved by the University of Guelph’s Animal Care Committee (AUP#09R022). The study was conducted between March and June of 2011. Pens consisted of an indoor sand-bedded pack area (3.6 m x 10.9 m; width x depth) and an outdoor concrete run (3.6 m x 16.4 m). Sand bedding was cleaned out and replenished once weekly throughout the experiment. Feed bunks were located along the front of each indoor pack area and varied in length depending on treatment. Water was available ad libitum to the heifers through a water bowl in each pen. Heifers were given ad libitum access to trace mineral salt blocks (Windsor TM Stock Salt, The Canadian Salt Company Limited, Pointe-Claire, Quebec, Canada).

5.2.2 Experimental Design

Prior to the start of the study, all heifers had previously been fed a TMR. Upon arrival, heifers were fed a high-concentrate TMR (Table 5.1) that was formulated, and
fed at a restricted level (2.0% of BW), to meet the nutrient requirements for a non-bred Holstein heifer growing at 0.9 kg/d (NRC, 2001). Over the 14-d adaptation period, heifers were fed straw (initially offered at 8.0 kg/pen as-fed) offered alongside the TMR. This long forage was provided to stimulate rumination, chewing, and resultant buffering (Beauchemin et al., 2008) to help transition the rumen to the high-concentrate TMR. The transition was done over 8 d; the amount of straw offered was gradually reduced by 1.0 kg/pen/d until heifers were only provided with the high concentrate, limit-fed TMR.

Following the adaptation period, groups of heifers were exposed to each of 4 treatments, in 21-d periods, using a 4x4 Latin square design with a 2x2 factorial arrangement of treatments. The treatments included arrangement of 2 feed delivery frequencies (1x/d at 1200 h and 2x/d at 1200 and 1400 h) and 2 levels of feed bunk space (adequate feed bunk space: 0.40 m/heifer and reduced feed bunk space: 0.29 m/heifer), resulting in 4 treatments. Each 21-d treatment period consisted of a 14-d adaptation period (to allow heifers to acclimate to the treatment), followed by a 7-d data collection period. The feed bunk was a fence-line feed bunk with a post and rail access. Once daily, dietary components were mixed in a TMR mixer wagon (Jaylor 4425, Jaylor Fabricating, Orton, ON, Canada). The appropriate amount of TMR was manually weighed out for each pen. Daily feed amounts for heifers fed twice per day were divided into two equal amounts with the second half of feed kept in an air conditioned room (12°C) in the barn until the second delivery. The total amount of feed offered to each pen was adjusted weekly, according to average pen BW.

5.2.3 Experimental Measurements

Group intakes were recorded daily throughout the study by weighing the amount of feed offered and amount of feed refused (if any). This data was used to calculate daily DMI (kg/d) on a pen basis. Heifers were weighed and measured on the same 2
consecutive days each week at 0700 h and these weekly weights were averaged for each heifer and used to calculate ADG.

Feeding and competitive behavior were monitored, using time-lapse video, continuously for the last 7-d of each treatment period. Heifers were recorded using 1 video camera (Panasonic WV-BP330; Osaka, Japan) per pen, a time-lapse video cassette recorder (Panasonic AG-6740) and a video multiplexer (Panasonic WJ-FS 616). Video cameras were mounted 3.2 m above each of the 4 feed bunks. Red lights (100W), mounted adjacent to the cameras, were used to facilitate recording at night. Digital photographs were taken showing a dorsal view of the back of each animal. These photos were used during video analysis to identify individuals within each pen. The amount of time spent feeding during the 7-d recording period was scored for individual heifers using instantaneous scan sampling every 1 min. For each scan, feeding was defined when a heifer had her head completely past the feed rail and over the feed. Total time spent feeding was then calculated for each heifer for each day of the 7-d recording period. To identify changes in diurnal feeding patterns between treatments, these scans were used to calculate the percentage of heifers feeding at the feed bunk over a 24-h period. Unrewarded time at the feed bunk (presence at the feed bunk when no feed was present) was also measured using 1-min scans. For each scan, unrewarded time at the feed bunk was defined when a heifer had her head completely past the feed rail and over an empty feed bunk. Total daily unrewarded time at the feed bunk was calculated for each day of the 7-d recording periods. Feeding competition, recorded as displacements from the feed bunk while feeding, was measured on d 16, 18 and 20 of each treatment period. A displacement was marked when a butt or a push from the actor (instigator) resulted in the complete withdrawal of the reactor’s head from beneath the feed rail (DeVries et al., 2004). These observations were used to calculate
the number of times each heifer was displaced from the feed bunk each day and also per minute of feeding time. Further, these data were used to calculate, according to DeVries et al. (2004), an index of success in agonistic interactions for each individual heifer. This index of success was calculated as follows: number of individuals subdominant to focal heifer / total number of individuals within a pen x 100%.

Lying behavior was recorded during the last 7-d of each treatment period. Daily lying times of individual heifers were collected using electronic data loggers (HOBO Pendant G Data Logger, Onset Computer Corporation, Pocasset, MA), previously validated by Ledgerwood et al. (2010). These devices were placed on the hind leg of each heifer using veterinary bandaging (Vetrap Bandaging Tape, 3M, St. Paul, MN) and measured the orientation of the leg at 1-min intervals. This data was summarized to calculate the average daily standing/lying time (min/d) as well as time spent standing without eating (e.g. daily standing time – feeding time).

5.2.4 Feed Sampling and Analysis

Representative grab samples of the TMR were taken for DM and chemical analysis during the last 7 d (recording weeks) of each treatment period. Samples of the dietary components were also taken for DM and chemical analysis once during each of these recording weeks. Duplicate samples of forage components were taken, once weekly during the last 7 d of each treatment period, for particle size separation. Grab samples of the TMR were also collected for particle size separation at the time of feed delivery twice weekly during the last 7 d of each treatment period. All samples were immediately frozen at -20°C until they were analyzed. Samples for particle size separation were thawed at a later date and separated using a 3-screen (19, 8, 1.18 mm) Penn State Particle Separator (PSPS; Kononoff et al., 2003). This separated the samples into 4 fractions: long (>19mm), medium (<19, >8mm), short (<8,>1.18mm) and
fine (<1.18mm) particles. After separation, DM of each separated fraction was determined by forced air drying at 55°C for 48 h. Samples taken for DM and chemical analysis were oven dried at 55°C for 48 h and then ground to pass through a 1 mm screen (Wiley Mill, Arthur H. Thomas Co., Philadelphia, PA). These samples were then sent to Cumberland Valley Analytical Services Inc. (Maugansville, MD, USA) for analysis of DM (135°C; AOAC, 2000: method 930.15), ADF (AOAC, 2000: 973.18), NDF with heat-stable α-amylase and sodium sulfite (Van Soest et al., 1991) and CP (N x 6.25) (AOAC 2000: method 990.03; Leco FP-528 Nitrogen Analyzer, Leco, St. Joseph, MI).

**5.2.5 Calculations and Statistical Analysis**

Data for intakes, growth (ADG), feeding time, unrewarded time, lying and standing behavior, and competitive behavior were averaged for each pen by treatment period. Variation within the pen in ADG (ADGv) was determined by calculating the standard deviation of weekly heifer gain values within treatment periods. Feeding time variability was determined by calculating the standard deviation of daily values for individual heifers within a pen within treatment periods and then averaging by period. Data for feed bunk attendance (percentage of heifers at the feed bunk when feed was and was not present) was summarized by hour for each pen for each treatment period.

Preliminary inspection of the data revealed that all dependent variables were normally distributed. To test for differences among treatments, DMI, ADG, ADGv, feeding and unrewarded time, feeding time variability, displacements, lying time, and time spent standing without eating were analyzed using the MIXED procedure of SAS (2008). The model included the fixed effects of period, feed delivery frequency, and bunk space, the random effect of pen, and the residual error. Interactions of period and treatments were also tested in the model. To test for the effect of treatment for diurnal feeding patterns, the data were analyzed using the MIXED procedure of SAS (2008).
treating hour as a repeated measure. The model included the fixed effects of period, feed delivery frequency, bunk space, hour, the random effect of pen, and the residual error. The covariance structure was autoregressive, according to the best-fit Schwarz’s Bayesian information criterion.

Overall, treatment responses were tested using the pen as the experimental unit; however, we had a specific prediction that certain heifers would respond differently in behavior based on their index of success. Thus, the effect of treatment on ADG, ADGv, feeding time, feeding time variability, unrewarded time, and lying behavior was also evaluated using heifer as the observational unit and the heifers’ index of success as a covariate. These data were analyzed using the MIXED procedure of SAS (SAS, 2009); the model included the index of success as a covariate, the fixed effects of period, feed delivery frequency, and bunk space, the random effect of heifer within pen, and the residual error.

All values reported are least squares means. Significance was declared as \( P \leq 0.05 \) and trends were reported if \( 0.05 < P \leq 0.10 \).

5.3 Results and Discussion

There were no orts throughout this study. As expected in a study with controlled feed amounts, DMI did not differ between treatments (Table 5.2). Although ADG was greater with increased feed bunk space and tended to be greater when delivering feed 1x/d, the variability of ADG within each pen did not differ (Table 5.2), indicating that treatment effects on growth were similar across animals within a pen. Heifers offered more feed bunk space may have been able to gain more than heifers with reduced bunk space due to the fact that increased space allows all heifers to feed simultaneously, without interference from their neighbors, and provides sufficient space to avoid
competition or more subtle social pressure (Albright, 1993; Collings et al., 2011). It is possible that this increase in ADG is due to a more stable rumen microbial population resulting from less overall fluctuation in rumen pH throughout the day, as observed by Greter et al. (submitted) through measurement of rumen temperature. Indeed, heifers provided with 0.40 m/heifer were more feed efficient than heifers provided with 0.29 m/heifer (Table 5.2). Heifers fed 1x/d were not, however, more efficient than heifers fed 2x/d. Similarly, Greter et al. (submitted) found that heifers fed 1x/d were not more efficient than heifers fed 2x or 4x/d. There was a tendency for an interaction between feed bunk space and frequency of feed delivery on the feed efficiency of limit-fed dairy heifers with heifers provided with restricted bunk space being less efficient when fed 2x/d. This may be due to day-to-day variability in individual DMI within a pen as the restricted bunk space in combination with smaller amounts of feed offered at each feed delivery may have increased the jostling at the bunk or impacted individual feeding rates. As we were unable to measure individual DMI or feeding rate in this study, further work on this is encouraged.

It must be noted that, although all efforts were made to consistently deliver feed at 1200 h, some day-to-day variability did occur, thereby impacting the diurnal feeding patterns (Figure 5.1). Analysis of the diurnal feed bunk attendance of heifers revealed no feed bunk space by hour interaction ($P=1.0$), but did show a feed delivery frequency by hour interaction (SE = 0.86, $P<0.001$; Figure 5.1a) when feed was available, indicating that feed bunk attendance differed as feed delivery frequency was altered with more heifers at the feed bunk immediately following feed delivery when fed 1x/d but not when fed 2x/d. This may have been due to the length of time between feed deliveries affecting the hunger status of the heifers. Overall, heifers fed 1x/d spent more time feeding per day; this is consistent with previous research (Greter et al., submitted) wherein heifers
fed 1x/d spent just over 1 h feeding while heifers fed 2x/d or 4x/d spent less than an hour feeding per day.

Heifers fed 1x/d were more competitive than heifers fed 2x/d (Table 5.2). When fed 1x/d, the time in between feedings was longer than when heifers were fed 2x/d. Following acclimation to treatment, heifers may have recognized that a single feed delivery is all that was to be provided on a daily basis and, therefore, if they did not consume large amounts of feed upon feed delivery, they would not be able to consume enough to meet their requirements. As such, these heifers would compete more for feed than heifers fed 2x/d in an effort to ensure maximal feed intake. This finding is, however, contrary to previous research that has indicated that frequency of feed delivery does not have an effect on displacements at the feed bunk in ad libitum-fed lactating dairy cows (Phillips and Rind, 2001; DeVries et al., 2005) or limit-fed dairy heifers (Greter et al., submitted). Thus, further research on feeding management factors and the social behavior of animals fed under limit feeding regimes is encouraged.

Restricted feed bunk space did not increase competition among heifers (Table 5.2). This is consistent with previous research wherein providing limit-fed heifers with varied amounts of feed bunk space did not alter competitive interactions (Chapter 4). However, the lesser amount of feed bunk space provided in that study allowed for 0.34 m/heifer of space, which was enough space to allow all animals to feed simultaneously. In the current study, although bunk space was restricted to 0.29 m/heifer, all heifers were still able to feed simultaneously, though with difficulty and in tight arrangement. It therefore seems that limit-fed heifers are so motivated to feed that they will forego competitive interactions in an effort to obtain as much feed as possible (Kitts et al., 2011). Longenbach et al. (1999) found that restricting feed bunk space (to as little as 0.15 m/heifer) for limit-fed heifers increased competition at the feed bunk and that this
competition decreased as feed bunk length increased. Those researchers restricted feed bunk space much more severely than was done in the present study. As such, competition would likely become more severe with increasingly restricted space allowance. Index of success did not affect an individual’s response to treatment and, therefore, will not be discussed further. Despite the lack of difference in displacements between treatments, less feed bunk space and delivering feed 1x/d did result in greater within-day variability in feeding time between heifers within a pen than more feed bunk space and delivering feed 2x/d (Table 5.2). It is likely that feeding time is related to social factors existing at the feed bunk. Cattle have established hierarchies in which subordinates do not feed near dominant animals (Manson and Appleby, 1990). When feed bunk space is restricted and feed is provided in a limited amount, it is likely that subordinate animals must alter their behavioral time budget in an effort to obtain sufficient feed for their metabolic requirements and growth. Aggression may increase in all heifers managed under conditions of restricted bunk space, resulting in increased jostling for position and pressure from conspecifics at the bunk (Rutter et al., 1987; Longenbach et al., 1999). The increased motivation of limit-fed animals may be such that complete displacements from the feed bunk (as defined by DeVries et al., 2004) do not occur as a result of feeding disruptions at the bunk (i.e. nudges or otherwise undetectable actions unable to be recognized through video analysis). Rather, this competitive activity may result in increased variability in feeding times among individuals within a pen. As we were unable to measure individual intakes, it is unclear whether heifers spending more time feeding also consumed more feed; thus we encourage further work in this area.

The amount of unrewarded time at the feed bunk (present at the bunk with no feed available) did not differ between treatments. This may have been an artifact of the
experimental design, with different treatments assigned to adjacent pens. As cattle are known to synchronize their behavior (Rook and Huckle, 1995), it appears that one heifer visiting the empty bunk would prompt other heifers to do the same. The majority of unrewarded visits surround feeding time (Figure 5.1a and b) and it seems that heifers fed 1x/d are still present at the empty bunk when heifers fed 2x/d are being fed the second time while heifers fed 2x/d spread their unrewarded visits more evenly across the feeding period, possibly reflecting dissatisfaction associated with lack of foraging and/or continuation of hunger (lack of satiety from smaller meals).

Heifers spent a similar daily amount of time lying down and standing without eating across all treatments (Table 5.2). This is similar to a previous study examining the effects of feed delivery frequency on the behavior of limit-fed heifers (Greter et al., submitted) and has also been demonstrated in studies with adult cattle (DeVries et al., 2005; Robles et al., 2007). The effect of feed bunk space on lying behavior has not been examined in previous work with limit-fed dairy heifers. Although this is the first study identifying no effect of feed bunk space on time spent standing without eating in limit-fed heifers, previous research examining frequency of feed delivery has shown that heifers fed more frequently (2x or 4x/d) spent more time standing without eating than heifers fed 1x/d (Greter et al., submitted). Heifers in the current study were more variable in their lying time than heifers in the study conducted by Greter et al. (submitted) and this translated into higher variability in time spent standing without eating. As such, despite a difference in feeding time, there was no overall effect of frequency of feed delivery on time spent standing without eating.

5.4 Conclusions

Overall, there were no interactions between feed bunk space and feed delivery frequency on the feeding behavior of limit-fed dairy heifers. However, providing 0.40 m
vs. 0.29 m of feed bunk space per heifer did increase ADG and minimize the variability in feeding time among individuals within a pen. Delivering feed 1x/d also contributed to a tendency for greater ADG and increased daily time spent feeding. However, delivering feed 1x/d also resulted in more variability in feeding time among heifers within a pen and an increase in competitive interactions at the feed bunk. From these results it is recommended to provide adequate feed bunk space to allow limit-fed heifers to comfortably feed simultaneously (i.e. not restricted or forced to feed in close proximity to dominant heifers). Furthermore, delivering a limit-fed ration 1x/d will enable the heifers to gain well and to spend more time feeding per day, but may also result in increased competition for feed.

5.5 Acknowledgements

We thank the staff and students at the University of Guelph, Kemptville Campus Dairy Education and Research Centre, particularly Megan Bruce, Bianca Kitts, Alexa Main, and Nancy Stonos for their technical help and support. Angela Greter was supported by a Natural Sciences and Engineering Research Council of Canada (NSERC, Ottawa, Ontario, Canada) Alexander Graham Bell Canadian Graduate Scholarship. This project was funded through an Ontario Ministry of Agriculture, Food and Rural Affairs (OMAFRA, Guelph, Ontario, Canada)/University of Guelph Production Systems research grant. This project was also supported through contributions from the Canadian Foundation for Innovation (CFI, Ottawa, Ontario, Canada)) and the Ontario Research Fund (Ministry of Research and Innovation, Toronto, Ontario, Canada).
Table 5.1. Chemical composition and particle size distribution (mean ± SD) of the treatment ration and the forages.

<table>
<thead>
<tr>
<th>Item</th>
<th>Treatment Ration¹,²</th>
<th>Corn Silage</th>
<th>Grass/Alfalfa Haylage</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Chemical composition³</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>DM, %</td>
<td>52.0 ± 3.7</td>
<td>44.1 ± 3.1</td>
<td>32.5 ± 4.2</td>
</tr>
<tr>
<td>OM, % of DM</td>
<td>93.1 ± 0.5</td>
<td>96.7 ± 0.2</td>
<td>87.1 ± 0.6</td>
</tr>
<tr>
<td>CP, % of DM</td>
<td>15.7 ± 0.9</td>
<td>8.2 ± 0.1</td>
<td>17.0 ± 1.6</td>
</tr>
<tr>
<td>ADF, % of DM</td>
<td>16.1 ± 1.4</td>
<td>22.9 ± 0.7</td>
<td>35.0 ± 2.7</td>
</tr>
<tr>
<td>NDF, % of DM</td>
<td>26.9 ± 2.4</td>
<td>37.2 ± 1.8</td>
<td>52.1 ± 4.6</td>
</tr>
<tr>
<td>NFC, % of DM</td>
<td>47.2 ± 2.5</td>
<td>49.0 ± 1.6</td>
<td>15.8 ± 2.6</td>
</tr>
<tr>
<td><strong>Particle Size⁴, %</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Long</td>
<td>5.5 ± 2.0</td>
<td>6.3 ± 0.8</td>
<td>39.6 ± 10.6</td>
</tr>
<tr>
<td>Medium</td>
<td>43.0 ± 4.5</td>
<td>54.7 ± 6.3</td>
<td>41.6 ± 8.5</td>
</tr>
<tr>
<td>Short</td>
<td>45.9 ± 3.6</td>
<td>35.5 ± 6.8</td>
<td>17.9 ± 5.2</td>
</tr>
<tr>
<td>Fine</td>
<td>5.7 ± 2.6</td>
<td>3.6 ± 2.0</td>
<td>0.9 ± 0.8</td>
</tr>
</tbody>
</table>

¹Ration composition (DM basis) was 20.0% corn silage, 20.0% grass/alfalfa haylage, 45.0% high moisture corn, and 15.0% protein supplement (Supplied by Dundas Feed and Seed (Winchester, ON, Canada), containing (on as-is basis): 25.6% corn gluten meal, 24.4% Tri-Pro Gold (Tri-County Protein Corp., Winchester, ON, Canada), 24.4% soybean meal, 10.0% canola meal, 4.8% ground limestone, 4.5% trace mineral/vitamin premix, 4.4% sodium bicarbonate, 1.9% cobaltized-iodized salt).

²Chemical composition of high moisture corn (DM basis) was 9.3 ± 0.0% CP, 4.0 ± 0.2% ADF, and 12.0 ± 0.5% NDF.

³Values were obtained from chemical analysis of TMR samples. NFC = 100 – (%CP + %NDF + %fat + %ash).

⁴Particle size determined by Penn State Particle Separator which has a 19 mm screen (long), 8 mm screen (medium), 1.18 mm screen (short) and a pan (fine).
Table 5.2. Intake and behavior measures from growing dairy heifers on experimental treatments.

<table>
<thead>
<tr>
<th>Item</th>
<th>1x 0.40 m</th>
<th>1x 0.29 m</th>
<th>2x 0.40 m</th>
<th>2x 0.29 m</th>
<th>SE</th>
<th>Bunk</th>
<th>Freq</th>
<th>Bunk x Freq</th>
</tr>
</thead>
<tbody>
<tr>
<td>DMI, kg/d</td>
<td>5.5</td>
<td>5.5</td>
<td>5.5</td>
<td>5.5</td>
<td>0.03</td>
<td>0.6</td>
<td>0.9</td>
<td>0.7</td>
</tr>
<tr>
<td>ADG, kg/d</td>
<td>1.0</td>
<td>0.9</td>
<td>1.0</td>
<td>0.8</td>
<td>0.04</td>
<td>0.008</td>
<td>0.1</td>
<td>0.2</td>
</tr>
<tr>
<td>ADGv, kg/d</td>
<td>0.4</td>
<td>0.4</td>
<td>0.3</td>
<td>0.2</td>
<td>0.08</td>
<td>0.6</td>
<td>0.2</td>
<td>0.5</td>
</tr>
<tr>
<td>Feed efficiency (DMI/ADG)</td>
<td>5.7</td>
<td>5.9</td>
<td>5.5</td>
<td>6.8</td>
<td>0.3</td>
<td>0.02</td>
<td>0.2</td>
<td>0.06</td>
</tr>
<tr>
<td>Feeding time, min/d</td>
<td>72.5</td>
<td>68.4</td>
<td>58.3</td>
<td>59.4</td>
<td>3.9</td>
<td>0.5</td>
<td>0.002</td>
<td>0.3</td>
</tr>
<tr>
<td>Feeding time variability, min/d</td>
<td>3.8</td>
<td>4.8</td>
<td>2.5</td>
<td>4.0</td>
<td>0.8</td>
<td>0.03</td>
<td>0.05</td>
<td>0.6</td>
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<td>22.3</td>
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Figure 5.1. Percentage of heifers present at the feed bunk over a 24-h period (summarized by hour) for 4 treatments when: a) feed was available, and b) no feed was available (unrewarded visits). Treatments were: 1) adequate bunk space (0.40 m/heifer) fed 1x (1200 h) each day (A1x), 2) adequate bunk space (0.40 m/heifer) fed 2x (1200 and 1400 h) each day (A2x), 3) restricted bunk space (0.29 m/heifer) fed 1x each day (R1x), and 4) restricted bunk space (0.29 m/heifer) fed 2x each day (R2x). Data were averaged across 7 d for 4 pens (4 heifers/pen) on each treatment.
CHAPTER 6: DO LIMIT-FED HEIFERS PREFER SUPPLEMENTARY LONG OR SHORT STRAW?

6.1 Introduction

Limit feeding has drawn attention in recent years as it offers much potential to reduce the costs associated with raising replacement dairy heifers. Feeding a nutrient-dense ration in a limited amount has the potential to reduce feed costs and nutrient/fecal excretion as well as improve feed efficiency (Hoffman et al., 2007; Lascano et al., 2009). Despite these benefits, limit feeding also poses a number of concerns. For instance, limit-fed heifers spend much less time feeding (~1-2 h; Chapter 4, Kitts et al., 2011) daily than ad libitum-fed heifers raised under intensive (3-5 h; Greter et al., 2008; DeVries and von Keyserlingk, 2009) or extensive (4-9 h; Hafez and Bouissou, 1975) environments. Additionally, Hoffman et al. (2007) found an increase in vocalizations, standing without eating, and aggressive “reaching” for feed in limit-fed heifers. Limit feeding cattle has also been associated with an increase in oral stereotypies such as bar biting, head nodding, and tongue rolling/playing (Redbo et al., 1996; Lindström and Redbo, 2000). These behavioral changes may be associated with hunger and frustration due to lack of satiety (Savory et al., 1993), or frustration due to lack of foraging substrate (Lindström and Redbo, 2000). Finally, limit-fed heifers may experience more severe depressions in rumen pH as a result of rapidly consuming a diet composed mainly of highly fermentable carbohydrates, thereby increasing the risk for development of sub-acute ruminal acidosis or other problems with rumen fermentation (Stone, 2004; Moody et al., 2007).

Providing supplementary straw alongside of a limit-fed TMR has recently been found to help alleviate some of these behavioral concerns. Heifers offered straw in addition to their TMR spent more time feeding and had higher DMI, while still gaining
benefit from feed efficiency, when compared to animals that are ad libitum-fed a high-forage diet (Chapter 4; Kitts et al, 2011). However, it is not clear whether animals were consuming straw due to hunger, a behavioral “foraging need”, or in an effort to ameliorate depressed rumen pH. Lindström and Redbo (2000) demonstrated that lactating dairy cows were motivated to spend time manipulating their feed, whether the rumen was full or not. These findings suggest that, even though the cattle may be physiologically “full”, they may have a behavioral need to perform feeding behaviors. Furthermore, Keunen et al. (2002) found that following induction of sub-acute ruminal acidosis, dairy cattle offered alfalfa pellets and long alfalfa hay altered their feeding in preference of the long hay. These researchers speculated that this was likely due to the increased buffering capacity of long hay due to increased chewing resulting in increased salivary secretion. Thus, the cows were selecting the alternative that would best attenuate the acidosis (Keunen et al., 2002). The objective of the experiment reported here was to determine whether limit-fed heifers will choose to consume long, rather than short, particles of a low-nutritive feedstuff (i.e. straw) to ameliorate rumen health and meet foraging needs. It was hypothesized that heifers will consume larger amounts of long straw in comparison to short straw.

6.2 Materials and Methods

6.2.1 Animals and Housing

Ten Holstein dairy heifers were used in this study. Heifers were 261.6 ± 39.2 (mean ± SD) d of age and weighed 303.3 ± 56.2 kg at the beginning of the study and weighed 316.2 ± 59.8 kg at the end of the study. Heifers were housed in a tie-stall barn at the University of Guelph, Kemptville Campus (Kemptville, Ontario, Canada) and were managed according to the guidelines set by the Canadian Council on Animal Care
(2009). Use of heifers was approved by the University of Guelph’s Animal Care Committee (AUP#09R022). The study was conducted between April and May of 2012. Each heifer was individually housed in a tie stall (203 x 124.5 x 91 cm; L x W x H), bedded with wood shavings, where she had ad libitum access to water (via her own water bowl) and access to a limit-fed ration via her own feed bunk (individual feed bunks were separated with dividers). Orts (if any) were cleaned out of feed bunks at 0900 h each day. Heifers were given a 2 h exercise period (0900 to 1100 h) each day in an outdoor dry lot pen. Heifers were given ad libitum access to trace mineral salt blocks while in the exercise yard (Windsor TM Stock Salt, The Canadian Salt Company Limited, Pointe-Claire, Quebec, Canada).

6.2.2 Experimental Design and Diets

The number of animals required per treatment was determined through power analysis (Morris, 1999) for primary response variables, including DMI and feeding behavior. Estimates of variation for these variables were based on previously reported values (Kertz and Chester-Jones, 2004; Greter et al., 2008; Kitts et al., 2011). Heifers were fed a high-concentrate TMR (Table 6.1) that was formulated to meet the nutrient requirements for a non-bred Holstein heifer growing at 0.9 kg/d (NRC, 2001) and fed at a restricted level (2.05% of BW). During the first 7-d, heifers were given extra haylage to facilitate gradual adaptation of rumen microbes to the high-concentrate ration. The amount of extra haylage was decreased by 5% each day over the 7-d period. Heifers then spent the additional 7 d of the 14-d adaptation period consuming solely the formulated nutrient-dense ration.

Following the adaptation period, heifers were exposed to each of 2 dietary treatments, in a random order, over two successive 7-d treatment periods using a
crossover design. This included a 4-d adaptation period and a 3-d data collection period. The treatments were: 1) provision of long particle oat straw (85% of particles >8 mm; LS) and 2) provision of short particle oat straw (45% of particles >8 mm; SS). Both treatments were offered following consumption of a limit-fed, high-concentrate TMR. Heifers in adjacent stalls were on different treatments. Short straw was chopped using a straw chopper with a 2.54 cm screen (New Holland 355 Grinder-Mixer, Smiths Farm Equipment Jasper Ltd., Jasper, ON, Canada). Straw was offered in 2 different bins with long straw available in a larger bin (64.1 x 41.9 x 38.7 cm; L x W x H) than short straw (76.8 x 52.1 x 41.9 cm). The location of each bin within the feed bunk was kept constant within heifers but alternated between heifers (i.e. heifer 1 – long straw on right side, short straw on left side, heifer 2 – long straw on left side, short straw on right side). The different sizes of the straw bins and the constant location were used to facilitate association of the straw length with its post-ingestive feedback and to avoid confusion (Forbes, 2007). Once daily, dietary components were mixed in a TMR mixer wagon (Jaylor 4425, Jaylor Fabricating, Orton, ON, Canada). The appropriate amount of TMR was manually weighed out for each heifer. The total amount of feed offered to each heifer was adjusted weekly, according to total heifer BW. The ration DM used to calculate feed amounts was adjusted every 2 wk. Heifers were fed their TMR at 1100 h and offered straw at 1600 h daily.

Following these 7-d periods of exposure to each type of straw (long and short), heifers were given access to both types of straw during an additional 2-d period to determine preference.

6.2.3 Experimental Measurements
Individual intakes were recorded daily throughout the study by weighing the amount of feed offered and amount of feed refused (if any) using a calibrated floor scale precise to the nearest 0.1 kg (Model 31-0851-T17430, Toledo Scale Company of Canada Ltd., Windsor, ON, Canada). This data was used to calculate daily DMI (kg/d). Heifers were weighed on the same 2 consecutive days each week and these weekly weights were averaged for each heifer and used to calculate BW to determine feed amounts.

Feeding behavior was monitored, using time-lapse video, continuously for the 7-d of each treatment period and the 2-d of the preference test. Heifers were recorded using 4 video cameras (Panasonic WV-BP330; Osaka, Japan), a time-lapse video cassette recorder (Panasonic AG-6740) and a video multiplexer (Panasonic WJ-FS 616). Each camera was positioned in front of the tie stalls (2.08 m off the floor and 0.53 m from the tie rail) such that 2 or 3 heifers could be recorded by each camera. The amount of time spent feeding during the 7-d recording period was scored for individual heifers using instantaneous scan sampling every 5 min. Although Kitts et al. (2011) found that 10 min scans were sufficient ($r = 0.95$ in correlation to continuous recording), we wished to increase the accuracy of this measurement by increasing our scanning frequency. For each scan, feeding was defined when a heifer had her head completely past the feed curb and over the feed. Total time spent feeding was then calculated by multiplying the number of scans by 5. Total time spent feeding was then calculated for each heifer for each day of the 7-d recording periods and the 2-d preference period. To identify changes in diurnal feeding patterns between treatments, these scans were used to calculate the percentage of heifers feeding at the feed bunk over a 24-h period.

Ruminal temperature was selected as a measurement for rumen health, as opposed to rumen pH, due to the lower level of invasiveness associated with
administration of rumen temperature boluses versus cannulation of the rumen for the placement of rumen pH probes that require regular calibration. The association between rumen temperature and rumen pH has been validated and discussed elsewhere (Alzahal et al., 2008; 2009). Ruminal temperature was recorded for the last 7 d of each treatment period using a telemetric acquisition system (SmartStock LLC, Pawnee, OK, USA), which was composed of the following: a telemetric ruminal bolus (3 cm in diameter and 8.5 cm in height, 120 g in weight), an antenna, a barn receiver unit, a base receiver unit, and a personal computer equipped with a software program for data logging, as validated by Alzahal et al. (2009). Ruminal temperature measurements were broadcasted through a radio frequency (0.3-3.0 GHz) from the bolus to the barn receiver unit through the antenna that was within 100 m of the heifers. The signal was then transmitted (0.9 GHz) from the barn receiver unit to the base receiver unit (located within 10 m), which was connected via a cable to the personal computer. The bolus was administered, using a bolus gun, to each heifer prior to the start of the study. The bolus was customized to transmit every minute and each transmission included 12 recordings.

Lying behavior was recorded during the 7-d of each treatment period. Daily lying times of individual heifers were collected using electronic data loggers (HOBO Pendant G Data Logger, Onset Computer Corporation, Pocasset, MA), previously validated by Ledgerwood et al. (2010). These devices were placed on the hind leg of each heifer using veterinary bandaging (Vetrap Bandaging Tape, 3M, St. Paul, MN) and measured the orientation of the leg at 1-min intervals. This data was summarized to calculate the average daily standing/lying time (min/d) as well as time spent standing without feeding (i.e. daily standing time – feeding time).

6.2.4 Feed Sampling and Analysis
Representative grab samples of the TMR were taken for DM and chemical analysis during the 7 d of each treatment period. Samples of the dietary components were also taken for DM and chemical analysis twice during each of these recording weeks. Duplicate samples of forage components were taken, once during each treatment period, for particle size separation. Grab samples of the TMR were also collected for particle size separation at the time of feed delivery twice weekly during the 7 d of each treatment period. All samples were immediately frozen at -20°C until they were analyzed.

Samples for particle size separation were thawed at a later date and separated using a 3-screen (19, 8, 1.18 mm) Penn State Particle Separator (PSPS; Kononoff et al., 2003). This separated the samples into 4 fractions: long (>19mm), medium (<19, >8mm), short (<8,>1.18mm) and fine (<1.18mm) particles. After separation, DM of each separated fraction was determined by forced air drying at 55°C for 48 h.

Samples taken for DM and chemical analysis were oven dried at 55°C for 48 h and then ground to pass through a 1mm screen (Wiley Mill, Arthur H. Thomas Co., Philadelphia, PA). These samples were then sent to Cumberland Valley Analytical Services Inc. (Maugansville, MD, USA) for analysis of DM (135°C; AOAC, 2000: method 930.15), ADF (AOAC, 2000: 973.18), NDF with heat-stable α - amylase and sodium sulfite (Van Soest et al., 1991) and CP (N x 6.25) (AOAC 2000: method 990.03; Leco FP-528 Nitrogen Analyzer, Leco, St. Joseph, MI).

6.2.5 Calculations and Statistical Analysis

For analyses of treatment effects, the heifer was considered the experimental unit. Preliminary inspection of the data revealed that all dependent variables were
normally distributed. A preliminary analysis of the effect of day within treatment period was conducted for the DMI, feeding behavior, and lying behavior data. For this analysis, data were analyzed using the MIXED procedure of SAS (SAS, 2009). The model included the fixed effect of treatment, day, and treatment by day interaction, the random effect of heifer within order, and the residual error. This analysis revealed a day effect; visual assessment of the data suggested that variability within treatments was greatest in the first 4 d of each treatment period, suggesting heifers were acclimating to their treatment rations. Due to this variability, the first 4 d of data from each period were excluded; further analysis of the last 3 d of each treatment period revealed no day effect. Thus, only data from the last 3 d of each treatment period were considered in further analyses. These data were averaged by treatment period for each heifer. To test for the effect of treatment, data were analyzed using the MIXED procedure of SAS (SAS, 2009). The model included the fixed effect of treatment, period, and order, the random effect of heifer within order, and the residual error.

The preference for type of straw was evaluated through a preference ratio that was calculated as the amount of long straw consumed during the test divided by the total amount of straw consumed. This preference ratio was then tested for a difference from 0.5 using PROC TTEST (SAS, 2009).

Heifers with less than 800 temperature recordings/d were removed from further analysis. Mean, minimum, and maximum daily ruminal temperatures were calculated from all available observations using PROC MEANS of SAS. The duration (min/d) that ruminal temperature was elevated above a given threshold (i.e. 38.0 °C, 38.2 °C, 38.4 °C, etc.) was computed to describe the magnitude of elevation in temperature in the rumen (Alzahal et al., 2008). The durations that ruminal temperatures were above a given temperature threshold were calculated for each day of recording using PROC
MEANS of SAS. Daily average ruminal temperature data (mean, maximum, minimum, and time (min/d) above a specific cut-off point) were analyzed using the MIXED procedure of SAS. The model included the fixed effects of period, treatment, and order, the random effect of heifer within order and the residual error. Interactions of the period with treatment were tested in the model and were not significant; therefore, these are not further reported.

All values reported are least squares means. Significance was declared as $P \leq 0.05$ and trends were reported if $0.05 < P \leq 0.10$.

6.3 Results

When heifers were fed LS they spent more time feeding throughout the day due to an increase in time spent consuming straw, rather than TMR, in comparison to when they were fed SS (Table 6.3). This difference in feeding time was due to heifers consuming the straw at a slower rate throughout the day when they were fed LS compared to when they were fed SS, whereas feeding rate of TMR did not differ between treatments (Table 6.3). The difference in feeding time can be seen in Figure 6.1 and analysis of diurnal patterns revealed a treatment effect ($P = 0.04$), further supporting our finding that when heifers were fed LS, they spent more time feeding throughout the day. Daily lying time and time spent standing without feeding were similar between treatments (Table 6.3).

Heifers completely consumed all offered TMR each day. Total DMI was similar between heifers, regardless of feed type (TMR or straw; Table 6.3).

The preference period showed a strong preference ratio for LS rather than SS (0.83; SE = 0.06, $P = 0.0003$) with heifers consuming $0.43 \pm 0.2$ kg/d of LS and $0.07 \pm$
0.1 kg/d of SS (mean ± SD). During the preference period, heifers spent 55.3 ± 18.9 min/d consuming LS and 14.6 ± 10.6 min/d consuming SS (mean ± SD).

Heifers maintained similar mean, minimum, and maximum rumen temperatures across treatments (Table 6.4). The amount of time that rumen temperature was elevated over 38.6°C, 39.0°C, and 39.4°C was also similar between treatments (Table 6.4).

6.4 Discussion

Previous research has suggested that providing a low-nutritive feedstuff alongside of a limit-fed TMR may help to reduce the negative behavioral effects associated with limit feeding (i.e. reduced time feeding, increased standing without eating) without compromising the feed efficiency to a similar level as ad libitum-fed heifers (Chapter 4; Kitts et al., 2011). It is not clear, however, what the animals are trying to accomplish by consuming the extra feed. Specifically, are they still experiencing hunger and wish to increase rumen fill; and/or do they desire a foraging substrate in an effort to address a behavioral need to forage or to increase the buffering capacity of the rumen?

The amount of time spent consuming the TMR as well as the rate at which the TMR was consumed were similar between treatments. These results were expected as heifers on either treatment received the same TMR. However, when heifers were fed LS they spent more time feeding throughout the day than when they were fed SS. This increase is due to the longer period of time required to consume the long straw particles, rather than the TMR. The physical characteristics (i.e. particle size) of feed directly affect how quickly a particular feedstuff may be consumed and it is no surprise that heifers spent more time consuming the long particles (Beauchemin et al., 2008). When heifers were fed LS they also consumed their feed at a slower rate across the day. This is
consistent with other research on feed particle size and feeding behavior. Kenney and Black (1984) found that increasing straw length from 10 to 30 mm decreased the rate of eating in sheep, a reflection of the increased time required to chew the longer particles.

Providing straw alongside of a limit-fed TMR increases overall time spent feeding (Chapter 4; Kitts et al., 2011) and helps to return feeding patterns to normal levels (3-5 h; Greter et al., 2008; DeVries and von Keyserlingk, 2009). This effect is enhanced when feeding LS as opposed to SS due to the increase in time spent feeding. We were unable to measure rumination in this study, but time spent ruminating is often similar to time spent feeding and it is likely that heifers fed LS would spend more time ruminating as well. Since feeding and rumination time make up feeding behavior, an increase in either of these factors would likely help to alleviate frustration due to lack of foraging as well as hunger from lack of rumen fill.

Despite this difference in feeding time, when heifers were fed LS they did not spend less time lying or standing without feeding compared to when they were fed SS. The variability in lying time among animals may account for this lack of difference in lying and standing behavior. Additionally, an increase in arousal is seen more often in animals that are feed restricted (Redbo et al., 1996; Redbo and Nordblad, 1997) and is a response that may be used as an indicator of hunger in other species (rats, Le Magnen, 1985; pigs, Terlouw et al., 1991). Heifers in this study were provided a limited amount of TMR but given ad libitum access to straw. Therefore, these heifers were not truly limit-fed, regardless of the particle size of the straw, and may not show a difference in lying or inactive activity as they had access to feed at all times.

The rumen temperature was similar between treatments and, overall, was rarely high enough to suggest acidotic rumen conditions (>39.2; AlZahal et al., 2009). Heifers that are limit-fed may experience a low pH following a feeding event as a nutrient-dense
TMR typically contains a high percentage of rapidly fermentable carbohydrates (5.3; Lascano and Heinrichs, 2009). Heifers in this study did not encounter this depression in pH, as measured by rumen temperature. This may be due to the relatively high forage:concentrate ratio of the limit-fed TMR (63:37) as well as to the straw offered ad libitum. Although there were a greater percentage of long particles in the LS, 40% of the particles from the SS were retained on the top 2 screens of the PSPS. As physically effective fiber (pef) is considered to be the long and medium particles from the PSPS, the SS was still fairly high in pef. Physically effective fiber helps to increase the buffering capacity of the rumen by increasing the bicarbonate and phosphate buffers through an increase in saliva secretion during chewing (Allen, 1997; Beauchemin et al., 2008). Krause and Combs (2003) also found that difference in particle size did not affect rumen pH. It was speculated that, similar to the current study, the difference in particle size between treatments was not drastic enough to impact rumen pH. Although rumen temperature was not negatively affected in this study, there is still the potential for problems to arise with rumen fermentation due to limit feeding. Care should be taken to ensure that the ration contains sufficient levels of pef and that heifers are transitioned over a period of 10-14 d (Lascano and Heinrichs, 2009). Further research examining the effect of a low forage:concentrate ratio in conjunction with a lack of pef is necessary to understand the impact of a nutrient-dense ration with supplementary roughage (i.e. straw) on the rumen.

As the TMR portion of the ration was provided in a controlled, limited amount, it is not surprising that the amount consumed did not differ between treatments. However, heifers consumed similar amounts of LS or SS, suggesting that they will eat whatever is available to them in an attempt to increase rumen fill or spend more time foraging. Continued arousal following feeding likely indicates that the animal is still experiencing
hunger and that either physical, metabolic, or both requirements have not been met by the feed consumed (D’Eath et al., 2009). Indeed, there is evidence to suggest that satiety in cattle is not only linked to the amount of feed consumed, but also to the duration of feeding as Lindström and Redbo (2000) found that, whether the rumen was full or not, lactating dairy cows were motivated to orally manipulate their feed, suggesting that the act of feed manipulation contributes to the negative feedback loop in decreasing the animals’ motivation to feed. In our study, heifers consumed LS or SS in similar amounts, indicating that either feedstuff was able to provide some satisfaction to their foraging needs. Kitts et al. (2011) found that the addition of straw to the diet of limit-fed heifers provided sufficient bulk and feeding time to decrease the heifers’ motivation to feed and these animals were assumed to be satiated. Similarly, heifers in this study were provided with straw ad libitum. It could be assumed that all animals’ foraging needs and/or gut fill were sufficiently satisfied when provided with either straw length.

Despite the fact that heifers will eat either straw length, they did show a very clear preference for the LS when given the choice. The LS contained a much greater percentage of long and medium particles than the SS (80 vs. 40%). It is possible that the heifers derived more satisfaction from the oral manipulation of these long particles, which required more chewing and subsequent rumination than the short particles. Additionally, the LS likely contributed much more to rumen fill (Allen, 1996) than the SS, helping heifers fed LS to feel physically full for a longer period of time. Castle et al. (1979) found that dairy cows will consume more DM and increase milk production when fed short particle silage in comparison to medium and long particle silage, but that they will eat significant amounts of the latter two foods when given a choice. Sheep have also been shown to consume considerable quantities of long forage (i.e. hay), even though it takes more time and effort to harvest, chew, and digest than a concentrate offered ad
libitum (Forbes and Kyriazakis, 1995). As limit-fed rations often contain a high percentage of ground forage and/or concentrate (short particles), it is possible that these animals prefer the LS in an effort to increase their consumption of longer forage particles. The SS was also higher in fine particles (dusty) than the LS. Jarridge et al. (1973) performed many measurements on voluntary intake and feeding behaviour of on chopped and ground forages and found that when the mean particle size of the forage was reduced to 0.75 mm or smaller, voluntary intake decreased. As such, heifers in the current study may have been showing an aversion to the higher proportion of very small particles found in the SS in this study, resulting in an exaggerated preference for LS.

Finally, although there were no differences in rumen temperature between treatments, it is possible that temperature increases (pH depressions) throughout the day were short in duration and would not be captured by the equipment as the boluses did not transmit as frequently when heifers were lying down. Animals have been shown to develop preferences for a feedstuff based on its ability to ameliorate malaise. Keunen et al. (2002) found that following induction of sub-acute ruminal acidosis, dairy cattle offered alfalfa pellets and long alfalfa hay altered their feeding in preference of the long hay. These researchers speculated that this was likely due to the increased buffering capacity of long hay due to increased chewing resulting in increased salivary secretion. Thus, the cows were selecting the alternative that would best attenuate the acidosis (Keunen et al., 2002).

6.5 Conclusions

Limit-fed heifers will choose to consume supplementary straw when provided to them in addition to a nutrient-dense TMR. Although they consume similar amounts of either LS or SS, they spend more time consuming the LS at a slower rate across the
day. Rumen temperature was not impacted by feeding either LS or SS, suggesting that both straw lengths contained sufficient levels of physically effective fiber. When given the choice, heifers prefer to consume LS, perhaps in an effort to maximize their foraging needs and/or to increase satiety through sufficient rumen fill. It is recommended to offer a supplementary low-nutritive feedstuff alongside of a TMR in order to allow limit-fed heifers to engage in normal feeding patterns and to alleviate frustration and hunger due to insufficient foraging or rumen fill.

6.6 Acknowledgements

We thank the staff and students at the University of Guelph, Kemptville Campus Dairy Education and Research Centre, particularly Megan Bruce, Morgan Overvest, and John Wynands for their technical help and support. Angela Greter was supported by a Natural Sciences and Engineering Research Council of Canada (NSERC) Alexander Graham Bell Canadian Graduate Scholarship. This project was funded through an Ontario Ministry of Agriculture, Food and Rural Affairs (OMAFRA)/University of Guelph Production Systems research grant and a Campbell Centre for the Study of Animal Welfare research grant. This project was also supported through contributions from the Canadian Foundation for Innovation (CFI) and the Ontario Research Fund.
Table 6.1. Ingredient, chemical composition, calculated nutrients, and particle size (mean ± SD) of the TMR.

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<tr>
<td></td>
<td>1.11 ± 0.02</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Calculated nutrients</th>
<th>NE_M, Mcal/kg</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>1.73 ± 0.02</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Particle size</th>
<th>Long</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>7.4 ± 3.3</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Particle size</th>
<th>Medium</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>46.2 ± 0.7</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Particle size</th>
<th>Short</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>39.1 ± 2.0</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Particle size</th>
<th>Fine</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>7.3 ± 0.7</td>
</tr>
</tbody>
</table>

^1 Supplied by Dundas Feed and Seed (Winchester, ON, Canada), containing (on as-is basis): 25.6% corn gluten meal, 24.4% Tri-Pro Gold (Tri-County Protein Corp., Winchester, ON, Canada), 24.4% soybean meal, 10.0% canola meal, 4.8% ground limestone, 4.5% trace mineral/vitamin premix, 4.4% sodium bicarbonate, 1.9% cobaltized-iodized salt.
Values were obtained from chemical analysis of TMR samples. NFC = 100 – (%CP + %NDF + %fat + %ash).

3 Calculated according to NRC (2001) equations.

4 Total digestible nutrients (calculated from ingredients).

5 Particle size determined by Penn State Particle Separator which has a 19 mm screen (long), 8 mm screen (medium), 1.18 mm screen (short) and a pan (fine).
Table 6.2. Chemical composition, calculated nutrients, and particle size distribution of the forages (mean ± SD; DM basis)

<table>
<thead>
<tr>
<th>Item</th>
<th>Grass/Alfalfa Haylage</th>
<th>Corn Silage</th>
<th>Long Straw</th>
<th>Short Straw</th>
</tr>
</thead>
<tbody>
<tr>
<td>Chemical composition¹</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>DM, %</td>
<td>40.5 ± 0.1</td>
<td>39.4 ± 0.1</td>
<td>87.8 ± 2.5</td>
<td>89.0 ± 1.5</td>
</tr>
<tr>
<td>CP, %</td>
<td>17.5 ± 1.1</td>
<td>8.3 ± 0.2</td>
<td>3.9 ± 0.2</td>
<td>5.5 ± 1.8</td>
</tr>
<tr>
<td>ADF, %</td>
<td>34.0 ± 0.4</td>
<td>18.2 ± 0.7</td>
<td>59.2 ± 0.6</td>
<td>55.4 ± 1.4</td>
</tr>
<tr>
<td>NDF, %</td>
<td>53.6 ± 0.2</td>
<td>31.0 ± 0.6</td>
<td>83.4 ± 0.0</td>
<td>80.8 ± 1.6</td>
</tr>
<tr>
<td>NFC², %</td>
<td>15.5 ± 0.7</td>
<td>54.7 ± 0.3</td>
<td>8.3 ± 0.4</td>
<td>8.9 ± 0.6</td>
</tr>
<tr>
<td>OM, %</td>
<td>88.5 ± 0.4</td>
<td>96.6 ± 0.1</td>
<td>95.4 ± 0.9</td>
<td>95.4 ± 0.5</td>
</tr>
<tr>
<td>Calculated nutrients</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>TDN, % of DM</td>
<td>59.2 ± 0.4</td>
<td>76.5 ± 0.0</td>
<td>47.5 ± 1.3</td>
<td>47.7 ± 0.1</td>
</tr>
<tr>
<td>ME, Mcal/kg</td>
<td>2.14 ± 0.02</td>
<td>2.76 ± 0.0</td>
<td>1.72 ± 0.05</td>
<td>1.72 ± 0.0</td>
</tr>
<tr>
<td>NE₉, Mcal/kg</td>
<td>0.72 ± 0.02</td>
<td>1.21 ± 0.0</td>
<td>0.34 ± 0.05</td>
<td>0.35 ± 0.0</td>
</tr>
<tr>
<td>NE₉, Mcal/kg</td>
<td>1.29 ± 0.02</td>
<td>1.83 ± 0.0</td>
<td>0.87 ± 0.05</td>
<td>0.88 ± 0.0</td>
</tr>
<tr>
<td>Particle size,³, %</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Long</td>
<td>40.8 ± 5.7</td>
<td>2.0 ± 1.3</td>
<td>66.1 ± 8.5</td>
<td>3.6 ± 1.3</td>
</tr>
<tr>
<td>Medium</td>
<td>39.6 ± 3.8</td>
<td>57.3 ± 0.2</td>
<td>20.8 ± 5.6</td>
<td>39.7 ± 7.0</td>
</tr>
<tr>
<td>Short</td>
<td>17.6 ± 0.8</td>
<td>39.4 ± 1.1</td>
<td>12.1 ± 3.0</td>
<td>49.8 ± 5.6</td>
</tr>
<tr>
<td>Fine</td>
<td>2.0 ± 1.1</td>
<td>1.3 ± 0.4</td>
<td>1.0 ± 0.4</td>
<td>6.9 ± 0.1</td>
</tr>
</tbody>
</table>

¹Values were obtained from chemical analysis of feed component samples.

²NFC = 100 – (% NDF + % CP + % ether extract + % ash).
Particle size determined by Penn State Particle Separator which has a 19 mm screen (long), 8 mm screen (medium), 1.18 mm screen (short) and a pan (fine).
Table 6.3. Intake and behavioral data from growing dairy heifers fed a high-concentrate TMR with either long or short straw.¹

<table>
<thead>
<tr>
<th>Item</th>
<th>Treatment²</th>
<th>SE</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>LS</td>
<td>SS</td>
<td></td>
</tr>
<tr>
<td>DMI, kg/d</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>6.7</td>
<td>6.7</td>
<td>0.4</td>
</tr>
<tr>
<td>TMR</td>
<td>6.3</td>
<td>6.3</td>
<td>0.4</td>
</tr>
<tr>
<td>Straw</td>
<td>0.34</td>
<td>0.37</td>
<td>0.06</td>
</tr>
<tr>
<td>Feeding time, min/d</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>197.7</td>
<td>175.2</td>
<td>5.7</td>
</tr>
<tr>
<td>TMR</td>
<td>137.9</td>
<td>141.0</td>
<td>4.8</td>
</tr>
<tr>
<td>Straw</td>
<td>59.8</td>
<td>34.2</td>
<td>5.0</td>
</tr>
<tr>
<td>Feeding rate, kg/min</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>TMR</td>
<td>0.05</td>
<td>0.05</td>
<td>0.003</td>
</tr>
<tr>
<td>Straw</td>
<td>0.006</td>
<td>0.01</td>
<td>0.001</td>
</tr>
<tr>
<td>Lying time, min/d</td>
<td>969.0</td>
<td>980.4</td>
<td>11.4</td>
</tr>
<tr>
<td>Standing without feeding, min/d</td>
<td>273.3</td>
<td>284.4</td>
<td>10.4</td>
</tr>
</tbody>
</table>

¹ Data for DMI, feeding times, feeding rates, lying time, and inactive standing time are averaged for 10 heifers over 3 d on each treatment period.

² Treatments were provision of: 1) long straw (LS) and 2) short straw (SS) at 1600 h following consumption of a high-concentrate TMR offered at 1100 h.
Table 6.4. Least squares means for ruminal temperature characteristics obtained by a telemetric monitoring system for growing dairy heifers fed a high-concentrate TMR with either long or short straw.¹

<table>
<thead>
<tr>
<th>Item</th>
<th>Treatment²</th>
<th>SE</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>LS</td>
<td>SS</td>
<td></td>
</tr>
<tr>
<td>Mean, °C</td>
<td>38.3</td>
<td>38.3</td>
<td>0.1</td>
</tr>
<tr>
<td>Maximum, °C</td>
<td>38.9</td>
<td>38.9</td>
<td>0.1</td>
</tr>
<tr>
<td>Minimum, °C</td>
<td>35.1</td>
<td>35.0</td>
<td>0.3</td>
</tr>
<tr>
<td>Duration, min/d</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>&gt;38.6°C</td>
<td>353.2</td>
<td>324.6</td>
<td>131.6</td>
</tr>
<tr>
<td>&gt;39.0°C</td>
<td>209.7</td>
<td>162.5</td>
<td>100.6</td>
</tr>
<tr>
<td>&gt;39.4°C</td>
<td>3.1</td>
<td>11.0</td>
<td>7.2</td>
</tr>
</tbody>
</table>

¹Data are averaged over 3 d on each treatment.

²Treatments were provision of: 1) long straw (LS) and 2) short straw (SS) at 1600 h following consumption of a high-concentrate TMR offered at 1100 h.
Figure 6.1. Hourly average for feeding time (min) of dairy heifers on 2 treatments. Treatments were provision of: 1) long straw (LS) and 2) short straw (SS) at 1600 h following consumption of a high-concentrate TMR offered at 1100h. Data are averaged across 3 d for 10 heifers on each treatment.
CHAPTER 7: MEASUREMENT OF FEEDING MOTIVATION IN LIMIT-FED DAIRY HEIFERS

7.1 Introduction

Limit feeding (feeding a nutrient-dense ration in a limited amount) is a feeding strategy that has been shown to effectively target ADG, reduce feed costs, decrease fecal/nitrogen excretion, and increase feed efficiency in replacement dairy heifers (Hoffman et al., 2007; Lascano et al., 2009). Despite these benefits, limit feeding has also been shown to reduce feeding and lying time, and increase time spent standing without eating, vocalizations, oral stereotypies, and the risk of development of sub-acute ruminal acidosis (SARA; Redbo et al., 1996; Hoffman et al., 2007; Moody et al., 2007; Kitts et al., 2011; Chapter 4).

Changes in behavior associated with limit feeding may be attributed to lack of satiety resulting from feed being available for a short duration and in a limited amount. Dairy cattle are grazers and, under natural grazing conditions, would engage in foraging behavior for 4 - 9 hours per day (Kilgour, 2012). Recent research has suggested that providing limit-fed heifers with a low-nutritive foraging substrate (i.e. straw) increases their feeding and rumination time, decreases time spent standing without eating, and maintain a targeted ADG (Kitts et al., 2011; Chapter 4). It is not clear whether, in these situations, the animals were consuming straw due to hunger, a “foraging need”, or in an effort to alleviate depressed rumen pH or other health concerns. Lindström and Redbo (2000) demonstrated that lactating dairy cows were motivated to spend time manipulating their feed, whether or not their rumens were full. These findings suggest that even though the cattle may be physiologically “full”, they may have a behavioral need to perform feeding behaviors. Restricted-fed sheep have been shown to work for a food reward by pushing against a panel (Jackson et al., 1999). Additionally, these sheep
worked harder following a period of feed deprivation ≥6 h. Verbeek et al. (2011) also found that feed restricted ewes would work for a higher number of rewards, walk a greater total distance, and approach the feeding station faster than un-restricted control animals. In a feeding motivation test conducted by Schutz et al. (2006) lactating dairy cattle grazed on pasture were shown to walk increasingly further distances within a few hours of feed deprivation. Cows have also been shown to push against increasingly heavier doors following periods of feed deprivation (Shore, 2003). These studies suggest that feed-deprived animals value access to additional feed, likely due to hunger or lack of rumen fill, and that they will work for a feed reward. Though receiving sufficient nutrients to achieve desired ADG (for maximal growth and frame size), limit-fed heifers may experience hunger as a result of lack of rumen fill or frustration due to lack of foraging substrate and limited time spent manipulating feed.

The objective of this experiment was to determine whether, and to what degree, limit-fed heifers were motivated to work, both immediately post-feeding and following a short-term period of feed deprivation, for parcels of a low-nutritive feedstuff (i.e. oat straw). It was hypothesized that heifers would work harder for feed following a short-term period of feed deprivation and that limit-fed heifers would work harder for extra feed than control heifers.

7.2 Materials and Methods

7.2.1 Animals and Housing

Ten Holstein dairy heifers were used in this study. Heifers were 291.6 ± 39.2 (mean ± SD) d of age and weighed 324.2 ± 61.2 kg at the beginning of the study and weighed 378.7 ± 63.0 kg at the end of the study. Heifers were housed in a tie-stall barn at the University of Guelph, Kemptville Campus (Kemptville, Ontario, Canada) and were
managed according to the guidelines set by the Canadian Council on Animal Care (2009). Use of heifers was approved by the University of Guelph’s Animal Care Committee (AUP#09R022). The study was conducted between May and June of 2012. Each heifer was individually housed in a tie stall (203 x 124.5 x 91 cm; L x W x H), bedded with wood shavings, where she had ad libitum access to water (via her own water bowl) and access to a limit-fed ration via her own feed bunk (individual feed bunks were separated with dividers). Orts (if any) were cleaned out of feed bunks at 0900 h each day. Heifers were given a 2 h exercise period (0900 to 1100 h) each day in an outdoor dry lot pen. Heifers were given ad libitum access to trace mineral salt blocks while in the exercise yard (Windsor TM Stock Salt, The Canadian Salt Company Limited, Pointe-Claire, Quebec, Canada).

7.2.2 Experimental Design and Diets

The number of animals required per treatment was determined through power analysis (Morris, 1999) for primary response variables, including DMI and feeding behavior. Estimates of variation for these variables were based on previously reported values (Kertz and Chester-Jones, 2004; Greter et al., 2008; Kitts et al., 2011). Heifers were fed either a low-forage TMR fed at a restricted level of 2.05% BW or a high-forage (control) TMR (Table 7.1) that was formulated to meet the nutrient requirements for a non-bred Holstein heifer growing at 0.9 kg/d (NRC, 2001). Heifers had been fed a low-forage TMR for 4 weeks before the start of this experiment.

Heifers were exposed to each of the 2 dietary treatments, in a random order, over two successive 26-d treatment periods using a crossover design. These periods included a 14-d adaptation period and a 12-d data collection period. The treatments were provision of: 1) a high-forage control TMR (60% DM; C) and 2) a limit-fed, low-
forage TMR (50% DM; LF). Heifers in adjacent stalls were on different treatments. Once daily, dietary components for both rations were mixed in a TMR mixer wagon (Jaylor 4425, Jaylor Fabricating, Orton, ON, Canada). A percentage of this mixture was unloaded at the barn and fed to heifers on the LF treatment. Additional haylage was added to the remainder of the mixture in the mixer wagon and fed to heifers on the C treatment. The appropriate amount of TMR was manually weighed out for each heifer using a floor scale. The total amount of feed offered to each heifer was adjusted weekly, according to total heifer BW. The ration DM used to calculate a feed amount was adjusted every 2 wk. Orts were removed at 0900 h and heifers were fed at 1100 h.

During adaptation periods, heifers switching from the C ration to the LF ration were adjusted over 14 d to facilitate gradual adaptation of rumen microbes to the low-forage ration. The amount of haylage was decreased by 5% each day over a 7-d period. Heifers then spent the additional 7 d of the 14-d adaptation period consuming the formulated low-forage ration.

Over the 12-d of the data collection period, heifers were tested for feeding motivation using a push-door apparatus at two time points: 3 h (immediately following consumption of all feed by LF heifers) and 21 h following feed delivery. Each heifer was tested 3 times at each time point on each treatment. The push-door was moveable and fitted to the dimensions of the feed bunk area in front of each tie-stall. The door was constructed of steel bars enabling the structure to support weight while leaving it open to allow the heifer to see clearly what was being placed in front of the door. The door weighed 8 kg without any weight on it and measured 1.2 x 1.3 m (L x H). Oat straw was offered in 0.5 kg increments behind the push-door. Each time a heifer accessed the straw reward, a 2.3 kg weight was added to the door. Additional reward parcels were added as needed. Weights were no longer added when the heifer either: 1) no longer
pushed the door at all after 10 min, or 2) the door was pushed but no straw was accessed. Heifers were given at least 24 h between subsequent tests and time points were alternated between consecutive tests. The motivational tests examined the amount of weight pushed, the amount of weight pushed as percentage of body weight, and the latency to access the door.

7.2.3 Experimental Measurements

Individual intakes were recorded daily throughout the study by weighing the amount of feed offered and amount of feed refused (if any) using a calibrated floor scale precise to the nearest 0.1 kg (Model 31-0851-T17430, Toledo Scale Company of Canada Ltd., Windsor, ON, Canada). This data was used to calculate daily DMI (kg/d). Heifers were weighed on the same 2 consecutive days each week and these weekly weights were averaged for each heifer and used to calculate BW to determine feed amounts.

Feeding behavior was monitored, using time-lapse video, continuously for the last 7-d of each treatment period. Heifers were recorded using 4 video cameras (Panasonic WV-BP330; Osaka, Japan), a time-lapse video cassette recorder (Panasonic AG-6740) and a video multiplexer (Panasonic WJ-FS 616). Each camera was positioned in front of the tie stalls (2.08 m off the floor and 0.53 m from the tie rail) such that 2 or 3 heifers could be recorded by each camera. The amount of time spent feeding during the 7-d recording period was scored for individual heifers using instantaneous scan sampling every 5 min. Although Kitts et al. (2011) found that 10 min scans were sufficient \((r = 0.95\) in correlation to continuous recording), we wished to increase the accuracy of this measurement by increasing our scanning frequency. For each scan, feeding was defined when a heifer had her head completely past the feed curb and over
the feed. Total time spent feeding was then calculated by multiplying the number of scans by 5. Total time spent feeding was then calculated for each heifer for each day of the 7-d recording periods. Recordings were also used to score time spent ruminating on d 2, 4, and 6 of each 7-d video recording period using instantaneous scan sampling every 5 min. To validate this scanning method we compared continuous video observations, for each heifer, with 5-min scan samples over a 24-hr period. The correlation was considered acceptable (r > 0.9) for all intervals less than 5 min. Total time spent ruminating was then calculated for each heifer for these 3 d by multiplying the number of scans by 5. To identify changes in diurnal feeding patterns between treatments, these scans were used to calculate the percentage of heifers feeding and ruminating over a 24-h period.

Ruminal temperature was selected as a measurement for rumen health, as opposed to rumen pH, due to the lower level of invasiveness associated with administration of rumen temperature boluses versus cannulation of the rumen for the placement of rumen pH probes that require regular calibration. The association between rumen temperature and rumen pH has been validated and discussed elsewhere (AlZahal et al., 2008; 2009). Ruminal temperature was recorded for the last 7 d of each treatment period using a telemetric acquisition system (SmartStock LLC, Pawnee, OK, USA), which was composed of the following: a telemetric ruminal bolus (3 cm in diameter and 8.5 cm in height, 120 g in weight), an antenna, a barn receiver unit, a base receiver unit, and a personal computer equipped with a software program for data logging, as validated by AlZahal et al. (2009). Ruminal temperature measurements were broadcasted through a radio frequency (0.3-3.0 GHz) from the bolus to the barn receiver unit through the antenna that was within 100 m of the heifers. The signal was then transmitted (0.9 GHz) from the barn receiver unit to the base receiver unit (located within
10 m), which was connected via a cable to the personal computer. The bolus was administered, using a bolus gun, to each heifer prior to the start of the study. The bolus was customized to transmit every minute and each transmission included 12 recordings.

7.2.4 Feed Sampling and Analysis

Representative grab samples of the TMR were taken for DM and chemical analysis during the 12 d of each treatment period. Samples of the dietary components were also taken (in duplicate) for DM analysis, chemical analysis, and particle size separation twice during each of these recording periods. Grab samples of the TMR were also collected for particle size separation at the time of feed delivery twice during the 12 d of each treatment period. All samples were immediately frozen at -20°C until they were analyzed.

Samples for particle size separation were thawed at a later date and separated using a 3-screen (19, 8, 1.18 mm) Penn State Particle Separator (PSPS; Kononoff et al., 2003). This separated the samples into 4 fractions: long (>19mm), medium (<19, >8mm), short (<8,>1.18mm) and fine (<1.18mm) particles. After separation, DM of each separated fraction was determined by forced air drying at 55°C for 48 h.

Samples taken for DM and chemical analysis were oven dried at 55°C for 48 h and then ground to pass through a 1mm screen (Wiley Mill, Arthur H. Thomas Co., Philadelphia, PA). These samples were then sent to Cumberland Valley Analytical Services Inc. (Maugansville, MD, USA) for analysis of DM (135°C; AOAC, 2000: method 930.15), ADF (AOAC, 2000: 973.18), NDF with heat-stable α-amylase and sodium sulfite (Van Soest et al., 1991) and CP (N x 6.25) (AOAC 2000: method 990.03; Leco FP-528 Nitrogen Analyzer, Leco, St. Joseph, MI).
7.2.5 Calculations and Statistical Analysis

For analyses of treatment effects, the heifer was considered the experimental unit. Preliminary inspection of the data revealed that all dependent variables were normally distributed. A preliminary analysis of the effect of day within treatment period was conducted for the DMI, feeding behavior, and rumen temperature data. For this analysis, data were analyzed using the MIXED procedure of SAS (SAS, 2009). The model included the fixed effect of treatment, day, and order, the random effect of heifer within order, and the residual error. This analysis revealed no day effects; therefore, data were averaged across days. Data were averaged by treatment period for each heifer. To test for the effect of treatment, data were analyzed using the MIXED procedure of SAS (SAS, 2009). The model included the fixed effect of period, treatment, and order, the random effect of heifer within order, and the residual error. To test for the effect of treatment on diurnal feeding patterns, the data were analyzed using the MIXED procedure of SAS (SAS, 2009) treating hour as a repeated measure. The model included the fixed effects of period, treatment, hour, and treatment by hour interaction and the random effect of heifer. The covariance structure was compound symmetry, according to the best-fit Schwarz’s Bayesian information criterion.

Preliminary analysis for the effect of test (1, 2, or 3 at each time point) within treatment periods was conducted for the motivation data using the MIXED procedure of SAS (SAS, 2009) treating test as a repeated measure. The model included the fixed effect of treatment, test, and test by treatment interaction, the random effect of heifer, and the residual error. This analysis revealed no test effect; therefore data for each time point was averaged across tests within each treatment period. To test for the effect of treatment and time, data were analyzed using the MIXED procedure of SAS (SAS, 2009) treating time as a repeated measure. The model included the fixed effect of period,
treatment, time, and order, the random effect of heifer within order, and the residual error.

Heifers with less than 800 temperature recordings/d were removed from further analysis. Mean, minimum, and maximum daily ruminal temperatures were calculated from all available observations using PROC MEANS of SAS. The duration (min/d) that ruminal temperature was elevated above a given threshold (i.e. 38.0 °C, 38.2 °C, 38.4 °C, etc.) was computed to describe the magnitude of elevation in temperature in the rumen (AlZahal et al., 2008). The durations that ruminal temperatures were above a given temperature threshold were calculated for each day of recording using PROC MEANS of SAS. Daily average ruminal temperature data (mean, maximum, minimum, and time (min/d) above a specific cut-off point) were analyzed using the MIXED procedure of SAS. The model included the fixed effects of period, treatment, and order, the random effect of heifer within order and the residual error.

All values reported are least squares means. Significance was declared as $P \leq 0.05$ and trends were reported if $0.05 < P \leq 0.10$.

7.3 Results

Heifers on the C treatment consumed more DM daily than heifers fed the LF treatment (Table 7.3). This is further reflected by the increase in time spent feeding and ruminating, and the decrease in feeding rate across the day when heifers were on the C treatment as opposed to the LF treatment (Table 7.3). These differences in feeding and rumination time are visualized in the diurnal patterns of heifers throughout the experiment (Figure 7.1a and b, respectively). Analysis of the diurnal patterns revealed a treatment x hour effect for feeding time ($P < 0.0001$) and rumination time ($P = 0.0001$),
suggesting that heifers on different treatments distributed their feeding and rumination time differently throughout the day.

Heifers maintained similar mean, minimum, and maximum rumen temperature across treatments (Table 7.4). The amount of time that rumen temperature was elevated over 38.8°C, 39.2°C, and 39.6°C was similar between treatments (Table 7.4).

All heifers, regardless of treatment, responded more to the test occurring 21 h after feed delivery than to the test occurring 3 h after feed delivery. There were distinct differences in the magnitude of response between treatments. Heifers were prepared to work harder during both testing time points (both total weight pushed and as a % of BW) for straw rewards when on the LF treatment than when on the C treatment (Table 7.3). Heifers pushed a greater amount of weight at the test occurring 21 h after feed delivery, the testing period that reflected a period of feed deprivation (LF treatment) or in which heifers only had access to smaller amounts of “stale” feed (C treatment). Individual responses when on both the LF and C treatments at 3 h and 21 h after feed delivery may be seen in Figure 7.2a and b, respectively. Individual responses when on both the LF and C treatments for the percentage of allotted time (10 min) used to access the push-door at 3 h and 21 h after feed delivery may be seen in Figure 7.3a and 7.3b, respectively. Despite these differences in the amount of work performed for a reward, the latency to access the push-door was similar between treatments (Table 7.3).

7.4 Discussion

This experiment aimed to evaluate and compare whether limit-fed dairy heifers will work, and to what extent, for a food reward (oat straw). A standard, ad libitum-fed heifer was included as a control treatment in an effort to provide a comparison of feeding motivation between conventional and limit-fed animals. Further comparison of the
feeding behavior between limit-fed and ad libitum-fed heifers was also completed to shed light on the difference in diurnal patterns of dairy heifers fed under these two feeding regimes.

As expected, when allowed ad libitum access to feed, when heifers were on the C treatment they consumed more DM than when they were on the LF treatment. This is consistent with previous work characterizing the feeding patterns of ad libitum and limit-fed dairy heifers in which ad libitum fed animals consumed considerably more feed than limit-fed heifers (Chapter 2).

When on the C treatment heifers spent more time feeding and ruminating throughout the day, likely due to both the increased quantity of feed consumed and to the higher percentage of fibrous feed in the ration (increased haylage). Cattle spend more time chewing and ruminating bulky feeds comprised mainly of long particles as this type of feed requires more mechanical breakdown to expose the surface to rumen microbes for digestion (Beauchemin et al., 2008). We previously found that heifers fed a high-forage control ration spent more time feeding throughout the day than heifers fed a limit-fed ration with ad libitum straw offered alongside the TMR (Chapter 2). It is evident from the diurnal pattern figures that when on the C treatment, heifers distributed their feeding time and, to a lesser extent, their rumination across the day much more than when they were on the LF treatment. Again, this is similar to our previous study examining the short-term diurnal patterns of limit-fed and ad libitum-fed heifers (Chapter 2), suggesting that these patterns do persist for some period of time following acclimation to treatment. The diurnal feeding and ruminating patterns of heifers on the C treatment are more reflective of natural feeding behavior, wherein cattle consume several small, short meals throughout the day and spend a similar period of time ruminating their feed (Hafez and Bouissou, 1975). The sharp peak in feeding time of
heifers on the LF treatment represents the short period of time in which they rapidly and completely consume their TMR. Limit-fed heifers spend approximately 2.5x less time feeding than ad libitum-fed heifers, and therefore may be unable to meet a behavioral need to forage for a significant portion of the day.

The increase in feeding time and feed intake translated into a reduction in feeding rate across the day for heifers on the C treatment. It has previously been found that restricted-fed animals will increase their rate of intake over ad libitum-fed counterparts (poultry, Sandilands et al., 2006; swine, Terlouw et al., 1991; rats, Le Magnen and Devos, 1980). This increase in feeding rate has been suggested to reflect increased feeding motivation as a result of an increasing discrepancy between desired and actual feed intake (Lawrence and Illius, 1989). In the current experiment, therefore, we can assume that when heifers were on the LF treatment, they were experiencing greater motivation to feed, thereby reflecting a greater level of hunger than when they were on the C treatment. When prolonged, this thwarted feeding motivation may contribute to frustration in the heifers, contributing to reduced welfare for heifers at this stage of life.

Although overall rumen temperature measurements were similar between treatments, the variability between individuals for time elevated over a given temperature was high. AlZahal et al. (2008) found that cows receiving a diet fed to induce SARA spent greater periods of time with rumen temperature elevated over 39.0°C and 39.2°C. These researchers further suggest that temperature over 39.2° correlates well with rumen pH < 6.0, a condition that may leave animals susceptible to development of SARA (AlZahal et al., 2009). In the current study, heifers on both treatments spent a considerable period of time with rumen temperature elevated over 39.2°C, suggesting that many animals may have been experiencing some degree of SARA or a disruption in
rumen function. A short period of time was also spent with rumen temperature elevated over 39.6°C, a threshold that would suggest that heifers may be experiencing problems with rumen fermentation and may be encountering discomfort during some parts of the day (Paton et al., 2006). Moody et al. (2007) found that limit-fed heifers spent a considerable period of time with rumen pH below 6.0 when fed a low-forage ration in comparison to a high-forage ration. However, these researchers also found that there was much variation in pH throughout the day and that this minimum pH and variation in pH were not affected by treatment, suggesting heifers on both treatments were rapidly ingesting readily fermentable feedstuffs. Both diets in our experiment were also high in rapidly fermentable carbohydrates and this may help to explain the lack of difference in rumen temperature between treatments. Additionally, the sample size in this experiment included only 10 heifers and, therefore, it is possible that with a larger sample size we may have seen different results. Further work on limit feeding and rumen health with a larger population is encouraged.

In support of our hypothesis, when heifers were on the LF treatment they pushed more weight (in general and as a %BW), regardless of time of test, than when they were on the C treatment. Verbeek et al. (2011) also found that feed restricted ewes would work harder for food rewards than un-restricted controls. Similarly, Jackson et al. (1999) found that feed restricted sheep would push through a push-door apparatus more often than control sheep. It is evident from these studies that limit-fed animals experience considerable hunger as they are willing to expend energy in exchange for additional food rewards. Indeed, the fact that heifers were willing to work for food immediately post-feeding (3 h after feed delivery) suggests that they were not satiated following intake of their daily TMR and that some feeling of hunger likely existed at that time point. Furthermore, these heifers were working to consume straw, which provides strong
evidence of hunger as straw is not considered by cattle to be a palatable feedstuff in comparison to many other forage sources (Baumont et al., 1996).

When heifers were on the LF treatment they worked considerably harder for a reward during the test occurring 21 h after feeding than during the test occurring 3 h after feeding, pushing nearly 10% of their BW to gain a reward. As the test occurring 21 h after feed delivery followed a short-term period of feed deprivation (approximately 19.5 h), it is likely that feelings of hunger and/or frustration at lack of foraging substrate were considerably higher at this time. Another explanation is that heifers on the LF treatment were experiencing more severe within-day depressions in rumen pH and that consumption of straw would help to ameliorate this effect by increasing salivary secretion (Beauchemin et al., 2008). Following various periods of feed deprivation (0, 3, 6, or 9 h), lactating dairy cattle were found to linearly increase the distance walked for a food reward, suggesting that these animals were motivated to consume feed after only moderate feed deprivation (Schutz et al., 2006). Contrary to our study, Savory et al. (1993) found that restricted fed broiler breeders will work similarly hard for extra feed, regardless of time since last meal. When pigs were fed to 1.0 or 0.8 of their ad libitum intake, they depressed rewards earned immediately post-feeding whereas when fed to 0.6 of ad libitum intake, the pigs worked for food rewards to the same extent regardless of time since last meal (Lawrence et al., 1988; Lawrence and Illius, 1989). The poultry and swine industries utilize limit feeding to a much greater extent than the cattle industry, with some birds receiving only 1/3 of what they would consume ad libitum (Savory et al., 1993) and many pigs receiving below 60% of ad libitum intake (Lawrence and Illius, 1989). Heifers managed under a limit feeding regime are typically fed to a level of 80-90% of ad libitum intake (Hoffman et al., 2007; Lascano and Heinrichs, 2009) and are, thus, likely not experiencing hunger to the same extent across the day as limit-fed
poultry or swine. However, although the rate of restriction is not as severe as is typically
done with restricted fed species, the current study imposed 26-d periods of continuous
feed deprivation on the heifers and utilized a restriction allowing less than 60% of ad libitum intake. As such, the continually high level of hunger (and possibly frustration) felt by these animals contributes to a negative affective state, which poses a major threat to their welfare.

Despite the increase in feeding motivation with respect to weight pushed, we did not find any effect of treatment on the latency to access the push-door. This result was surprising, as previous research with feed restricted sheep has shown that restricted animals access a push-door faster than controls (Jackson et al., 1999) or approached the feeding station faster than un-restricted sheep (Verbeek et al., 2011). Our results show a high degree of variability between individuals in response to treatment as well as large numerical differences in latency measures. Thus, it is possible that a larger sample size or a longer period of testing may reflect a difference between treatments in latency to access the push-door. Further research is encouraged to quantify this response.

7.5 Conclusions

When on the C treatment, heifers consumed more DMI, spent more time feeding throughout the day at a slower rate of intake, and displayed more natural diurnal patterns than when they were on the LF treatment. When on the LF treatment, heifers worked harder for additional food rewards (oat straw parcels) by pushing more weight and by pushing more weight as a %BW. When on the LF treatment heifers also worked harder during the test occurring 21 h after feed delivery, suggesting that a short-term period of feed deprivation (approx. 19.5 h without feed) had a greater impact on hunger than the test occurring immediately post-feeding (3 h after feed delivery). Overall, heifers
managed under a limit feeding strategy are likely experiencing considerable hunger
and/or frustration due to lack of rumen fill and/or foraging substrate and will work for
access to extra feed. It is recommended to feed a low-nutritive feedstuff alongside of a
limit-fed TMR in an effort to meet this behavioral need and to satisfy normal feeding
behavior and diurnal feeding patterns.

7.6 Acknowledgements

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Maarten Prinsen, and John Wynands for their technical help and support. Angela Greter
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(OMAFRA)/University of Guelph Production Systems research grant and a Campbell
Centre for the Study of Animal Welfare research grant. This project was also supported
through contributions from the Canadian Foundation for Innovation (CFI) and the Ontario
Research Fund.
Table 7.1. Ingredient, chemical composition, calculated nutrients, and particle size (mean ± SD) of the rations.

<table>
<thead>
<tr>
<th>Composition</th>
<th>Limit-fed TMR</th>
<th>Control TMR</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ingredient, %DM</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Corn silage</td>
<td>20</td>
<td>20.0</td>
</tr>
<tr>
<td>Grass/alfalfa haylage</td>
<td>20</td>
<td>50.4</td>
</tr>
<tr>
<td>High moisture corn</td>
<td>43</td>
<td>22.1</td>
</tr>
<tr>
<td>Protein supplement(^1)</td>
<td>17</td>
<td>7.5</td>
</tr>
<tr>
<td>Chemical composition(^2)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>DM, %</td>
<td>59.3 ± 2.7</td>
<td>49.1 ± 2.8</td>
</tr>
<tr>
<td>OM, % of DM</td>
<td>92.6 ± 0.3</td>
<td>92.1 ± 0.8</td>
</tr>
<tr>
<td>CP, % of DM</td>
<td>16.9 ± 0.6</td>
<td>16.8 ± 0.3</td>
</tr>
<tr>
<td>ADF, % of DM</td>
<td>12.6 ± 1.3</td>
<td>18.1 ± 0.8</td>
</tr>
<tr>
<td>NDF, % of DM</td>
<td>22.4 ± 1.9</td>
<td>29.9 ± 2.3</td>
</tr>
<tr>
<td>NFC, % of DM</td>
<td>49.6 ± 1.7</td>
<td>42.2 ± 3.0</td>
</tr>
<tr>
<td>Calculated nutrients(^3)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>TDN(^4), % of DM</td>
<td>78.0 ± 0.5</td>
<td>73.3 ± 0.5</td>
</tr>
<tr>
<td>ME, Mcal/kg</td>
<td>2.82 ± 0.02</td>
<td>2.65 ± 0.02</td>
</tr>
<tr>
<td>NE(_G), Mcal/kg</td>
<td>1.24 ± 0.01</td>
<td>1.11 ± 0.01</td>
</tr>
<tr>
<td>NE(_M), Mcal/kg</td>
<td>1.88 ± 0.01</td>
<td>1.73 ± 0.02</td>
</tr>
<tr>
<td>Particle size(^5), %</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Long</td>
<td>2.5 ± 1.5</td>
<td>6.8 ± 2.7</td>
</tr>
<tr>
<td>Medium</td>
<td>35.0 ± 0.5</td>
<td>43.0 ± 3.9</td>
</tr>
<tr>
<td>Short</td>
<td>50.5 ± 2.1</td>
<td>42.0 ± 4.2</td>
</tr>
<tr>
<td>Fine</td>
<td>12.1 ± 0.7</td>
<td>8.3 ± 2.7</td>
</tr>
</tbody>
</table>

\(^1\) Supplied by Dundas Feed and Seed (Winchester, ON, Canada), containing (on as-is basis): 25.6% corn gluten meal, 24.4% Tri-Pro Gold (Tri-County Protein Corp., Winchester, ON, Canada), 24.4% soybean meal, 10.0% canola meal, 4.8% ground limestone, 4.5% trace mineral/vitamin premix, 4.4% sodium bicarbonate, 1.9% cobaltized-iodized salt.
Values were obtained from chemical analysis of TMR samples. NFC = 100 – (%CP + %NDF + %fat + %ash).

3 Calculated according to NRC (2001) equations.

4 Total digestible nutrients (calculated from ingredients).

5 Particle size determined by Penn State Particle Separator which has a 19 mm screen (long), 8 mm screen (medium), 1.18 mm screen (short) and a pan (fine).
Table 7.2. Chemical composition, calculated nutrients, and particle size distribution of the forages (mean ± SD; DM basis)

<table>
<thead>
<tr>
<th>Item</th>
<th>Grass/Alfalfa Haylage</th>
<th>Corn Silage</th>
</tr>
</thead>
<tbody>
<tr>
<td>Chemical composition</td>
<td></td>
<td></td>
</tr>
<tr>
<td>DM, %</td>
<td>36.4 ± 4.9</td>
<td>41.9 ± 2.9</td>
</tr>
<tr>
<td>CP, %</td>
<td>18.6 ± 2.4</td>
<td>8.7 ± 0.0</td>
</tr>
<tr>
<td>ADF, %</td>
<td>32.1 ± 0.7</td>
<td>18.6 ± 1.9</td>
</tr>
<tr>
<td>NDF, %</td>
<td>49.0 ± 5.0</td>
<td>31.6 ± 1.3</td>
</tr>
<tr>
<td>NFC², %</td>
<td>19.5 ± 3.9</td>
<td>53.5 ± 1.3</td>
</tr>
<tr>
<td>OM, %</td>
<td>88.5 ± 0.3</td>
<td>96.3 ± 0.1</td>
</tr>
<tr>
<td>Calculated nutrients</td>
<td></td>
<td></td>
</tr>
<tr>
<td>TDN, % of DM</td>
<td>60.0 ± 0.8</td>
<td>76.1 ± 0.6</td>
</tr>
<tr>
<td>ME, Mcal/kg</td>
<td>2.2 ± 0.00</td>
<td>2.7 ± 0.0</td>
</tr>
<tr>
<td>NE₉, Mcal/kg</td>
<td>0.7 ± 0.00</td>
<td>1.2 ± 0.0</td>
</tr>
<tr>
<td>NEₘ, Mcal/kg</td>
<td>1.3 ± 0.00</td>
<td>1.8 ± 0.0</td>
</tr>
<tr>
<td>Particle size,³ %</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Long</td>
<td>41.2 ± 10.5</td>
<td>2.3 ± 1.6</td>
</tr>
<tr>
<td>Medium</td>
<td>44.1 ± 7.3</td>
<td>57.0 ± 5.4</td>
</tr>
<tr>
<td>Short</td>
<td>13.8 ± 3.1</td>
<td>37.5 ± 5.2</td>
</tr>
<tr>
<td>Fine</td>
<td>1.0 ± 0.7</td>
<td>3.1 ± 1.3</td>
</tr>
</tbody>
</table>

¹Values were obtained from chemical analysis of feed component samples.

²NFC = 100 – (% NDF + % CP + % ether extract + % ash).
Particle size determined by Penn State Particle Separator which has a 19 mm screen (long), 8 mm screen (medium), 1.18 mm screen (short) and a pan (fine).
Table 7.3. Intake and behavioral data from growing dairy heifers provided a limit-fed, low-forage TMR at 2.05% BW or a control, high-forage TMR.¹

<table>
<thead>
<tr>
<th>Item</th>
<th>Treatment²</th>
<th>SE</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>LF</td>
<td>C</td>
<td></td>
</tr>
<tr>
<td>DMI, kg/d</td>
<td>7.2</td>
<td>12.9</td>
<td>0.6</td>
</tr>
<tr>
<td>Rumination, min/d</td>
<td>318.3</td>
<td>452.2</td>
<td>15.8</td>
</tr>
<tr>
<td>Feeding time, min/d</td>
<td>82.4</td>
<td>209.3</td>
<td>6.2</td>
</tr>
<tr>
<td>Feeding rate, kg/min</td>
<td>0.09</td>
<td>0.06</td>
<td>0.004</td>
</tr>
<tr>
<td>Weight pushed, kg</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>3 h after feed delivery</td>
<td>15.3</td>
<td>6.7</td>
<td>3.0</td>
</tr>
<tr>
<td>21 h after feed delivery</td>
<td>30.6</td>
<td>9.9</td>
<td>3.0</td>
</tr>
<tr>
<td>Weight pushed, %BW</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>3 h after feed delivery</td>
<td>4.5</td>
<td>1.9</td>
<td>1.0</td>
</tr>
<tr>
<td>21 h after feed delivery</td>
<td>9.3</td>
<td>2.8</td>
<td>1.0</td>
</tr>
<tr>
<td>Latency, sec</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>3 h after feed delivery</td>
<td>82.0</td>
<td>145.6</td>
<td>45.0</td>
</tr>
<tr>
<td>21 h after feed delivery</td>
<td>48.0</td>
<td>145.3</td>
<td>45.0</td>
</tr>
</tbody>
</table>

¹ Data for DMI, feeding time, and feeding rate are averaged for 10 heifers over 7 d on each treatment. Data for rumination are averaged for 10 heifers over 3 d on each treatment period. Motivation data are averaged for 10 heifers over 3 tests per heifer per time point each treatment period.

² Treatments were provision of: 1) a limit-fed, low-forage TMR (LF) and 2) a control, high-forage TMR (C).
Table 7.4. Least squares means for ruminal temperature characteristics obtained by a telemetric monitoring system for growing dairy heifers provided a limit-fed, low-forage TMR at 2.05% BW or a control, high-forage TMR.¹

<table>
<thead>
<tr>
<th>Item</th>
<th>Treatment²</th>
<th>SE</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>LF</td>
<td>C</td>
<td></td>
</tr>
<tr>
<td>Mean, °C</td>
<td>38.2</td>
<td>38.3</td>
<td>0.1</td>
</tr>
<tr>
<td>Maximum, °C</td>
<td>39.0</td>
<td>39.1</td>
<td>0.1</td>
</tr>
<tr>
<td>Minimum, °C</td>
<td>35.0</td>
<td>35.1</td>
<td>0.3</td>
</tr>
<tr>
<td>Duration, min/d</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>&gt;38.8°C</td>
<td>203.0</td>
<td>233.8</td>
<td>83.8</td>
</tr>
<tr>
<td>&gt;39.2°C</td>
<td>67.5</td>
<td>78.3</td>
<td>46.7</td>
</tr>
<tr>
<td>&gt;39.6°C</td>
<td>17.9</td>
<td>0.0</td>
<td>9.2</td>
</tr>
</tbody>
</table>

¹Data are averaged for 10 heifers over 7 d on each treatment. forage TMR (C).
Figure 7.1. Hourly average for: a) feeding time (min), and b) rumination time (min) of dairy heifers on 2 treatments. Treatments were provision of: 1) a limit-fed, low-forage TMR at 2.05% BW and 2) a control, high-forage TMR. Data are averaged across 7 d (feeding time) or 3 d (rumination time) for 10 heifers on each treatment.
Figure 7.2. Weight pushed by individual heifers at: a) 3 h and b) 21 h after feed delivery by heifers on 2 treatments. Treatments were provision of: 1) a limit-fed, low-forage TMR and 2) a control, high-forage TMR. Data are averaged across 3 tests per treatment at each time point.
Figure 7.3. Percentage of allotted time (10 min) taken to access push-door by individual heifers at: a) 3 h and b) 21 h after feed delivery by heifers on 2 treatments. Treatments...
were provision of: 1) a limit-fed, low-forage TMR and 2) a control, high-forage TMR. Data are averaged across 3 tests per treatment at each time point.
CHAPTER 8: GENERAL DISCUSSION

8.1 Important Findings

There is rising public concern regarding the environmental impact of intensive livestock operations and the welfare of the animals housed in such facilities. Additionally, rising costs of production, particularly feed costs, have had a detrimental economic impact on livestock producers. With respect to the raising of replacement dairy heifers, some new feeding management strategies have been developed in an effort to stem the impact of rising feed costs and to reduce the environmental risks associated with production. Limit feeding is a feeding strategy that can potentially reduce feed costs due to providing smaller amounts of a nutrient-dense ration and can reduce fecal excretion (and thereby reduce nitrogen excretion) via improvement of feed efficiency (Hoffman et al., 2007; Lascano et al., 2009; Kitts et al., 2011). However, this feeding strategy alters the behaviour of dairy heifers in several ways that pose risks to the animals’ welfare, including a reduction in feeding time, a major change in diurnal feeding patterns, decreased lying time and increased time standing without eating, increased oral stereotypies or abnormal behaviour, and a potential to depress rumen pH (Lindstrom and Redbo, 2000; Moody et al., 2007; Kitts et al., 2011). The research described in this dissertation was conducted to better understand the effect of limit feeding on the feeding motivation, feeding behaviour and growth of dairy heifers. I sought to determine management factors affecting the feeding behaviour associated with this feeding strategy while also examining the feeding motivation of limit-fed heifers. The results from this thesis represent some of the first experiments to quantify the feeding motivation of a limit-fed dairy heifer and to shed some light on the behavioural patterns associated with this feeding practice.
Feeding a high-concentrate, limit-fed ration to dairy heifers in comparison to feeding a high-forage ration ad libitum resulted in a decrease in DMI, feeding time, rumination, and lying time while increasing feeding rate and time spent standing without eating (Chapter 2 and 6). The diurnal feeding patterns of limit-fed heifers were characterized by a peak in feeding activity immediately following feeding, lasting for 1-3 h, and no further activity throughout the day whereas ad libitum-fed heifers consumed several small, short meals throughout the day and distributed rumination time similarly. The feeding patterns of ad libitum-fed heifers observed were much more similar to those of cattle housed under more natural extensive systems, wherein the animals will spend 4-9 h feeding throughout the day and a similar period of time ruminating (Hafez and Bouissou, 1975).

Increasing the frequency of feed delivery artificially split the meals of limit-fed heifers into 2-4 feeding bouts (Chapter 3), which is desirous as far as an attempt to return to natural diurnal patterns. However, each of these meals was so small and short in duration that the heifers were likely not feeling physically (and possibly metabolically) satisfied after any of these meals, resulting in a continued and thwarted feeding motivation as demonstrated by an increase in time spent standing without eating. The divergence from natural feeding patterns shown by limit-fed heifers is of concern as these animals may spend lengthy periods of time feeling frustrated or bored by the lack of available foraging substrate or foraging activity, thereby affecting their ability to express normal behaviours and impacting their welfare.

Limit-fed heifers were found to compete less for feed than ad libitum-fed heifers (Chapter 4) and, although less competition is desirable from a management viewpoint, it is indicative of a high level of feeding motivation in the limit-fed heifers. As this feeding motivation was very likely left unsatisfied by a lack of sufficient rumen fill or foraging
time, this decrease in competition is positive with some caveats. Indeed, an increase in feed bunk space resulted in very little change in the behaviour of these animals as they continued to spend a similar period of time feeding, competing, lying, and standing without eating regardless of bunk space allowance (Chapter 4). A combination of increased feeding frequency and increased bunk space was not found to impact the behaviour of limit-fed dairy heifers, though this may be due to the fact that all heifers were able to feed simultaneously, despite the restriction in feed bunk space that was imposed during the experiment (Chapter 5). However, the restriction in feed bunk space did result in an increase in variability between heifers within a pen for feeding time and a decrease in ADG for heifers fed at restricted bunks.

Limit-fed heifers did not appear to be negatively affected by the feeding strategy with respect to their rumen health (as measured by rumen temperature). Increased frequency of feeding had very little effect on the rumen temperature of the heifers (Chapter 3) and did not result in an increase in temperature that would be indicative of depressed rumen pH and sub-acute ruminal acidosis. Provision of short or long straw provided the same effect on the rumen temperature of limit-fed heifers (Chapter 6). This lack of difference between treatments may, however, have been due to provision of a TMR that was still relatively high in forage and/or to the fact that the short straw still contained particles that were considered physically effective (approximate 40% of particles). There also appeared to be a high level of variation between individuals on the control and limit-fed treatments in Chapter 7 as the numerical differences and the standard error were considerably high. Animals on both of these treatments, however, may have encountered some depression in rumen pH, altered rumen fermentation patterns, and/or discomfort associated with a high rumen temperature (low rumen pH) as
animals in this experiment spent a considerable period of time with rumen temperature elevated over 39.4°C.

As several management factors failed to return the behaviour of limit-fed dairy heifers to more natural patterns, I sought to determine whether limit-heifers desired supplementary feed in addition to their TMR and whether they would work for this extra feed. Regardless of the particle length of supplementary oat straw, heifers consumed similar amounts when offered this feed following consumption of their limit-fed TMR, suggesting that they were not physically satisfied as they would consume either straw length when offered (Chapter 6). However, in that same study, when offered a choice between straw of different lengths, the heifers consistently chose the longer particle straw (>80% long and medium particles), indicating that they preferred this feedstuff. This may have been due to the increase in physical fill that was felt by consumption of the longer, bulky fibre of long straw; the desire to ameliorate any drop in post-prandial pH encountered through consumption of the limit-fed TMR, which contained large quantities of rapidly fermentable carbohydrates; or to the increased time required for foraging, mastication, and rumination of the long particles, which may have helped the animals to satisfy a behavioural need to forage. Furthermore, in Chapter 7 heifers were found to work considerably for their extra feed as, when evaluated with a weighted push-door apparatus, limit-fed heifers pushed nearly 10% of their BW to access feed following a 21 h period following initial feed delivery. Indeed, even immediately post-feeding the limit-fed heifers would work against nearly 5% of their BW for extra straw, suggesting that they were not satiated immediately following consumption of the TMR. The results of Chapters 6 and 7 suggest that limit-fed heifers are not able to meet many of their basic freedoms (i.e. feeling hunger, inability to express normal behaviours) when not provided with a low-nutritive, supplementary feed. They are likely still hungry, may be
frustrated or bored, and cannot express normal feeding patterns under this feeding regime. Providing straw or a similar low-nutritive feedstuff significantly helps in returning behaviours and feeding patterns to more normal levels.

The studies throughout this dissertation utilized an aggressive limit feeding strategy that limited the animals to 1.93-2.05% BW. As such, the results obtained in these studies are specific to more severe feed restriction. Other strategies offer a slightly higher forage ration at a level that is 80-90% of ad libitum intake. These altered programs will allow heifers to feed for considerably longer periods throughout the day. Although these strategies are not without their own problems, they may be an alternative method that helps to optimize the welfare of growing dairy heifers.

8.2 Future Research

These studies have gone a long way to characterize the behavioural problems associated with limit feeding (at a level of 1.93-2.05% BW) and potential solutions to these problems. However, more work is needed to understand the relationship between feeding management and the development of these behaviours, as well as the influence of these behaviours on the health and welfare of these animals.

The idea of “normal” or “natural” feeding behaviour may require further research with respect to what this may entail. In my experiments, I compared the behaviour of intensively raised ad libitum heifers to that of intensively raised limit-fed heifers. It must be noted, however, that the “normal” behaviour of intensively raised dairy heifers does not compare to the “natural” feeding behaviour of grazing cattle, although it is much closer to these patterns than the behaviour of limit-fed animals. To truly assess whether dairy heifers wish to engage in more normal, natural behaviour, it would be necessary to ask this question of the animal. Allowing a choice between indoor housing and outdoor
grazing may help in determining a preference. Further experimentation may include requiring the heifer to work for access to outdoor grazing, if a preference was suggested.

It must be noted that there was a wide range in the age of heifer used in these studies. The strategy of limit feeding has been suggested for use in heifers from weaning to 30-45 d before calving (Zanton and Heinrichs, 2008). Heifers have similar requirements for growth and maintenance throughout the rearing phase (with extra needs after breeding; NRC, 2001) and it is possible that these results may be extrapolated to all heifers from weaning to breeding. Heifers in this age range are likely more stable with relation to their behavioural development (Greter et al., 2008) and management factors that may affect this development. Provided that a dairy producer considers such management factors as stocking density, feed bunk space, and feeding level required to meet the needs of a growing heifer, the results from these experiments would likely be similar across the heifer rearing phase.

Additionally, the level of feed restriction in these studies was considerably severe, with some heifers receiving only 60% of what they would consume ad libitum (Chapter 7). The current recommendations for utilization of this feeding strategy are to feed heifers to 80-90% of ad libitum intake (Zanton and Heinrichs, 2008), which would likely result in less severe discrepancies in behaviour between ad libitum and limit-fed heifers. The higher level of feed provided may be achieved by diluting the ration somewhat with forages. This would have significant benefit to returning the feeding behaviour of the heifer to a pattern more conducive to a grazing animal. However, caution must be taken here as cattle are adept at manipulating and sorting their feed and this leaves a growing heifer susceptible to other problems associated with sorting behaviour (Section 1.2.2).
Rumen temperature was measured in 3 of the experiments in this dissertation (Chapters 3, 6, and 7). The cut-off point for inclusion of daily data from each heifer was 800 recordings/d. This was believed to be representative of the animal’s daily mean, maximum, and minimum rumen temperature as per AlZahal et al. (2008 and 2009). However, it was not possible to directly measure pH or to develop an algorithm by which we may determine daily flux in temperature and the potential impact this would have on the animals’ welfare. As within-day bouts of sub-acute ruminal acidosis largely develop as a result of the rapid intake of a large meal (Krause and Oetzel, 2006), it is still possible that limit-fed heifers in these experiments were experiencing short-term bouts of SARA. Results of rumen temperature must be interpreted with caution in these studies as they do not reflect the within-day fluctuation that may have been occurring. Direct measurement of rumen pH could have provided more concrete evidence of moderate to severe depression in rumen pH in either limit-fed or ad libitum-fed heifers and whether there is considerable variability in individuals or between treatments. Thus, future research is needed to completely understand the effect of limit feeding on rumen pH and whether these effects would negatively affect growth and development of these animals.

Although a decrease in overall competitive behaviour was found in these studies in comparison to ad libitum-fed heifers, it should be noted that limit-fed heifers that were individually housed (Chapter 6 and 7) spent considerably more time consuming their TMR than heifers that were group-fed. This indicates that some amount of social pressure is present at the feed bunk of group-fed animals and is likely impacting the behaviour and welfare of conspecifics within a pen. Further research examining the relationship between feeding behaviour and social factors within a pen of limit-fed heifers may help in minimizing or eliminating some of the detrimental effects on feeding behaviour observed in these animals.
Time spent standing without eating was a consistent response to feed restriction in these studies. I was unable to record behaviour throughout the pen and thus, it is unclear whether the heifers were engaging in appetitive or restless behaviours (perhaps due to discomfort associated with hunger or some other form of dissatisfaction) or whether they simply have a requirement to stand. Heifers still spent sufficient periods of time lying down so it cannot be stated that they were at any particular risk for development of hoof pathologies over ad libitum-fed heifers. Additionally, cattle generally prefer to ruminate while lying down. As limit-fed heifers had much less feed to ruminate, they may have simply chosen to stand for longer periods of time. Whether this would be detrimental to their well-being has yet to be determined. Experiments examining the trade-offs that heifers would choose between activities (i.e. feeding and lying) may shed some light on what the animals’ needs are with respect to standing, lying, feeding, etc.

As the heifers had been shown to prefer the long straw particles in Chapter 6, I chose to use long straw during the motivation testing in Chapter 7. It is important to determine, however, whether limit-fed heifers will work for short straw as well and whether they will work to a greater extent for long or for short straw. This may further aid in determining the importance of supplementary long fibre to the animals with respect to meeting foraging needs and ameliorating low rumen pH. Further, if heifers will work for the short straw or some other short particle feedstuff, would they work for inert bulk as well? Perhaps these animals are so motivated to continue feeding that they will work for any extra substance that will induce feelings of physical satiety in addition to metabolic satiety that is likely felt from consumption of the nutrient-dense TMR. These experiments would help to tease apart the ultimate goal of limit-fed heifers with respect to consumption of a supplementary feedstuff.
There is also a possibility that restricted-fed animals are not conscious of feelings of hunger until they are presented with visual cues or external stimuli reminiscent of feeding. Perhaps the mere visualization of the stockperson, tractor, or feed bunk may stimulate feelings of hunger where the animal was otherwise not feeling any discomfort. This “out of sight, out of mind” idea may be discovered by measuring physiological indicators of hunger and satiety (such as specific hormonal levels) without presenting any external stimuli. Although this type of experiment would be difficult to design, it would help to gain insight into this problem.

Overall, further research on the effect of limit feeding on the feeding behaviour and growth of dairy heifers should consider both earlier time periods in the life of the animal and also future behaviour and production potential in adult life. Most heifer calves are limit-fed milk or milk replacer from the beginning of life. The feeding patterns between ad libitum and limit-fed calves are different (Miller-Cushon et al., in press) and may have some impact on how limit-fed heifers respond to this feeding strategy. Previous research has shown that behavioural patterns may persist across ration changes and through time (Greter et al., 2010). Thus, it is possible that the altered diurnal patterns and increased feeding rate found in limit-fed heifers may negatively impact the behaviour of these animals once they reach the lactating herd. For example, in lactating cattle, cows that consume their feed at a slower rate and spend more time eating ultimately increase their total daily saliva secretion, thereby helping to prevent the development of sub-acute ruminal acidosis (SARA; Beauchemin et al., 2008). In contrast, cattle that rapidly consume a highly fermentable ration are more likely to encounter depressions in rumen pH, leading to acidotic conditions (Ostergaard and Grohn, 2000; Beauchemin et al., 2002). The future productivity of dairy cattle may be affected by metabolic disease, such as SARA, and thus setting the animals up to
develop feeding patterns that may lead to problems with rumen function is of concern. Effects of limit feeding (both with and without provision of supplementary low-nutritive forage) on the growth, development, production potential, and future behaviour of dairy animals should be examined.

The aim of this future research would be to develop feeding management strategies for growing dairy heifers that will best utilize their ability to learn proper feeding behaviours and patterns in an effort to prevent growth, production, and health problems. In better understanding the feeding behaviour, feeding motivation, and factors affecting satiety of young dairy heifers, we are more likely to find practical, effective solutions to minimize or eliminate the development of undesirable or detrimental feeding patterns and to maximize the welfare of these animals.

8.3 Implications

Based on these results, it is recommended that, when employing a limit feeding strategy, dairy producers should provide a supplementary low-nutritive feedstuff to their growing dairy heifers alongside a nutrient-dense TMR. Additionally, feeding group-fed animals from a feed bunk with sufficient space for all animals to feed simultaneously is essential to maintaining growth and minimizing competition. This will enable these animals to grow well while maintaining feed efficiency above the level of ad libitum-fed heifers, distribute their feeding activity throughout the day, reduce feed bunk competition, express normal feeding behaviours, and help satisfy feeding motivation, resulting in satiety and improved welfare for replacement dairy heifers.
CHAPTER 9: REFERENCES


CCAC. 2009. Guidelines on: The care and use of farm animals in research, teaching and testing. Canadian Council on Animal Care, Ottawa, ON, Canada.


