Interaction of Sorting Behavior with the Health and Production of Early Lactation Dairy Cows

by
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ABSTRACT

INTERACTION OF SORTING BEHAVIOR WITH THE HEALTH AND PRODUCTION OF EARLY LACTATION DAIRY COWS

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Through a study where 41 multiparous Holstein cows were fed 1 of 2 diets varying in wheat straw particle size immediately after calving, this thesis sought to identify the effect of straw particle size on the feed sorting behavior, health, and production of early lactation dairy cows. It was hypothesized that a reduction in the wheat straw particle size would minimize sorting behavior, which would maintain intake and rumination while stabilizing reticulorumen pH. Cows fed the shorter wheat straw particle size sorted the ration to increase intake of physically-effective fiber, which may have contributed to a more stable reticulorumen pH and a tendency for greater milk production. Whereas cows fed the longer straw particle size sorted against the most physically-effective components of the diet and suffered from greater variability in time spent below a reticulorumen pH of 5.8 as well as time spent ruminating. In a secondary analysis, cows were further categorized by risk for SARA as either high risk or low risk. Cows fed longer straw sorted against long particle regardless of risk category, while cows fed shorter straw did not sort these particles. High risk cows fed the shorter straw diet sorted to increase intake of physically-effective fiber, possibly in an attempt to ameliorate the effects of low reticulorumen pH. This study suggests that reducing wheat straw particle size in the diets of early

lactation dairy cows may stabilize reticulorumen pH and increase milk production by minimizing sorting behavior, which maintains intake and rumination.

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TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

LIST OF ABBREVIATIONS

ADF – acid detergent fiber
AUC – Area under the curve
BCS – body condition score
BHB – beta-hydroxybutyrate
BW – body weight
DM – dry matter
DMI – dry matter intake
ECM – energy-corrected milk
FCM – 4% fat-corrected milk
MUN – milk urea nitrogen
NEB – negative energy balance
NDF – neutral detergent fiber
OFT – Optimal Foraging Theory
peNDF – physically-effective neutral detergent fiber
PSPS – Penn State Particle Separator
SARA – subacute ruminal acidosis
SCC – somatic cell count
SCK – sub-clinical ketosis
TDR – top-dressed ration
TMR – total mixed ration
VFA – volatile fatty acid

Chapter specific terminology

Chapter 2:

Long – Treatment diet with wheat straw particle size of 5.08 cm
Short – Treatment diet with wheat straw particle size of 2.54 cm

Chapter 3:

HR – high risk for SARA
LR – low risk for SARA

CHAPTER 1. INTRODUCTION

The transition period in dairy cows is defined as the 3 wk prior to calving and 6-8 wk after giving birth (Staples et al., 1990; Drackley, 1999). The high-producing dairy cow is vulnerable during this time because of the high-energy demand required for producing a calf and beginning lactogenesis (Goff and Horst, 1997; McArt et al., 2012).

For an early lactation dairy cow, nutrition and feeding behavior play important roles in the recovery from pregnancy and calving and the initial surge in milk production. A decrease in dry matter intake (DMI) prior to parturition contributes to the vulnerability of the transition cow to health disorders, such as ketosis (Bertics et al., 1992; Grummer et al., 2004). This decrease in DMI has been attributed to systemic inflammation stimulated by a cytokine response (Dantzer and Kelley, 2007). High energy demand, coupled with a decrease in DMI, increases the risk of going into a state of negative energy balance (NEB; Grant and Albright, 1995; Sovani et al., 2000). Excessive negative-energy balance in early lactation makes dairy cows vulnerable to disease, particularly subclinical ketosis (SCK; Esposito et al., 2014). Subclinical ketosis is the result of increasing energy demands for lactation combined with a delayed increase in DMI after calving (Herdt, 2000; Gordon et al., 2013). If SCK becomes severe enough, a dairy cow may experience clinical ketosis symptoms such as decreased DMI and milk production, as well as a rapid loss of body condition (Baird, 1982).

The rumen environment is particularly susceptible to changes in diet and feeding behavior such as that endured by fresh cows during the transition period (Zebeli et al., 2015). A decrease in DMI pre-calving and delayed onset of increased DMI post-calving are examples of changes in feeding behavior observed in early lactation dairy cows (Goldhawk et al., 2009). Changes in diet include the transition from a low-energy, forage-rich dry diet to a high-energy lactating diet composed primarily of concentrate components (Zebeli et al., 2015). This transition puts early lactation dairy cows are at an increased risk of developing subacute ruminal acidosis (SARA; Zebeli et al., 2015). Penner et al. (2007) found that the incidence and severity of ruminal acidosis increased soon after calving in primiparous cows. An abrupt dietary transition at calving can result in a buildup of volatile fatty acids (VFA) if the concentrate component of the ration is over-consumed relative to the physically-effective forage components in the diet (Enemark, 2009). The rumen of a recently-calved cow is not adapted to a rapidly fermentable lactating diet; this is because of two factors: 1) underdeveloped mucosal papillae that cannot absorb the VFAs efficiently and 2) cellulolytic ruminal microflora adapted to ferment a forage-based, dry diet instead of a lactating diet (Kleen et al., 2003). A buildup of VFA can depress rumen pH and result in SARA symptoms such as decreased DMI, diarrhea, reduced milk production, and an increased risk of laminitis and liver abscesses (Zebeli et al., 2012). To prevent such problems, dairy cows in early lactation must be fed a diet that adapts the mucosal papillae and ruminal microflora to a higher concentrate diet, while also meeting energy demands for lactation (Kleen et al., 2003; Enemark, 2009).

A ration that meets energy requirements, while maintaining a stable rumen environment, are not the only considerations to be made when formulating diets for fresh cows. The diet must also be presented to cows in a manner that limits their ability to sort the feed, thus preventing consumption of an unbalanced ration (Miller-Cushon and DeVries, 2017a). When provided a mixed ration, cows typically sort their feed, selectively consuming the smaller concentrate components and selecting against the longer, forage components (Leonardi and Armentano, 2003; Leonardi et al., 2005a; DeVries et al., 2007). Palatability, particle size, management, and metabolic status all contribute to sorting behavior, making it a difficult behavior to control (Miller-Cushon and DeVries, 2017a).

This review will describe SARA and its specific effects on early lactation dairy cows. Feed sorting behavior will also be outlined, with a focus placed on the relationship between this feeding behavior, SARA, and forage particle size in the diet.

1.1 SUBACUTE RUMINAL ACIDOSIS

Subacute ruminal acidosis is a common disorder observed in the transition dairy cow (Krause and Oetzel, 2006). Modern dairy cows are genetically predisposed to high milk production, as a result, diets must be high in concentrates and lower in forages (Plaizier et al., 2009). Subacute ruminal acidosis occurs when ruminants are fed a diet too high in rapidly fermentable carbohydrates (Krause and Oetzel, 2006). Another contributing factor to SARA is an inadequate amount of physically-effective fiber in the diet, which is necessary to ensure proper adaptation of the mucosal papillae and ruminal microflora and efficient absorption of VFAs (Enemark, 2009; Zebeli et al., 2012). When an overconsumption of rapidly fermentable carbohydrates occurs, VFAs build up in the rumen causing a depression of rumen pH (Plaizier et al., 2009). To be classified as SARA, and not simply natural variation in rumen pH, the pH of the rumen must be depressed for prolonged periods of time over the course of the day (Kleen et al., 2003). Subacute ruminal acidosis has negative consequences for the dairy cow, including decreased DMI and milk production (Stone, 1999; Kleen et al., 2003). Also, ruminal digestibility is negatively affected and conditions such as lameness and liver abscesses are seen in cows afflicted with the disorder (Plaizier et al., 2009). Garrett et al. (1997) found that 19% of early lactation dairy cows experienced SARA in a study of 15 Wisconsin farms.

Traditionally, time spent below a pH threshold of 5.8-5.6 has been used as an indicator of SARA in dairy cows (Kleen et al., 2003; Yang and Beauchemin, 2006a). Some researchers have used an acidosis index for classifying cows as either higher or lower risk for developing SARA (Penner et al., 2009a; Macmillan et al., 2017). The severity of acidosis is determined by calculating the area under the threshold (difference in units below pH threshold × min spent at that pH) and standardizing it for DMI (area below pH 5.8/DMI; Gao and Oba, 2014, 2016; Macmillan et al., 2017). Steers classified as lower risk for SARA using the index had lower VFA concentrations than high risk steers, possibly because of either higher VFA absorption or lower VFA production (Schlau et al., 2012). In a recent study, cows were categorized for risk of SARA using the acidosis index and then assessed for differences in feeding behavior (Macmillan et al., 2017). It was determined that feeding 3×/d versus 1×/d resulted in decreased severity of acidosis for high risk cows and had no effect on low risk cows (Macmillan et al., 2017). As opposed to rapidly consuming their feed at the time of feeding, high risk cows demonstrated a more evenly distributed feeding pattern over the course of the day when fed 3×/d as compared to when fed 1×/d (Macmillan et al., 2017). Thus, nutritional management should be focused on reducing the risk of SARA in early lactation through incorporation of adequate dietary fiber and sufficient energy in the diet for lactation.

1.2 THE ROLE OF CHEWING AND FIBER IN MAINTENANCE OF RUMEN PH

Buffering acids that accumulate in the rumen as a result of rapid fermentation of carbohydrates is in part accomplished by salivation (Owens et al., 1998). The remainder of buffering occurs by bicarbonate and phosphate buffering systems and VFA absorption (Bergman, 1990; Owens et al., 1998; Krause and Oetzel, 2006). Thirty to forty percent of the neutralization of acids that occurs in the rumen is completed by buffers contained in saliva

(Allen, 1997). Rate of salivation can be manipulated through feed composition and DMI (Yang and Beauchemin, 2006a). The rate of salivation is affected by chewing; more chewing results in a greater flow of saliva (Maekawa et al., 2002). Chewing, and the resultant increase in salivary flow, occurs most frequently during eating and rumination (Maekawa et al., 2002).

Once swallowed, feed must be further broken down through rumination before readily passing through the reticulo-omasal orifice (Welch, 1982). Two ration characteristics that influence time spent ruminating are the neutral detergent fiber (NDF) content and the physically-effective nature (i.e. structural features that require further chewing to digest) of the ration (Zebeli et al., 2012). These two characteristics are related in terms of stimulating rumination; NDF is a chemical constituent of the feed that provides the basis for physically-effective fiber (peNDF), which is the portion of the feed that stimulates chewing and rumination (Kononoff et al., 2003a; Zebeli et al., 2012). Most of the NDF in rations fed to dairy cows comes from forages (Oba and Allen, 1999). Longer forage components of the diet increase the time spent chewing and ruminating and contribute to the peNDF content of the feed (Kononoff et al., 2003a). A limitation of NDF is that too much NDF in the diet may decrease DMI because NDF fills the rumen (Oba and Allen, 1999).

The peNDF portion of feed was originally calculated by multiplying the amount of feed particles greater than 1.18mm in length by the NDF content of the total ration (Mertens, 1997). This calculation remains correct except that minimum particle length has changed to more accurately reflect digesta passage rates (Maulfair et al., 2011; Heinrichs, 2013). Originally, a particle size of 1.18mm was used because it was established as the smallest particle size that is retained in the rumen and not readily digested (Poppi and Norton, 1980). The Penn State Particle Separator (PSPS) was invented to practically measure the proportion of particles of different sizes in a ration (Lammers et al., 1996). Originally, the PSPS used 2 sieves with holes of a diameter of 19mm and 8mm to sort the feed by particle size (Lammers et al., 1996). Kononoff et al. (2003b) developed a 3rd sieve with a hole diameter of 1.18mm. Since that time, research by Maulfair et al. (2011) has suggested that the minimum particle size that is still physically-effective is 4mm. As a result, a new PSPS has been developed, consisting of 3 sieves with the following diameter holes: 19mm, 8mm, and 4mm (Maulfair et al., 2011; Heinrichs, 2013). To maintain sufficient intake of peNDF, diets must be formulated to ensure adequate distribution of particles in the ration by size.

1.3 FEED SORTING

While proper formulation of the feed is a necessity for maintaining the health of the animal, another important factor to consider is feed selection (sorting) behavior. Feed sorting occurs when a cow selectively consumes certain feed components in greater quantities than expected and avoids others (DeVries et al., 2007). A total mixed ration (TMR) is a homogenized feed mixture designed to make feed sorting more difficult (Coppock et al., 1981). When fed a TMR, cows typically sort in favor of smaller concentrate feed components and against longer forage components (Leonardi and Armentano, 2003; DeVries et al., 2007). Sorting can result in consumption of an unbalanced diet relative to that predicted and increase the risk of SARA due to an overconsumption of the grain component of the diet and an under-consumption of physically-effective fiber (DeVries et al., 2008). Preventing feed sorting helps to maintain rumen function, ingesta flow, and adequate intake of fiber (Coppock et al., 1981).

DeVries et al. (2011) found greater efficiency of milk production (kg of milk/kg of DMI) was associated with sorting against the longer forage components. In that study it was speculated that ingestion of less forage would increase feed conversion efficiency, resulting in greater efficiency of milk production (Yang and Beauchemin, 2006b; DeVries et al., 2011). Sova et al. (2013) found that reduced sorting, at a herd level, against the long particles of a TMR resulted in a decrease in 4% fat-corrected milk (FCM) and energy-corrected milk (ECM). The researchers in that study suggested that this finding was the result of stabilization of the rumen environment due to intake of longer forage components (Sova et al., 2013). A more stable rumen pH may have resulted in increased fiber digestibility, which then resulted in increased 4% FCM and ECM yield (Sova et al., 2013). Rumen pH variation and low rumen pH in particular are responsible for reduced rumen functionality and inhibition of lipogenesis, which leads to reduced milk yield and milk fat production (Griinari et al., 1998; Alzahal et al., 2010). Sova et al. (2013) also found that reduced sorting in favor of fine particles resulted in an increase in milk yield. Again, this finding may be attributed to less rumen pH variation and resultant increased feed digestibility, this time as a result of a reduced intake of easily fermentable carbohydrates (Sova et al., 2013).

Milk fat production is also affected by sorting in favor of the concentrate components and against the longer forage components (Miller-Cushon and DeVries, 2017). Milk fat percentage has been shown in several studies to decrease with increased sorting against long ration particles (DeVries et al., 2011; Fish and DeVries, 2012; Miller-Cushon and DeVries, 2017b). Similar to milk production, decreases in milk fat production are likely related to a depression of the rumen pH, provoked by reduced intake of physically-effective fiber (Fish and DeVries, 2012).

1.3.1 Development of Sorting Behavior

Early experiences have a formative effect on the feeding behavior and feed preferences of ruminants (Arnold and Maller, 1977; Nolte et al., 1990). Arnold and Maller (1977) reported that when introduced to a new environment, sheep initially demonstrate varying dietary preferences and DMI from their con-specifics, for whom the environment is not novel (Arnold and Maller, 1977). Miller-Cushon and DeVries (2011) reported that calves will sort in favor of ration components that they were exposed to early in life. An initial aversion to a novel ration component may be attributed to under-developed motor skills necessary for digesting the new food (Miller-Cushon and DeVries, 2011). Another possibility is that neophobia results because of the unfamiliar taste, smell, or texture of the novel ration components (Miller-Cushon and DeVries, 2011).

Miller-Cushon et al. (2013) studied the effect of particle size of the forage component of the feed fed during the pre-weaning stage of life on later sorting behavior and dietary preferences in dairy calves. Calves that had been fed a finely chopped hay (FN) in the pre-weaning stage and coarsely-chopped hay (CRS) after weaning, consumed less NDF and acid detergent fiber (ADF) and more non-fiber carbohydrates (NFC) than calves fed only CRS (Miller-Cushon et al., 2013). Calves initially fed the FN also sorted their feed against the coarsely-chopped hay and in favor of concentrate more so than the calves fed only CRS (Miller-Cushon et al., 2013). This sorting behavior remained consistent over the 3 wk post-weaning (Miller-Cushon et al., 2013). These findings were not supported by Overvest et al. (2016), who found no difference in sorting post-weaning between calves offered their feed in different ways prior to being weaned. In support of feeding a more homogenized mixture to ensure a balanced diet, studies have demonstrated that feeding heifers a TMR as opposed to a top-dressed ration (TDR) helps reduce sorting in favor of the concentrate component (DeVries and von Keyserlingk, 2009; Greter et al., 2010). Given what is known about the development of sorting behavior, it is possible that the behavior is not simply an innate instinct fueled by feed efficiency, palatability, or particle size, but also an action motivated by early experience and affective states.

1.3.2 Internal Factors Affecting Sorting Behavior

1.3.2.1 Optimal Foraging Theory

The Optimal Foraging Theory (OFT) states that all animals will forage for food in a manner that minimizes energy output and maximizes energy input to increase their reproductive fitness (Krebs and McCleery, 1984). This theory is supported by studies where it has been found that dairy cows sort in favor of the energy-rich grain component and against the longer forage components when there is an energy demand for lactation (DeVries et al., 2008, 2011). DeVries et al. (2011) demonstrated that lactating cows would sort against ADF and in favor of crude protein at 53 days in milk (DIM). Acid detergent fiber is not easily digestible, so sorting against it when experiencing a high energy demand for milk production is logical (DeVries et al., 2011). Long forage components take more time to chew and digest and are also less energy-dense (Allen, 2000), so it makes sense to avoid these components based on the OFT (Krebs and McCleery, 1984). However, cows do not exclusively sort their feed in favor of high-energy, easily consumed foods, contrary to the OFT (DeVries et al., 2008). Knowing this, there must be other factors that contribute to the motivations for sorting behavior in cows.

1.3.2.2 Post-ingestive Feedback

Recently, researchers have shown that selection for physically-effective feed particles stimulates post-ingestive feedback and may influence feed preferences (Keunen et al., 2002; DeVries et al., 2008, 2011). DeVries et al. (2008) demonstrated that cows at a high risk for developing severe acidosis will sort a TMR in favor of the longer forage components when experiencing a severe bout of induced ruminal acidosis. Beauchemin and Yang (2005; 2006b) also observed increased sorting in favor of long particles by cows fed low-fiber diets. These researchers suggested that this sorting was an effort to attenuate the negative effects of low ruminal pH (Beauchemin and Yang, 2005). This sorting may stimulate greater chewing and salivation, which would help buffer rumen pH and mitigate the effects of acidosis (Keunen et al., 2002; DeVries et al., 2008). When subjected to a ruminal acidosis challenge, beef steers sorted increasingly in favor of long particles and medium particles with a greater change in rumen pH < 5.5 (DeVries et al., 2014a). These animals also sorted increasingly in favor of long particles and against fine particles with a greater change in area of pH < 5.5 (DeVries et al., 2014a). Similarly, DeVries et al. (2014b) showed Angus heifers fed a high-grain diet for 34 d prior to an acidosis challenge were better adapted for the challenge than those fed the high-grain diet for only 8 d prior. The cows given less time to adapt to the high-grain diet did not sort in favor of the long and medium particles and against the fine particles to the same extent as cows given more time to adapt, indicating that a longer adaption period helped to better acclimate the heifers to a high-grain diet and minimize rumen pH variation (DeVries et al., 2014b). All of these studies demonstrate that cows will sort a ration to optimize intake of physically-effective fiber in order to cope with low rumen pH. Nonetheless, rumen pH may be negatively affected by feed sorting, becoming depressed as a result of a higher consumption of highly fermentable carbohydrates and a lower consumption of fiber than expected (DeVries et al., 2008).

1.3.3 External Factors Affecting Sorting Behavior

1.3.3.1 Management

In addition to internal factors, feed sorting may also be related to the management and composition of the feed provided. The amount of feed offered to dairy cows is an example of how intensive management of dairy cattle affects sorting behavior. In a study by Miller-Cushon and DeVries (2010), when more feed (18% orts (HFA – Higher Feed Amount) and 11.5% orts (LFA – Lower Feed Amount)) was offered to lactating cows there was an increase in sorting in favor of medium particles on the HFA diet. Cows also sorted their feed against the small particles on the HFA diet (Miller-Cushon and DeVries, 2010). Higher DMI by cows on the HFA diet resulted in similar intake of NDF and starch as cows on the LFA diet, despite the fact that the HFA diet promoted greater sorting (Miller-Cushon and DeVries, 2010). Similar results were seen in a herd-level study by Sova et al. (2013), where a higher refusal rate was positively associated with sorting in favor of small particles and against longer forage components. Greter and DeVries (2011) also looked at how feeding amount affects sorting behavior and found that cows sorted against the long particles and tended to sort in favor of the short particles, regardless of amount fed. However, there was only a tendency for a difference in orts between the 2 treatment diets and as such there were no differences observed for feeding rate or time or DMI (Greter and DeVries, 2011). These results suggest that feeding for a minimal refusal rate may reduce the amount of sorting and ensure a more balanced nutritional intake (Miller-Cushon and DeVries, 2017a).

Miller-Cushon and DeVries (2017a) suggested that cows provided more time to sort a TMR will do so; increasing delivery frequency will minimize sorting of the TMR. Decreasing the degree of sorting for group-fed dairy cows is important because dominant cows will sort the feed, leaving the remainder of the feed to be nutritionally unbalanced (DeVries et al., 2005). As the feed becomes more sorted the nutritive value decreases for submissive cows that eat later in the day (DeVries et al., 2005). DeVries et al. (2005) found that increasing feed delivery frequency from 1x to 2x per day resulted in reduced sorting behavior and more equal access to fresh feed for all cows. These results are consistent with more recent studies of commercial dairy farms (Endres and Espejo, 2010; Sova et al., 2013).

DeVries et al. (2004) found that increasing the head space provided at feed bunks from 0.5 to 1 m per cow reduced displacements at feeding time, allowing more cows to access fresh, unsorted feed. Hosseinkhani et al. (2008) found that competition tended to affect sorting behavior 4 h after feeding; competitively-fed cows did not sort the ration while non-competitively-fed cows sorted against medium particles. There was also an increase in feeding rate with increased stocking density (Hosseinkhani et al., 2008). Crossley et al. (2017) also found that feeding rate increased with increased competition, however, stocking density did influence sorting behavior. Cows that did not have to compete for access to sorted their feed in favor of the long particles, while those that faced competition sorted against this particle fraction and in favor of the medium particles (Crossley et al., 2017). It is clear that stocking density and available space to feed affect social competition, which may have an effect on sorting behavior.

1.3.3.2 Nutrition

In addition to management, the composition and presentation of the feed itself should be considered when attempting to minimize sorting behavior. The degree of sorting of a TMR may be related to the DM concentration of the ration. Leonardi et al. (2005) found that adding water to a dry ration (80.8% DM vs. 64.4% DM) resulted in reduced sorting against long particles and in favor of shorter particles. Fish and DeVries (2012) found that for a 60% DM diet containing corn silage and haylage forage sources, sorting against the long particles occurred regardless of whether water was added or not, but that adding water contributed to reduced sorting for the fine particles. Alternatively, it has been observed that cows will sort their feed more, and have a reduced DMI, when water is added to an already high-moisture TMR (~60% DM), containing only corn silage and haylage as forages (Miller-Cushon and DeVries, 2009; Felton and DeVries, 2010). Interestingly, adding a molasses-based liquid feed supplement may change the particle size distribution of the feed and sweeten the ration, making it more palatable and also minimizing sorting of the feed against long particles (DeVries and Gill, 2012a).

The ratio of forage to concentrate in the diet may influence sorting behavior (Miller-Cushon and DeVries, 2017a). In dairy calves and heifers, sorting against the longest fraction of the diet has been observed when greater amounts of straw were included in mixed diets (Greter et al., 2008; Groen et al., 2015). However, when provided a ration higher in forage content, lactating cows exhibited less sorting against long forage components and in favor of small particles (DeVries et al., 2007). The researchers in that study suggested that when fed a ration lower in forage content, cows sort against the long particles and for the smaller components because the smaller particles are more proportionately abundant. For the early lactation dairy cow, this sorting behavior is concerning because it could lead to an excessive intake of the finest particles in the diet and a refusal of the peNDF content in the diet, particularly when coupled with the rapid increase in DMI post-calving (DeVries et al., 2007).

One of the primary nutritional factors influencing feed sorting is forage particle size. It is well documented that cows will sort against the longest particles in the diet and in favor of the smaller components (Leonardi and Armentano, 2003; Leonardi et al., 2005a; DeVries and von Keyserlingk, 2009). Consistent with the OFT, the small particle size of the grain component allows cows to eat faster and without feeling as satiated as when long particles high in NDF are consumed (Oba and Allen, 1999; Miller-Cushon et al., 2013). There may also be anatomical factors that contribute to the degree of sorting that occurs (DeVries et al., 2011). It has been suggested that primiparous cows sort their feed more than multiparous cows because they have smaller mouthparts (DeVries et al., 2011). The mouthparts of the smaller, younger cows are better able to sort the small grain particles from the longer forage components (DeVries et al., 2011).

The challenge associated with forage particle size as it pertains to sorting is ensuring a balanced intake of physically-effective fiber without exacerbating sorting against the longest particles in the diet. Studies have shown that moderately reducing the forage particle length can have positive effects for limiting sorting behavior, increasing DMI, and maintaining rumen health (Kononoff et al., 2003a; Kmicikewycz et al., 2015; Shaani et al., 2017). However, in diets containing large amounts of concentrate (50-60% DM), forage particle size should not be reduced below 4-6mm, otherwise the particles are no longer physically-effective and can negatively affect time spent ruminating as well as ruminal fermentation (Zebeli et al., 2012). In support of reducing forage particle size, Kononoff et al. (2003a) found that while time spent eating and ruminating were not different, DMI and NDF intake increased with a reduction in corn silage particle size (from 8.8 to 7.4mm). Additionally, sorting increased with an increase in corn silage particle size (Kononoff et al., 2003a). Kmicikewycz et al. (2015) found similar results when subjecting mid-lactation dairy cows to a SARA challenge. There was an increase in DMI with decreasing corn silage particle size (62.7 to 5.33mm; Kmicikewycz et al., 2015). Cows fed the short corn silage particle size sorted in favor of these particles when experiencing SARA, whereas cows fed the longer corn silage particle size continued to sort against them (Kmicikewycz et al., 2015). Leonardi et al. (2005b) also found a tendency for increased sorting against long particles with an increase in oat silage particle size. Decreasing oat silage particle size was associated with increased DMI, milk yield, and milk protein percentage and yield (Leonardi et al., 2005b). More recently, Shaani et al. (2017) demonstrated that increasing wheat hay particle size resulted in decreased DMI and increased sorting against the longest fraction of the ration in non-lactating cows fed for a restricted intake. In contrast, cows fed the short wheat hay particle size spent more time ruminating and had a higher mean rumen pH (Shaani et al., 2017). These results support the theory that a reduction in forage particle size results in less sorting, a more balanced nutritive intake, and a healthier rumen environment (Shaani et al., 2017).

Taking into consideration the results of the studies in the previous paragraph, there are still conflicting conclusions about the effect of forage particle size on sorting behavior as it relates to NDF intake and rumen health. Kononoff and Heinrichs (2003) showed that with increasing corn silage particle length, eating and ruminating efficiency (expressed as minutes per kilogram of NDF intake) increased. However, an increase in sorting was also observed with increasing corn silage particle length. The researchers in this study concluded that perhaps particle size and the physical-effectiveness of the roughage plays only a minor role in rumen pH and fermentation (Kononoff and Heinrichs, 2003). Similarly, Maulfair and Heinrichs (2013) found that increasing corn silage particle size actually tended to increase mean and max pH and that ruminal fermentation was unaffected by corn silage particle size. Despite significant sorting over the course of the day, cows consumed a balanced ration by the end of a 24-h period, meaning that cumulatively, little sorting occurred (Maulfair and Heinrichs, 2013). While increasing corn silage particle size decreased DMI, the researchers concluded that the size of the corn silage particles did not have an effect on rumen fermentation or rumen pH even when cows sorted throughout the day (Maulfair and Heinrichs, 2013). Lastly, Zebeli et al. (2008) found no effect of reducing corn silage particle length on ruminal VFA concentration or rumen pH despite observing an increase in sorting against long particles with increasing corn silage particle size. There was however an increase in ruminal enzyme activity and non-fiber degradation for cows fed the medium corn silage particle size (Zebeli et al., 2008). The conflicting results of these studies, and those mentioned previously, suggest that more research is needed to elucidate the relationship between forage particle size, sorting behavior, and rumen health.

A variety of forage sources have been studied in relation to particle size and sorting behavior, although most studies focus on either corn silage or alfalfa (Leonardi and Armentano, 2003; Krause and Combs, 2003; Kahyani et al., 2013). To our knowledge, only one study has been conducted on the effect of reducing straw particle size to minimize sorting behavior. Suarez-Mena et al. (2013) studied differences in sorting behavior and rumen health between cows fed diets with varying oat straw particle sizes (80.37 to 10.16mm). No biologically significant differences were detected for feeding behavior or rumen pH, although sorting against the long particles increased with increasing straw particle size (Suarez-Mena et al., 2013). It should be noted that this study was conducted on dry cows, who do not have the same energy demands as early lactation dairy, nor are they at the same increased risk for SARA.

Determining the optimal forage particle size is challenging because increasing forage particle size has two opposing outcomes: 1) increased particle size reduces risk of SARA by increasing intake of peNDF and 2) increased particle size reduces DMI and feed digestibility by increasing rumen fill (Zebeli et al., 2012). In addition to these conflicting outcomes, the tendency to sort against the physically-effective components of the diet is disadvantageous for early lactation dairy cows because they are already at risk for SARA (Steele et al., 2016). A moderate reduction of the forage particle length may prevent sorting behavior by homogenizing the forage particle size with the rest of the TMR while still maintaining its physically-effective nature (Zebeli et al., 2008; Shaani et al., 2017).

1.4 OBJECTIVES AND HYPOTHESES

The overall objective of this thesis was to determine if a relationship existed between sorting behavior in early lactation dairy cows and their health and production. This objective was addressed in a study of early lactation dairy cows, fed 1 of 2 diets differing in the particle size of the straw component. A specific objective (Chapter 2) was to identify how early lactation dairy cows sort a TMR differing in straw particle size and determine the resultant effects on reticulorumen health and milk production. It was hypothesized that a diet lower in straw particle size would maintain intake and rumination activity, prevent feed sorting, and stabilize reticulorumen pH. It was also hypothesized that a diet with a longer wheat straw particle size would result in increased sorting against the longest particles in the diet. Reticulorumen pH would decrease as a consequence of this sorting behavior and a destabilization of the rumen pH would then reduce DMI, rumination, and milk yield. A second specific objective (Chapter 3) was to determine if low reticulorumen pH affects sorting behavior and to determine whether straw particle size in the diet further impacts this. It was hypothesized that cows experiencing chronically low reticulorumen pH would sort their feed to minimize the negative consequences of this condition and experience a decrease in rumination and milk production. It was also predicted that this behavior would be more apparent in cows fed a diet that is more easily sorted.

CHAPTER 2: Effect of straw particle size on the behavior, health, and production of early lactation dairy cows

2.1 INTRODUCTION

When provided a TMR, dairy cows often sort their feed, typically favoring small grain components and avoiding longer forage particles (Leonardi and Armentano, 2003; Leonardi et al., 2005; DeVries et al., 2007). Particle size of forages is one of the primary factors influencing feed sorting, with smaller particles being less easily sorted than longer particles (Miller-Cushon and DeVries, 2017a). Additionally, high palatability of the concentrate components of TMR motivates cows to sort in favor of these smaller components and against the longer forage ingredients (Nombekela et al., 1994; Miller-Cushon and DeVries, 2017a).

Intake of dietary forage is important, as it primarily contributes the physically-effective NDF (peNDF) portion of the diet, which is responsible for stimulating rumination and improving fiber digestibility (Yang and Beauchemin, 2006a; Zebeli et al., 2012). Sorting behavior can result in an over-consumption of easily fermented carbohydrates and an under-consumption of peNDF (Miller-Cushon and DeVries, 2017a). This unbalanced diet, as a result of sorting, may lead to an increased risk of subacute ruminal acidosis (SARA; DeVries et al., 2008); an overconsumption of grains relative to peNDF can cause a buildup of VFAs and a resultant prolonged depression of the rumen pH (<5.6-5.8 for multiple hours per day) characteristic of SARA (Steele et al., 2016). Subacute ruminal acidosis can lead to decreased DMI, milk production, and fiber digestibility (Plaizier et al., 2009). Fresh cows are especially vulnerable to SARA because of the abrupt transition from a low-energy diet to a more highly-fermentable diet at calving (Steele et al., 2016). It is important that transition cows are not able to easily sort their feed during this time, as this behavior likely exacerbates the condition through over consumption of easily fermented carbohydrates and under consumption of effective fiber.

Nutritional management of fresh cows can be difficult because there are competing demands for nutrient-dense concentrate components to sustain lactation and for sufficient forage to maintain good rumen function. It is important that the dietary fiber in the diet be presented in a manner that discourages sorting to ensure a balanced diet that stimulates rumination. Reducing the particle size of the dietary fiber source increases NDF intake and decreases sorting against the long particles and for fine particles (Miller-Cushon and DeVries, 2017a). A forage particle size that is large enough to stimulate rumination, yet small enough to prevent sorting against the forage component may, thus, reduce the risk of SARA in early lactation dairy cows.

The objective of this research was to determine if reducing the particle size of wheat straw in a fresh-cow diet would have positive impacts on cow health and production in early lactation. It was hypothesized that a diet with a smaller particle size wheat straw would minimize sorting behavior, while maintaining DMI levels and rumination, resulting in a stabilization of reticulorumen pH. Alternatively, it was hypothesized that cows fed a diet with longer particle size wheat straw would sort against the longest particles, resulting in reduced rumination time, and less stabilized reticulorumen pH, thus decreasing DMI and milk production.

2.2 MATERIALS AND METHODS

2.2.1 Animals and Housing

Forty-one multiparous Holstein cows (parity=2.8±1.1; mean ± SD) were used in this study, which took place at the University of Guelph Livestock Research and Innovation Centre Dairy Facility (Elora, Ontario, Canada). At approximately 17 d from calving, cows were enrolled in the study; this time frame allowed for a minimum 3-d training period in addition to 2 wk of baseline data collection prior to calving. During this time, cows were housed in a close-up pen and trained to eat out of individual automated feed bins (Insentec B.V., Marknesse, the Netherlands). Each cow was assigned her own bin, and trained to eat only out of that bin during the 3-d period. At approximately 2 wk prior to calving (d -13.4 ± 4.9), cows had an average BW of 847.7 ± 77.6 kg and an average BCS of 3.7 ± 0.34. The close-up pen had 12 automated feed bins, 24 free stalls, and 2 water troughs. There were never more than 12 cows in the close-up pen at one time, ensuring that each cow had access to her own individual feed bin. Cows spent 3.1 ± 3.2 d in the maternity pen prior to calving and 5.2 ± 3.3 d in the maternity pen after calving. The maternity pens were individual box stalls (3.5 × 4.9 m) with access to feed and water. After calving, cows were milked in their maternity pens using a portable milking system.

Dry cows were fed a dry cow TMR (Table 2.1, 2.2) 1x/d between 1000 and 1100 h. The total amount of feed offered was adjusted daily to target approximately 10% refusals per bin (actual = 14.7 ± 12.3 %). The dry cow feed bins were cleaned out each day at approximately 0930 h every morning. After calving and following exit from the maternity pens, cows were moved to a lactating pen and again assigned to an individual feed bin. The lactating cow pen had 15 automated feed bins, 30 free stalls, and 2 water troughs. There were never more than 15 cows in the lactating pen at one time, ensuring that each cow had access to her own individual feed bin. Lactating cows were fed 1x/d between 1300 and 1400 h. The total amount of feed offered was adjusted daily to target approximately 10% refusals per bin (actual = 13.0 ± 15.6 %). Cows were milked 2x/d at 0500 and 1700 h in a milking parlor.

The use of cows and experimental procedures complied with the guidelines of the Canadian Council on Animal Care (2009) and were approved by the University of Guelph Animal Care Committee (Animal Use Protocol #2518).

2.2.2 Experimental Design

Sample size and power analyses were used to calculate (as per Morris, 1999) the minimum number of replicates needed per treatment (n=20) to detect a 10% level of observed difference for the primary outcome variables, including DMI, rumination, sorting, and milk production. Estimates of variation for these variables were based on previously reported values (DeVries et al., 2007; DeVries and Gill, 2012a). Due to the likelihood of technical complications associated with the monitoring equipment used in this study, more than 20 cows per treatment were initially enrolled in this study to ensure the target sample size was achieved.

Upon calving, cows were randomly assigned to 1 of 2 dietary treatments (Table 2.1, 2.2), a TMR that differed in the length of the wheat straw component in that diet: 1) 2.54 cm straw length (**Short**; n=21) or 2) 5.08 cm straw length (**Long**; n=20). Cows were fed these treatment diets from 1 to 28 DIM. Treatment allocation was balanced for parity and milk production in the previous lactation (10,334.4 ± 1,955.7 kg). Straw was chopped using a bale processor (Haybuster Model H-1150, Jamestown, ND, USA) using a 5.08-cm screen for the Long diet and a 2.54-cm screen for the Short diet. Each day, the basal diet, without straw, was prepared using a TMR mixer (Jaylor Model 5572, Jaylor Fabricating, Orton, ON, Canada). Feed was then transferred to a feed cart (Super Data Ranger; American Calan, Northwood, NH, USA) and the appropriate amount of straw was added and mixed prior to delivery. Cows remained on their respective treatments for 4 wk post-calving. The lactating cow TMR was formulated to, at minimum, meet the nutrient requirements of dairy cows producing 36 kg/d (NRC, 2001).

2.2.3 Behavioral Data Collection

Feeding behavior and DMI were monitored using the automated feed bins, as validated by Chapinal et al. (2007). 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 calculated. These data were then summarized to calculate daily DMI (kg/d), daily time spent feeding (min/d), and average feeding rate (kg/min). Individual feeding bouts were combined and separated into meals using a meal criterion (i.e. the minimum duration of time between meals) calculated for each cow. Meal criteria for the dry period and lactating period were calculated for each cow using methods described by DeVries et al. (2003); a software package (MIX 3.1.3; MacDonald and Green, 1988) was used to fit normal distributions to the frequency of $\log_{10}$ transformed intervals of time between feeding visits. Meal frequency (no./d) was determined for each cow by summarizing the number of intervals between feeding events that exceeded their meal criterion. Meal length (min/meal) was calculated as the time between the start of the first feeding visit, until the end of the last visit within the meal criterion. Meal size (kg DM/meal) was calculated as DMI divided by meal frequency.

An electronic monitoring system (HR-TAG-LD, SCR Engineers Ltd., Netanya, Israel) was used in this study to monitor rumination activity, as validated by Schirmann et al. (2009). Rumination data loggers, attached to a nylon neck collar, were fitted to each cow 2 wk prior to their expected calving date. Until the cow gave birth, and then for the first 4 wk of lactation, rumination activity was monitored 24 h/d. The system contained a radio frequency reader, which allowed data from the collars to be continuously uploaded to the control unit. This data, stored in 2-hour intervals, was used to determine total time spent ruminating throughout each day.

2.2.4 Health Data Collection

At the time of enrolment, cow BW was recorded (I-20W scale, Ohaus, Dundas, ON, Canada) and BCS was recorded using the five-point scale, as described by Wildman et al. (1982). Assessment of BW and BCS occurred once during the dry period at enrollment and every 14 d starting on Day +4 post-calving for a total of 3 measurements including a final weighing on the last of the trial period. To ensure accuracy for body condition scoring, inter-observer reliability testing was conducted between 3 individuals, with a resultant 85% accuracy rate. One of these individuals conducted the body condition scoring for the entirety of the trial.

Wireless telemetry boluses (eBolus, eCow Ltd., Devon, UK) were used to measure reticulorumen pH to assess rumen health (as validated by Falk et al., 2016). At time of enrollment, the boluses were administered orally using a balling gun. Data consisted of reticulorumen pH data points on 15 min intervals 24 h/d throughout the trial period. Data was downloaded 3 d/wk during the dry period and every 3 d post-calving. Data was amalgamated into a continuous record for each individual cow. Time spent below a pH threshold of 5.8 was then calculated along with daily mean, minimum, and maximum pH values. An absolute value for area below pH 5.8 was calculated by subtracting the pH value from 5.8 and multiplying it by the time spent at that pH value. These values were summed by day creating a single value for area under the curve (**AUC**) for each day for each cow. These values were also standardized for intake by dividing by DMI for each day for each cow. Lastly, values were averaged for the first 28 DIM, creating a single value or AUC index for each cow.

Blood BHB was assessed weekly prior to calving starting on the day of enrolment and post calving every 4 d starting on d+4 after calving. For blood sampling, cows were restrained in a stall to obtain a small blood sample (~0.5 mL) from the coccygeal vein. Blood BHB was measured using an electronic hand-held device (FreeStyle Precision Neo, Abbott Diabetes Care, Saint Laurent, QC, Canada), as validated by Kanz et al. (2015). Blood BHB concentrations >1.2 mmol/L were deemed indicative of SCK (Geishauser et al., 1998; McArt et al., 2012). Testing of

BHB occurred just before feeding (0930 h for dry cows and 1230 h for lactating cows) throughout the study.

2.2.5 Milk Yield and Components

Milk yield was recorded at every milking in the milking parlor (using DelPro software, DeLaval, Peterborough, ON, Canada) for the 4 wk cows were observed on treatment. Milk samples were collected from each cow, at each milking, on 2 consecutive days each week starting at d +6. These samples were sent to a DHI testing laboratory (CanWest DHI, Guelph, ON, Canada) for component analysis (fat, protein, MUN, and SCC) using a Fourier Transform Infrared full spectrum analyzer (Milkoscan FT+ and Milkoscan 6000; Foss, Hillerød, Denmark). One value per cow on each sampling day was obtained by calculating the average across milkings.

Milk composition samples were used to determine the yield of 4% FCM (kg/d), calculated as: (0.4 × milk yield (kg/d)) + (15.0 × fat yield (kg/d)) (NRC, 2001). Energy-corrected milk was calculated as: ECM (kg/d) = (0.327 × kg of milk) + (12.95 × kg of fat) + (7.2 × kg of protein) (Tyrrell and Reid, 1965). Efficiency of milk production was calculated as kilograms of milk, 4% FCM yield, and ECM yield per kg of DMI per cow.

2.2.6 Feed Sampling and Analysis

Throughout the study, 2 fresh feed samples were collected 3x/wk: 1 sample was collected to determine DM and chemical composition, and the other sample was collected to determine particle size distribution. Samples of the TMR components were also collected once monthly to be analyzed for DM and chemical composition. During the dry period, orts samples were collected 3x/week to determine particle distribution for calculation of sorting. Orts samples were collected every 3 d during the lactating period starting on day +4 for the same purpose. All samples were immediately frozen at -20ºC until further analysis.

After a 12-h period of thawing, fresh and orts TMR samples collected for particle size analysis were processed using a 4-screen Penn State Particle Separator (PSPS; Maulfair et al., 2011; Heinrichs, 2013), which separates the sample into 4 fractions based on particle size: long (> 19 mm), medium (< 19 > 8 mm), short (< 8 > 4 mm), and fine (< 4 mm). Separated samples were then oven dried at 55^0C for 48 h.

The sorting of each PSPS fraction was calculated (as per Leonardi and Armentano, 2003) by dividing the actual amount of feed consumed of each fraction by the predicted amount of feed consumed of that fraction and expressing it as a percentage. For each fraction, the actual amount consumed was calculated by subtracting the DM refused from the DM offered, as determined by the PSPS analysis. The predicted amount consumed for each fraction was calculated as the product of the DMI of the total diet multiplied by the DM percentage of that fraction in the fed TMR. If the sorting value equalled 100 %, then no sorting of the particle fraction occurred, a value <100 % indicated sorting against that particle size fraction, while a value > 100 % indicated sorting in favor of that particle fraction. The greater the difference from 100 %, the more the feed was sorted, either for or against that particle size fraction.

Feed samples collected for nutrient composition analysis included the fresh TMR samples, feed components, and fresh TMR PSPS fractions. These samples were oven dried at 55^0C for 48 h and then were ground to pass through a 1-mm screen (Model 4 Wiley Laboratory Mill, Thomas Scientific, Swedesboro, NJ, USA). Ground samples, pooled by week, 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: method 973.18), NDF with heat-stable α-amylase and sodium sulfite (Van Soest et al., 1991), CP (N x 6.25; AOAC 2000: method 990.03; Leco FP-528 Nitrogen Analyzer, Leco, St. Joseph, MI), and starch (Hall, 2009).

2.2.7 Statistical Analyses

All statistical analyses were conducted using SAS 9.4 software (SAS Institute Inc., 2013). Due to technical failures of 3 reticulorumen pH boluses, all analyses involving reticulorumen pH were conducted using a sample size of 38 cows (Long, n=19; Short, n=19). Remaining analyses were conducted using a sample size of 41 cows (Long, n=20; Short, n=21). Significance was declared at $P \leq 0.05$ and tendencies were reported if $0.05 < P \leq 0.10$. If the P-value of an interaction term was ≤ 0.05 it was considered, otherwise interaction terms were disregarded. Prior to analyses, data were tested for normality using the UNIVARIATE procedure of SAS; all assumptions of normality were met for all data.

To investigate the effect of dietary treatment on feed sorting, feeding behavior, DMI, rumination behavior, reticulorumen pH, and milk production and composition, data were summarized by week of lactation (1 to 4) and analysed using the MIXED procedure of SAS, treating week as a repeated measure. The model included the fixed effects of week, treatment, and the week × treatment interaction. The subject of the repeated statement was cow. Compound symmetry was selected as the covariance structure on the basis of best fit according to Schwarz's Bayesian information criterion. The PDIFF procedure was used in the LS MEANS statement for analyzing differences between the week × treatment interactions when differences were detected. Covariates from the dry period were used for detecting differences between treatments for DMI, meal frequency, meal size, and intervals between meals. To determine the occurrence of sorting within treatments, the summarized data for each particle size was tested for a difference from

100 using t-tests. Differences in sorting were analyzed using the previously described MIXED procedure model.

Given the known impact of pre-calving BCS on risk of elevated BHB post-calving (Gillund et al., 2001), cows were categorized as having a normal dry-period BCS if their dry period BCS (collected at enrolment) was ≤3.5 (Long: n=8, Short: n=6) or as having a high dry-period BCS if their dry period BCS was >3.5 (Long: n=12, Short: n=15). To test the effect of treatment on blood BHB concentration, data were analysed using the MIXED procedure of SAS, treating day of blood sampling as a repeated measure. The model included the fixed effects of day of blood sampling, treatment, dry period BCS category, and the dry period BCS category × treatment interaction. The subject of the repeated statement was cow. Compound symmetry was selected as the covariance structure based on best fit according to Schwarz's Bayesian information criterion.

To model the changes in DMI, rumination time, milk yield, mean reticulorumen pH, and time spent below a reticulorumen pH over the 28-d post-calving period, an analysis of covariance was conducted to determine if there were significant linear, quadratic, or cubic effects of day. To accomplish this, data were analyzed using the MIXED procedure of SAS. The fixed effects of treatment, day, treatment × day, day^2, treatment × day^2, day^3, and treatment × day^3 were tested with the random effect of cow. Starting with the highest order term, those terms that were non-significant ($P > 0.05$) were removed from the model in a stepwise manner until only significant ($P \leq 0.05$) terms remained in the model. When an interaction was significant, the lower order term was removed.

2.3 RESULTS

Cows sorted the longest ration particles (>19mm) differently by treatment (Table 2.3); cows on the Long treatment sorted against the longest particles while cows on the Short treatment did not sort for or against those particles. Cows on the Long treatment did not sort for or against the medium particles (<19mm, >8mm), while cows on the Short treatment sorted in favor of those particles; a difference between treatments in sorting of the medium fraction was not, however, detected. Cows on the Long treatment did not sort for or against the short particles (<8mm, >4mm), while cows on the Short treatment selected in favor of those particles; however, there was no detected difference in sorting for the short particles between treatments. Cows tended to sort the fine fraction (<4mm) differently by treatment; cows on the Long treatment did not sort for or against the fine fraction, while cows on the Short treatment sorted against this fraction.

There were no differences between treatments detected in DMI or feeding behavior during the 4-wk study period (Table 2.4). The only exception was a treatment × week interaction (P=0.02) for within meal interval length (min). In the first week post-calving, cows on the Long treatment tended to spend more time not eating within a meal than cows on the Short treatment (5.7 ± 0.8 vs. 3.6 ± 0.8 min; P=0.06); there were no difference in within meal interval length between treatments for wk 2 to 4 post-calving (P=1.0). No effect of treatment was detected on time spent ruminating per day during the study period (Table 2.4).

There were no differences detected between treatments for mean, min, or max daily reticulorumen pH during the 4-wk study period (Table 2.5). There was also no difference between treatments in daily time spent below a reticulorumen pH of 5.8 or AUC for a reticulorumen pH of 5.8 (Table 2.5). Daily milk production, composition, component yield, and milk efficiency were not affected by treatment (Table 2.6). However, the cumulative milk yield for the 4-wk study period tended to be different between treatments (P = 0.07); cows on the Short treatment tended to produce more milk (1,013.0 ± 28.1 kg) than cows on the Long treatment (937.8 ± 28.8 kg).

Eighteen cows on the Long treatment (n=20) and 20 cows on the Short treatment (n=21) had at least one blood BHB concentration reading of 1.2 mmol/L or greater during the study period. No differences were detected between treatments for mean BHB concentration (Long: 1.6 ± 0.15 mmol/L; Short: 1.5 ± 0.15 mmol/L; P = 0.66), days to first diagnosis (Long: 8.3 ± 1.2 d; Short: 8.1 ± 1.2 d; P=0.87), and number of diagnoses (Long: 4.9 ± 0.6; Short: 4.3 ± 0.5; P = 0.46). There were also no differences detected between treatments for cows classified as either a normal or high dry period BCS (P = 0.30).

Changes in DMI, reticulorumen pH, time spent below a reticulorumen pH of 5.8, rumination time, and milk production over the 28-d study period were modelled. The fitted data indicated treatment differences between linear slopes (P < 0.001) for daily DMI (Fig 2.1). The fitted data indicated treatment differences between y-intercepts (P = 0.05), quadratic coefficients (P < 0.001), and cubic coefficients (P < 0.001) for daily rumination time (Fig 2.4). The fitted data for mean daily reticulorumen pH indicated treatment differences between linear slopes (P < 0.001) (Fig 2.2). Treatment differences were noted between the linear slopes (P < 0.001), quadratic coefficients (P < 0.001), and cubic coefficients (P < 0.001) for fitted data for mean daily time spent below a reticulorumen pH of 5.8 (Fig 2.3). The fitted data indicated treatment differences between the linear slopes (P < 0.001), quadratic coefficients (P < 0.001), and cubic coefficients (P < 0.001) for daily milk yield (Fig 2.5).

2.4 DISCUSSION

Particle size of the wheat straw included at 9% DM to a fresh cow diet had an impact on the feed sorting behavior of early lactation cows in this study. The Short diet did not prevent feed sorting as hypothesized. Instead, cows on the Short diet sorted their feed to a small degree in favor of the medium and short particles and sorted against the fine particles. Sorting in favor of some of the longer dietary particles and against the finest particles is unusual because cows typically sort in the opposite direction: favoring the shorter particles and avoiding the longer particles (Miller-Cushon and DeVries, 2017a). Comparatively, cows fed the diet with longer straw particles sorted against the longest ration particles. Greater sorting against these long particles on that diet was hypothesized, as researchers have revealed that cows demonstrate more sorting against long particles when particle size is more easily distinguished (Oelker et al., 2009; DeVries and Gill, 2012b). There was also greater NDF content in the longest particle fraction of the Long ration compared to that in the Short ration, which was expected given the differences in particle size of the straw in both diets. As cows will sort against NDF (DeVries et al., 2007), this greater proportion of NDF further explains why cows on the Long treatment sorted against the longest particle fraction to a greater extent. Differences in sorting between the 2 diets would have contributed to a lesser intake of the most physically-effective fiber on the Long diet and, potentially, a less stable rumen environment.

Although there were no detectable differences between treatments for mean pH, max pH, and min pH, summarized on a weekly basis, cows on the Long treatment experienced a greater linear decrease in reticulorumen pH during the first 7 to 10 DIM than cows on the Short treatment. Sorting against the long particles by cows on the Long treatment may have contributed to a less stable reticulorumen environment. DeVries et al. (2008) demonstrated that sorting against long particles was associated with depressed rumen pH. Similarly, despite no treatment differences in mean time (min/d) spent below a reticulorumen pH of 5.8, the regression analysis revealed differences in how that time spent below a reticulorumen pH of 5.8 varied by treatment across the first 28 DIM. During the first 10 DIM, cows on the Long treatment exhibited a greater linear increase in time spent below a reticulorumen pH of 5.8 than cows on the Short treatment. Between 10 and 23 DIM, the opposite effect was observed, with Long cows experiencing a greater decrease in time spent below a reticulorumen pH of 5.8. Cows on the Long diet sorted against the longest, and most physically-effective components, of the feed; this may have altered the buffering capacity of the reticulorumen (Zebeli et al., 2012) and, thus, contributed to greater fluctuations in reticulorumen pH. This degree of fluctuation is atypical for healthy cows; the pH of fluid in the reticulum, where the bolus was mostly situated, fluctuates to a lesser degree than ruminal fluid pH (Falk et al., 2016). Falk et al. (2016) suggested that the reason reticular pH is more stable is because the reticulum is smaller than the rumen and the contents are less varied. A smaller reticular volume likely minimizes movement of the bolus; this stabilization may provide more consistent measurements (Falk et al., 2016). Additionally, the reticulum has a higher pH than the rumen (Sato et al., 2012). Sato et al. (2012) suggested that a reticular pH of 6.3 or less was indicative of SARA, but did not provide a minimum time requirement for this threshold. It is possible that more pronounced differences between treatments in measures of pH in the current study may have been observed if measurement of pH were taken within the ventral rumen sac.

Despite a lack of a detectable difference in weekly mean DMI between treatment groups, cows on the Short diet had a greater linear daily increase in DMI over the duration of the trial than cows on the Long diet. A plateau in DMI was observed for both treatment groups between approximately 10 and 20 DIM, which is unusual for fresh cows, who typically experience a rapid increase in DMI in early lactation (Kertz et al., 1991). To ensure that any differences between the 2 treatment diets were discernible, the straw content of the diets was higher than what would allow for maximal DMI in cows averaging 122 ± 62 DIM (9% vs 5% DM; Eastridge et al., 2017). It is, thus, possible that the DMI of cows on both trial diets were limited at around 10 DIM because of the high straw content. The straw may have filled the rumen and prevented rapid digestion, as was speculated in a recent study using a diet with 10% (DM) wheat straw (Eastridge et al., 2017). However, McCarthy et al. (2015a; b) suggested that DMI was limited by rumen-fill when NDF intake reached 1.1% of BW in the first 3 wk of lactation for cows fed a diet with 11.5% DM wheat straw (McCarthy et al., 2015c). At no time in the present study did mean daily NDF intake, as a percentage of BW, reach 1.1%, suggesting that DMI was not limited only by the amount of straw in the diets. Dry matter intake may also be limited by propionate production via hepatic oxidation, which may be exacerbated in early lactation (Allen, 2014).

An alternative explanation for the plateau in DMI is that it is related to the rapid decline in reticulorumen pH observed in the first 10 DIM. The beginning of the plateau in DMI immediately follows the peak in time spent below a reticulorumen pH of 5.8 at 7 to 10 DIM, when cows on the Long treatment spent 267 min/d below this pH threshold. While there are no definitive parameters for how long reticulorumen pH must be depressed to diagnose SARA, it is generally accepted that pH must be below 5.8 for several hours per day (Plaizier et al., 2009). With this threshold in mind, it seems plausible that cows on both treatments were experiencing SARA in the first 10 DIM, the symptoms of which include decreased DMI and milk production (Plaizier et al., 2009). In support of this theory, cows on the Short treatment tended to produce 76 kg more milk over the first 28 DIM than cows on the Long treatment, possibly due to consumption of a more consistent diet (relative to that predicted) and greater stability in their reticulorumen environment. These results are supported by Sova et al. (2013), who observed that group-level sorting against long particles was associated with decreased fat-corrected milk yield, possibly as a result of a less stable reticulorumen environment. While there was no detectable difference in weekly mean milk yield between treatments, there was a 4.7% numerical increase on the Short treatment. In a post-hoc analysis, it was determined that an increase in milk yield of 3.25 kg/d (8.9%) would have been needed to detect significant differences between treatments (Morris, 1999).

Aside from time spent not eating within a meal, feeding behavior was unaffected by treatment. This was not expected, as changing forage particle size has been shown to affect time spent chewing for oat silage, corn silage, and alfalfa hay (Kononoff and Heinrichs, 2003; Leonardi et al., 2005b; Kahyani et al., 2013). However, Suarez-Mena et al. (2013) did not find a difference in time spent eating, chewing, or ruminating for non-lactating cows fed a diet differing in straw particle size, which is consistent with the findings of the current study. It may be that the difference between treatments in straw particle size, in both studies, was not large enough to elicit a response in chewing time. In the first week of lactation cows on the Long treatment tended to spend more time not eating within a meal than cows on the Short treatment. It is possible that the transition to a diet with a longer straw particle size initially required more chewing (Nasrollahi et al., 2016), which resulted in more time spent not actively consuming feed from the bin and greater within meal intervals. After a period of adaptation, cows on the long treatment may have then been able to reduce the time spent not eating within a meal. These results are, however, not supported by Yang and Beauchemin (2006), who found that increasing the forage particle size of barley silage did not result in more chewing within a meal. The discrepancy between this study and the current study may be the result of differences in forage sources. It is possible that straw takes longer within a meal to chew than silage; support for this is provided by Sudweeks and Ely (1979) and Sudweeks et al. (1981) who demonstrated that oat straw has a higher chewing time/kg DM than other forage sources such as hay and various silages.

While there were no detected treatment differences in weekly mean time spent ruminating, treatment did affect how time spent ruminating evolved over the first 28 DIM. Cows on the Short treatment showed less fluctuation in rumination time after 10 DIM. Cows on the Short treatment had a lower initial daily rumination time after calving than cows on the Long treatment but recovered faster in the first 10 DIM. These results are consistent with the stabilization of reticulorumen pH in cows on the Short treatment, likely due to the difference in sorting behavior on that diet, as discussed above. Stimulating rumination by feeding forage is important for maintaining a stable reticulorumen environment because rumination results in increased rates of salivation, which then buffers the reticulorumen pH (Oba and Allen, 1999).

Milk yield evolved differently by treatment across the first 28 DIM. During the first 10 DIM, cows on Long treatment exhibited a greater linear increase in milk yield. However, after this initial surge in milk yield, cows on both treatments experienced a plateau in daily milk yield, which slightly declined until 23 DIM. A high incidence rate of SCK across treatments may explain why a plateau in milk yield occurred for both treatment groups. Of the 41 cows used in this trial, 38 (Long, n=18; Short, n=20) were diagnosed with SCK at least once during the trial period. There is an association between decreased milk production and increased BHB concentrations post-calving (Ospina et al., 2010). Thus, this higher than expected incidence rate of SCK may have contributed to the plateau in milk yield observed in this study (Ospina et al., 2010; McArt et al., 2012). It is also interesting to note that from 10 to 23 DIM, cows on the Long treatment experienced greater declines in milk yield compared to cows on the Short treatment. This difference is likely not the result of SCK, as there were no treatment differences in measures of BHB concentration. It is more plausible that greater fluctuations in reticulorumen pH and rumination during the same time period, created by sorting against the longest particles in the ration, resulted in more variable milk yield for cows on the Long treatment over the first 28 DIM.

CONCLUSIONS

Over the first 28 DIM cows sorted in favor of the medium and short particles and against the finest ration particles when fed a diet containing shorter chopped wheat straw. In contrast, cows sorted against the longest ration particles during that time period when the diet contained longer chopped wheat straw. These differences in sorting behavior explain why cows fed the diet with the shorter chopped straw demonstrated more stability in their rumination and reticulorumen pH over the first 28 days of lactation. As consequence, cows fed a diet with a shorter straw particle size exhibited more stability in their milk production, and tended to produce more milk, over that time period than cows fed a diet with the longer straw particle size. These results highlight the importance of managing forage particle size in early lactation diets to promote consistency in intake, relative to that predicted, and resultant stability in reticulorumen health and milk yield.

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Table 2.1. Ingredient and chemical composition (mean ± SD) of the dry cow and experimental total mixed rations.

Composition	Dry Diet	Long Lactating Diet[1]	Short Lactating Diet[2]
Ingredient, %DM			
Corn Silage[3]	42	34	34
Wheat Straw[4]	21	9[5]	9[6]
Alfalfa Haylage[7]	16	26	26
High Moisture Corn	-	19	19
Dry Cow Supplement[8]	21	-	-
Lactating Cow Supplement[9]	-	12	12
Chemical Composition[10]			
DM, %	48.3 ± 2.9	49.0 ± 3.9	49.0 ± 4.8
OM, % of DM	92.2 ± 0.5	91.8 ± 0.8	92.1 ± 0.8
CP, % of DM	13.6 ± 1.0	15.9 ± 2.0	15.5 ± 1.8
ADF, % of DM	30.5 ± 1.3	22.4 ± 1.9	22.7 ± 1.7
NDF, % of DM	44.1 ± 1.8	32.6 ± 2.7	33.0 ± 2.5
Starch, % of DM	17.0 ± 1.2	26.1 ± 2.0	26.4 ± 2.4
NFC, % of DM	34.6 ± 1.8	43.3 ± 2.3	43.5 ± 3.0
Ca, % of DM	1.0 ± 0.1	1.1 ± 0.2	1.1 ± 0.1
P, % of DM	0.4 ± 0.0	0.5 ± 0.1	0.5 ± 0.1
NE_L, Mcal/kg of DM	1.52 ± 0.02	1.63 ± 0.03	1.63 ± 0.03

[1]Straw in long lactating diet was cut to measure approximately 5.08 cm.
[2]Straw in short lactating diet was cut to measure approximately 2.54 cm.
[3]Corn silage had a DM of 41.1± 8.0% and chemical composition (DM basis) 7.3 ± 0.6% CP, 18.6 ± 1.5% ADF, 30.6 ± 1.8% NDF, and 40.8 ± 0.7% starch.
[4]Straw had a DM of 90.0 ± 2.9% and chemical composition (DM basis) 4.8 ± 0.7% CP, 55.6 ± 1.1% ADF, and 77.4 ± 1.1% NDF.
[5]Straw particle size distribution (% of DM) for long treatment = 14.1 ± 6.9 % long (>19mm), 39.8 ± 4.7 % medium (<19, > 8 mm), 17.4 ± 1.0 % short (< 8, > 4 mm), and 28.6 ± 10.5 % fine (< 4 mm) particles.
[6]Straw particle size distribution (% of DM) for short treatment = 1.4 ± 0.8 % long (>19mm), 25.0 ± 4.8 % medium (<19, > 8 mm), 24.8 ± 1.7 % short (< 8, > 4 mm), and 48.9 ± 3.4 % fine (< 4 mm) particles.
[7]Alfalfa (80%) and timothy grass (20%) haylage had a DM of 39.7 ± 6.3% and chemical composition (DM basis) 17.6 ± 1.5% CP, 35.3 ± 0.5% ADF, and 41.4 ± 2.3% NDF.
[8]Supplied by Floradale Feed Mill Ltd (Floradale, Ontario, Canada) including ingredients (as is); 34.0% Soy Plus, 18.0% soy hulls (ground), 17% canola, 13.6% wheat shorts,8.5% soybean meal, 2% Diamond V Yeast XP, 1.9% limestone calcium carbonate, 1.3% magnesium oxide, 1.0% vitamin E, 1.0% fine salt, 1.0% tallow, 0.5% FFM Org Ruminant Micro PRX, 0.07% Alkosel 2000, 0.05% Rumensin, and 0.01% Rovimix Biotin 20,000.
[9]Supplied by Floradale Feed Mill Ltd (Floradale, Ontario, Canada) including ingredients (as is); 44.5% Soy Plus, 32.5% soybean meal, 4.2% limestone calcium carbonate, 3.9% sodium sesquicarbonate, 3.6% fine salt, 3.5% fish meal (Herring), 2.7% monocalcium phosphate, 2.0%

magnesium oxide, 1.8% Diamond V Yeast XP, 0.9% FFM Org Ruminant Micro P, 0.4% Metasmart, 0.05% Rumensin, and 0.03% Selplex 2000.

[10]Values were obtained from chemical analysis of TMR samples. OM = 100 - % ash. NFC = 100 – (% CP + % NDF + % fat + % ash). NE_L was calculated based on NRC (2001) equations.

Table 2.2. Particle size distribution[1] (%DM) and chemical composition of experimental lactating cow diets (mean ± SD).

	Long Lactating Diet[2]	Short Lactating Diet[3]
% of DM		
Long	6.4 ± 4.2	4.2 ± 3.6
Medium	38.9 ± 5.7	39.4 ± 6.2
Short	19.3 ± 2.3	20.5 ± 3.4
Fine	35.4 ± 7.0	36.0 ± 7.0
ADF, % of screen DM[4]		
Long	31.5 ± 1.0	28.9 ± 1.2
Medium	26.6 ± 0.9	25.8 ± 1.0
Short	18.6 ± 0.5	19.5 ± 1.0
Fine	13.9 ± 0.5	15.2 ± 1.2
NDF, % of screen DM[4]		
Long	45.2 ± 1.2	40.7 ± 1.9
Medium	37.7 ± 1.6	36.6 ± 2.0
Short	28.2 ± 1.0	29.4 ± 1.8
Fine	21.2 ± 1.0	23.7 ± 1.7

[1]Particle size determined by Penn State Particle Separator which has a 19 mm screen (long), 8 mm screen (medium), 4 mm screen (short), and a pan (fine).
[2]Straw in long lactating diet was cut to measure approximately 5.08 cm.
[3]Straw in short lactating diet was cut to measure approximately 2.54 cm.
[4]Values were obtained from chemical analysis of separated TMR samples.

Table 2.3. Effect of straw particle size on the sorting (%)[1] of lactating dairy cows in the first 28 d of lactation (mean ± SE)[2].

Sorting of particle fractions, %[3]	Treatment[4]		P-Value
	Long	Short	
Long	94.2 ± 1.9*	99.7 ± 1.9	0.049
Medium	100.0 ± 0.8	101.8 ± 0.8*	0.12
Short	100.7 ± 0.5	101.3 ± 0.5*	0.44
Fine	99.8 ± 1.2	96.8 ± 1.2*	0.08

[1]Sorting = DMI Refused/ DMI predicted for each fraction. Sorting % = 100 means no sorting occurred, sorting % $<$ 100 means sorting occurred against, and sorting % $>$ 100 means sorting occurred in favor.
[2]Long, n=20 cows, Short, n=21 cows
[3]Particle size determined by Penn State Particle Separator which has a 19mm screen (long), 8 mm screen (medium), 4 mm screen (short) and a pan (fine).
*Difference in sorting values from 100% expressed as: $P < 0.05$; all other values are $P > 0.05$.
[4]Long: TMR with long (5.08 cm) wheat straw particles; Short: TMR with short (2.54 cm) wheat straw particles.

Table 2.4. Effect of straw particle size on the DMI and feeding behavior of lactating dairy cows[1] in the first 28 d of lactation (mean ± SE).

	Treatment[2]		
Item	Long	Short	*P*-Value
DMI, kg/d	19.0 ± 0.5	19.0 ± 0.5	0.98
DMI as a percentage of BW, %	2.6 ± 0.08	2.6 ± 0.08	0.86
Feeding time, min/d	163.0 ± 8.7	163.3 ± 8.5	0.98
Feeding rate, kg DM/min	0.14 ± 0.006	0.14 ± 0.006	0.90
Meal frequency, meals/d	9.5 ± 0.5	9.2 ± 0.5	0.66
Within meal intervals, min	4.5 ± 0.6	4.1 ± 0.6	0.65
Meal length, min/meal	23.4 ± 1.6	23.1 ± 1.5	0.89
Meal size, DMI/meal	2.2 ± 0.1	2.3 ± 0.1	0.50
Intervals between meals, min	142.8 ± 8.4	148.1 ± 8.0	0.65
Meal criterion, min	18.2 ± 2.8	18.0 ± 2.7	0.97
Rumination time, min/d	295.4 ± 10.4	296.8 ± 10.1	0.92

[1] Long: n=20 cows; Short: n=21 cows

[2] Long: TMR with long (5.08 cm) wheat straw particles; Short: TMR with short (2.54 cm) wheat straw particles.

Table 2.5. Effect of straw particle size on the reticulorumen pH of lactating dairy cows in the first 28 d of lactation (mean ± SE)[1].

Item	Treatment[2] Long	Short	*P*-Value
Mean reticulorumen pH	6.2 ± 0.05	6.2 ± 0.05	0.63
Maximum reticulorumen pH	6.6 ± 0.05	6.6 ± 0.05	0.50
Minimum reticulorumen pH	5.9 ± 0.05	5.9 ± 0.05	0.60
Mean time below reticulorumen pH 5.8, min/d	140.9 ± 47.8	97.6 ± 48.1	0.53
AUC[3] pH <5.8, pH × min/d	19.9 ± 9.0	15.2 ± 9.1	0.72
AUC/DMI[4]	1.0 ± 0.5	1.1 ± 0.5	0.96

[1]Long, n=19 cows; Short, n=19 cows
[2]Long: TMR with long (5.08 cm) wheat straw particles; Short: TMR with short (2.54 cm) wheat straw particles.
[3]AUC = area under curve
[4]AUC/DMI = area under curve pH <5.8 (pH × min/d) divided by DMI (kg/d)

Table 2.6. Effect of straw particle size on the milk production and composition of lactating dairy cows in the first 28 d of lactation (mean ± SE)[1,2].

Item	Treatment[3]		*P*-Value
	Long	Short	
Milk production, kg/d			
Milk	36.4 ± 1.1	38.1 ± 1.1	0.31
4% FCM	44.4 ± 1.7	46.2 ± 1.6	0.45
ECM	48.5 ± 2.9	52.2 ± 3.0	0.38
Milk composition, %			
Fat	5.1 ± 0.1	5.2 ± 0.1	0.79
Protein	4.1 ± 1.0	4.3 ± 0.94	0.90
Milk component yield, kg/d			
Fat	1.9 ± 0.08	2.0 ± 0.1	0.56
Protein	1.5 ± 0.4	1.8 ± 0.4	0.62
Efficiency of milk production, kg/kg			
Milk/DMI	2.0 ± 0.06	2.1 ± 0.06	0.49
4% FCM/DMI	2.3 ± 0.09	2.5 ± 0.08	0.27
ECM/DMI	2.5 ± 0.2	2.8 ± 0.2	0.27
MUN, mg/dL	10.4 ± 0.5	10.0 ± 0.4	0.62
Log SCC[4]	4.7 ± 0.2	4.7 ± 0.3	0.98

[1]Long, n=20 cows; Short, n=21 cows

[2]Milk composition and efficiency were averaged over 5 d.

[3]Long: TMR with long (5.08 cm) wheat straw particles; Short: TMR with short (2.54 cm) wheat straw particles.

[4]Somatic cell counts (cells/mL) were log-transformed, given that they did not meet the assumption of normality.

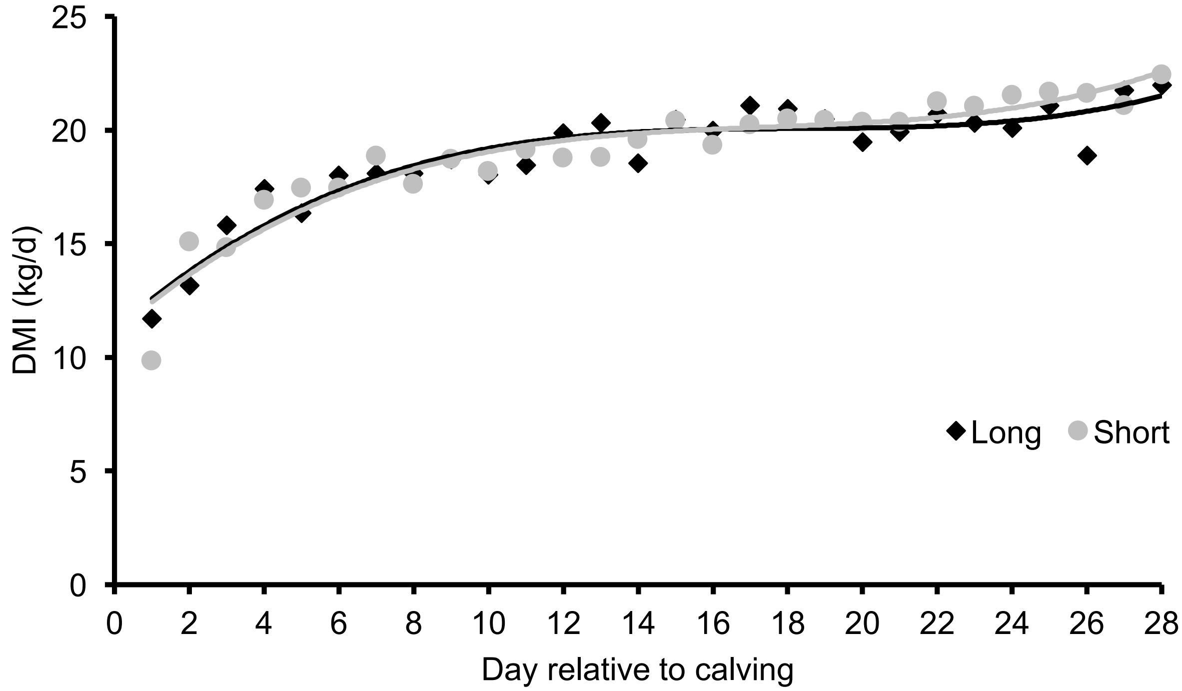

Figure 2.1. Daily DMI (kg/d) for cows fed 1 of 2 dietary treatments differing in the length of the wheat straw component: 1) 2.54 cm straw length (Short: n = 21 cows) or 2) 5.08 cm straw length (Long: n = 20 cows). Trend lines were constructed using analysis of covariance: y=1.42d-0.080d^2+0.0015d^3+11.44 for the Long treatment; y=1.46d-0.080d^2+0.0015d^3+11.15 for the Short treatment.

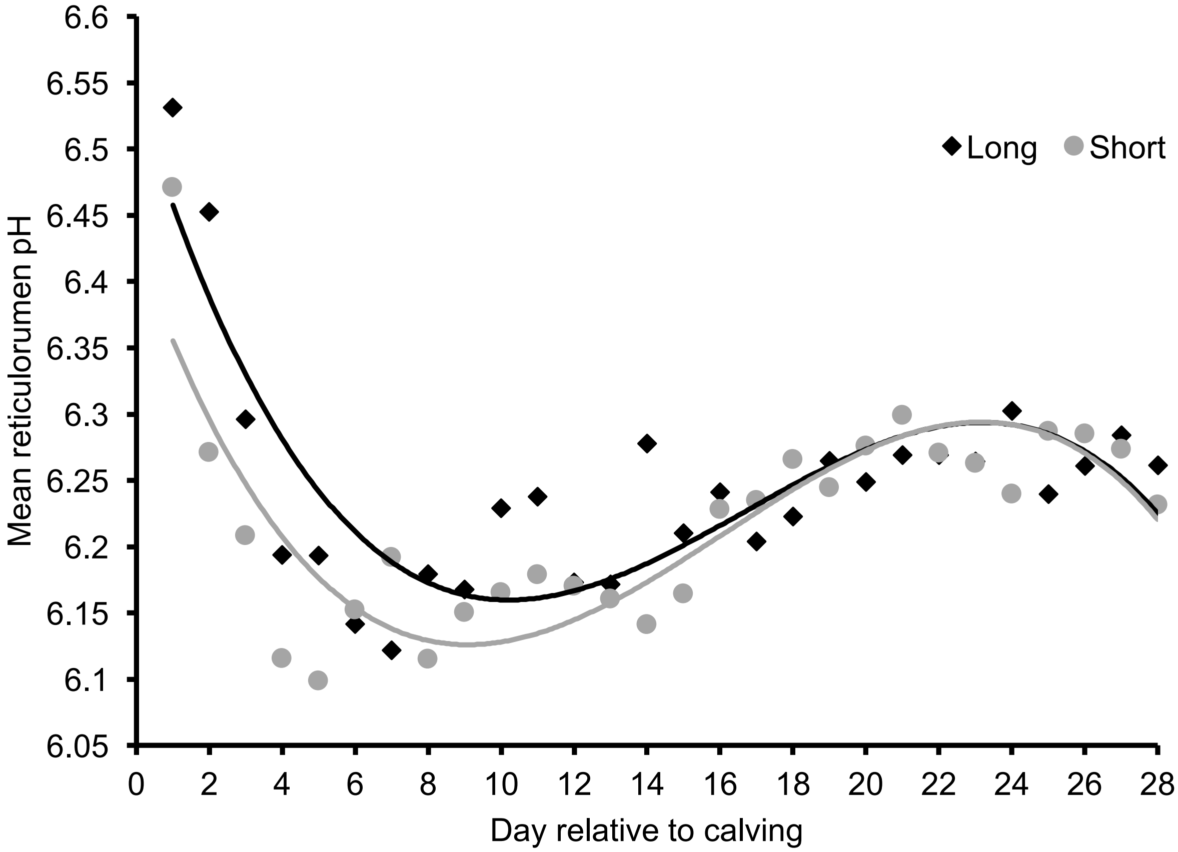

Figure 2.2. Mean daily reticulorumen pH for cows fed 1 of 2 dietary treatments differing in the length of the wheat straw component: 1) 2.54cm straw length (Short, n = 19 cows) or 2) 5.08cm straw length (Long, n = 19 cows). Trend lines were constructed using analysis of covariance: $y=-0.083d+0.0060d^2-0.00012d^3+6.51$ for the Long treatment; $y=-0.080d+0.0060d^2-0.00012d^3+6.43$ for the Short treatment.

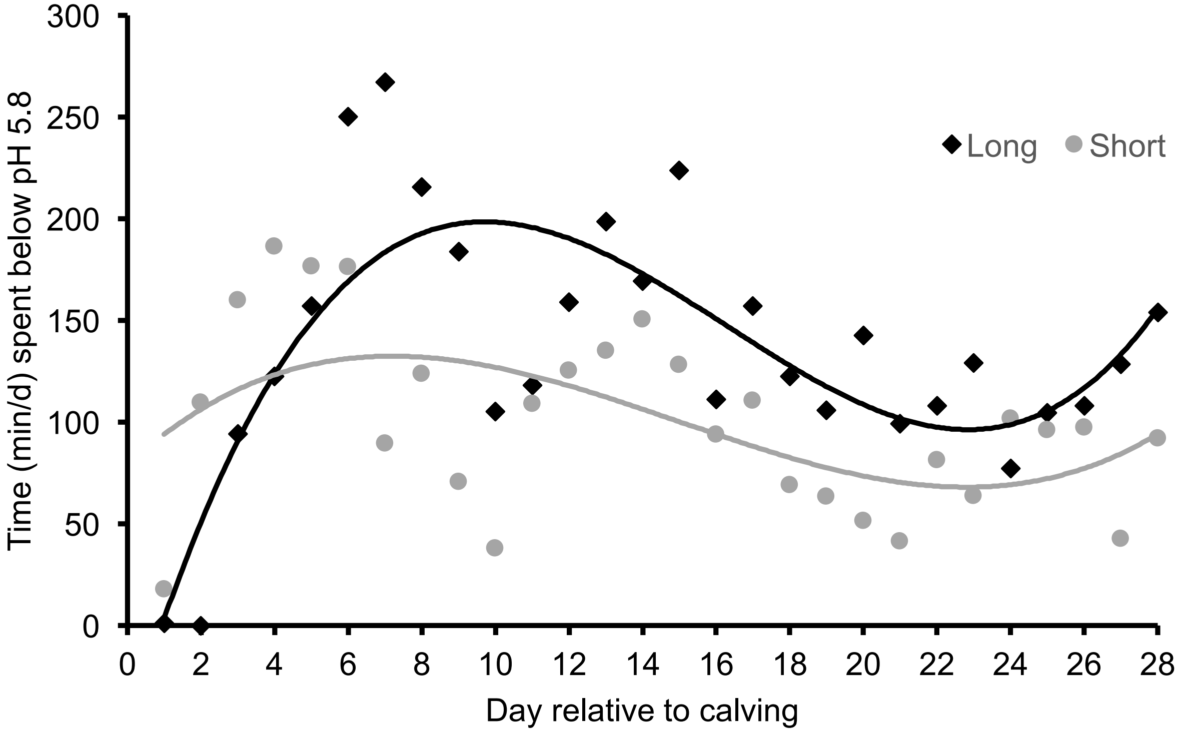

Figure 2.3. Mean time (min/d) spent below a reticulorumen pH of 5.8 for cows fed 1 of 2 dietary treatments differing in the length of the wheat straw component: 1) 2.54cm straw length (Short, n = 19 cows) or 2) 5.08cm straw length (Long, n = 19 cows). Trend lines were constructed using analysis of covariance: $y=61.11d-4.53d^2+0.091d^3-33.73$ for the Long treatment; $y=16.13d-1.50d^2+0.032d^3+93.88$ for the Short treatment.

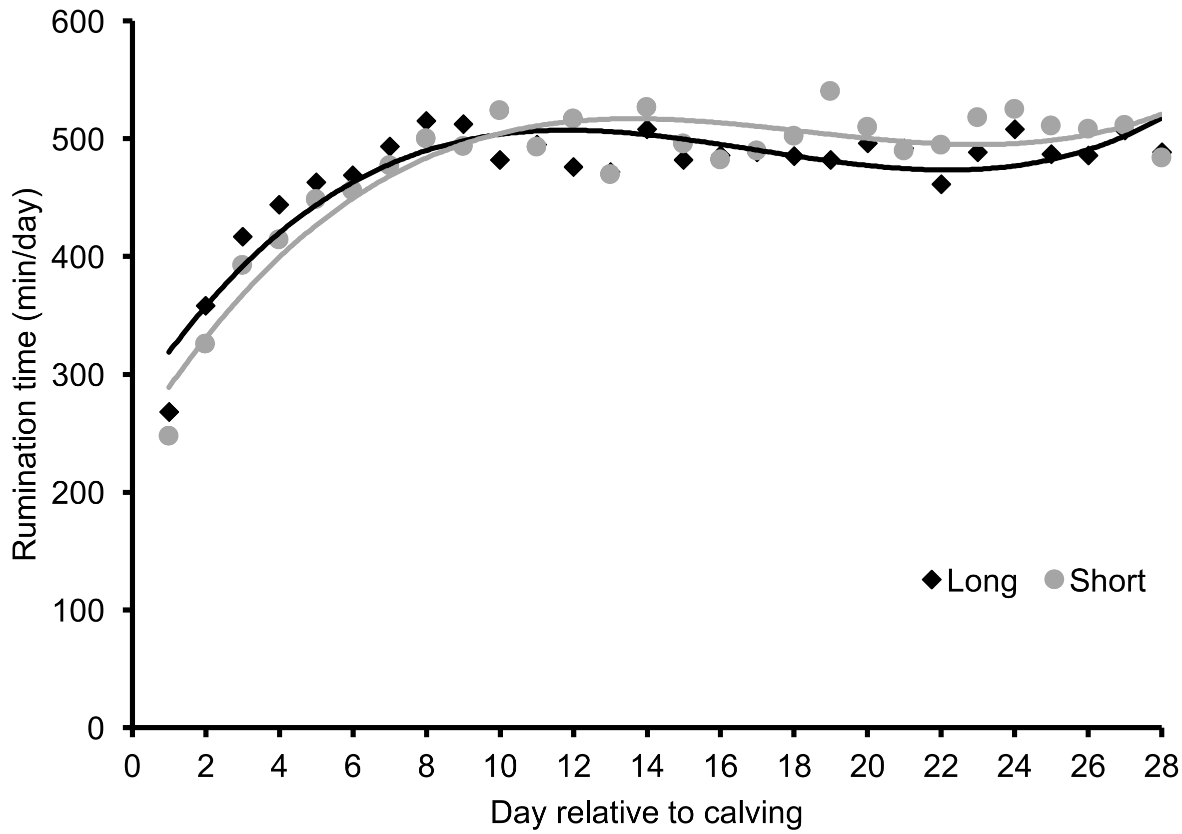

Figure 2.4. Mean rumination time (min/d) post-calving for cows fed 1 of 2 dietary treatments differing in the length of the wheat straw component: 1) 2.54cm straw length (Short, n = 21 cows) or 2) 5.08cm straw length (Long, n = 20 cows). Trend lines were constructed using analysis of covariance: $y = 47.85d - 3.12d^2 + 0.061d^3 + 278.43$ for the Long treatment; $y = 47.85d - 2.67d^2 + 0.047d^3 + 229.93$ for the Short treatment.

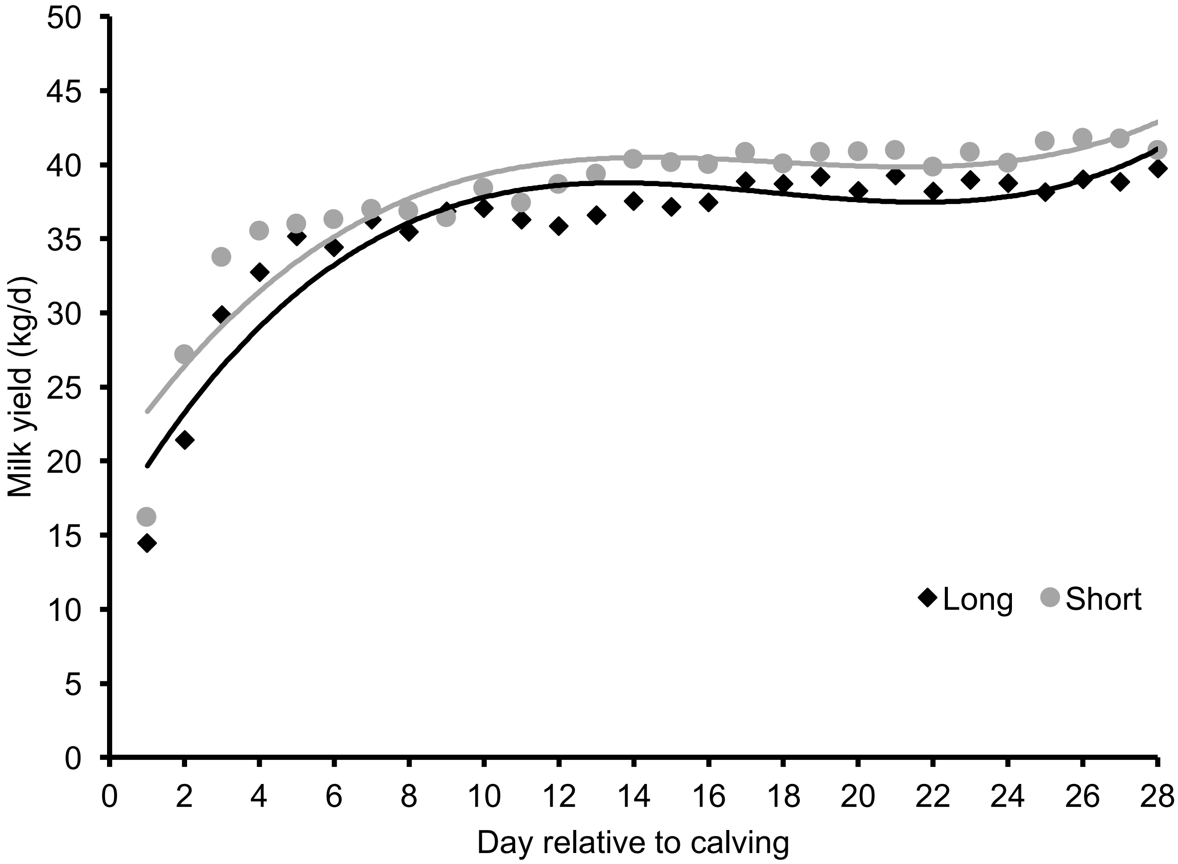

Figure 2.5. Mean daily milk yield for cows fed 1 of 2 dietary treatments differing in the length of the wheat straw component: 1) 2.54cm straw length (Short, n = 21 cows) or 2) 5.08cm straw length (Long, n = 20 cows). Trend lines were constructed using analysis of covariance: $y=4.081d-0.24d^2+0.0044d^3+17.44$ for the Long treatment; $y=3.13d-0.18d^2+0.0032d^3+22.34$ for the Short treatment.

CHAPTER 3: Risk of subacute ruminal acidosis affects the feed sorting behavior and production of early lactation cows

3.1 INTRODUCTION

Subacute ruminal acidosis (SARA) is a disorder characterized by prolonged depression of rumen pH and can result in decreased DMI and milk production (Plaizier et al., 2009). This disorder is caused by an over-consumption of easily fermentable carbohydrates, resulting in an accumulation of VFA in the rumen (Plaizier et al., 2009). Alternatively, greater chewing and rumination, as a result of greater intake of physically-effective fiber, increases saliva production which helps to neutralize the acidity of the rumen and prevent SARA (Zebeli et al., 2012).

Early lactation dairy cows are particularly susceptible to SARA because of the abrupt transition from a dry diet composed of primarily forages and minimal grain to a high-energy lactation diet containing a greater proportion of readily-digestible concentrate components (Steele et al., 2016). Adequate forage consumption is necessary for early lactation cows; forages may increase rumination and saliva production and, thus, reduce risk of SARA, but will also limit intake if the inclusion rate is too high (Allen, 1996). Limiting intake is undesirable for early lactation dairy cows because they already experiencing a lag in DMI compared to milk production and need to maximize intake to meet energy demands for lactation (Dado and Allen, 1995; Allen, 1996).

The risk of SARA is not only linked to the diet provided, but also the diet consumed by the cow. Sorting of a TMR by dairy cows can result in the ration actually consumed by cows being greater in fermentable carbohydrates than intended and lesser in effective fiber, thereby increasing the risk of depressed rumen pH (DeVries et al., 2008). It is noteworthy that lactating dairy cows experiencing low ruminal pH may change their sorting behavior, sorting in favor of the longest, most physically-effective particles in the diet (Beauchemin and Yang, 2005; Yang and Beauchemin, 2006). DeVries et al. (2008) demonstrated that individual risk of SARA may impact such sorting behavior, finding that early lactation cows (39 to 78 DIM) at higher risk for SARA sorted their feed to maximize intake of physically-effective fiber after experiencing an acute bout of acidosis more so than mid-lactation cows (52 to 232 DIM) at lower risk for SARA.

To date, there is no data on the association of SARA risk and sorting in cows in the immediate post-partum period. Thus, the primary objective of this research was to determine how risk of developing SARA affects the behavior and production of early lactation cows. It is known that long forage particles are more easily discernible and, therefore, more often sorted against than smaller forage particles (Oelker et al., 2009; DeVries and Gill, 2012b). Reducing particle size of forages for early lactation cows should, thus, reduce the imbalance of nutrients consumed by those cows at highest risk for SARA. A secondary objective of this research was to determine if straw particle size in the diet of early lactation dairy cows further impacts these outcomes, depending on SARA risk. It was predicted that cows experiencing chronically low reticulorumen pH would sort their feed to minimize the negative consequences of this condition and that this behavior would be more apparent in cows fed a diet that is more easily sorted. It was also predicted that cows suffering from chronically low reticulorumen pH would spend less time ruminating and also experience decreased milk production.

3.2 MATERIALS AND METHODS

This research is part of a larger study aimed at identifying the effect of straw particle size on the behavior, health, and production of early lactation dairy cows. For this reason, detailed descriptions of the methodology are presented in Chapter 2.

3.2.1 Animals and Housing

The study took place at the University of Guelph Livestock Research and Innovation Centre Dairy Facility (Elora, Ontario, Canada), where 41 multiparous cows were housed and milked 2x/day at 0500 and 1700 h in a milking parlor. The use of cows and experimental procedures complied with the guidelines of the Canadian Council on Animal Care (2009) and were approved by the University of Guelph Animal Care Committee (Animal Use Protocol #2518).

3.2.2 Experimental Design

The treatments consisted of feeding cows 1 of 2 fresh cow diets, randomly assigned at calving, and fed for the following 4 wk. Diets only differed in 1 aspect, the length of the wheat straw component in that diet: 1) 2.54 cm straw length (**Short**; n=21) or 2) 5.08 cm straw length (**Long**; n=20). The Long and Short diets were identical in ingredient composition (on a DM basis): 34% corn silage, 9% wheat straw, 26% alfalfa haylage, 19% high moisture corn, and a 12% supplement. Treatment allocation was balanced for parity and milk production in the previous lactation (10,334.4 ± 1,955.7 kg). The lactating cow TMR was formulated to, at minimum, meet the nutrient requirements of dairy cows producing 36 kg/d (NRC, 2001).

3.2.3 Health Data Collection

At the time of enrolment, cow BW was recorded (I-20W scale, Ohaus, Dundas, ON, Canada). Assessment of BW occurred once during the dry period at enrollment and every 14 d starting on Day +4 post-calving for a total of 3 measurements including a final weighing on the last of the trial period.

Wireless telemetry boluses (eBolus, eCow Ltd., Devon, UK) were used to measure reticulorumen pH to assess rumen health (as validated by Falk et al., 2016). At time of enrollment, the boluses were administered orally using a balling gun. Data consisted of reticulorumen pH data points on 15 min intervals 24 h/d throughout the trial period that was then amalgamated into a continuous record for each individual cow.

3.2.4. Feed Sampling and Analysis

Throughout the study, 2 fresh TMR samples were collected 3x/wk: 1 sample was collected to determine DM and chemical composition, and the other sample was collected to determine particle size distribution. Orts samples were collected every 3 d during the experimental period starting on d +4. All samples were immediately frozen at -20ºC until further analysis.

Fresh and orts TMR samples collected for particle size analysis were processed using a 4-screen Penn State Particle Separator (PSPS; Maulfair et al., 2011; Heinrichs, 2013), which separates the sample into 4 fractions based on particle size: long (> 19 mm), medium (< 19 > 8 mm), short (< 8 > 4 mm), and fine (< 4 mm). Separated samples were then oven dried at 55^0C for 48 h.

Sorting of each PSPS fraction was calculated (as per Leonardi and Armentano, 2003) by dividing the actual amount of feed consumed of each fraction by the predicted amount of feed consumed of that fraction and expressing it as a percentage. For each fraction, the actual amount consumed was calculated by subtracting the DM refused from the DM offered, as determined by the PSPS analysis. The predicted amount consumed for each fraction was calculated as the product of the DMI of the total diet multiplied by the DM percentage of that fraction in the fed TMR. If the sorting value equalled 100 %, then no sorting of the particle fraction occurred, a value <100 % indicated sorting against that particle size fraction, while a value > 100 % indicated sorting in favor of that particle fraction.

Feed samples collected for nutrient composition analysis included the fresh TMR samples and TMR PSPS fractions. These samples were oven dried at 55^{0}C for 48 h and then were ground to pass through a 1-mm screen (Model 4 Wiley Laboratory Mill, Thomas Scientific, Swedesboro, NJ, USA). Ground samples, pooled by week, 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: method 973.18), NDF with heat-stable α-amylase and sodium sulfite (Van Soest et al., 1991), CP (N x 6.25; AOAC 2000: method 990.03; Leco FP-528 Nitrogen Analyzer, Leco, St. Joseph, MI), and starch (Hall, 2009).

3.2.5 Milk yield and components

Milk yield was recorded at every milking in the milking parlor (using DelPro software, DeLaval, Peterborough, ON, Canada) for the 4 wk cows were observed on treatment. Milk samples were collected from each cow, at each milking, on 2 consecutive days each week starting at d +6. These samples were sent to a DHI testing laboratory (CanWest DHI, Guelph, ON, Canada) for component analysis (fat, protein, MUN, and SCC) using a Fourier Transform Infrared full spectrum analyzer (Milkoscan FT+ and Milkoscan 6000; Foss, Hillerød, Denmark). One value per cow on each sampling day was obtained by calculating the average across milkings. Milk composition samples were used to determine the yield of 4% FCM (kg/d), calculated as: (0.4 × milk yield (kg/d)) + (15.0 × fat yield (kg/d)) (NRC, 2001).

3.2.6 Categorization based on reticulorumen health

Due to technical failures of 3 reticulorumen pH boluses, all analyses involving reticulorumen pH were conducted using a sample size of 38 cows (Long, n=19; Short, n=19). The boluses collected pH data every 15 min 24 h/d. Time below pH 5.8 was determined by summing time spent below pH 5.8 for each cow for each day. An absolute value for area below pH 5.8 was calculated by subtracting the pH value from 5.8 and multiplying it by the time spent at that pH value. These values were summed by day creating a single value for area under the curve (AUC) for each day for each cow. These values were normalized by dividing by DMI for each day for each cow (Penner et al., 2009b). The normalized values were averaged by cow, creating a single value for each cow. An acidosis index was created from the values to classify cows based on risk of acidosis (Penner et al., 2009b). Macmillan et al. (2017) defined risk of acidosis using an acidosis index threshold of 1.0 or greater. The pH was measured in the rumen fluid from the ventral rumen sac. In the present study pH boluses were located in the ruminoreticulum (Falk et al., 2016). The reticulum has a higher mean pH; it has been suggested that the SARA threshold for reticular fluid is 6.3 versus 5.8 for rumen fluid (Sato et al., 2012; Falk et al., 2016). As such, we chose to define our acidosis risk threshold to be an AUC/DMI value of 0.5 or greater. This classification was based on the distribution of this index for all cows; cows were classified as being low risk (**LR**) for developing SARA if AUC/DMI averaged less than 0.5 for the duration of the trial (n=25; Long: n=12, Short: n=13). If the acidosis index averaged greater than or equal to 0.5 for the duration of the trial, a cow was classified as being high risk (**HR**) (n=13; Long: n=7, Short: n=6).

3.2.7 Statistical Analyses

All statistical analyses were conducted using SAS 9.4 software (SAS Institute Inc., 2013). Significance was declared at $P \leq 0.05$ and tendencies were reported if $0.05 < P \leq 0.1$. If the P-value of an interaction term was ≤ 0.1 it was considered, otherwise interaction terms were disregarded. Prior to all analyses, data were tested for normality using the UNIVARIATE procedure of SAS; all assumptions of normality were met for all data.

To investigate the effect of dietary treatment within SARA risk categories (high vs. low) on feed sorting, rumination, reticulorumen pH, DMI, DMI by BW, AUC, AUC/DMI, milk yield, FCM (4%), milk fat percentage, milk protein percentage, and milk fat yield, data were summarized by week of lactation (1 to 4) and analysed in a general linear mixed model using the MIXED procedure of SAS, treating week as a repeated measure. The model included the fixed effects of week, treatment, SARA risk, and the SARA risk × treatment interaction. The subject of the repeated statement was cow. Compound symmetry was selected as the covariance structure on the basis of best fit according to Schwarz's Bayesian information criterion. The PDIFF procedure was used in the LSMEANS statement for analyzing differences between the SARA risk × treatment interactions when differences were detected.

To determine the occurrence of sorting within treatments, the summarized data for each particle size was tested for a difference from 100 using t-tests. Differences in sorting were analyzed using the previously described MIXED procedure model.

3.3 RESULTS AND DISCUSSION

Cows categorized as LR had higher daily mean, max, and min reticulorumen pH than HR cows (Table 3.2). Again, LR cows also had lower daily time spent below a reticulorumen pH of 5.8 and AUC than HR cows. These results are supported by Macmillan et al. (2017) and Nasrollahi et al. (2017), both of whom found that the pH variables measured were consistently lower in value, duration, and AUC for HR cows than for LR cows. Risk of developing SARA had no effect on DMI or DMI as a percentage of BW (Table 3.2), which is consistent with the findings of Gao and Oba (2016) who found no difference in DMI between higher and lower risk mid-lactation cows fed a high-grain diet (30% forage) (Gao and Oba, 2016).

While there were no detectable differences between groups for daily milk yield, LR cows produced more 4% FCM per day than HR cows (Table 3.2). Lower risk cows produced more milk fat than higher risk cows (Table 3.2). Although there was no detected difference in milk fat percentage between HR and LR cows (Table 3.2), a milk fat percentage × treatment interaction was detected. Within cows on the Long treatment, LR cows had higher milk fat percentage than HR cows (5.4 ± 0.2 vs. 4.7 ± 0.2 %; P=0.1). All of these findings are consistent with the milk fat depression seen in cows experiencing low rumen pH (Griinari et al., 1998; Enjalbert et al., 2008; Alzahal et al., 2010). Consequently, there is usually a decrease in milk and milk fat production in cows suffering from SARA (Krause and Oetzel, 2006). Cows categorized as LR had numerically higher milk yield and milk fat percentage than HR cows, possibly leading to greater 4% FCM yield and milk fat yield.

As presented in Chapter 2, cows on the Long treatment sorted against the longest ration particles, while cows on the Short treatment did not sort this fraction of the diet. A SARA risk × treatment interaction for sorting of all particle fractions ($P \leq 0.05$) suggests that the sorting behavior of the cows varied depending on SARA risk category within treatment. Higher risk cows on the Long treatment sorted against the long particles (93.7 ± 2.0 %, P=0.004), while HR cows on the Short treatment did not sort this fraction of the diet (105.5 ± 3.4 %, P=0.12) (Figure 3.1). The straw in the Long diet may have been long enough (at 5.08 cm) that it was easily sorted against (Oelker et al., 2009; DeVries and Gill, 2012a). As discussed in Chapter 2, sorting against the long particles by cows on the Long treatment may have resulted in a greater linear decrease in reticulorumen pH and a greater fluctuation in time spent below a reticulorumen pH of 5.8. It is, thus, possible that the sorting behavior against the easily sorted long particles, high in ADF and NDF (Table 3.1), exhibited by HR cows on the Long treatment, reduced their intake of physically-effective fiber and increased their risk of SARA. This increased risk of SARA likely explains why these cows also had lower milk fat percentage than LR cows on the Long treatment (Krause and Oetzel, 2006). Cows on the Short treatment, regardless of SARA risk, did not sort the long particles (Figure 3.1), possibly because the straw particle size was small enough to be more homogenous with the ration and less easily distinguished.

Higher risk cows on the Long treatment did not sort the medium particles (97.8 ± 1.4 %, P=0.11), while HR cows on the Short treatment tended to sort in favor of these particles (102.7 ± 1.5 %, P=0.09) (Figure 3.2). Only HR cows on the Short treatment sorted the short and fine particles; sorting in favor of the short particles (102.7 ± 1.5 %, P<0.001; Figure 3.3) and against the fine particles (93.4 ± 2.2, P=0.005; Figure 3.4). Although not significantly different from 100%, HR cows on the Short treatment demonstrated a positive numerical difference from 100 % for sorting of the long particles, suggesting that these cows may even have been sorting in favor of this particle size fraction, in addition to the medium and short particles, to ameliorate the effects of low reticulorumen pH. All of these particles (long, medium, and short) are physically-effective, requiring further mastication and, thus, also stimulating further rumination (Maulfair et al., 2011). When experiencing an acute bout of ruminal acidosis, cows have been shown to sort in favor of the physically-effective portion of the diet to alleviate the symptoms of low rumen pH, likely as a result of post-ingestive feedback (DeVries et al., 2008). Beef cattle have similarly been shown to sort in favor of the longest dietary particles when induced with an acute bout of ruminal acidosis (DeVries et al., 2014a), with those animals experiencing the greatest degree of acidosis demonstrating the greatest selection for long, fibrous particles (DeVries et al., 2014b; a). Thus, it is possible that HR cows on the Short treatment sorted in favor of the medium and short fractions and against the fine particles to ameliorate the negative effects of low reticulorumen pH. With the exception of the sorting in favor of the short particles, these results are consistent with DeVries et al. (2008), who found that cows with a high risk of SARA sorted in favor of the medium particles and against the short and fine particles.

Aside from the long particles, which LR cows on the Long treatment sorted against, LR cows on both treatments did not sort any of the remaining 3 fractions ($P \geq 0.12$; Figure 3.1, 3.2, 3.3, and 3.4). Minimal sorting would have resulted in a more balanced intake of nutrients, which may have contributed to a reduced risk of SARA in those cows (DeVries et al., 2008).

There was a SARA risk × treatment interaction detected for daily rumination time ($P=0.1$); LR cows on the Long treatment tended to have higher daily rumination time than HR cows on the Long treatment (493.2 ± 19.6 vs. 432.3 ± 25.7 min/d, $P=0.07$). This may be explained by the sorting direction of cows on the Long treatment. Both HR and LR cows on the Long treatment sorted against long particles (Figure 3.1), as shown in Chapter 2. Cows sorted the medium particles differently by SARA risk within the Long treatment ($P=0.03$); HR sorted against the medium particles (97.8 ± 1.4%), while LR cows did not sort this fraction (101.7 ± 1.1%) (Figure 3.2). In sorting against both the long and medium particles, HR cows on the Long treatment reduced their intake of physically-effective fiber and subsequently would have ruminated less (Zebeli et al., 2012; Shaani et al., 2017).

CONCLUSIONS

Cows categorized as HR on the Long treatment sorted against the longest particles in the diet, which reduced their time spent ruminating and subsequently, decreased their milk fat percentage. Low reticulorumen pH may have resulted in decreased lipogenesis, leading to lower 4% FCM and milk fat yield for HR cows compared to LR cows. In contrast, HR cows on the Short treatment may have attempted to ameliorate the effects of low reticulorumen pH by maximizing intake of physically-effective fiber through not sorting the long particles, favoring the medium and short particles, and avoiding the fine particles. Aside from sorting the longest particles on the Long treatment, LR cows did not sort their ration, regardless of treatment, and maintained a more balanced intake of nutrients.

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Table 3.1. Particle size distribution[1] (%DM) and chemical composition of experimental lactating cow diets (mean ± SD).

	Long Lactating Diet[2]	Short Lactating Diet[3]
% of DM		
Long	6.4 ± 4.2	4.2 ± 3.6
Medium	38.9 ± 5.7	39.4 ± 6.2
Short	19.3 ± 2.3	20.5 ± 3.4
Fine	35.4 ± 7.0	36.0 ± 7.0
ADF, % of screen DM[4]		
Long	31.5 ± 1.0	28.9 ± 1.2
Medium	26.6 ± 0.9	25.8 ± 1.0
Short	18.6 ± 0.5	19.5 ± 1.0
Fine	13.9 ± 0.5	15.2 ± 1.2
NDF, % of screen DM[4]		
Long	45.2 ± 1.2	40.7 ± 1.9
Medium	37.7 ± 1.6	36.6 ± 2.0
Short	28.2 ± 1.0	29.4 ± 1.8
Fine	21.2 ± 1.0	23.7 ± 1.7

[1]Particle size determined by Penn State Particle Separator which has a 19 mm screen (long), 8 mm screen (medium), 4 mm screen (short), and a pan (fine).
[2]Straw in long lactating diet was cut to measure approximately 5.08 cm.
[3]Straw in short lactating diet was cut to measure approximately 2.54 cm.
[4]Values were obtained from chemical analysis of separated TMR samples.

Table 3.2. Effect of SARA risk on the reticulorumen pH, DMI, and milk production and composition[1] for lactating dairy cows in the first 28 d of lactation (mean ± SE).

	SARA Risk[2,3]		
Item	Low Risk	High Risk	*P*-Value
Mean reticulorumen pH	6.3 ± 0.03	6.1 ± 0.04	<0.001
Maximum reticulorumen pH	6.7 ± 0.03	6.5 ± 0.05	<0.001
Minimum reticulorumen pH	6.0 ± 0.03	5.7 ± 0.04	<0.001
Mean time below reticulorumen pH 5.8, min/d	21.9 ± 31.7	309.2 ± 44.7	<0.001
Area pH <5.8, pH × min/d	1.5 ± 6.5	49.4 ± 9.2	<0.001
Acidosis index, pH × min/DMI	0.08 ± 0.4	3.0 ± 0.5	<0.001
DMI, kg/d	19.4 ± 0.5	18.4 ± 0.7	0.22
DMI as a percentage of BW, %	2.6 ± 0.08	2.5 ± 0.1	0.32
Milk production, kg/d			
Milk	38.2 ± 1.1	35.7 ± 1.5	0.18
4% FCM	47.2 ± 1.5	41.9 ± 2.1	0.05
Milk composition, %			
Fat	5.2 ± 0.1	5.0 ± 0.2	0.26
Protein	3.2 ± 0.6	4.5 ± 0.8	0.20
Milk component yield, kg/d			
Fat	2.1 ± 0.1	1.8 ± 0.1	0.04

[1]Data were collected over 28 d (pH, milk yield, DMI) or 2 d/wk (4% FCM and milk composition)

[2]Risk of developing SARA was calculated using an acidosis index of 0.5, calculated by determining area below pH 5.8 and dividing by DMI, averaged across the trial period. Low risk cows had an acidosis index less than 0.5 while High risk cows had an acidosis index greater than 0.5.

[3]Low Risk, n=25; High Risk, n=13

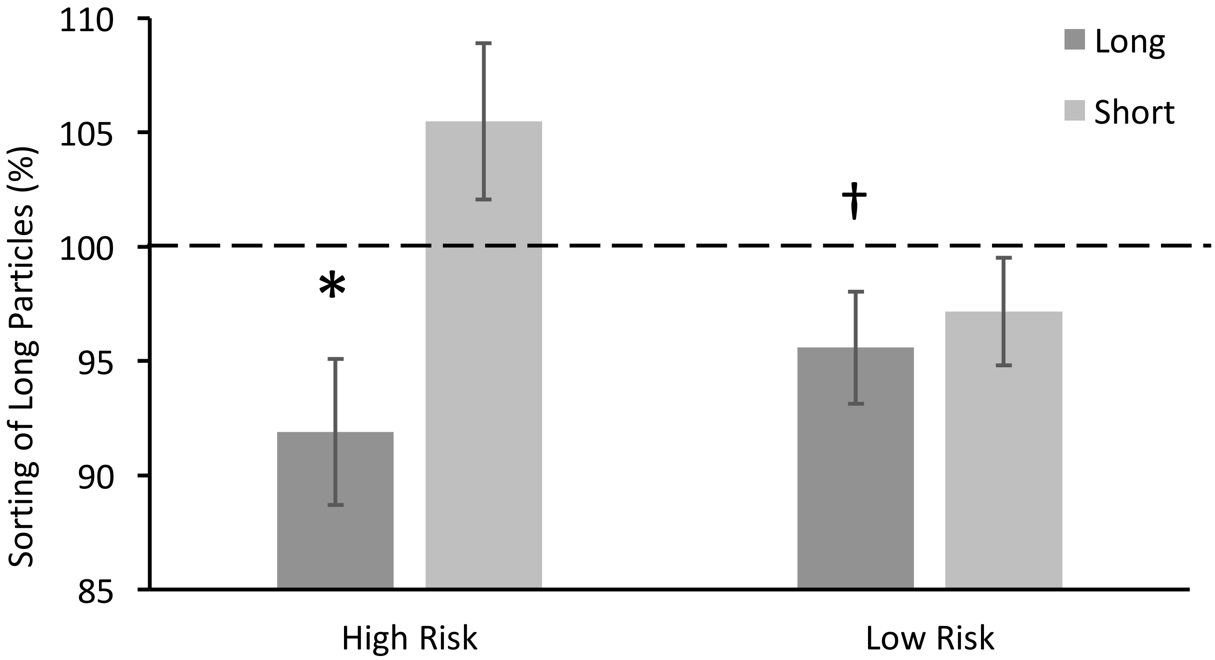

Fig 3.1. Sorting (%) of long particles (>19mm) for cows fed 1 of 2 dietary treatments differing in the length of the wheat straw component: 1) 2.54 cm straw length (Short: n = 19 cows) or 2) 5.08 cm straw length (Long: n = 19 cows). Cows were also categorized by reticulorumen pH status: 1) Low reticulorumen pH for cows with an acidosis index (area under the curve for pH < 5.8 ((pH × min/d)/DMI)) greater than 0.5 (n = 13; Long: n = 7; Short: n = 6) and 2) Normal reticulorumen pH for cows with an acidosis index less than 0.5 (n = 25; Long: n = 12; Short: n = 13).
* Indicates a sorting value different from 100 % ($P \leq 0.05$).
† Indicates a tendency for a sorting value to be different from 100% ($0.05 < P \geq 0.1$).

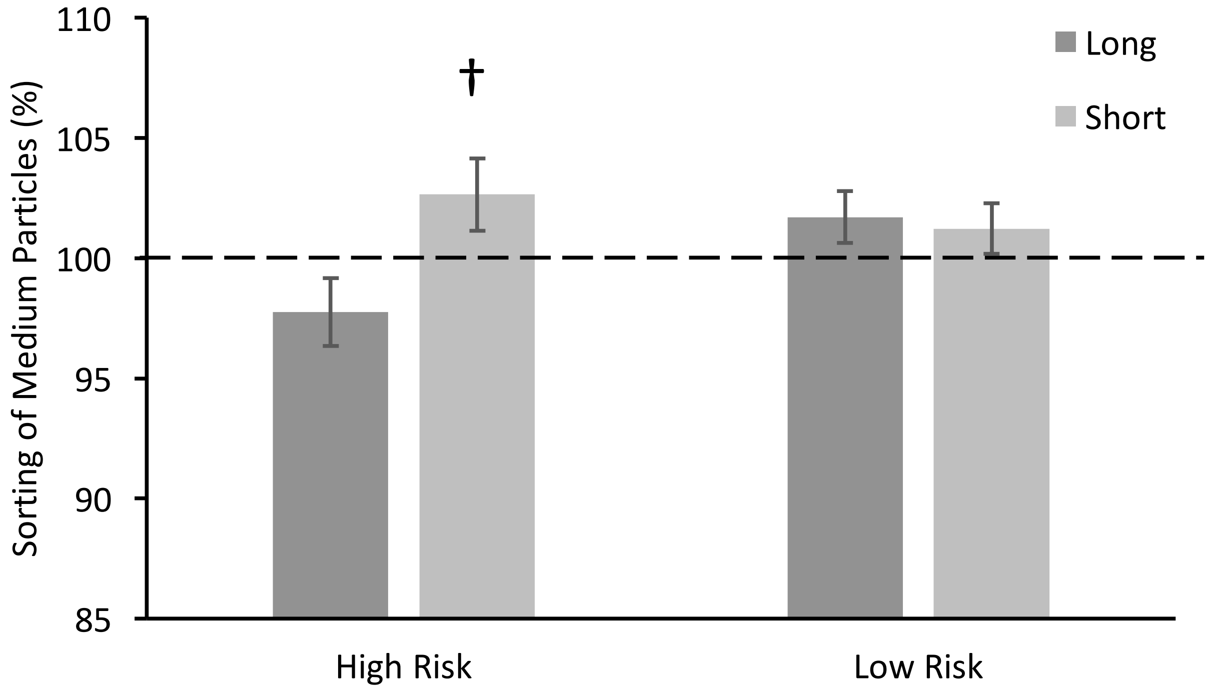

Fig 3.2. Sorting (%) of medium particles (<19mm,>8mm) for cows fed 1 of 2 dietary treatments differing in the length of the wheat straw component: 1) 2.54 cm straw length (Short: n = 19 cows) or 2) 5.08 cm straw length (Long: n = 19 cows). Cows were also categorized by reticulorumen pH status: 1) Low reticulorumen pH for cows with an acidosis index (area under the curve for pH < 5.8 ((pH × min/d)/DMI)) greater than 0.5 (n = 13; Long: n = 7; Short: n = 6) and 2) Normal reticulorumen pH for cows with an acidosis index less than 0.5 (n = 25; Long: n = 12; Short: n = 13).
* Indicates a sorting value different from 100 % ($P \leq 0.05$).
† Indicates a tendency for a sorting value to be different from 100% ($0.05 < P \geq 0.1$).

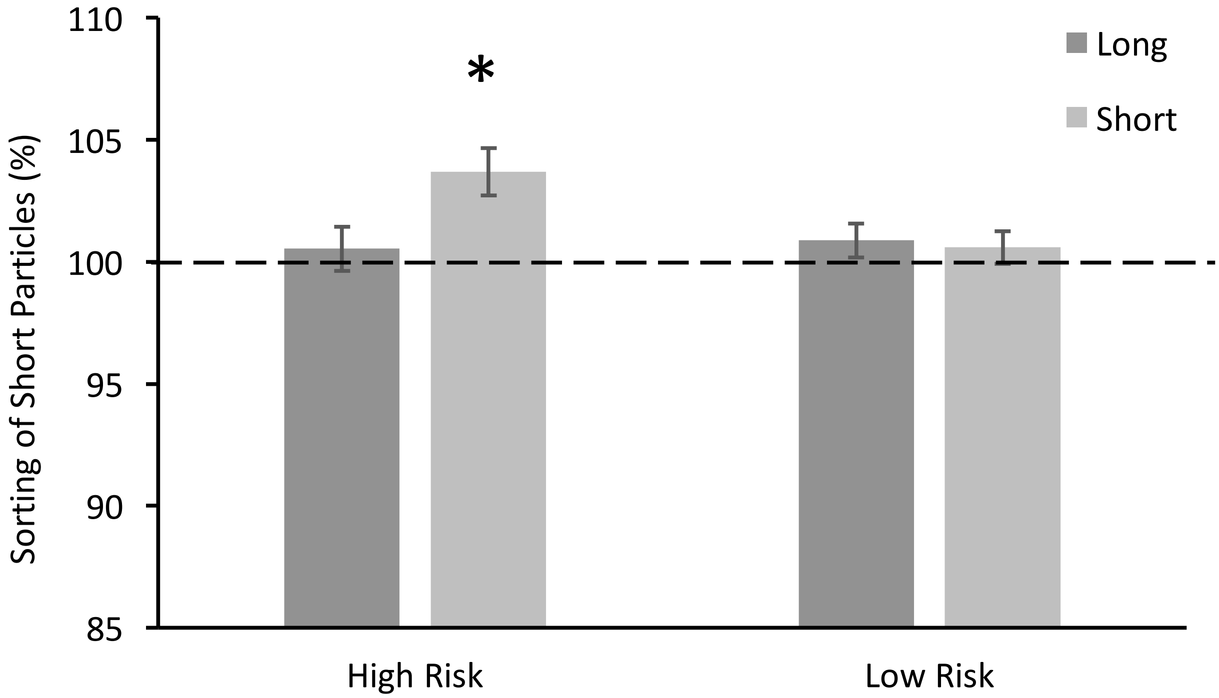

Fig 3.3. Sorting (%) of short particles (<8mm,>4mm) for cows fed 1 of 2 dietary treatments differing in the length of the wheat straw component: 1) 2.54 cm straw length (Short: n = 19 cows) or 2) 5.08 cm straw length (Long: n = 19 cows). Cows were also categorized by reticulorumen pH status: 1) Low reticulorumen pH for cows with an acidosis index (area under the curve for pH < 5.8 ((pH × min/d)/DMI)) greater than 0.5 (n = 13; Long: n = 7; Short: n = 6) and 2) Normal reticulorumen pH for cows with an acidosis index less than 0.5 (n = 25; Long: n = 12; Short: n = 13).
* Indicates a sorting value different from 100 % ($P \leq 0.05$).
† Indicates a tendency for a sorting value to be different from 100% ($0.05 < P \geq 0.1$).

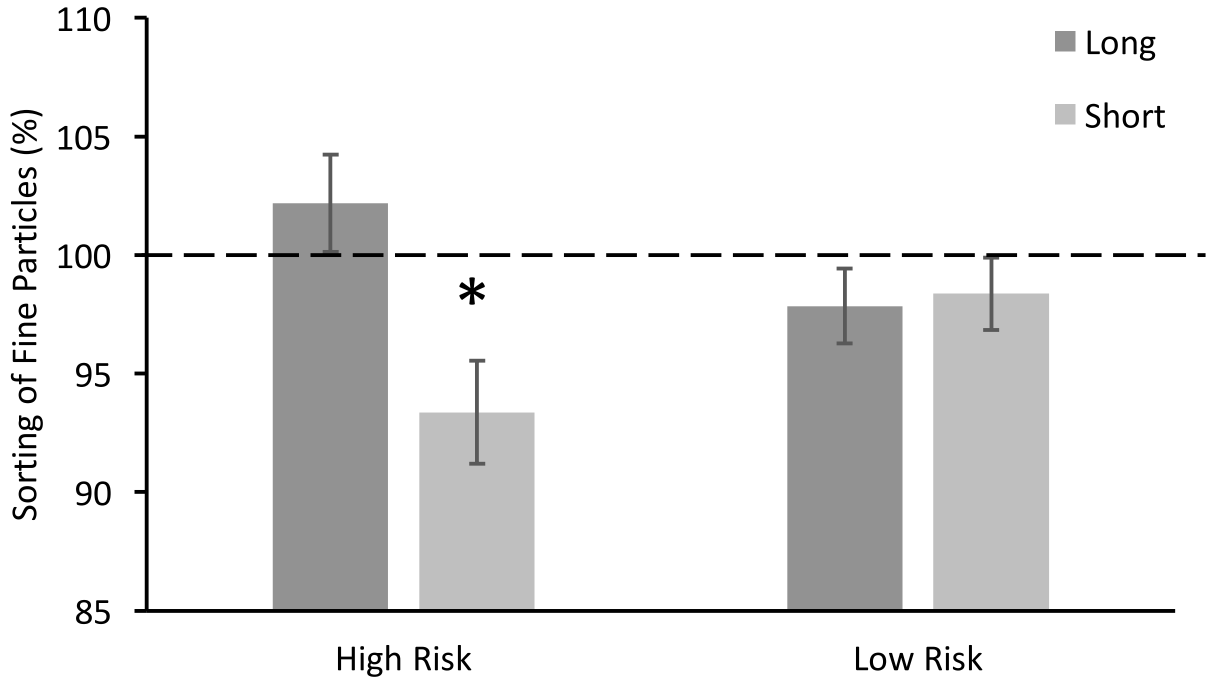

Fig 3.4. Sorting (%) of fine particles (<4mm) for cows fed 1 of 2 dietary treatments differing in the length of the wheat straw component: 1) 2.54 cm straw length (Short: n = 19 cows) or 2) 5.08 cm straw length (Long: n = 19 cows). Cows were also categorized by reticulorumen pH status: 1) Low reticulorumen pH for cows with an acidosis index (area under the curve for pH < 5.8 ((pH × min/d)/DMI)) greater than 0.5 (n = 13; Long: n = 7; Short: n = 6) and 2) Normal reticulorumen pH for cows with an acidosis index less than 0.5 (n = 25; Long: n = 12; Short: n = 13).
* Indicates a sorting value different from 100 % (P≤0.05).
† Indicates a tendency for a sorting value to be different from 100% (0.05<P≥0.1).

CHAPTER 4. GENERAL DISCUSSION

4.1 Important Findings

Diets fed to early lactation cows are typically high-energy, containing a substantial amount of concentrate required to support milk production (Plaizier et al., 2009). Unfortunately, this type of diet may also exacerbate sorting behavior, particularly for early lactation cows that are transitioning from a low-energy, forage-based dry diet to a nutrient-dense lactating cow diet (DeVries et al., 2008). By sorting in favor of readily-digestible concentrate components and against longer forage particles, dairy cows reduce their intake of physically-effective fiber and have greater risk of depressed rumen pH (DeVries et al., 2008) and SARA. To prevent transition cow disorders like SARA, while maintaining high production, it is important that the ration fed to early lactation dairy cows is formulated to minimize sorting behavior, stimulate rumination, and promote DMI.

The aim of this thesis was to determine the impact of sorting behavior on the rumen health and milk production of early lactation dairy cows. This aim was addressed in a study where fresh dairy cows were exposed for the first 28 DIM to 1 of 2 TMR, differing in the length of the wheat straw component immediately post-calving: 1) 2.54 cm straw length (**Short**) (n=21) or 2) 5.08 cm straw length (**Long**) (n=20).

In Chapter 2, the objective was to determine how early lactation dairy cows sort a TMR with varying wheat straw particle size, and determine the impact on feeding behavior, reticulorumen health, and milk production. It was demonstrated that cows on the Long treatment did in fact sort against the long particles in the diet while cows on the Short treatment did not sort this fraction. This is consistent with previous studies that show cows will sort against easily distinguishable forage particles (Oelker et al., 2009; DeVries and Gill, 2012a). Although cows fed the Long diet did not differ in terms of averages of measures of rumen health, behavior, and milk production from cows fed the Short diet, there were differences between treatments in how these variables evolved over the first 28 DIM. Mean reticulorumen pH decreased to a greater extent for cows on the Long treatment, particularly in the first 10 DIM. This drop in reticulorumen pH coincided with a rise in time spent below a reticulorumen pH of 5.8 for cows on the Long treatment and greater fluctuation in this variable over the course of the trial. Not surprisingly, greater fluctuation over the 28-d trial period in time spent ruminating was also observed for cows on the Long treatment compared with those on the Short treatment. By sorting against the longest particles in the diet, and thus reducing intake of physically-effective fiber, cows on the Long treatment may have experienced a destabilized reticulorumen environment. This is consistent with the hypothesis that a diet with longer forage particle size would increase sorting and increase reticulorumen pH variability.

Comparatively, cows on the Short treatment may have had greater intake of physically-effective fiber by not sorting the longest particles, favoring the medium and short particles, and sorting against the finest particles (Chapter 2). This sorting behavior likely contributed to a more stabilized reticulorumen environment, contributing to the greater linear increase in DMI over the course of the trial (Sova et al., 2013). A stable reticulorumen environment may have also resulted in a tendency for cows on the Short treatment to produce 76 kg more milk in the first 28 DIM than cows on the Long treatment. Sova et al. (2013) speculated that increased feed digestibility and milk production could be achieved through reduced sorting against long particles and decreased rumen pH variability, as was seen in the present study for cows on the Short treatment. These results support the hypothesis that a diet with a shorter straw particle size would reduce sorting and lead to a more stable reticulorumen environment, which would maintain DMI and improve milk production.

In Chapter 3, cows were categorized as either higher (HR) or lower risk (LR) for developing SARA. Similar to the results of Chapter 2, cows on the Long treatment sorted against long particle regardless of risk of SARA, whereas HR cows on the Short treatment did not sort this fraction. Instead, HR cows on the Short treatment sorted in favor of medium and short particles and sorted against fine particles. These results suggest that HR cows on the Short treatment were attempting to ameliorate the effects of low reticulorumen pH by sorting to maximize their intake of effective fiber. This type of sorting may be stimulated by negative post-ingestive feedback triggered by chronic low reticulorumen pH (DeVries et al., 2008). Milk yield and composition were affected by SARA risk. Regardless of treatment, HR cows produced lower 4% FCM and had lower milk fat yield than LR cows. These findings might be related to decreased feed digestibility resulting from SARA (Krause and Oetzel, 2006). The results also indicate that LR cows benefitted from a stable reticulorumen environment through increased 4% FCM and milk fat yield, possibly, in part, as a result of reduced sorting of their TMR.

4.2 Future Research

More apparent differences between treatments in terms of particle size distribution may have emphasized the results seen in this study. Researchers that have previously studied the effect of forage particle size on sorting behavior used forage particle sizes that were more different in length than the present study (Kononoff and Heinrichs, 2003; Zebeli et al., 2008; Suarez-Mena et al., 2013). However, aside from similar sorting results to those reported in this dissertation, differences between treatments for DMI, ruminal pH, and milk production were minimal in those studies.

In addition to greater differences in wheat straw particle size between treatments, a further reduction of the straw particle size in the Short treatment would shed light on the minimum straw particle size that would still stimulate chewing and rumination without aggravating sorting behavior. Only one other study has been conducted on how sorting behavior and reticulorumen health are affected by straw particle lengths less than 2.54 cm (Suarez-Mena et al., 2013). Suarez-Mena et al. (2013) found a linear increase in sorting against long particles with increasing straw particle size and a quadratic effect for time spent eating in minutes per kg of DMI.

In the present study, a higher inclusion rate of straw than what would allow for maximal DMI (9 vs. 5% DM; Eastridge et al., 2017) was used to accentuate any discernible differences between treatments. However, a lower inclusion rate of wheat straw in the diet of early lactation dairy cows would be more reasonable for ensuring maximal DMI (Eastridge et al., 2017). Decreasing the inclusion rate of wheat straw might also prevent rumen fill if the plateau in DMI observed in the present study was in fact due to the bulky nature of the wheat straw (McCarthy et al., 2015c). If the plateau was not due to rumen fill, then further research is needed to investigate the causes of the plateau in both DMI and milk yield. It seems plausible that these trends might be related to the decrease in reticulorumen pH experienced in the first ~10 DIM. Alternatively, the majority of the cows in this study experienced SCK (38 of 41 cows) during the trial period. Decreased DMI and milk production are symptoms of this disorder and may also explain the plateau in these metrics seen in this study (Allen et al., 2009; Esposito et al., 2014). Differences between ketotic and non-ketotic cows would be of interest to study because of the relationship between the transition diet, SCK, and feeding behavior (Drackley and Cardoso, 2014).

A tendency for cows on the Short treatment to produce more cumulative milk during the first 28 DIM than cows on the Long treatment was observed in this study (Chapter 2). Due to greater variability than expected, the sample size calculations made prior to this study were not accurate enough to predict sample sizes necessary to detect significant differences between treatments. While a 4.7% numerical difference in milk yield was detected between treatments (Chapter 2), a power analysis using estimates of variation from the data collected suggested that a difference in milk yield of 8.9% would have been necessary to detect a significant difference. Similarly, given that only a 1.0% numerical difference in milk fat percentage was observed in this study between treatments (Chapter 2), a difference of 7.8% would have been needed to detect a significant difference between treatments. Lastly, a 44.3% numerical difference was observed between treatments for time (min/d) spent below pH threshold of 5.8 (Chapter 2). According to a power analysis, a 139.4% difference would have been needed to detect a significant difference between treatments. Thus, estimates of variation of the outcome measures from this study should be used in future studies for *a priori* sample size calculations.

4.3 Implications

These results highlight the importance of forage particle size and its relationship to sorting behavior and reticulorumen health in early lactation dairy cows. Moderately reducing wheat straw particle size from 5.08 to 2.54 cm helped to minimize sorting behavior, which in turn stabilized reticulorumen pH and time spent ruminating over the first 28 DIM. For cows on the Short treatment, a stable rumen environment may have led to a greater linear increase in DMI and a tendency for greater cumulative milk production. Cows at higher risk for SARA, on the Long treatment, sorted against the longest ration particles despite suffering from chronic low reticulorumen pH. This highlights the need to offer diets that minimize sorting and, thus, increase intake of physically-effective fiber, as was seen in HR cows on the Short treatment as well as amongst LR cows. Overall, the results suggest that dietary components should be managed such that feed sorting is minimized, without decreasing the physically-effective nature of the ration, to ensure consumption of a balanced diet by early lactation dairy cows, and thus promote a stable rumen environment and greater milk production.

CHAPTER 5. REFERENCES

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