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Interaction Of Stocking Density And The Feeding Environment In Lactating Holstein Dairy Cows

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INTERACTION OF STOCKING DENSITY AND THE FEEDING ENVIRONMENT IN LACTATING HOLSTEIN DAIRY COWS

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ABSTRACT

Stocking density serves as a sub-clinical stressor impacting natural behavior and affective state of dairy cows. However, cows rarely experience stocking density as an isolated stressor. Understanding the effects of stocking density with additional management stressors such as low-fiber diets or feed restriction is the next step in alleviating stress and improving the well-being of lactating dairy cows housed in freestall barns. The overall goal of this dissertation was to evaluate the interaction of stocking density and the feeding environment on short-term production, behavioral, ruminal fermentation, and stress responses of lactating dairy cattle.

The first two studies (Chapter 2 and 3) served as preliminary research for the main studies of this dissertation. The first study objective was to evaluate the effectiveness of using chopped wheat straw to reduce sub-acute ruminal acidosis (SARA) in order to formulate diets for the first main study. Treatments were low straw (0 kg dry matter (DM)/d; LS) and high straw (1.36 kg DM/d; HS). High straw appeared to effectively reduce SARA by lowering time below pH 5.8 with minimal impact on feed intake and rumination. The second study objective was to evaluate the effect of type of blood collection tube on haptoglobin concentration across two commercially-available haptoglobin assays and evaluate assay agreement in order to determine haptoglobin concentrations for the main studies. Lithium heparinized, sodium heparinized, and K_2-EDTA plasma resulted in increased haptoglobin concentrations compared to serum using the Tri-Delta colorimetric assay, but no differences were observed using the Life Diagnostics ELISA assay. However, there was a lack of agreement between assays and further identification of a gold-standard assay is needed before analyzing haptoglobin for the main studies.

The third study (Chapter 4) investigated the interaction of stocking density (100% and 142% of freestalls and headlocks) and source of forage fiber (no added straw and added straw at 3.5% ration DM). Treatments did not impact feed intake, but straw diets tended to reduce milk production. Increasing stocking density reduced lying time but increased efficiency of stall use. Though feeding and rumination times were unaffected, overstocking shifted the location of rumination away from the freestall. Increased stocking density tended to increase stress responses. Both greater stocking density and no straw diets increased SARA, and the combination of these stressors tended to exacerbate this pH response. Adding straw to the diet reduced the negative impacts of overstocking on ruminal pH.

The fourth study (Chapter 5) evaluated the interaction of stocking density (100% and 142%) and feed access (5-h reduced feed access and no reduced feed access). Treatments had minimal impact on short-term feed intake and production. Overstocking affected behavior similar to responses observed in Chapter 4. Reducing feed access decreased feeding time, though cows altered feeding and rumination responses to maintain daily rumination. Both treatments shifted priorities for feeding and lying behavior, though increased stocking density had the larger impact. Though reduced feed access did not impact ruminal pH, an exacerbated response was observed when combined with increased stocking density.

The combination of stocking density and feeding environment stressors exacerbate negative effects on biological function and should be avoided.

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CHAPTER 1: LITERATURE REVIEW

Introduction

The interest in animal welfare has grown markedly over the past few decades. Although the European Union continually adopts new and more specific regulations on dairy cattle welfare, the United States' Animal Welfare Act fails to provide specific welfare standards for the dairy industry (USDA, 2017). Therefore, it is imperative for the dairy industry itself to understand the definition, criteria, and on-going research in the field of dairy welfare.

The former approach to defining welfare consisted of providing animals with the "Five Freedoms": freedom from hunger and thirst, freedom from discomfort, freedom from pain, injury or disease, freedom from fear and distress, and freedom to express normal behaviors (Webster, 2001). However, these welfare components lack levels of clarity and reality as to whether domesticated animals can exhibit true freedom within each component. Therefore, three components of welfare (natural living, biological function, and affective state) have been established within the dairy industry with the goal of managing animals to maintain high standards within each component (Fraser et al., 1997; von Keyserlingk et al., 2009). Each of these components plays an important role in maintaining basal function and health while optimizing production. More importantly, these components often interact, and factors that influence one component may have significant impacts upon another.

Economics play an important role in driving the use of various management practices within the dairy industry. While a management practices such as overstocking

may ultimately increase milk production, resulting in greater economic return, this practice can expose production livestock to multiple stressors that affect each of the three welfare components.

Primary Stressor: Overstocking

Stocking density represents the ratio of animals to area or resources in a given pen. In order to maximize the use of fixed costs and increase overall farm production, producers tend to overstock their pens with cows (Bewley et al., 2001; De Vries et al., 2016). Overstocking, as defined by Grant and Albright (2001), is the management practice of providing less than one stall per cow, providing less than 0.6 m of bunk space for each cow, or a combination of the two.

National standards for stocking densities do not exist, allowing the industry to regulate itself. For example, the National Dairy FARM (Farmers Assuring Responsible Management) Program simply recommends that "all animals within a pen receive adequate nutrition and water without competitive pressure. In best practice, all animals have access to a sanitary and comfortable place to rest" (NMPF, 2017). However, overstocking is common throughout the U.S. dairy industry and continually growing in use. According to the USDA National Animal Health Monitoring Service (NAHMS), a survey of freestall based dairy producers reported that 58% of farms provided less than the recommended 0.6 m of feed bunk space and 43% of farms provided less than one stall per cow (USDA, 2010). These numbers continue to increase as evidenced by von Keyserlingk et al. (2012) who reported that feed bunk stocking density ranged from 58 to

228%. In the northeast U.S. particularly, feed bunk stocking densities averaged 142% with 78% overstocking prevalence and freestall stocking densities ranged from 71 to 197% with 60% prevalence of overstocking (von Keyserlingk et al., 2012).

Overstocking remains a commonly used management tool in the dairy industry due to increased economic incentive. De Vries et al. (2016) identified that optimal stall densities and economic returns occurred at greater than 100% and greater than 120% stocking density in 67% and 42% of modeled scenarios, respectively. However, the economic benefit from overstocking also depends on milk pricing; with reduced prices (decreased income over feed costs) shifting greatest economic return towards lower stocking levels (De Vries et al., 2016). Further, depending on the level of overstocking in the herd, greater economic advantages may be gained from building new facilities as opposed to heavily overstocking existing facilities (De Vries et al., 2016).

However, stocking density can significantly reduce the cow's ability to perform natural behaviors (Wechsler, 2007). Although economic return first and foremost guides the use of management practices, a balance needs to be achieved between productive efficiency and animal health and well-being.

Effect of Stocking Density on Lying/Standing Behavior

High stocking density has shown consistent negative consequences on lying behavior in cattle as demonstrated in several studies. A negative linear relationship was identified between lying time and increasing stocking densities from 100 to 150% (Fregonesi et al., 2007) given a freestall environment. Both Hill et al. (2009) and

Krawczel et al. (2012b) also reported this linear decrease in lying time with increasing stocking densities, specifically at stocking densities greater than 113%. Wang et al. (2016) observed no differences in lying time across stocking densities from 82% to 129%, highlighting a breakpoint for stocking density that exists around 113 to 130% before the occurrence of reductions in lying time. Although there was no significant correlation between lying time and stocking density in a large, commercial farm study by Charlton et al. (2014), the authors reported that farms over 100% stocking density were unable to achieve lying times of 12 h/d or greater, a benchmark of natural lying time suggested by Jensen et al. (2005).

Although several studies have observed significant impacts upon lying time of increased stocking density, total number or the duration of lying bouts remained unaffected (Krawczel et al., 2012b; Wang et al., 2016). Furthermore, Solano et al. (2015) observed no differences in bout frequency or bout duration of lying time in a field study comparing 141 Canadian, freestall-housed, Holstein farms with less than 1 stall/cow or greater than 1 stall/cow. Lack of differences for lying bout frequency and bout duration demonstrated the inelasticity of lying behavior of dairy cows under various stocking conditions, with cows unable to make up for reduced lying times by altering bout characteristics.

Due to the reduction in stall resources, higher stocking densities increase idle standing time in the alley (Fregonesi, 2007; Hill et al., 2009, Falk et al., 2012). These idle standing observations were similar to those observed by Krawczel et al. (2008), as stall use index (SUI; number of cows lying in stalls/number of cows not actively feeding;

Overton et al., 2002) decreased linearly with increasing stocking density whereas cow comfort index (CCI; number of cows lying in stalls/number of cows in stalls; Nelson, 1996) and stall standing index (SSI; number of cows standing in stalls/number of cows in stalls; Cook et al., 2005) were not affected.

Cows place their highest priority on lying time, greater than either feeding or social interactions (Metz, 1985; Munksgaard et al., 2005). Cooper et al. (2007) reported an increase in lying behavior for the first 8 h following lying deprivation of either 2 or 4 h. Further, Falk et al. (2012) observed no differences in lying time across various indoor stocking density levels (100%, 150%, 300%, and no available stalls) when cows were given access to pasture, illustrating their motivation to adapt to surrounding conditions by altering lying location to meet their daily requirement. Due to increased motivation during restricted access (lack of available resources in overstocked conditions), cows will alter their lying behavior to maximize the available resource. Wang et al. (2016) observed increases in SUI and CCI during peak lying hours (2300 to 0400 h) with higher stocking density, likely due to shifts in feeding behavior of sub-dominant cows resulting from a lack of resources and greater stall use efficiency during overstocked conditions. Further, variation in stall use decreased with higher stocking density, through greater and more uniform use throughout the day (Fregonesi et al., 2007). Reduced variation in stall use was also confirmed by Ito et al. (2014), as increasing stocking density by 10% increments resulted in -0.08 ± 0.03 (h/d) reductions in the standard deviation of lying time. Other studies have observed this indirectly through a decrease in stall use for standing or

perching behavior at higher stocking densities (Wirenga and Hopster, 1990; Hill et al., 2009; Falk et al., 2012).

Reducing the cow's ability to meet daily lying requirements in overstocked conditions also leads to increased aggression, with higher stall displacements associated with higher stocking densities (Fregonesi et al. 2007). Friend and Polan (1974) observed a positive relationship between bodyweight and hierarchy, indicating competition between parities for freestall access would most likely affect younger cows. This highlights the importance of separating first lactation cows in overstocked conditions. For example, time spent lying outside freestalls increased for primiparous cows compared to multiparous cows at 200% stocking density (González et al., 2003). While these relationships with parity and cow size approximate hierarchy, they may not always be indicative of social status within the herd, such as the case with dominant primiparous cows or timid multiparous cows. Further research is needed to identify other cow characteristics related to pen dominance structures.

In relation to overstocking at the feedbunk, cows exhibited a shorter latency to lay down following milking at 150% stocking density compared to 100% (Fregonesi et al., 2007). This latency is important for udder health, as observed by Watters et al. (2014), where cows with post-milking standing durations around 90 to 120 minutes were at reduced risk for coagulase-negative staphylococci intra-mammary infections than shorter latencies. However, measuring pen averages of lying latency can skew the usability of the data, particularly due to the high variability in overstocked conditions. Therefore, future research needs to evaluate the cow's desire to lie down immediately following return

from the parlor, specifically focusing on the proportion of the pen performing eating or lying behaviors.

Effect of Stocking Density on Lameness and Cleanliness

Increased standing time puts cows at greater risk for leg injuries, claw lesions, and lameness (Greenough and Vermunt, 1991), suggesting that increased stocking density, which increases idle standing time in alleys, may significantly contribute to herd lameness. Lame cows spend significantly more time lying down (Walker et al., 2008), indicating further concern of placing clinically lame cows or those developing various hoof health issues in overstocked pens where there may be limited access to freestalls to relieve pressure on the limbs. This is particularly important given the 25% prevalence of lameness in herds in the U.S. (Espejo et al., 2006). Increased stocking density is also significantly correlated with increased probability of culling (Bach et al., 2008), likely due to the increases in standing time and risk of lameness. Leonard et al. (1996) observed higher foot lesion scores and clinical lameness when housing primiparous cows at 200% during the first 2 months post-partum compared to 100%. Using herd-level risk factor studies, Barrientos et al. (2013) and King et al. (2016) identified significant increases in overall hock injuries and severe lameness with increasing stocking densities, respectively. Furthermore, increased time away from the pen was positively correlated with increased prevalence of lameness (Espejo and Endres, 2007), suggesting that increased stocking density, which may alter the cow's time-budget through increased time spent out of the pen, may play a further indirect role in lameness prevalence. However, more research is

needed to identify long-term effects of varying stocking density on the prevalence of lameness.

Due to the increased manure output per given space in overstocked pens and idle standing time in alleys, cows in overstocked pens may be at greater risk for lowered hygiene. Leg hygiene decreased above 131% but stocking density showed no effect on udder hygiene (Krawczel et al., 2012b). However, this could be due to the 2-h continuous alley scraping during the study. Farms that scrape alleys less often may increase the chance for stocking density to affect leg and udder hygiene.

Effect of Stocking Density on Reproduction

While many farms tend to overcrowd medium to high producing pens as opposed to transition pens, particularly pens with cows 60 to 150 days in milk, overstocking may play a key role in affecting reproductive function and time to breed back. Schefers et al. (2010) observed reduced conception rates with overstocking and lower service rates tended to be associated with increased stocking densities. Further, Caraviello et al. (2006) identified bunk space per cow as a key factor influencing pregnancy status at 150 days post-partum, with probability of pregnancy increasing quadratically with increased bunk space. While overstocking may influence physiological factors concerning pregnancy, the lack of headlocks for every cow during overstocked condition may also lead to reduced ease of access to service cows, leading to reduced service and conception rates (Schefers et al., 2010). Further research is needed in the area of reproduction during overstocked

conditions, particularly to determine whether a breakpoint exists with increasing stocking density before reproductive efficiency is reduced.

Effect of Stocking Density on Feeding Behavior

While reducing linear space per cow at the feedbunk, such as comparing 3-row barns to 2-row barns, has demonstrated increased bunk utilization (Mentink and Cook, 2006), limiting access to the resource due to competition alters feeding behavior. Cows experience significant motivation to feed, particularly following fresh feed delivery and milking (DeVries et al., 2003), resulting in agonistic interactions at the feedbunk. Even under non-competitive conditions (feedbunk, 0.6 m/cow; freestalls, 1 stall/cow), 87.6% of displacements experienced by the cow throughout the day can be attributed to competition at the feedbunk (Val-Laillet et al., 2008). Several studies have reported positive, linear relationships between stocking density and bunk aggression. Collings et al. (2011) noted greater than a two-fold increase in bunk aggressions with mean values of 4.8 and 11.2 at 100% and 200% stocking densities, respectively. Other studies have described similar outcomes, concluding that increasing space at the feedbunk reduces the number of aggressive interactions (DeVries et al., 2004; Krawczel et al., 2012b). However, Telezhenko et al. (2012) did not report changes in bunk aggression when evaluating ranges from 25% to 100% stocking density. This suggests that there is little added benefit concerning feedbunk aggression of reducing stocking density below 100%. However, Talebi et al. (2014) identified reductions in feedbunk displacements at these levels with reduced stocking density when regrouping cattle, suggesting benefit on farms

with high levels of regrouping throughout the lactation. Furthermore, the correlation between competitive success at the feedbunk compared to other resources is low (Val-Laillet et al., 2008), indicating complex social dominance structures within pens that differ between resources. With overstocked conditions, dominant cows at the feedbunk may shift this aggression towards other resources, particularly toward increased freestall interactions (Fregonesi et al., 2007).

Alterations in feeding time vary with the intensity of stocking density. Increasing stocking density from 86% to 142% resulted in no difference in feeding time (Hill et al., 2009; Krawczel et al., 2012; Wang et al., 2016). However, other studies have found reductions in feeding time with stocking densities of 200% (Collings et al., 2011), 300% (Huzzey et al., 2006; Crossley et al., 2017), and 400% (Olofsson, 1999). Therefore, a break point likely occurs between 142% and 200% stocking density before consistent decreases in feeding time are observed. Further, DeVries et al. (2004) reported a 14% increase in feeding activity, 10% increase in daily feeding time, and increased post-milking feeding activity when increasing the space from 0.5 m to 1 m per cow at the feedbunk. This suggests some benefit to feeding behavior when cows are housed with greater than the recommended 0.6 m/cow at the feedbunk (Grant and Albright, 2001).

Regardless of reduced feeding time, overstocked cows consistently maintain dry matter intake (DMI) across various levels of stocking density as measured in several short-term studies (142%, Krawczel et al., 2012b; 200%, Collings et al., 2011; 300%, Crossley et al., 2017). In order to maintain DMI at higher levels of stocking density, cows will alter their feeding behavior. Cows increased their feeding rates at 200% (Collings et

al., 2011), 300% (Crossley et al., 2017), and 400% (Olofsson, 1999) stocking densities. Increases in feeding rate were particularly pronounced following fresh feed delivery and upon return from milking (Huzzey et al., 2006; Collings et al., 2011; Crossley et al., 2017), where motivation to feed increases (DeVries et al., 2003). While many of these studies solely looked at changes in competition at the feedbunk, studies where freestall access was also restricted found similar changes in feeding behavior. Cows spent greater time eating during lying deprivation, but reduced their eating time during the first 8 h post-deprivation, as cows shifted their behavioral needs to recuperate lost lying time (Cooper et al., 2007). Furthermore, Krawczel et al. (2012b) noted no changes in the portion of cows feeding over a 24-h period, suggesting the cows adjust their feeding patterns, seeking to maintain more uniform feedbunk use in overstocked situations.

Stocking density has limited effects on other meal characteristics. Similar to the break point relationship with feeding time, meal length increased at high levels of competition (300%, Crossley et al., 2017) due to greater non-feeding time within each meal, but there were no differences in meal length, frequency of feedbunk visits, and time between visits across stocking densities from 100% to 142% (Black et al., 2016). It appears that cows can adjust feeding behaviors easier than lying behavior in situations of higher stocking density. Feeding behavior can be adjusted through altering the timing of feeding at stocking densities from 100% to 142%, and rate of feeding above 142% stocking density, whereas lying time remains more inelastic with no differences in total bouts or bout duration. These differences are likely due to the lower daily requirement of 5 to 6 h for feeding time compared to the 12 to 14 h requirement for lying time (Grant

and Albright, 2001), leaving cows less time and opportunity to make up lost lying time. This inelastic need for resting was further demonstrated by Jensen et al. (2005), as cows continuously worked in demand-reward systems to maintain 12 to 13 h lying time. However, surveys over a large range of commercial farms in the northeast U.S. indicated less than 30% of farms achieved average lying times of at least 10 h, with only 1-2 farms falling into the 12-13 h range. With cows unable to achieve these inelastic lying time needs, other behavior, production, and stress responses may occur.

Effect of Stocking Density on Rumination Behavior

Limited research has looked at the effects of stocking density on rumination. Batchelder (2000) reported rumination averages of 37% for pens at 100% stocking density compared to only 28% for pens at 130% stocking density. Further, Cooper et al. (2007) reported a decrease in rumination behavior during lying deprivation of either 2 or 4 h. However, more recent stocking densities studies reported no effects of stocking densities between 100% and 142% on rumination time (Krawczel et al., 2012b; Wang et al., 2016). However, location of rumination behavior seems to be affected above 113% stocking density, with reduced rumination performed within the freestall (Krawczel et al., 2012b). With minimal research in this field, further research should be conducted to evaluate the impact of level of stocking density on rumination as well as the effects of altered rumination location within the pen.

Effect of Stocking Density on Milk Production

Bach et al. (2008) identified a positive linear relationship between milk production and the number of stalls per cow, accounting for 38% of the variation in milk production with this single factor across 47 dairy herds controlling for feed and genetic influence. Higher stocking density was associated with lower *de novo* fatty acid output (Woolpert et al., 2016), which has been associated with decreased milk fat and protein content (Barbano et al., 2014). Further, increasing feedbunk space was associated with increases in milk yield (Deming et al., 2013), increases in milk fat percentage, and decreases in somatic cell count (Sova et al., 2013).

In contrast to herd-level associated studies, controlled studies altering levels of stocking density suggest little short-term impact upon milk yield and composition. Altering stocking densities between 100% and 142% resulted in no significant changes in milk yield, milk composition, or milk fatty acids (Krawczel et al., 2012b), particularly the trans-10, cis-12 CLA which has been linked to milk fat depression through alterations in the biohydrogenation pathway within the rumen (Bauman and Griinari, 2003). Furthermore, somatic cell count (SCC) did not differ between stocking densities from 100% to 142%. However, higher stocking densities during the dry period were correlated with increases in SCC (Green et al., 2008). Similar outcomes were observed by both Wang et al. (2016) and Collings et al. (2011), though Collings et al. (2011) maintained 100% stocking density at the freestalls and identified no difference in daily lying time, lying bouts, or non-feeding standing time. Two to four h of lying time deprivation also did not affect milk yield (Cooper et al., 2007). This is most likely due to a shift in

behavior to recuperate the lost lying time as observed when cows regained 40% of their lost lying time by 40 h post-lying deprivation. Although Hill et al. (2007) identified a 0.2% reduction in milk fat for cows at 142% stocking density, treatment periods were only 7-d long and treatment differences may change with longer exposure and greater rumen adaptation.

Periods of high feedbunk competition (300%) reduced milk protein yield and were also associated with greater variability in milk yield, milk fat percentage, and milk fat yield, due to increased feeding rate and decreased lying time (due to greater time spent competing for feed) when compared to 100% or 200% feedbunk competition (Crossley et al., 2017). Greater variability in milk outputs at higher levels of competition emphasize the differing impact on cows within the same pen, particularly the subdominant cows (typically primiparous in a mixed pen setting) which are likely to have greater production losses than dominant, multiparous cows under the same conditions (Friend and Polan, 1974; Crossley et al., 2017).

However, it is vital to state that published research in the field of stocking density has been limited to evaluating the short-term impacts on production, usually with periods of two or less weeks during the study. Larger impacts of overstocking are typically observed during large field studies, where cows experience greater, long-term exposure to overstocking as well as variations in other management practices. Therefore, our best estimates of the impacts of overstocking on production and milk quality come from herd-level association field studies. Further long-term, controlled research studies are needed

to isolate the effects of various stocking densities on production measures as well as to identify how management practices interact in the field.

Effect of Stocking Density on Transition Cows

Understanding the effects of stocking density during the dry period has become of increased interest throughout the last 10 years, with many of the same effects on lying time and feeding behavior consistent with lactating cows.

There was no relationship between DMI and displacement index (instigated displacements compared to overall displacements) in both primiparous and multiparous cows, but a negative relationship existed between displacement index and feeding rate (sub-dominant cows ate more quickly) for multiparous cows (Proudfoot et al., 2009). Similar stocking density levels in Hosseinkhani et al. (2008) also resulted in altered feeding behaviors, with no differences in feeding time, but increased feeding rates, reduced feedbunk visits, and a tendency toward increased meal size and length in order to maintain DMI. Further, there were no differences among high, medium, and low success cows in terms of feeding time or time to approach the feed (Huzzey et al., 2012). Multiparous cows also showed a significant increase in rate of feed intake 2 weeks post-calving, highlighting the ability of overstocking during the dry period to affect feeding behavior in their next lactation. Interestingly, increasing competition at the feedbunk didn't alter sorting behavior at 4 h or 12 h post-feed delivery (Hosseinkhani et al., 2008), though differences may occur with lactating cows due to differences in dietary composition.

Research has shown behavioral and production benefits to under-stocking during the prepartum period. Cows housed at 80% stocking density experienced less feedbunk displacements and spent greater time lying prior to parturition (Lobeck-Luchterhand et al., 2015). However, there were no effects on metabolite concentrations (non-esterified fatty acids and β-hydroxybutyrate), culling rate, reproductive parameters, or milk production up to 155 DIM for either primiparous or multiparous cows (Silva et al., 2014). Furthermore, Cook and Norlund (2004) observed a 0.73 kg/d decrease in milk production for primiparous cows through 83 days in milk with each 10% increase in stocking density above 80% in the pre-fresh pen.

Although a common practice in the industry, further research is needed to identify whether there are benefits to maintaining stocking density at 100% or below for transition cows, particularly as farms go through cyclic periods of overstocking throughout the year.

Effect of Stocking Density on Stress Markers in Lactating Cows

Due to the stress of altered behavioral responses, overstocked cows can exhibit changes in neuroendocrine function through activation of the hypothalamic-pituitary-adrenal (HPA) axis. During HPA axis activation, the hypothalamus releases corticotrophin releasing hormone (CRH), acting on the pituitary to release adrenocorticotrophic hormone (ACTH), which finally acts on the adrenal gland to release glucocorticoids such as cortisol, a primary stress measurement in cattle. Elevated

concentrations of cortisol help the cow mobilize energy to manage stress, such as the fight/flight response (Moberg, 2000).

Due to altered behavioral changes in sub-dominant cows, overstocking at 200% resulted in greater blood cortisol concentrations for primiparous cows compared to mature cows at 60 and 90 min following an ACTH administration (González et al., 2003). There was also a positive correlation between reduced time spent feeding and blood cortisol concentrations in primiparous cows (likely due to high displacement rates at the feedbunk) as well a positive correlation between time spent lying in the alley (likely due to reduced access to freestalls) and cortisol concentrations in multiparous cows (González et al., 2003).

Cows housed above 150% stocking density experienced greater plasma glucocorticoid responses to ACTH challenges and experienced greater response curves, indicating greater adrenal responsiveness to the stressor (Friend et al., 1979). Although there were significant differences in stress levels between stocking densities in this study, there was no effect on milk production. Munksgaard and Lovendahl (1993) identified a reduction in growth hormone for 5 h following an ACTH challenge in cows deprived of lying time for 14 h/d. This demonstrates a reduction in growth and lactation hormones due to the inability to meet daily lying requirements. Dry cows also experienced similar responses with reduced access to lying (through space reduction) as exhibited by increased dehydroepiandrosterone (DHEA) concentrations and cortisol concentrations prior to calving (Fustini et al., 2017).

Reductions in lying access increased basal blood cortisol concentrations within 5 days and reduced ACTH and cortisol responses to CRH challenges, indicating down-regulation of the HPA axis with no significant negative feedback mechanisms on the pituitary gland (Fisher et al., 2002). Down-regulation of the HPA axis was also identified with a longer exposure (23 d) to reductions in lying access (Munksgaard and Simonsen, 1996). Overstocked cows will likely experience greater losses in biological reserves due to consistently higher basal cortisol levels. Due to chronic down-regulation of the HPA axis, overstocked cows may also experience desensitization to overstocking but increased sensitization to additional stressors.

Further, increasing lying deprivation in cows resulted in adverse behavioral responses such as an increase in grooming and head-pressing behavior, suggesting greater psycho-social responses in addition to physiological responses (Munksgaard and Simonsen, 1996). This demonstrates the need for future research to focus on the affective and cognitive state of dairy cows during high stress, overstocked environments in addition to production responses.

Krawczel et al. (2012b) did not find any significant differences in 11-oxoetiocholanolone (fecal cortisol metabolite) for cows stocked between 100 and 142%. In contrast, Huzzey et al. (2012) identified that dry cows with low displacement success had increased peak insulin responses, 11,17-di-oxoandrostane concentrations, and tended to have greater higher non-esterified fatty acids responses compared to high success cows at 200% stocking density. The increase in peak insulin responses could place these cows at greater risk for insulin resistance which could contribute to decreases in DMI in the

subsequent lactation. As noted by the authors, all cows on this study experienced displacements, but the overload of the low displacement success rates in the low group likely contributed to the greater stress responses. However, there were no differences in cortisol following ACTH challenges or alterations in glucose response curves following glucose tolerance testing, signifying reduced neuro-endocrine and metabolic sensitivity.

Further research is needed to evaluate the use of other stress markers such as acute phase proteins, LPS, or behavioral indicators within overstocked herds in order to develop field tools to evaluate stress responses and improve cow well-being.

Role of Stocking Density as a Sub-Clinical Stressor

Understanding the cow's environment and other stressors the cow is experiencing may change the economic benefit of using this management practice. For example, modeling the economic outcome of stocking density was based on a 0.50, 0.75, or 1.00 kg/d milk loss per cow with every 10% increase in stocking density (De Vries et al., 2016). While controlled research studies have continuously shown little to no effect on milk production, authors included these effects to counter unquantified effects on lameness and milk quality. Likely, under conditions of multiple stressors, effects may be more pronounced and unequally affect cows in the pen, lowering the economic incentive to overstock in combinations with other stressors.

In order to evaluate the role that stocking density plays as a stressor for dairy cows, it is necessary to determine the type of stress the cow is experiencing. Through evaluation of the behavioral and physiological effects from literature, stocking density

can be defined as a sub-clinical stressor. According to Moberg (2000), sub-clinical stress does not shift enough biological resources to cause changes in biological function, thus very little to no clinical signs are observed. Therefore, subdominant cows may experience changes in behaviors that do not always result in clinical or visible outcomes such as lower milk production or altered health status. Although these clinical signs are not always identified, the stressor reduces biological reserves in the cow, diminishing her effectiveness against additional stressors, placing her in a state of distress (Moberg, 2000). Cows housed at higher stocking densities experience intermittent stress throughout the day as cows compete for resources (ie. space at the feedbunk or water trough, access to freestalls, and hierarchy when entering the milking parlor or footbath). Chronic subjection to these relatively acute stressors would result in long term exposure to sub-clinical stress. This stress would continue until the cow is removed from the environment or the environment changes to shift the herd hierarchy in favor of the subdominant cow experiencing the stress.

Sensitization, desensitization, and normalization may also play important roles in the cow's ability to cope with higher stocking density situations. Defined by Moberg (2000):

Sensitization – an increase in intensity of a physiological response from repeated exposure of stressor

Desensitization – a decrease in intensity of a physiological response from repeated exposure of stressor

Normalization – The elimination of a physiological stress response due to mechanistic suppression opposed to adaptation to a stressor

Research has indicated that animals may use multiple strategies when encountering stress responses. For example, the low-intensity stress of tethering in swine resulted in desensitization to the tethering, but increased sensitization to ACTH through altered cortisol responses (von Borell and Ladewig, 1989). From the sub-clincial stress endured during high stocking density periods, cows may desensitize to the acute behavioral changes from stocking density but increase sensitization to additional stressors. Further, cows may desensitize parts of the HPA axis through down-regulations (Munksgaard and Simonsen, 1996; Fisher et al., 2002), but increase sensitization to additional cortisol responses regardless of increased basal cortisol concentrations (Fisher et al., 2002). Future research should focus upon the cow's ability to normalize to stocking density, given her environment prior to lactation as well as the consistency of stocking density throughout the entire lactation.

In addition to overstocking, previous research demonstrated that two factors significantly influencing cow well-being and efficiency of production are nutrition and feed availability (Bach et al., 2008). Rarely does one management practice occur on a farm in isolation; rather, combinations of these practices are being used simultaneously. No research has been conducted on the interactions of these stressors. Therefore, it is vital to further understand the interaction between overstocking and other important management factors affecting production to determine the impact they may have on rumen function, behavior, production, and stress in dairy cows.

Secondary Stressor: Feeding Low Physically Effective Neutral Detergent Fiber

Physically effective neutral detergent fiber (peNDF) is a value calculated from both the chemical analysis and particle size determination of the feedstuff. The resulting value is the product between the neutral detergent fiber (NDF) concentration of the analyzed sample (theoretical scale from 0 to 100; expressed as a percentage of dry matter) and the physical effectiveness factor (pef; theoretical scale from 0-1) which represents the diet or feed's ability to stimulate chewing activity and maintain a floating ruminal digesta mat (Mertens, 1997). The pef value can be obtained from two methods including the wet sieving technique using the Penn State Particle Separator (PSPS; Lammers et al., 1996) with a modified 4-mm screen (Cotanch et al., 2010) as well as dry (forced-air) sieving technique using a Ro-Tap (Ro-Tap testing sieve shaker model B; W. S. Tyler Combustion Engineering, Inc., Mentor, OH) instrument (Mertens, 1997). The modified PSPS and Ro-Tap techniques characterize pef values for the percentage of feed retained on or above a 4-mm and 1.18-mm sieve, respectively. In contrast to previously used effective neutral detergent fiber (eNDF) or chewing activity per kg of DM systems, peNDF minimizes cow or production variation and creates a consistent measure based solely on the physical characterization of the forage (Mertens, 1997).

Increasing peNDF content in the diet is associated with linear increases in eating, rumination, and total chewing time (Beauchemin and Yang, 2005, Yang and Beauchemin, 2006). Beauchemin and Yang (2005) observed moderate correlations for eating ($r = 0.41$) and total chewing ($r = 0.37$) and quadratic increases in the number of

meals throughout the day with increasing dietary peNDF. Linear increases in rumination and total chewing were also observed by Teimouri Yansari et al. (2004) through increases in dietary peNDFs of 15.2%, 20.5%, and 23.4%, although eating time increased only between the 15.2% and the 23.4% treatments when altering alfalfa particles. Increased physically effective fiber in the diet increases saliva production, through increased chewing (eating and rumination), which allows for greater buffering of the rumen (Cassida and Stokes, 1986). However, feeding practices in the field often include high levels of concentrate or feeding inadequate levels of physically effective fiber, which can increase the cow's risk for sub-acute ruminal acidosis (Stone, 2004).

Relationship Between peNDF and Sub-Acute Ruminal Acidosis

While sub-actue ruminal acidosis (SARA) can be defined as a certain amount of time spent below a determined pH, these values are quite variable in the literature. For this review, SARA will be defined as the time in hours spent below pH 5.8 (Beauchemin et al., 2003). Furthermore, this definition can be broken down into two sub-sections; concern for risk of the effects of SARA effects and increased risk of the effects of SARA. Zebeli et al. (2008) identified that 3 to 5 h spent below pH 5.8 placed cows at concern for SARA as this time frame represents altered periods of ruminal pH but did not affect the population size or activity of cellulolytic bacteria (Zebeli et al., 2007a). However, when cows experienced greater than 5.2 h below pH 5.8, they were at greater risk of adverse effects from SARA. Stone (2004) summarized the basic relationship of dietary peNDF with associated risk of SARA. Risk of SARA is greater when peNDF is below 21% and

lower when it is above 23% of DM. While higher dietary peNDF increases ruminal pH (Zebeli et al., 2006), diets should be formulated for an optimal balance of peNDF at approximately 21 to 23% as excessive peNDF may constrain DMI and feed efficiency (Stone, 2004). Teimouri Yansari et al. (2004) observed a decrease in mean ruminal pH due to decreases in dietary peNDF from 20.5% to 15.2% using varied alfalfa particle length. Furthermore, decreasing mean particle size of the diet increased time spent below pH 5.8 and area under the curve below pH 5.8 (Krause et al., 2002b).

Balancing energy-dense diets for maximizing milk production, while maintaining optimum ruminal health, has become a large challenge for the dairy industry (Zebeli et al., 2011). Garrett et al. (1997) identified that upwards of 19% of early lactation cows and 26% of mid-lactation cows show signs of SARA, though it is likely that these numbers have increased. Increased SARA, due to insufficient peNDF, can alter ruminal microbiota populations, leading to adverse effects on feed efficiency and production as well as inflammatory responses.

Effect of SARA on Ruminal Microbiota

Due to reduced chewing activity and consequently lowered buffering capabilities, feeding lower peNDF diets that result in SARA can lead to periods of rapid VFA production, drops in ruminal pH, and alterations in microbial communities (Nocek, 1997). In contrast to acute acidosis, lactic acid-producing bacteria and lactic acid-utilizing bacteria maintain balance under SARA conditions (Plaizier et al., 2008). Similarly, Nagaraja and Titgemeyer (2007) observed increases in Lactobacillus spp.,

lactic acid-producers, and lactic acid-utilizers with normal ruminal lactic acid levels when comparing SARA to acute acidosis in beef cattle.

Khafipour et al. (2009) investigated the effects of severe SARA (time below pH 5.6 was 5.6 h/d) and mild SARA (time below pH 5.6 was 3.6 h/d) on ruminal microbiota using grain-induced SARA treatments. Though the pH threshold in this study was 5.6 rather than 5.8 as defined in this review, these two treatments correlate well to the concern for risk (3 to 5 h/d) and increased risk (> 5 h/d) of SARA categories defined by Zebeli et al. (2008). The phylum *Firmicutes* increased while the phylum *Bacteriodetes* decreased when comparing severe SARA to mild SARA. Species diversity was significantly lower for mild SARA cows and tended to be lower for severe SARA cows when compared to controls. Mild SARA populations were higher in *Megasphaera elsdenii, Selenomonas ruminantium, Prevotella bryantii* and *Anaerovibrio lipolytica* at 0-h post-feeding compared to increased populations of *M. elsdenii, Succinivibrio dextrinisolvens, P. bryantii*, and *Ruminococcus flavefaciens* at 6-h post-feeding. In contrast, severe SARA resulted in increased *Escherichia coli, Streptococcus bovis, M. elsdenii*, and *Lactobacillus spp.* at 0-h post-feeding while populations of *M. elsdenii* and *S. bovis* dominated at 6-h post-feeding. The high abundance of *E. coli* and *M. elsdenii*, both gram negative bacteria, during severe SARA may be indicative of greater lysing or LPS shedding, resulting in greater risk of increased inflammatory responses from the cow. Furthermore, the high presence of lactic acid-utilizing bacteria such as *M. elsdenii* indicates the control of lactic acid build-up during SARA opposed acute acidosis. However, it is important to note that samples from this study were taken solely from the

ventral sac and strained through four layers of cheesecloth during collection which could significantly reduce the number of cellulolytic bacteria present, though treatment differences would remain consistent (Khafipour et al., 2009).

Hook et al. (2011b) observed a decrease in bacterial density associated with the digesta solids fraction during a grain-induced SARA challenge as well as a decrease in bacterial diversity over time as the rumen adapted to SARA. Similar to Khafipour et al. (2009), Hook et al. (2011b) also observed an increase in *Firmicutes* and a reduction in *Bacteriodetes* phylum. Populations of *S. ruminantium* increased during adaptation to SARA but *Ruminococcus spp.* were present for the entirety of the study, opposed to only occurring during mild SARA conditions induced by Khafipour et al. (2009). This difference may be due to reduced severity of SARA induced in Hook et al. (2011b) where time spent below pH 5.6 was only 4.6 h for the first week and declined throughout the study as cows adjusted to the diets.

Sub-acute rumianl acidosis also resulted in increased populations of protozoa during grain-induced challenges and reduced populations upon recovery of the SARA bout (Hook et al., 2011a). However, while SARA did not alter the density of methanogen populations, diversity and community structures were altered (Hook et al., 2011a).

There are limited data on the effects of non-grain-induced SARA on ruminal microbiota population densities and diversity. Further research is needed to identify the effects on microbiota of SARA induced through manipulations of feeding behavior or the feeding environment and how these changes compare to grain-induced studies.

Effect of SARA on Feed Digestibility

Due to alterations in microbial populations, adverse effects on feed efficiency can occur with cows experiencing SARA. This is primarily due to the decline in cellulolytic populations associated with low pH during SARA bouts. *In vitro* studies with altered buffer pH observed a decrease in rate and lag of NDF digestion for various forages (Grant and Mertens, 1992). Krajcarski-Hunt et al. (2002) identified a significant decrease in 24-h and 48-h NDF digestibility of grass hay, legume hay, and corn silage when subjected to an *in situ*, pellet-induced SARA challenge. Further, Zebeli et al. (2010) established a breakpoint relationship at approximately 3.5 h/d below pH 5.8, where greater time spent in SARA resulted in reduced ADF digestibility. These alterations in digestibility may also be explained by a reduction in attachment sites for microbes by replacement of cations with hydrogen ions at lower pH (Allen and Mertens, 1988).

Effect of SARA on Milk Production

Stone (1999) identified a 2.7 kg/d reduction in milk yield, a 0.3% reduction in milk fat content, and a 0.12% reduction in milk protein content associated with SARA in a field study. However, these effects on production in controlled studies are quite variable.

Krause and Oetzel (2005) observed a decrease in milk yield during a grain-induced SARA challenge following a day of restricted feed intake. However, milk fat increased during the challenge period compared to the baseline with little effects on milk

protein. This outcome was also observed by Krause et al. (2009) where both control and buffer-treated groups exhibited greater milk fat percentage but decreased milk production during the SARA challenge compared to the baseline. Due to the design of these studies (SARA challenges following one day of feed restriction), there is the possibility that cows mobilized adipose tissue due to the nutrient restriction which may be accounting for an increased milk fat percentage (increased preformed fatty acids) on the day of the SARA challenge.

While Gozho et al. (2007) did not observe a treatment difference for milk yield or composition, ruminal pH only increased from 3.11 to 5.15 h/d below pH 5.6. The lack of altered milk yield and composition may be explained by a lack of shifts in microbial populations as these cows remained in the concern for risk time frame. Further, Gao and Oba (2014) observed no effects of SARA on milk yield or composition during a continuous grain feeding studies with differences between tolerant and susceptible cows to SARA exhibiting <1 h and 9.2 h spent below pH 5.8, respectively. Though the ruminal pH differences and levels of concentrate fed were much larger than Gozho et al. (2007), the longer adaptation period (17 vs. 5 d) may have allowed for greater dietary adjustment. This suggests that slow dietary change over longer periods may reduce effects of SARA on production, although production may eventually be impacted with long-term SARA exposure. Although Khafipour et al. (2009) observed a linear decrease in milk yield and composition over a 6-wk period of increasing alfalfa pellets to induce SARA, cows were late in lactation and no covariate was used in statistical analysis of the milk production or composition data. Therefore, it is unclear how much of the variation in milk yield and

composition can be attributed to the SARA treatment or natural change in production across lactation.

Alterations in ruminal pH and microbial populations have been linked to milk fat depression through alterations in the biohydrogenation pathway towards formation of the trans-10, cis-12 CLA isomer (Bauman and Griinari, 2003). While Coleman et al. (2013) observed SARA treatments resulting in approximately 5.8 h/d below 5.8, no difference were observed with this isomer. However, as treatments in this study were on the edge of the increased risk category, greater hours of SARA may result in shifts in the biohydrogenation pathway. Further research should identify the effects of longer periods or more frequent bouts of SARA on milk fatty acid composition, specifically the trans-10, cis-12 isomer.

Effect of SARA on Inflammatory Responses

Sub-acute ruminal acidosis can have quite variable effects on cow health depending on severity and effects on rumen function. Due to the decrease in ruminal pH, osmotic pressure in the rumen is altered, resulting in increased risk of damage to the ruminal epithelium (Nocek, 1997). This damage can further affect barrier functions and allow lipopolysaccharide (LPS) to translocate into the bloodstream. Lipopolysaccharide, an endotoxin component of the cell wall in gram-negative bacteria, can have significant impact on cow health through increased risk for laminitis, acute phase protein inflammatory responses, and liver abscesses (Nocek, 1997; Gozho et al., 2005; Plaizier et al., 2008). However, although many studies observed increases in ruminal LPS, LPS

translocation appears limited during SARA conditions (Gozho et al., 2005; Gozho et al., 2007; Li et al., 2012). The lack of LPS translocation in both of these studies is likely due to the severity of SARA induced during these studies. For example, Gozho et al. (2007) observed SARA treatments resulting in a mean of 5.15 h/d below pH 5.8 while both treatments in Li et al. (2012) resulted in approximately 3.75 and 5 h for alfalfa pellet and grain-induced SARA, respectively. These time spans fall within the concern for risk of SARA category described by Zebeli et al. (2008) and may not have caused significant damage to ruminal epithelium to allow LPS to enter the bloodstream. Therefore, under SARA conditions, translocation of LPS to the peripheral bloodstream may be limited. These findings also suggest that dietary conditions before episodes of SARA may play an important role in the health consequences for the cow following SARA (Plaizier et al., 2008). However, in more severe or more frequent episodes of SARA, or the addition of other stressors on rumen function or environment, risk of LPS translocation may be increased. Further, Khafipour et al. (2009) observed similar LPS responses from both grain and alfalfa pellet induced SARA, but only the grain diets resulted in inflammatory responses. This would indicate that other factors are playing roles in the susceptibility to SARA rather than LPS alone. Nocek (1997) noted that polyamine production such as histamine decarboxylation was associated with SARA and increased risk of laminitis. Histamine absorption, through reduced barrier function in the rumen epithelium, can then lead to greater systemic consequences such as regulation of feed and water intake and cardiovascular damage (Underwood, 1992; Rossi et al., 1998; Plaizier et al., 2008). Further research is needed to identify secondary compounds produced during SARA and

their effects on the rumen and systemic health of the cow. In addition, greater research is needed to identify differences between diet-induced SARA and altered feeding behavior-induced SARA with effects on rumen function and the risk of inflammatory consequences.

In addition to an increase in LPS, SARA can have significant effects on acute phase protein production. Positive acute phase proteins, such as haptoglobin or serum amyloid-A (SAA), are produced in the liver and increase in response to tissue damage and inflammation. Acute phase proteins work to fight bacterial infections by removing necessary resources for bacterial production and activating repair systems to heal the cow and prevent further injury (Baumann and Gauldie, 1994). In response to SARA challenges, cows increase haptoglobin and SAA production (Gozho et al., 2005; Khafipour et al., 2006). Gozho et al. (2007) reported increases in SAA, but not haptoglobin during grain-induced SARA challenges. Furthermore, Zebeli et al. (2012) observed a breakpoint relationship between dietary concentrate level and plasma SAA, with linear increases in SAA concentrations above 44.1% grain inclusion in the diet. In addition, a positive linear relationship was observed between time spent below pH 6.0 and SAA concentrations (Zebeli et al., 2012). However, differences in acute phase protein production can demonstrate differences in stress responses, with SAA more representative of acute inflammation while haptoglobin can be more useful to identify chronic inflammation (Horadagoda et al., 1999). This indicates that the severity and length of SARA bouts may dictate differences in acute phase production.

Reduced Feed Access

Nutrition models calculate nutrient requirements assuming cows have *ad libitum* access to feed and are not overstocked. The reality is that the majority of dairy cows in the US are fed within overstocked conditions – and increasingly producers are feeding for lower amounts of daily feed refusals in an effort to minimize wastage of expensive feed (USDA-ERS, 2014). To ensure feed was available for 24 h/d, providing 5% more feed than the predicted requirements was recommended (Grant and Albright, 2001; NRC, 2001; NFACC, 2009). However, a survey of western US dairy farms suggested a growing number of producers are targeting 0% feed refusals, commonly referred to as feeding to a "slick or clean bunk" (Silva-del-Rio et al., 2010). Feed costs comprise 55% of total operating costs (USDA-ERS, 2014) and their continued increase has driven producers to move away from feeding for refusals. Consequently, further understanding is needed concerning the interaction of stocking density and feed availability as it influences feeding behavior, rumination, production efficiency, and rumen function.

Effect of Reduced Feed Access on Intake and Feeding Behavior

Feeding for 0% refusals may limit feed intake as it results in periods of the day when little to no feed is available. However, cows became hungry and were highly motivated to eat after only 3 h of feed restriction (Schutz et al., 2006). Collings et al. (2011) also observed a high motivation of cows to eat following a 10-h temporal restriction with 1.5x increase in DMI 2 h following feed delivery. While a 12-h temporal

feed restriction resulted in no differences in daily DMI (Munksgaard et al., 2005), cow tended to have lower daily DMI with 10-h temporal feed restriction (Collings et al., 2011). Further, increasing access to feed (8 to 20 h/d) increased daily feed intake (Erdman et al., 1989). The ability of the cow to maintain DMI may depend on other feeding environment factors (feeding frequency, frequency of restriction) as well as expected intake based on production.

Cows consistently adjust feeding behavior in order to maintain DMI under reduced feed access conditions. A 12-h temporal restriction on feed availability reduced feeding time and increased feeding rate (Munksgaard et al., 2005). Similarly, Collings et al. (2011) observed reduced feeding times, fewer meals, and higher feeding rates under a 10-h temporal restriction. Further, changes in feeding behavior peaked following feed delivery, with increased feeding time, meals, and displacements at the feedbunk (Collings et al., 2011). Cows denied access to a total mixed ration during the night, due to pasture-based housing, also compensated by spending more time eating in the first 3 h and increasing displacement activities when a total mixed ration became available (Chapinal et al., 2010a).

Effect of Reduced Feed Access on Efficiency and Production

While limit feeding increased feed efficiency in pregnant heifers (Hoffman et al., 2007), there is no evidence to suggest the same is true for lactating dairy cows. Routine feed push-up and feeding to ensure feed availability increased milk production by 4 kg/d versus cows with restricted access to feed (Bach et al., 2008). Both 10-h and 12-h feed

restrictions did not affect milk production when compared to 24-h access (Collings et al., 2011; Munksgaard et al., 2005). Similarly, Erdman et al. (1989) observed no effect on milk production, but linear decreases in FCM efficiency, milk protein percentage, and milk protein yield with increasing access to feed from 8 h/d to 20 h/d. However, while feed intake increased with feed access, intake as a percent of body weight was not affected due to increasing body weights with increased access to feed (Erdman et al., 1989). This indicates the possibility for cows with reduced access to feed to compensate for production through body stores and would likely have elevated non-esterified fatty acid levels compared to non-restricted cows. Furthermore, no differences in milk fat are likely explained by the ability of cows to maintain overall chewing activity in order to maintain adequate buffer production for ruminal function and biohydrogenation (Erdman et al., 1989).

Effect of Reduced Feed Access on Ruminal Fermentation

Limited research has been conducted focusing on the effect of reduced feed access on ruminal metabolism, particularly in dairy cattle. Erickson et al. (2003) observed no differences in daily mean ruminal pH or area under the curve below pH 5.6 when comparing finishing beef steers subjected to 24-h access vs. 10-h reduced access to feed. However, temporally restricted steers did exhibit greater variation in ruminal pH than 24-h access steers. While DMI was consistent between treatment groups, temporally restricted steers had higher feeding rates, longer and less frequent meals, and numerically higher ruminal pH values prior to feed delivery, allowing more buffering capacity to

large meal consumptions following feed delivery (Erickson et al., 2003). However, under different management conditions (reduced time budget due to milking) for dairy cows, changes in feeding and resting behaviors may result in different ruminal fermentation outcomes.

Interaction of Reduced Feed Access and Stocking Density

One study has investigated the interaction between stocking density (100% vs. 200% of feedbunk) and temporal feed restriction (14 h vs. 24 h) on feeding behavior (Collings et al., 2011). Reduced feed access during overstocked conditions resulted in increased feeding rates and displacements, both daily and 2 h following feed delivery. Further, an interaction was found between stocking density and feed access for feeding time 2 h following feed delivery, with temporally restricted cows at 200% stocking density having the highest feeding time while temporally restricted, overstocked cows had greater displacements during this time period. Generally, the negative effects of temporal feed restriction and overstocking on feeding behavior are similar because both limit the cow's access to feed. It is possible that restricting access to feed in an effort to improve overall feed efficiency may actually accentuate the negative consequences of overstocking.

JUSTIFICATION OF OBJECTIVES

Although several research studies have evaluated the effects of stocking density as a sole stressor on behavioral, production, and stress responses, there is virtually no

understanding concerning the effects of stocking density when secondary stressors are present on the farm. Further, there is no research on the effects of stocking density on ruminal pH and fermentation dynamics. Evaluating ruminal conditions in these studies will provide novel additions to previous stocking density research. Understanding the cumulative effects of stocking density in conjunction with the feeding environment, such as diet composition and access to feed, is the next vital step for alleviating stress and improving the well-being and long-term productive efficiency of lactating dairy cows.

In order to ensure different ruminal responses between low and high peNDF diets, preliminary research is needed to test the effects of dietary peNDF sources and their associated risks of SARA. Further, there is a lack of evidence concerning proper blood tube type collection in order to evaluate haptoglobin levels among commercially-available assays. In order to identify the effects of stocking density and feeding environment interactions on stress metabolites, preliminary research is needed to confirm similar haptoglobin concentrations among blood tube types.

While previous research has investigated the interaction of stocking density and temporal feed restriction on behavioral responses, severity of treatments imposed extend beyond typical commercial settings. Overstocking of the feed bunk at 200% far exceeds the industry average for the northeast U.S. at 142% (von Keyserlingk et al., 2012). In addition, Collings et al. (2011) created overstocked conditions of 200% through the assignment of two cows to the same feed bin. Further research is needed to understand how the entire herd dynamic influences behaviors with more than one-to-one competition for feed bunk space. In addition, constraints in feed availability would likely be less than

10 h/d, but no published survey work quantifies the extent of this practice. Feeding for 2.5% feed refusals at 18 h post-feeding, or blocking access to the feed bunk for 5 to 6 h/d, resulted in “clean bunk” management (French et al., 2005). This degree of feed restriction would be a logical level to evaluate the interaction between overstocking and restricted feed availability under conditions representative of commercial farms.

In order to address these interactions, the following objectives were addressed in this dissertation:

1. To measure the effectiveness of using chopped wheat straw to reduce sub-acute ruminal acidosis (SARA) when formulating diets for future studies comprising this dissertation.

2. To determine the effect of type of blood collection tube on haptoglobin concentration across several, commercially available haptoglobin assays when analyzing stress metabolites for future studies comprising this dissertation.

3. To determine the effect of stocking density and source of forage fiber on short-term responses in ruminal fermentation, behavior, production, and stress responses of lactating Holstein dairy cows.

4. To determine the effect of stocking density and reduced feed access on short-term responses in ruminal fermentation, behavior, production, and stress responses of lactating Holstein dairy cows.

REFERENCES

Allen, M. S., and D. R. Mertens. 1988. Evaluating constraints on fiber digestion by rumen microbes. J. Nutr. 118:261-270.

Bach, A., N. Valls, A. Solans, and T. Torrent. 2008. Associations between nondietary factors and dairy herd performance. J. Dairy Sci. 91:3259-3267.

Barbano, D. M., C. Melilli, and T. R. Overton. 2014. Advanced use of FTIR spectra of milk for feeding and health management. Pages 105-113 in Proc. Cornell Nutrition Conf., Syracuse, NY. Cornell University, Ithaca, NY.

Barrientos, A. K., N. Chapinal, D. M. Weary, E. Galo, and M. A. G. von Keyserlingk. 2013. Herd-level risk factors for hock injuires in freestall-housed dairy cows in the northeastern United States and California. J. Dairy Sci. 96:3758-3765.

Batchelder, T. L. 2000. The impact of head gates and overcrowding on production and behavior patterns of lactating dairy cows. Pages 325-330 in Dairy Housing and Equipment Systems. Managing and Planning for Profitability. Publ. 129. Nat. Resour., Agric., and Eng. Serv., Camp Hill, PA.

Bauman, D. E., and J. M. Griinari. 2003. Nutritional regulation of milk fat synthesis. Annu. Rev. Nutr. 23:203-227.

Baumann, H., and J. Gauldie. 1994. The acute phase response. Imm. Today. 15:74-80.

Beauchemin, K. A., and W. Z. Yang. 2005. Effects of physically effective fiber on intake, chewing activity, and ruminal acidosis for dairy cows fed diets based on corn silage. J. Dairy Sci. 88:2117-2129.

Beauchemin, K. A., W. Z. Yang, and L. M. Rode. 2003. Effects of particle size of alfalfa-based dairy cow diets on chewing activity, ruminal fermentation, and milk production. J. Dairy Sci. 86:630-643.

Bewley, J., R. W. Palmer, and D. B. Jackson-Smith. 2001. A comparison of free-stall barns used by modernized Wisconsin dairies. J. Dairy Sci. 84:528-541.

Black, R. A., R. J. Grant, and P. D. Krawczel. 2016. Short communication: Short-term changes in stocking density did not alter meal characteristics of lactating Holstein dairy cattle. J. Dairy Sci. 99:6572-6577.

Caraviello, D. Z., K. A. Weigel, M. Craven, D. Gianola, N. B. Cook, K. V. Nordlund, P. M. Fricke, and M. C. Wiltbank. 2006. Analysis of reproductive performance of lactating cows on large dairy farms using machine learning algorithms. J. Dairy Sci. 89:4703-4722.

Cassida, P. E., and M. R. Stokes. 1986. Eating and resting salivation in early lactation dairy cows. J. Dairy Sci. 69:1282-1292.

Chapinal, N., D. M. Weary, J. Rushen, A. M. de Passille, and M. A. G. von Keyserlingk. 2010a. Short communication: Effects of temporal restriction in availability of the total mixed ration on feeding and competitive behavior in lactating dairy cows. Livest. Sci. 137:282-286.

Charlton, G. L., D. B. Haley, J. Rushen, and A. M. de Passillé. 2014. Stocking density, milking duration, and lying times of lactating cows on Canadian freestall dairy farms. J. Dairy Sci. 97:2694-2700.

Coleman, E., E. Khafipour, B. Vlaeminck, B. De Baets, J. C. Plaizier, and V. Fievez. 2013. Grain-based versus alfalfa-based subacute ruminal acidosis induction experiments: Similarities and differences between changes in milk fatty acids. J. Dairy Sci. 96:4100-4111.

Collings, L. K. M., D. M. Weary, N. Chapinal, and M. A. G. von Keyserlingk. 2011. Temporal feed restriction and overstocking increase competition for feed by dairy cattle. J. Dairy Sci. 94:5480-5486.

Cook, N. B., and K. V. Nordlund. 2004. Behavioral needs of the transition cow and considerations for special needs facility design. Vet. Clin. Food Anim. 20:495-520.

Cook, N. B., T. B. Bennett, and K. V. Nordlund. 2005. Monitoring indices of cow comfort in free-stall-housed dairy herds. J. Dairy Sci. 88:3876-3885.

Cooper, M. D., D. R. Arney, and C. J. C. Phillips. 2007. Two- or four-hour lying deprivation on the lying behavior of lactating dairy cows. J. Dairy Sci. 90:1149-1158.

Cotanch, K. W., J. D. Darrah, C. S. Ballard, and R. J. Grant. 2010. Modification of the Penn State Particle Separator with 3.18- or 4.76-mm perforated steel sieves to measure physically effective fiber. J. Dairy Sci. 88(E-Suppl. 1):147. (Abstr.)

Crossley, R. E., A. Harlander-Matauschek, and T. J. DeVries. 2017. Variability in behavior and production among dairy cows fed under differing levels of competition. J. Dairy Sci. 100:1-14.

Deming, J. A., R. Bergeron, K. E. Leslie, and T. J. DeVries. 2013. Associations of housing, management, milking activity, and standing and lying behavior of dairy cows milked in automatic systems. J. Dairy Sci. 96:344-351.

DeVries, T. J., M. A. G. von Keyserlingk, and D. M. Weary. 2004. Effects of feeding space on the inter-cow distance, aggression, and feeding behavior of free-stall housed lactating dairy cows. J. Dairy Sci. 87:1432-1438.

DeVries, T. J., M. A. G. von Keyserlingk, and K. A. Beauchemin. 2003. Short communication: Diurnal feeding pattern of lactating dairy cows. J. Dairy Sci. 86:4079-4082.

De Vries, A., H. Dechassa, and H. Hogeveen. 2016. Economic evaluation of stall stocking density of lactating dairy cows. J. Dairy Sci. 99:3848-3857.

Erdman, R. A., T. W. Moreland, and W. R. Stricklin. 1989. Effect of time of feed access on intake and production in lactating dairy cows. J. Dairy Sci. 72:1210-1216.

Erickson, G. E., C. T. Milton, K. C. Fanning, R. J. Cooper, R. S. Swingle, J. C. Parrott, G. Vogel, and T. J. Klopfenstein. 2003. Interaction between bunk management and monensin concentration on finishing performance, feeding behavior, and ruminal metabolism during an acidosis challenge with feedlot cattle J. Anim. Sci. 81:2869-2879.

Espejo, L. A., and M. I. Endres. 2007. Herd-level risk factors for lameness in high-producing Holstein cows housed in freestall barns. J. Dairy Sci. 90:306-314.

Espejo, L. A., M. I. Endres, and J. A. Salfer. 2006. Prevalence of lameness in high-producing Holstein cows housed in freestall barns in Minnesota. J. Dairy Sci. 89:3052-3058.

Falk, A. C., D. M. Weary, C. Winckler, M. A. G. von Keyserlingk. 2012. Preference for pasture versus freestall housing by dairy cattle when stall availability indoors is reduced. J. Dairy Sci. 95:6409-6415.

Fisher, A. D., G. A. Verkerk, C. J. Morrow, and L. R. Matthews. 2002. The effects of feed restriction and lying deprivation on pituitary-adrenal axis regulations in lactating cows. Livest. Prod. Sci. 73:255-263.

Fraser, D., D. M. Weary, E. A. Pajor, and B. N. Milligan. 1997. A scientific conception of animal welfare that reflects ethical concerns. Anim. Welfare 6:187-205.

Fregonesi, J. A., C. B. Tucker, and D. M. Weary. 2007. Overstocking reduces lying time in dairy cows. J. Dairy Sci. 90:3349-3354.

French, P., J. Chamberlain, and J. Warntjes. 2005. Effect of feed refusal amount on feeding behavior and production in Holstein cows. J. Anim. Sci. 83(E-Suppl.1): 175. (Abstr.).

Friend, T. H., and C. E. Polan. 1974. Social rank, feeding behavior, and freestall utilization by dairy cattle. J. Dairy Sci. 57:1214-1220.

Friend, T. H., F. C. Gwazdauskas, and C. E. Polan. 1979. Change in adrenal response from free stall competition. J. Dairy Sci. 62:768-771.

Fustini, M., G. Galeati, G. Gabai, L. E. Mammi, D. Bucci, P. A. Accorsi, and A. Formigoni. 2017. Overstocking dairy cows during the dry period affects dehydroepiandrosterone and cortisol secretion. J. Dairy Sci. 100:620-628.

Gao, X., and M. Oba. 2014. Relationship of severity of subacute ruminal acidosis to rumen fermentation, chewing activities, sorting behavior, and milk production in lactating dairy cows fed a high-grain diet. J. Dairy Sci. 97:3006-3016.

Garrett, E. F., K. V. Norlund, W. J. Goodger, and G. R. Oetzel. 1997. A cross-sectional field study investigating the effect of periparturient dietary management on ruminal pH in early lactation dairy cows. J. Dairy Sci. 80(Suppl. 1):169. (Abstr.).

González, M., A. K. Yabuta, and F. Galindo. 2003. Behaviour and adrenal activity of first parturition and multiparous cows under a competitive situation. Appl. Anim. Behav. Sci. 82:259-266.

Gozho, G. N., D. O. Krause, and J. C. Plaizier. 2007. Ruminal lipopolysaccharide concentration and inflammatory response during grain-induced subacute ruminal acidosis in dairy cows. J. Dairy Sci. 90:856-866.

Gozho, G. N., J. C. Plaizier, D. O. Krause, A. D. Kennedy, and K. M. Wittenberg. 2005. Subacute ruminal acidosis induces ruminal lipopolysaccharide endotoxin release and triggers and inflammatory response. J. Dairy Sci. 88:1399-1403.

Grant, R. J., and D. R. Mertens. 1992. Influence of buffer pH and raw corn starch addition on in vitro fiber digestion kinetics. J. Dairy Sci. 75:2762-2768.

Grant, R. J., and J. L. Albright. 2001. Effect of animal grouping on feeding behavior and intake of cattle. J. Dairy Sci. 84(E-Suppl.):E156-E163.

Green, M. J., A. J. Bradley, G. F. Medley, and W. J. Brown. 2008. Cow, farm, and herd management factors in the dry period associated with raised somatic cell counts in early lactation. J. Dairy Sci. 91:1403-1415.

Greenough, P.R., and J. J. Vermunt. 1991. Evaluation of subclinical laminitis in a dairy herd and observations on associated nutritional and management factors. Vet Rec 128:11-17.

Hill, C. T., P. D. Krawczel, H. M. Dann, C. S. Ballard, R. C. Hovey, and R. J. Grant. 2007. Effect of stocking density on the short-term behavior of dairy cows. J. Dairy Sci. 90(Suppl. 1):244. (Abstr.).

Hill, C. T., P. D. Krawczel, H. M. Dann, C. S. Ballard, R. C. Hovey, W. A. Falls, and R. J. Grant. 2009. Effect of stocking density on the behavior of dairy cows with differing parity and lameness status. Appl. Anim. Behav. Sci. 117:144-149.

Hoffman, P. C., C. R. Simson, and M. Wattiaux. 2007. Limit feeding of gravid Holstein heifers: Effect on growth, manure nutrient excretion, and subsequent early lactation performance. J. Dairy Sci. 90:946-954.

Hook, S. E., M. A. Steele, K. S. Northwood, A-D. G. Wright, and B. W. McBride. 2011a. Impact of high-concentrate feeding and low ruminal pH on methanogens and protozoa in the rumen of dairy cows. Environ. Microb. 62:94-105.

Hook, S. E., M. A. Steele, K. S. Northwood, J. Dijkstra. J. France, A-D. G. Wright, and B. W. McBride. 2011b. Impact of subacute ruminal acidosis (SARA) adaptation and recovery on the density and diversity of bacteria in the rumen of dairy cows. Microb. Ecol. 78:275-284.

Horadagoda, N. U., K. M. G. Knox, H. A. Gibbs, S. W. J. Reid, A. Horadagoda, S. E. R. Edwards, P. D. Eckersall. 1999. Acute phase proteins in cattle: discrimination between acute and chronic inflammation. Vet. Rec. 144:437-441.

Hosseinkhani, A., T. J. DeVries, K. L. Proudfoot, R. Valizadeh, D. M. Veira, and M. A. G. von Keyserlingk. 2008. The effects of feed bunk competition on the feed sorting behavior of close-up dry cows. J. Dairy Sci. 91:1115-1121.

Huzzey, J. M., R. J. Grant, and T. R. Overton. 2012. Short communication: Relationship between competitive success during displacements at an overstocked feed bunk and measures of physiology and behavior in Holstein dairy cattle. J. Dairy Sci. 95:4434-4441.

Huzzey, J. M., T. J. DeVries, P. Valois, and M. A. G. von Keyserlingk. 2006. Stocking density and feed barrier design affect the feeding and social behavior of dairy cattle. J. Dairy Sci. 89:126-133.

Ito, K., N. Chapinal, D. M. Weary, and M. A. G. von Keyserlingk. 2014. Associations between herd-level factors and lying behavior of freestall-housed dairy cows. J. Dairy Sci. 97:2081-2089.

Jensen, M. B., L. J. Pedersen, and L. Munksgaard. 2005. The effect of reward duration on demand functions for rest in dairy heifers and lying requirements as measured by demand functions. Appl. Anim. Behav. Sci. 90:207-217.

Khafipour, E., D. O. Krause, and J. C. Plaizier. 2006. Influence of grain induced sub-acute ruminal acidosis (SARA) on lipopolysaccharide endotoxin (LPS) and acute phase proteins. Can. J. Anim. Sci. 86:577. (Abstr.).

Khafipour, E., S. Li, J. C. Plaizier, and D. O. Krause. 2009. Rumen microbiome composition determined using two nutritional models of subacute ruminal acidosis. Appl. Env. Micro. 75:7115-7124.

King, M. T. M., E. A. Pajor, S. J. LeBlanc, and T. J. DeVries. 2016. Associations of herd-level housing, management, and lameness prevalence with productivity and cow behavior in herds with automated milking systems. J. Dairy Sci. 99:9069-9079.

Krajcarski-Hunt, H., J. C. Plaizier, J. P. Walton, R. Spratt, and B. W. McBride. 2002. Short communication: Effect of subacute ruminal acidosis on in situ fiber digestion in lactating dairy cows. J. Dairy Sci. 85:570-573.

Krause, K. M., D. K. Combs, and K. A. Beauchemin. 2002b. Effects of forage particle size and grain fermentability in midlactation cows II: Ruminal pH and chewing activity. J. Dairy Sci. 85:1947-1957.

Krause, K. M., D. V. Dhuyvetter, and G. R. Oetzel. 2009. Effect of a low-moisture buffer block on ruminal pH in lactating dairy cattle induced with subacute ruminal acidosis. J. Dairy Sci. 92:352-364.

Krause, K. M., and G. R. Oetzel. 2005. Inducing subacute ruminal acidosis in lactating dairy cows. J. Dairy Sci. 88:3633-3639.

Krawczel, P. D., C. T. Hill, H. M. Dann and R. J. Grant. 2008. Effect of stocking density on indices of cow comfort. J. Dairy Sci. 91:1903-1907.

Krawczel, P. D., L. B. Klaiber, R. E. Butzler, L. M. Klaiber, H. M. Dann, C. S. Mooney, and R. J. Grant. 2012b. Short-term increases in stocking density affect the lying and social behavior, but not the productivity, of lactating Holstein dairy cows. J. Dairy Sci. 95:4298-4308.

Lammers, B. P., D. R. Buckmaster, and A. J. Heinrichs. 1996. A simple method for the analysis of particle sizes of forage and total mixed rations. J. Dairy Sci. 79:922-928.

Leonard F. C., J. M. O'Connell, and K. J. O'Farrell. 1996. Effect of overcrowding on claw health in firstcalved friesian heifers. Br. Vet. J. 152:459-472.

Li, S., E. Khafipour, D. O. Krause, A. Kroeker, J. C. Rodreiguez-Lecompte, G. N. Gozho, and J. C. Plaizier. 2012. Effects of subacute ruminal acidosis challenges on fermentation and endotoxins in the rumen and hindgut of dairy cows. J. Dairy Sci. 95:294-303.

Lobeck-Luchterhand, K. M., P. R. B. Silva, R. C. Chebel, and M. I. Endres. 2015. Effect of stocking density on social, feeding, and lying behavior of prepartum dairy animals. J. Dairy Sci. 98:240-249.

Mentink, R. L., and N. B. Cook. 2006. Short communication: Feed bunk utilization in dairy cows housed in pens with either two or three rows of free stalls. J. Dairy Sci. 89:134-138.

Mertens, D. R. 1997. Creating a system for meeting the fiber requirements of dairy cows. J. Dairy Sci. 80:1463-1481.

Metz, J. H. M. 1985. The reaction of cows to a short-term deprivation of lying. Appl. Anim. Behav. Sci. 13:301-307.

Moberg, G. P. 2000. Biological response to stress: Implications for animal welfare. In: Moberg, G. P. and J. A. Mench (eds) The Biology of Animal Stress. CAB. International, Wallingford, UK, pp. 1-21.

Munksgaard, L., and H. B. Simonsen. 1996. Behavioral and pituitary adrenal-axis responses of dairy cows to social isolation and deprivation of lying down. J. Anim. Sci. 74:769-778.

Munksgaard, L., M. B. Jensen, L. J. Pedersen, S. W. Hansen, and L. Matthews. 2005. Quantifying behavioural priorities - Effects of time constraints on behavior of dairy cows, Bos Taurus. Appl. Anim. Behav. Sci. 92:3-14.

Munksgaard, L., and P. Lovendahl. 1993. Effects of social and physical stressors on growth hormone levels in dairy cows. Can. J. Anim. Sci. 73:847-853.

Nagaraja, T. G., and E. C. Titgemeyer. 2007. Ruminal acidosis in beef cattle: The current microbiological and nutritional outlook. J. Dairy Sci. 90(E. Suppl.):E17-E38.

National Milk Producers Federation (NMPF). 2017. National Dairy FARM Animal Care Manual. Available at http://www.nationaldairyfarm.com/animal-care-resources.html.

National Research Council. 2001. Nutrient Requirements of Dairy Cattle. 7th rev. ed. National Academy Press. Washington, DC.

Nelson, A. J. 1996. On-farm nutrition diagnostics. Pages 76-85 in Proc. 29th Annu. Conf. Am. Bovine Pract., San. Diego, CA. Am. Assoc. Bovine Pract., Rome, GA.

NFACC. 2009. Code of practice for the care and handling of dairy cattle. Dairy Farmers of Canada and the National Farm Animal Care Council, Ottawa, Ontario, Canada.

Nocek, J. E. 1997. Bovine acidosis: implications on laminitis. J. Dairy Sci. 80:1005-1028.

Olofsson, J. 1999. Competition for total mixed diets fed for ad libitum intake using one or four cows per feeding station. J. Dairy Sci. 82:69-79.

Overton, M. W., W. M. Sischo, G. D. Temple, and D. A. Moore. 2002. Using time-lapse video photography to assess dairy cattle lying behavior in a free-stall barn. J. Dairy Sci. 85:2407-2413.

Plaizier, J. C., D. O. Krause, G. N. Gozho, and B. W. McBride. 2008. Subacute ruminal acidosis in dairy cows: The physiological causes, incidence, and consequences. Vet. J. 176:21-31.

Proudfoot, K. L., D. M. Veira, D. M. Weary, and M. A. G. von Keyserlingk. 2009. Competition at the feed bunk changes the feeding, standing, and social behavior of transition dairy cows. J. Dairy Sci. 92:3116-3123.

Rossi, R., E. Del Prete, and E. Scharrer. 1998. Effects of histamine H1 receptors on the feeding and drinking patterns in pygmy goats. J. Dairy Sci. 81:2369-2375.

Schefers, J. M., K. A. Weigel, C. L. Rawson, N. R. Zwald, and N. B. Cook. 2010. Management practices associated with conception rate and service rate of lactating Holstein cows in large, commercial herds. J. Dairy Sci. 93:1459-1467.

Schutz, K., D. Davison, and L. Matthews. 2006. Do different levels of moderate feed deprivation in dairy cows affect feeding motivation? Appl. Anim. Behav. Sci. 101:253-263.

Silva, P. R. B., A. R. Dresch, K. S. Machado, J. G. N. Moraes, K. Lobeck-Luchterhand, T. K. Nishimura, M. A. Ferreira, M. I. Endres, and R. C. Chebel. 2014. Prepartum stocking density: Effects on metabolic health, reproductive, and productive responses. J. Dairy Sci. 97:5521-5532.

Silva-del-Rio, N., J. M. Heguy, and A. Lago. 2010. Feed management practices on California dairies. J. Dairy Sci. 93(E-Suppl. 1):773. (Abstr.).

Solano, L., H. W. Barkema, E. A. Pajor, S. Mason, S. J. LeBlanc, C. G. R. Nash, D. B. Haley, D. Pellerin, J. Rushen, A. M. de Passillé, E. Vasseur, and K. Orsel. 2015. Associations between lying behavior and lameness in Canadian Holstein-Friesian cows housed in freestall barns. J. Dairy Sci. 99:2086-2101.

Sova, A. D., S. J. LeBlanc, B. W. McBride, and T. J. DeVries. 2013. Associations between herd-level feeding management practices, feed sorting, and milk production in freestall dairy farms. J. Dairy Sci. 96:4759-4770.

Stone, W. C. 1999. The effect of subclinical rumen acidosis on milk components. Pages 40-46 in Cornell Nutr. Conf. Feed Manuf., Proc. 1999, Cornell Univ., Ithaca, NY.

Stone, W. C. 2004. Nutritional approaches to minimize subacute rumen acidosis and laminitis in dairy cattle. J. Dairy Sci. 87(E-Suppl.):E13-E26.

Talebi, A., M. A. G. von Keyserlingk, E. Telezhenko, and D. M. Weary. 2014. Reduced stocking density mitigates the negative effects of regrouping in dairy cattle. J. Dairy Sci. 97:1358-1363.

Teimouri Yansari, A., R. Valizadeh, A. Naserian, D. A. Christensen, P. Yu, and F. Eftekhari Shahroodi. 2004. Effects of alfalfa particle size and specific gravity on chewing activity, digestibility, and performance of Holstein dairy cows. J. Dairy Sci. 87:3912-3924.

Telezhenko, E., M. A. G. von Keyserlingk, A. Talebi, and D. M. Weary. 2012. Effect of pen size, group size, and stocking density on activity in freestall-housed dairy cows. J. Dairy Sci. 95:3064-3069.

Underwood, W. J. 1992. Rumen lactic acidosis. Part II. Clinical signs, diagnosis, treatment, and prevention. Compendium for continuing education for the practicing veterinarian. Food Anim. 14:1265-1270.

USDA. 2010. Facility characteristics and cow comfort on U.S. dairy operations, 2007. US Dept. Agric.-Anim. Plant Health Insp. Serv.-Vet. Serv., Cent. Epidemiol. Anim. Health, Fort Collins, CO.

USDA-ERS. 2014. Monthly cost of production estimates. USDA-Economic Research Service, Washington, DC. Accessed April 14, 2014. http://www.ers.usda.gov/data-products/milk-cost-of-productionestimates.aspx.

USDA-NAL. 2017. Animal Welfare Act and Regulations. Accessed March 11, 2017. https://www.nal.usda.gov/awic/animal-welfare-act

Val-Laillet, D., D. M. Veira, and M. A. G. von Keyserlingk. 2008. Short communication: Dominance in free-stall-housed dairy cattle is dependent upon resource. J. Dairy Sci. 91:3922-3926.

von Borell, E., and J. Ladewig. 1989. Altered adrenocortical response to acute stressors or ACTH(l-24) in intensively housed pigs. Domest. Anim. Endocrinol. 6:299-309.

von Keyserlingk, M. A. G., A. Barrientos, K. Ito, E. Galo, and D. M. Weary. 2012. Benchmarking cow comfort on North American freestall dairies: lameness, leg injuries, lying time, facility design, and management for high-producing Holstein dairy cows. J. Dairy Sci. 95:7399-7408.

von Keyserlingk, M. A. G., J. Rushen, A. M. de Passillé, and D. M. Weary. 2009. The welfare of dairy cattle – Key concepts and the role of science. J. Dairy Sci. 92:4101-4111.

Walker, S. L., R. F. Smith, J. E. Routly, D. N. Jones, M. J. Morris, and H. Dobson. 2008. Lameness, activity time-budgets, and estrus expression in dairy cattle. J. Dairy Sci. 91:4552-4559.

Wang, F. X., D. F. Shao, S. L. Li, Y. J. Wang, A. Azarfar, and Z. J. Cao. 2016. Effects of stocking density on behavior, productivity, and comfort indices of lactating dairy cows. J. Dairy Sci. 99:3709-3717.

Watters, M. E. A., H. W. Barkema, K. E. Leslie, M. A. G. von Keyserlingk, and T. J. DeVries. 2014. Relationship between postmilking standing duration and risk of intramammary infection in freestall-housed dairy cows milked 3 times per day. J. Dairy Sci. 97:3456-3471.

Webster, A. J. 2001. Farm animal welfare: the five freedoms and the free market. Vet. J. 161:229-237.

Wechsler, B. 2007. Normal behavior as a basis for animal welfare assessment. Anim. Welfare 16:107-110.

Wierenga, H.K., and H. Hopster. 1990. The significance of cubicles for the behaviour of dairy cows. Appl. Anim. Behav. Sci. 26:309-337.

Woolpert, M. E., H. M. Dann, K. W. Cotanch, C. Melilli, L. E. Chase, R. J. Grant, and D. M. Barbano. 2016. Management, nutrition, and lactation performance are related to bulk tank milk de novo fatty acid concentration on northeastern US dairy farms. J. Dairy Sci. 99:8486-8497.

Yang, W. Z., and K. A. Beauchemin. 2006. Increasing the physically effective fiber content of dairy cow diets may lower efficiency of feed use. J. Dairy Sci. 89:2694-2704.

Zebeli, Q., B. U. Metzler-Zebeli, and B. N. Ametaj. 2012. Meta-analysis reveals threshold level of rapidly fermentable dietary concentrate that triggers systemic inflammation in cattle. J. Dairy Sci. 95:2662-2672.

Zebeli, Q., D. Mansmann, B. N. Ametaj, H. Steingab, and W. Drochner. 2010. A model to optimise the requirements of lactating dairy cows for physically effective neutral detergent fibre. Arch. Anim. Nut. 64:265-278.

Zebeli, Q., J. Dijkstra, M. Tafaj, H. Steingass, B. N. Ametaj, and W. Drochner. 2008. Modeling the adequacy of dietary fiber in dairy cows based on the responses of ruminal pH and milk fat production to composition of the diet. J. Dairy Sci. 91:2046-2066.

Zebeli, Q., M. Tafaj, H. Steingass, B. Metzler, and W. Drochner. 2006. Effects of physically effective fiber on digestive processes and milk fat content in early lactating dairy cows fed total mixed rations. J. Dairy Sci. 89:651-668.

Zebeli, Q., S. M. Dunn, and B. N. Ametaj. 2011. Perturbations of plasma metabolites correlated with the rise of rumen endotoxin in dairy cows fed diets rich in easily degradable carbohydrates. J. Dairy Sci. 94:2374-2382.

Zebeli, Q., V. Ölschläger, M. Tafaj, W. Vahjen, B. Junck, O. Simon, and W. Drochner. 2007a. Evaluation of counts of ruminal fibrolytic bacteria and enzyme activities in response to corn silage particle size in high-yielding dairy cows. J. Dairy Sci. 90(Suppl. 1):618-619. (Abstr.).

CHAPTER 2: PRELIMINARY RESEARCH: EVALUATING THE ADDITION OF DIETARY STRAW TO REDUCE SUB-ACUTE RUMINAL ACIDOSIS IN LACTATING DAIRY COWS

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ABSTRACT

The objective of this preliminary study was to measure the effectiveness of using chopped wheat straw to reduce sub-acute ruminal acidosis (SARA) when formulating diets for future studies. Four multiparous, lactating cannulated Holstein cows (2.3 ± 0.5 parity), 158 ± 21 days in milk (DIM), and 51.3 ± 5.7 kg/d milk production; mean ± standard deviation (SD) were used to test the effects of two diets designed to affect SARA: low straw (LS) and high straw (HS). Diets contained 32.4% corn silage and either 14.1% haycrop silage with 0.7% of wheat straw (LS) or 11.3% haycrop silage with 3.5% of wheat straw (HS; dry matter (DM) basis). Cows were assigned randomly to diets (n = 2/treatment) for one week. Cows then switched treatments for one additional week. Samples of TMR and orts were collected three times per treatment period, composited, and analyzed for particle size distribution, physical effectiveness, and sorting. Ruminal pH measurements were recorded each day using indwelling pH loggers and averaged across each weekly treatment period. Similarly, dry matter intake (DMI) and rumination were monitored daily and averaged across each weekly treatment period. Data were analyzed using descriptive statistics (mean ± SD). The physically effective neutral detergent fiber (peNDF) averaged 22.0 ± 0.1 and 19.5 ± 0.9% of DM for LS and HS, respectively. The lower peNDF associated with HS was likely due to the smaller physical effectiveness factor of straw compared to haycrop silage that resulted from the hammer mill processing technique used on the straw. Nonetheless, hours below pH 5.8 and area under the curve below pH 5.8 appeared to be less in three of the four cows on HS versus LS treatment. However, HS resulted in marginally lower DMI, more sorting, and lower

rumination. Replacement of haycrop silage with chopped straw effectively reduced SARA. Future dietary treatments designed to manipulate degree of SARA should limit the inclusion rate of chopped straw to 1.36 kg of DM to minimize reductions in DMI and rumination and to avoid sorting.

Key words: physically effective NDF, ruminal pH, SARA

INTRODUCTION

Physically effective neutral detergent fiber (peNDF) is calculated from the neutral detergent fiber (NDF) concentration of the analyzed sample and its physical effectiveness factor (pef), measuring the feed's ability to stimulate chewing activity and maintain a floating ruminal digesta mat (Mertens, 1997). Stone (2004) documented the basic relationship of dietary peNDF with associated risk of sub-acute ruminal acidosis (SARA); higher risk of SARA when peNDF is below 21% and lower risk when it is above 23% of dry matter (DM). While higher dietary peNDF increases ruminal pH (Zebeli et al., 2006), diets should be formulated for an optimal balance of peNDF at approximately 21 to 23% as excessive peNDF may constrain DMI and feed efficiency (Stone, 2004).

To formulate dietary treatments for future studies investigating the effects of stocking density and source of forage fiber on ruminal pH responses, this preliminary study was necessary to test the effects of dietary peNDF sources and their associated risks of SARA.

It was hypothesized that a one-to-one replacement (DM basis) of haycrop silage with chopped straw would increase dietary peNDF and daily mean pH, reduce time below pH 5.8 (defined as SARA) and maintain DMI. Therefore, the objective of this study was to evaluate the effectiveness of replacing haycrop silage with chopped wheat straw to alter the dietary peNDF content to simulate high- and low-risk SARA conditions.

MATERIALS AND METHODS

Animals, Housing, and Management

Four multiparous cannulated Holstein cows were housed in a naturally ventilated, sand bedded 4-row freestall barn at the William H. Miner Agricultural Research Institute (Chazy, NY) from August 7, 2014 to August 25, 2014. Cows averaged 2.3 ± 0.5 (mean ± standard deviation) parity, 158 ± 21 days in milk (DIM), 51.3 ± 5.7 kg/d milk production, and 759 ± 67 kg body weight prior to the start of the study. Cows were milked 3 times daily in a double-12 parallel parlor (Xpressway Parallel Stall System; Bou-Matic, Madison, WI). Total mixed rations were mixed and delivered for *ad libitum* intake once daily at approximately 0800h with a Calan broadbent feeding system (American Calan, Inc., Northwood, NH). Animal care and handling protocols were approved by the William H. Miner Agricultural Research Institute Animal Care and Use Committee.

Experimental Design and Treatments

Cows (n = 2/treatment) were assigned to one of two treatments: low straw (LS) or high straw (HS) for 7 d. Following 7 d, cows were then switched to the other treatment for another 7 d. Diets were similar except that a portion of haycrop silage was replaced with either 0.7 or 3.5% (dry matter (DM) basis) of chopped wheat straw (Table 2.1). Each diet was formulated using NDS Professional© based on the Cornell Net Carbohydrate and Protein System model (v. 6.1; RUM&N Sas, Reggio Emilia, Italy).

Dry Matter Intake

The dry matter intake (DMI) was measured daily for each cow and averaged across each weekly treatment period. Samples of TMR and orts were collected three times per week. In order to determine DM, samples were dried in a forced-air oven at 105°C for 24 h. Data were calculated for DMI (kg/d), DMI (% of BW), neutral detergent fiber (NDF) intake (kg/d), and NDF intake (% of BW).

Particle Size Distribution and Physical Effectiveness

Sub-samples of individual diets and orts were assessed for particle size distribution using the Penn State Particle Separator (as-fed basis; Lammers et al., 1996) with a 4-mm screen modification and dry (forced-air oven at 55°C for 48-h) vertical sieving (Ro-Tap testing sieve shaker model B, DM basis; W. S. Tyler Combustion Engineering, Inc., Mentor, OH) using a 1.18-mm sieve (Mertens, 1997). Particles retained above the 1.18-mm sieve were ground (2-mm grind; Cyclone Sample Mill; UDY

Corporation, Fort Collins, CO) and analyzed for NDF (ash-corrected) using the ANKOM A200 Fiber Analyzer filter bag technique (ANKOM Technology Corp., Fairport, NY; Van Soest et al., 1991) with α-amylase and sodium sulfite. The resultant values were used with a $pef_{1.18}$ (Ro-Tap) value to determine peNDF (Mertens, 2002).

Rumination

Each cow was fitted with a neck collar (SCR, Netanya, Israel; Schirmann et al., 2009) prior to the start of the study to monitor rumination (min/d). Rumination data were collected daily for each cow and averaged across each weekly treatment period.

Ruminal pH

Ruminal pH was measured using an indwelling ruminal pH/ORP/REDOX measurement system (Penner et al., 2006; LRCpH; Dascor, Escondido, CA) at 1-min intervals and averaged into 10-min intervals for each day of the study. Ruminal pH data were summarized as mean pH, time spent below pH 5.8 (h/d), and area under the curve below pH 5.8 (AUC, units x pH; Bauer et al., 1995). Daily ruminal pH measurements were averaged across each weekly treatment period for each cow.

Statistical Analysis

Due to time and sample size limitations inherent in this preliminary study, proper experimental design and power could not be achieved for analysis of variance. However, descriptive statistics are presented as mean ± standard deviation.

RESULTS AND DISCUSSION

Dietary evaluation of NDF, particle size distribution, and peNDF of each diet are presented in Table 2.2. Despite intentions to increase peNDF for the HS diet, the HS diet lowered NDF (29.5 ± 0.3 versus 27.9 ± 1.5% of DM, LS and HS, respectively) and dietary peNDF (22.0 ± 0.1 versus 19.5 ± 0.9 of DM, LS and HS respectively). Although individual feed ingredients were not analyzed for this study, it is likely that the processing technique (hay-busting; hammer-mill action) for the chopped straw decreased the pef value of the straw, placing greater NDF in the non-peNDF contributing fraction ($< pef_{1.18}$). This was evidenced in NDF analysis of fractions greater than $pef_{1.18}$ for each diet which resulted in $NDF_{>1.18}$ values of 35.9 ± 0.7 versus 31.0 ± 0.6 for LS and HS, respectively. Although pef (% as-fed) values of the total diet decreased slightly with HS, this is likely due to the low inclusion rate of the straw compared to other dietary ingredients.

Ruminal responses to dietary treatments are presented in Table 2.3. The addition of straw appeared to reduce hours below pH 5.8 (6.52 ± 3.03 versus 2.02 ± 1.37 for LS and HS, respectively) and area under the curve below pH 5.8 (1.33 ± 0.82 versus 0.29 ± 0.25 for LS and HS, respectively) as well as increase overall mean pH (5.99 ± 0.08 versus

6.16 ± 0.07 for LS and HS, respectively) for three of the four cows. Cow 3 seemed to exhibit ruminal responses opposite to the other three cows, but this is likely due to normal cow-to-cow variation as well as confounding factors including adverse health events and estrus that the cow exhibited during the study. The DMI and NDF intake responses to dietary treatments are presented in Table 2.4. The DMI (kg/d and % of BW) appeared to marginally decrease for cows fed HS compared to LS. Further, the combination of lower intake and a lower NDF percentage of the HS diet resulted in lower NDF intake, both as kg/d and as a percentage of BW. Sorting activity (actual intake as a percentage of predicted intake; Leonardi and Armentano, 2003; Table 2.5) was minimal (within 10%; Miller-Cushon and DeVries, 2017). However, it appeared that cows sorted for concentrate on HS with a greater standard deviation (5.3%) above 100% of predicted intake. It also appeared that cows sorted against longer particles with HS, as observed by both Ro-Tap pef values (0.63 ± 0.02 versus 0.66 ± 0.01 for LS and HS, respectively) and PSPS pef values (0.63 ± 0.02 versus 0.66 ± 0.03 for LS and HS, respectively). The combination of reduced NDF intake, lower peNDF values, and sorting appeared to result in small reductions in rumination on the HS treatment (Table 2.6), although the drop in rumination did not affect ruminal pH enough to counteract the positive effect of the straw on ruminal pH.

CONCLUSIONS

The objective of this preliminary study was to evaluate the effectiveness of replacing haycrop silage with chopped straw in order to manipulate dietary peNDF levels

and influence ruminal pH responses so that we would be able to better formulate diets that predictably affected ruminal pH. In contrast to the hypothesis that using chopped straw would increase dietary peNDF percentage, the HS treatment appeared to lower dietary NDF and peNDF content, likely due to shifts in particle size distribution of straw towards shorter particles with processing. Nonetheless, the addition of straw to the diet effectively reduced SARA as evidenced by increased daily mean pH and reductions in both time and severity below pH 5.8. Further research is needed to understand the effects of altering source of dietary fiber on difference in ruminal pH responses as the responses weren't due to increased chewing activity. Due to the possible adverse effects on DMI and rumination, as well as the increased risk of sorting, 1.36 kg DM/d of chopped straw appeared to be the upper limit for dietary inclusion. In order to maximize differences in ruminal pH responses while minimizing intake and sorting risks, diets used in the main studies of this dissertation to simulate increased versus decreased risk of SARA (low straw inclusion versus high straw inclusion) used approximately 1.36 kg DM/d or 3.5% of the total ration DM.

REFERENCES

Bauer, M. L., D. W. Herold, R. A. Britton, R. A. Stock, T. J. Klopfenstein, and D. A. Yates. 1995. Efficacy of laidlomycin propionate to reduce ruminal acidosis in cattle. J. Anim. Sci. 73:3445-3454.

Lammers, B. P., D. R. Buckmaster, and A. J. Heinrichs. 1996. A simple method for the analysis of particle sizes of forage and total mixed rations. J. Dairy Sci. 79:922-928.

Leonardi, C., and L. E. Armentano. 2003. Effect of quantity, quality, and length of alfalfa hay on selective consumption by dairy cows. J. Dairy Sci. 86:557-564.

Mertens, D. R. 1997. Creating a system for meeting the fiber requirements of dairy cows. J. Dairy Sci. 80:1463-1481.

Mertens, D. R. 2002. Physical and chemical characteristics of fiber affecting dairy cow performance. Pages 125-144 in Cornell Nut. Conf., Proc. 2002., Dept. Anim. Sci., Cornell Univ., Ithaca, NY.

Miller-Cushon, E. K., and T. J. DeVries. 2017. Feed sorting in dairy cattle: Causes, consequences, and management. J. Dairy Sci. 100:4172-4183.

Penner, G. B., K. A. Beauchemin, and T. Mutsvangwa. 2006. An evaluation of the accuracy and precision of a stand-alone submersible continuous ruminal pH measurement system. J. Dairy Sci. 89:2132-2140.

Schirmann, K., M. A. G. von Keyseylingk, D. M. Weary, D. M. Veira, and W. Heuwieser. 2009. Technical note: Validation of a system for monitoring rumination in dairy cows. J. Dairy Sci. 92:6052-6055.

Stone, W. C. 2004. Nutritional approaches to minimize subacute rumen acidosis and laminitis in dairy cattle. J. Dairy Sci. 87(E-Suppl.):E13-E26.

Van Soest, P. J., J. B. Robertson, and B. A. Lewis. 1991. Methods for dietary fiber, neutral detergent fiber, and nonstarch polysaccharides in relation to animal nutrition. J. Dairy Sci. 74:3583-3597.

Zebeli, Q., M. Tafaj, H. Steingass, B. Metzler, and W. Drochner. 2006. Effects of physically effective fiber on digestive processes and milk fat content in early lactating dairy cows fed total mixed rations. J. Dairy Sci. 89:651-668.

Table 2.1. Ingredient composition (dry matter (DM) basis) of TMR samples for low straw (LS) and high straw (HS) diets.

Item	LS	HS
Ingredient, % of DM		
Conventional corn silage	32.4	32.4
Haycrop silage	14.1	11.3
Wheat straw, chopped[1]	0.7	3.5
Concentrate mix[2]	52.8	52.8

[1]Hay-busted; hammer-mill chopping technique; mo. #H1100, Duratech Industries Inc., Jamestown, North Dakota.

[2]Concentrate mix was composed of the following (% of DM): corn meal (34.18), citrus pulp (14.26), soybean meal, 48% CP (13.14), canola meal, solvent (9.56), AminoMax (Afgritech, LLC. Watertown, NY; 7.76), wheat, red dog (5.84), blood meal (3.31), molasses (2.89), Berga fat (Berg+ Schmidt GmbH & Co., Hamburg, Germany; 2.55), calcium carbonate (2.13), sodium sesquicarbonate (1.69), Salt (0.80), magnesium oxide (0.65), urea (0.40, trace minerals (contained 6.73% S, 2.73% Ca, 1.46% K, 0.34% P, 0.31% Mg, 0.23% Cl, 0.03% Na, 54,693 mg/kg Zn, 48,078 mg/kg Mn, 18,419 mg/kg Fe, 7,313 mg/kg Cu, 1,031 mg/kg Co, 733 mg/kg I, and 320 mg/kg Se which contained 147 mg/kg organic Se; 0.23), calcium phosphate dicalcium (0.19), Smartamine M (Adisseo USA Inc., Alpharetta, GA; 0.13), ClariFly, 0.67% (Central Garden and Pet Company, Schaumburg, IL; 0.08), vitamin A, D, and E premix (contained 24,097 kIU/kg vitamin A, 92.7 kIU/kg vitamin E, 5,553 kIU/kg vitamin D3, 29.2% Ca, and 0.98% Mg; 0.06), Availa 4 (Zinpro Corporation, Eden Prairie, MN; 0.06), vitamin E premix (contained 88.18 kIU vitamin E, 7.08 mg/kg Cu; 0.02), Rumensin 90 (Elanco Animal Health, Indianapolis, IN; 0.02), and biotin (0.02).

Table 2.2. The NDF content and physical characterization of TMR samples for low straw (LS) and high straw (HS) diets.

Item	LS	HS
NDF, % of dry matter (DM)	29.5 ± 0.3[1]	27.9 ± 1.5
Particle size distribution, % as-fed		
>19.0 mm	5.2 ± 1.3	5.7 ± 1.0
8.0 to 19.0 mm	42.7 ± 2.4	42.3 ± 2.4
4.0 to 8.0 mm	14.2 ± 0.8	13.5 ± 1.0
<4.0 mm	37.9 ± 3.2	38.5 ± 2.1
Particle size distribution, % of DM		
>19.00 mm	0.3 ± 0.4	0.4 ± 0.4
13.20 to 19.00 mm	0.7 ± 0.3	0.2 ± 0.2
9.50 to 13.20 mm	2.0 ± 0.3	1.6 ± 0.4
6.70 to 9.50 mm	6.9 ± 1.1	7.1 ± 1.5
4.75 to 6.70 mm	9.7 ± 1.3	9.2 ± 1.7
3.35 to 4.75 mm	11.2 ± 0.1	11.8 ± 1.2
2.36 to 3.35 mm	10.0 ± 0.7	9.8 ± 0.4
1.18 to 2.36 mm	21.0 ± 0.2	20.7 ± 0.4
0.60 to 1.18 mm	18.4 ± 1.6	18.8 ± 1.0
0.30 to 1.60 mm	13.3 ± 0.9	13.6 ± 0.6
<0.30 mm	6.5 ± 0.2	6.8 ± 0.3
pef[2], % of DM	0.62 ± 0.03	0.61 ± 0.02
peNDF[3], % of DM	22.0 ± 0.1	19.5 ± 0.9

[1]Mean ± standard deviation.
[2]Physical effectiveness factor.
[3]Physically effective neutral detergent fiber.

Table 2.3. Daily ruminal pH responses of cows fed low straw (LS) and high straw (HS) diets.

Variable	LS	HS
pH < 5.8, h/d		
Cow 1	10.43 ± 3.97[1]	6.21 ± 1.86
Cow 2	6.07 ± 5.05	0.93 ± 1.29
Cow 3	0.00 ± 0.00	0.76 ± 1.87
Cow 4	9.57 ± 3.11	0.17 ± 0.44
Average	6.52 ± 3.03	2.02 ± 1.37
AUC < pH 5.8, units x pH[2]		
Cow 1	2.33 ± 1.34	0.95 ± 0.52
Cow 2	0.81 ± 0.72	0.02 ± 0.03
Cow 3	0.00 ± 0.00	0.11 ± 0.29
Cow 4	2.17 ± 1.20	0.06 ± 0.16
Average	1.33 ± 0.82	0.29 ± 0.25
Mean pH		
Cow 1	5.84 ± 0.09	5.94 ± 0.07
Cow 2	6.00 ± 0.10	6.14 ± 0.03
Cow 3	6.27 ± 0.05	6.22 ± 0.10
Cow 4	5.86 ± 0.07	6.33 ± 0.09
Average	5.99 ± 0.08	6.16 ± 0.07

[1]Mean ± standard deviation.
[2]AUC; area under the curve below pH 5.8.

Table 2.4. Feed intake responses of cows fed low straw (LS) and high straw (HS) diets.

Variable	LS	HS
DMI[1], kg/d		
Cow 1	25.6 ± 1.5[2]	25.3 ± 1.5
Cow 2	30.2 ± 2.5	26.9 ± 2.8
Cow 3	28.1 ± 1.8	26.3 ± 2.6
Cow 4	26.2 ± 2.3	25.4 ± 3.1
Average	27.5 ± 2.0	26.0 ± 2.5
DMI, % of BW		
Cow 1	3.37 ± 0.20	3.30 ± 0.20
Cow 2	3.69 ± 0.30	3.19 ± 0.33
Cow 3	3.83 ± 0.25	3.61 ± 0.35
Cow 4	3.69 ± 0.33	3.62 ± 0.44
Average	3.65 ± 0.27	3.43 ± 0.33
NDF[3] intake, kg/d		
Cow 1	7.6 ± 0.5	6.8 ± 0.4
Cow 2	9.0 ± 0.7	7.2 ± 0.7
Cow 3	8.2 ± 0.5	7.6 ± 0.8
Cow 4	7.7 ± 0.7	7.4 ± 0.9
Average	8.1 ± 0.6	7.3 ± 0.7
NDF intake, % of BW		
Cow 1	1.00 ± 0.06	0.88 ± 0.05
Cow 2	1.10 ± 0.09	0.86 ± 0.09
Cow 3	1.12 ± 0.07	1.05 ± 0.10
Cow 4	1.08 ± 0.10	1.05 ± 0.13
Average	1.08 ± 0.08	0.96 ± 0.09

[1]Dry matter intake.
[2]Mean ± standard deviation.
[3]Neutral detergent fiber.

Table 2.5. Sorting responses (actual intake as a percentage of predicted intake) of cows fed low straw (LS) and high straw (HS) diets.

Variable	LS	HS
Ro-Tap[1]		
>19.00 mm	100.20 ± 0.24[2]	100.33 ± 0.10
13.20 to 19.00 mm	100.47 ± 0.23	99.61 ± 0.56
9.50 to 13.20 mm	100.35 ± 0.33	99.62 ± 1.06
6.70 to 9.50 mm	99.90 ± 0.75	99.24 ± 1.75
4.75 to 6.70 mm	99.37 ± 0.63	98.08 ± 1.40
3.35 to 4.75 mm	98.84 ± 1.01	99.00 ± 0.28
2.36 to 3.35 mm	99.64 ± 0.34	98.54 ± 0.76
1.18 to 2.36 mm	99.72 ± 0.31	100.32 ± 0.78
0.60 to 1.18 mm	99.54 ± 0.42	102.53 ± 2.40
0.30 to 0.60 mm	100.98 ± 0.82	101.76 ± 2.40
<0.30 mm	100.99 ± 0.92	100.98 ± 0.18
Average pef[3]	0.63 ± 0.02	0.66 ± 0.01
PSPS[4]		
>19.0 mm	101.1 ± 1.3	98.8 ± 1.6
8.0 to 19.0 mm	97.8 ± 2.1	97.5 ± 4.2
4.0 to 8.0 mm	99.9 ± 1.0	99.4 ± 0.5
<4.0 mm	101.3 ± 3.9	104.3 ± 5.3
Average pef[5]	0.63 ± 0.02	0.66 ± 0.03

[1]Ro-Tap testing sieve shaker (model B, W. S. Tyler Combustion Engineering, Inc., Mentor, OH).
[2]Mean ± standard deviation.
[3]Proportion of TMR (DM basis) > 1.18 mm.
[4]Penn State Particle Separator
[5]Proportion of TMR (as-fed basis) > 4 mm; Penn State Particle Separator physical effectiveness factor.

Table 2.6. Rumination responses of cows fed low straw (LS) and high straw (HS) diets.

Variable	LS	HS
Rumination, min/d		
Cow 1	454 ± 48[1]	422 ± 28
Cow 2	513 ± 23	470 ± 39
Cow 3	433 ± 30	449 ± 55
Cow 4	599 ± 24	564 ± 34
Average	500 ± 31	476 ± 39

[1]Mean ± standard deviation.

CHAPTER 3: PRELIMINARY RESEARCH: EFFECTS OF TYPE OF BLOOD TUBE AND ASSAY ON HAPTOGLOBIN CONCENTRATIONS FROM LACTATING DAIRY COWS

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ABSTRACT

Haptoglobin, an acute phase protein, serves as a biomarker for stress and inflammation in dairy cows. Consequently, obtaining an accurate value for haptoglobin is vital for research and on-farm management decisions. Blood collection methods reported in peer-reviewed articles differ greatly when similar assays are performed. The objective of this study was to determine the effect of type of blood collection tube on haptoglobin concentration across two commercially-available haptoglobin assays and to evaluate agreement between assays. Coccygeal blood was obtained from 21 early lactation, 9 unhealthy, and 30 late lactation dairy cows from three farms in order to obtain a range in haptoglobin concentrations. For each cow, blood was collected into four separate 10-mL BD Vacutainer tubes: serum separator, lithium heparin, sodium heparin, and K_2-EDTA. Blood was then processed according to tube type. Plasma and serum were analyzed for haptoglobin concentration using a colorimetric assay (Tri-Delta Development Ltd; Maynooth, Ireland) and an ELISA assay (Life Diagnostics, Inc., West Chester, PA). Intra-assay and inter-assay CV were 3.2% and 4.3% for the colorimetric assay and 12.7% and 5.0% for the ELISA assay, respectively. Data were reduced into a smaller dataset using distanced-based redundancy analysis, logarithmically transformed, and analyzed using a MIXED model in JMP with cow as the experimental unit. In order to assess bias, data were analyzed for agreement between tubes and agreement between assays using the Bland-Altman method with the serum separator tube serving as the gold-standard. A maximum allowable difference was set at the largest variation (intra-assay) reported by each manufacturer at ± 0.15 mg/mL for the Tri-Delta colorimetric assay, ± 0.20 mg/mL

for the Life Diagnostics ELISA assay, and ± 0.20 mg/mL for the assay comparison. Haptoglobin concentrations were lower for serum compared with lithium heparinized, sodium heparinized, and K_2-EDTA plasma using the Tri-Delta colorimetric assay. Compared to serum, there was a lack of agreement with lithium heparinized, sodium heparinized, and K2-EDTA plasma with mean and slope biases. These results indicated greater disagreement among tubes at higher haptoglobin concentrations. The use of lithium heparinized, sodium heparinized, and K_2-EDTA plasma for haptoglobin analysis using the Tri-Delta colorimetric assay overestimated haptoglobin concentrations due to interference with assay reagents and isn't recommended. There were no differences between blood tube types using the Life Diagnostics ELISA assay with adequate agreement amongst blood tubes. Heparinized or K_2-EDTA should be analyzed using the Life Diagnostics assay as opposed to the Tri-Delta colorimetric assay. However, there was a lack of agreement between assays with mean and slope biases, indicating haptoglobin concentrations are higher with the Tri-Delta colorimetric assay. Therefore, further research is needed to identify which assay serves as a gold-standard and haptoglobin will not be analyzed for the main studies in this dissertation.

Key words: haptoglobin, inflammation, vacutainer

INTRODUCTION

Haptoglobin, a positive acute phase protein, is produced in the liver and increases in response to tissue damage and inflammation (Baumann and Gauldie, 1994). Normal dairy cattle haptoglobin concentrations are typically less than 0.1 mg/mL while disease

conditions such as mastitis, metritis, and respiratory infections result in concentrations greater than 1.0 mg/mL (Skinner et al., 1991; Nazifi et al., 2008).

In response to digestive tract infections, such as sub-acute ruminal acidosis (SARA), cows will exhibit increased concentrations of haptoglobin of 0.6 mg/mL (Gozho et al., 2005; Khafipour et al., 2006; Nazifi et al., 2008) and haptoglobin concentration can be useful to identify chronic inflammation in dairy cattle as a result of SARA (Horadagoda et al., 1999). With future studies in this dissertation focusing on stocking density, marginal physically effective fiber, and reduced feed access, cows are likely to experience SARA and haptoglobin concentrations may be a useful biomarker in determining effects of these variables on stress responses.

However, based on exploratory research for this dissertation, there appeared to be differences in the clarity of samples in the wells of the Tri-Delta PHASE colorimetric haptoglobin assay when using heparinized plasma. Despite several published studies utilizing sodium heparinized plasma to analyze haptoglobin concentrations with the Tri-Delta colorimetric assay (Huzzey et al., 2011; Yasui et al., 2014; McCarthy et al., 2016), and a claim of equivalence between serum and lithium heparinized plasma from Tri-Delta Development Ltd (Maynooth, Ireland), the exploratory research prompted the need to evaluate haptoglobin concentrations derived from serum and plasma.

Based on the exploratory research, it was hypothesized that there would be differences and lack of agreement between the heparinized and K2¬-EDTA plasma haptoglobin concentrations compared to serum haptoglobin concentrations when using the Tri-Delta ELISA assay. Furthermore, we hypothesized that there would be no

difference or lack of agreement between the assays. The primary objective of this study was to determine the effect of type of blood collection tube on haptoglobin concentrations across two commercially-available haptoglobin assays to identify an appropriate assay for heparinized or K_2-EDTA plasma samples taken for the main studies in this dissertation. The secondary objective of this study was to evaluate agreement between the commercially-available haptoglobin assays used in this study.

MATERIALS AND METHODS

Animal Descriptions

The study was conducted in February 2016 at the William H. Miner Agricultural Research Institute (Chazy, NY; farm A) and two additional local dairy farms (Champlain, NY; farm B and Chazy, NY; farm C). The cows in this study were cared for and subjected to sampling protocols approved by the William H. Miner Agricultural Research Institute's Animal Care and Use Committee. A total of 60 Holstein dairy cows were sampled across the three locations (n = 20 samples/farm). To obtain a range in haptoglobin concentrations, samples were taken from 21 early lactation cows (9.2 ± 9.1 days in milk, 2.3 ± 1.6 parity; mean ± standard deviation), 9 unhealthy cows (178.1 ± 119.1 DIM, 2.9 ± 1.8 parity), and 30 late lactation cows (307.1 ± 33.5 DIM, 2.0 ± 1.1 parity).

Blood Measurements

Coccygeal blood samples were collected from each cow into four types of 10-mL vacutainer tubes (BD, Franklin Lakes, NJ): serum separator, lithium heparinized plasma (158 USP spray-coated), sodium heparinized plasma (158 USP spray-coated), and K_2-EDTA plasma (18 mg spray-coated) in order as listed. Samples of lithium heparinized plasma, sodium heparinized plasma, and K_2-EDTA plasma were immediately placed on ice until processing. Plasma samples were centrifuged at 1200 x *g* for 20 min at 4° C. Serum samples were allowed to clot for one hour at room temperature before processing. Serum samples were centrifuged at 1200 x *g* for 20 min at 22°C. Following centrifugation, all samples were aliquoted (sample for each of the two assays) into 2-mL cryogenic vials (Fisher Scientific, Pittsburgh, PA) and stored at -20°C until analysis. Processing of samples was completed within 2 h following collection.

Tri-Delta Colorimetric Assay

The colorimetric assay was performed according to the procedures provided by the manufacturer (Tri-Delta PHASE haptoglobin assay, cat. no. TP-801; Tri-Delta© Development Ltd; Maynooth, Ireland). Plate absorbances were read in a microplate reader (BioTek Synergy 2; Winooski, VT) at 630 nm OD. All samples were analyzed in duplicate. The intra-assay and inter-assay CV were calculated at 3.2% and 4.3%, respectively.

Life Diagnostics ELISA Assay

The ELISA assay was performed according to the procedures provided by the manufacturer (Life Diagnostics Cow Haptoglobin ELISA assay, cat. no. HAPT-11; Life Diagnostics, Inc., West Chester, PA). Plate absorbances were read in a microplate reader (BioTek Synergy 2; Winooksi, VT) at 450 nm OD. All samples were analyzed in duplicate. The intra-assay and inter-assay CV were calculated at 12.7% and 5.0%, respectively.

Statistical Analysis

Tube Type Comparison

Due to high similarity of low haptoglobin responses among samples, data exhibited a large right tail skew. Therefore, a new dataset was created using distanced based redundancy analysis (Euclidean distances obtained through Principal Component Analysis matrices (Legendre and Anderson, 1999) using JMP (SAS Institute Inc., Cary, NC) on data obtained from serum samples. Samples were systematically reduced (lowest to highest squared Euclidian distance) until data met normality assumptions ($W \geq 0.90$; "$P < W$" ≥ 0.05) using the Shapiro-Wilk test (Shapiro and Wilk, 1965). This resulted in the removal of half of the observations from the data set with a squared Euclidian distance of less than 39.7. Remaining samples were equally representative of each farm, with 10 samples from farm A (4 early lactation cows, 4 sick cows, and 2 late lactation cows; 108 ± 106 DIM and 1.7 ± 0.8 parity), 9 samples from farm B (7 early lactation cows and 2 late lactation cows; 73 ± 141 DIM and 1.9 ± 1.7 parity), and 11 samples from

farm C (5 early lactation cows, 3 sick cows, and 3 late lactation cows; 174 ± 170 DIM and 2.7 ± 1.6 parity). Data were transformed using Box Cox Y transformations with Λ value of 0.4 (Box and Cox, 1964). Transformed data were analyzed using a MIXED model in JMP using the following model:

$$Y_{ij} = \mu + S_i + C_j + E_{ij}$$

where Y_{ij} is the dependent variable (haptoglobin concentration), μ is the overall mean, S_i is the fixed effect of tube type, C_j is the random effect of cow, and E_{ij} is the residual error. Means separation was conducted using Tukey's HSD procedure with significance declared at $P \leq 0.05$ and trends at $0.05 < P \leq 0.10$.

Data were analyzed for agreement between tube types using the Bland-Altman method (Bland and Altman, 1986). Maximum allowable differences (MAD) were determined at ± 0.15 mg/mL and ± 0.20 mg/mL for Tri-Delta colorimetric assay and Life Diagnostics ELISA assay, respectively, based on the larger variation (intra-assay; Life Diagnostics) reported by both manufacturers. Serum separator tubes served as the gold-standard in comparison with the plasma tubes due to its accepted used in both assays. Differences in slopes were determined using linear regression analysis in JMP. Mean biases were determined using JMP as the mean of the difference responses. Significance was declared at $P \leq 0.05$ and trends at $0.05 < P \leq 0.10$.

Assay Comparison

Data from serum separator tubes served as the gold-standard for assay comparison due to its accepted use in both assays. Data were analyzed for agreement between assays

using the Bland-Altman method (Bland and Altman, 1986) with a maximum allowable difference (MAD) at ± 0.20 mg/mL, based on the larger variation (intra-assay variation) reported by both manufacturers. Difference in slope was determined using linear regression analysis in JMP. Significance was declared at $P \leq 0.05$ and trends at $0.05 < P \leq 0.10$.

RESULTS AND DISCUSSION

Tube Type Comparison

Tube type comparison data for each assay is shown in Table 3.1. Haptoglobin concentrations from lithium heparinized, sodium heparinized, and K_2-EDTA plasma were greater compared to serum for the Tri-Delta colorimetric assay. Upon visual assessment (Figure 3.1), wells with heparinized plasma or K_2-EDTA appeared cloudier than those with serum. Due to the colorimetric methodology used in the Tri-Delta assay, increases in sample cloudiness would result in greater light absorption, interference with the optical density, and elevated haptoglobin concentrations. The increase in sample cloudiness followed the addition of reagent two, indicating possible interactions among the chemicals in reagent two with the heparin or K_2-EDTA from each of the plasma blood tubes. Due to company proprietorship, the specific chemical in reagent two causing the interference is unknown. Further communication with the company revealed changes in the chemical reagents in 2009, suggesting that differences between blood tubes may only affect studies using this assay following the change in 2009 (Personal communication, Paul Mitchell; Tri-Delta, Ltd.).

Agreements between blood tubes for the Tri-Delta colorimetric assay are shown in Figures 3.2 to 3.4. Lithium heparinized, sodium heparinized, and K_2-EDTA plasma exhibited a lack of agreement when compared to the gold-standard of serum, exceeding the positive MAD set at ± 0.15 mg/mL. Each comparison resulted in positive slope biases ($P < 0.01$), indicating greater disagreement between blood tubes at high concentrations of haptoglobin. Slope biases were highly correlated, but only explained 71%, 73%, and 72% of the variation between lithium heparinized, sodium heparinized, and K_2-EDTA plasma with serum, respectively. Furthermore, each comparison resulted in positive mean biases of 0.86 mg/mL, 0.82 mg/mL, and 0.86 mg/mL for lithium heparinized, sodium heparinized, and K_2-EDTA plasma, respectively. Due to the existence of mean biases and slope biases and 27-29% unexplained variation, correction factors between the various plasma haptoglobin concentrations and serum haptoglobin concentrations could not be developed. As mean and slope biases exceeded the difference between healthy cattle and diseased cattle (Skinner et al., 1991), differences due to blood tube type would result in biologically meaningful differences.

There were no differences observed between blood tubes for the Life Diagnostics ELISA assay (Table 3.1; $P = 0.16$). Agreements between blood tubes for the Life Diagnostics ELISA assay are shown in Figures 3.5 to 3.7. Majority of data fell within the MAD for each comparison indicating adequate agreement between heparinized blood tubes and serum. However, it was noted that comparisons began to exceed the MAD with higher averages, indicating increasing disagreement with higher concentrations of haptoglobin. Mean biases were minimal and fell within the MAD at 0.02 mg/mL, -0.02

mg/mL, and 0.01 mg/mL for lithium heparinized, sodium heparinized, and K_2-EDTA plasma, respectively. There were no significant slope biases for any comparison (P = 0.27, lithium heparinized plasma; P = 0.20, sodium heparinized plasma; and P = 0.56, K_2-EDTA plasma). With no differences and adequate agreement between the plasma blood tubes and the serum blood tube, it is recommended that blood samples taken into lithium heparinized, sodium heparinized, or K_2-EDTA plasma tubes be analyzed using the Life Diagnostics ELISA assay instead of the Tri-Delta colorimetric assay.

Assay comparison

Agreement between haptoglobin assays is shown in Figure 3.8. There was a lack of agreement between the Tri-Delta colorimetric assay and the Life Diagnostics ELISA assay with a positive mean bias of 0.24 mg/mL, exceeding the MAD set at ± 0.20 mg/mL to exceed manufacturer intra- and inter-assay variation for both assays. This indicates that haptoglobin concentrations were elevated with the Tri-Delta colorimetric assay compared to the Life Diagnostics ELISA assay. In addition, the disagreement resulted in a positive slope bias ($P < 0.01$) indicating greater lack of agreement at higher concentrations of haptoglobin in the sample. However, the slope regression only explained 74% of the variation between assays. Due to the existence of both mean and slope biases and 26% unexplained variation, a correction factor between the assays could not be determined. Though slope and mean biases were smaller than the tube type comparison with the Tri-Delta assay, the differences are still likely to affect whether samples are biologically

different. Therefore, further research is needed to identify which of the commercially-available haptoglobin assays serves as the gold-standard for haptoglobin analysis.

CONCLUSIONS

With lack of agreement and differences in haptoglobin concentrations between blood tubes using the Tri-Delta colorimetric assay, it is not recommended to use lithium heparinized, sodium heparinized, or K_2-EDTA plasma with this assay. The Life Diagnostics ELISA assay demonstrated agreement between blood tubes with no differences in haptoglobin concentrations, indicating it as an appropriate assay to analyze haptoglobin concentrations derived from lithium heparinized, sodium heparinized, or K_2-EDTA plasma. Further analysis revealed a lack of agreement between the two assays. Due to this lack of agreement, additional research is needed to identify a gold-standard assay to analyze future samples obtained from the main studies of this dissertation. Therefore, haptoglobin concentrations will not be reported in later studies.

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REFERENCES

Baumann, H., and J. Gauldie. 1994. The acute phase response. Imm. Today. 15:74-80.

Bland, J. M., and D. G. Altman. 1986. Statistical methods for assessing agreement between two methods of clinical measurement. Lancet. 327:307-310.

Box, G. E. P., and D. R. Cox. 1964. An analysis of transformations. J. Royal Statistical Society, Series B, 26:211-252.

Gozho, G. N., J. C. Plaizier, D. O. Krause, A. D. Kennedy, and K. M. Wittenberg. 2005. Subacute ruminal acidosis induces ruminal lipopolysaccharide endotoxin release and triggers and inflammatory response. J. Dairy Sci. 88:1399-1403.

Horadagoda, N. U., K. M. G. Knox, H. A. Gibbs, S. W. J. Reid, A. Horadagoda, S. E. R. Edwards, P. D. Eckersall. 1999. Acute phase proteins in cattle: discrimination between acute and chronic inflammation. Vet. Rec. 144:437-441.

Huzzey, J. M., D. V. Nydam, R. J. Grant, and T. R. Overton. 2011. Associations of prepartum plasma cortisol, haptoglobin, fecal cortisol metabolites, and nonesterified fatty acids with postpartum health status in Holstein dairy cows. J. Dairy Sci. 94:5878-5889.

Khafipour, E., D. O. Krause, and J. C. Plaizier. 2006. Influence of grain induced sub-acute ruminal acidosis (SARA) on lipopolysaccharide endotoxin (LPS) and acute phase proteins. Can. J. Anim. Sci. 86:577 (Abstr.).

Legendre P., and M. J. Anderson. 1999. Distance-based redundancy analysis: Testing multispecies responses in multifactorial ecological experiments. Ecol. Monogr. 69:1-24.

McCarthy, M. M., T. Yasui, M. J. B. Felippe, and T. R. Overton. 2016. Associations between the degree of early lactation inflammation and performance, metabolism, and immune function in dairy cows. J. Dairy Sci. 99:680-700.

Nazifi, S., A. Rezakhani, M. Koohimoghadam, M. Ansari-Lari, and Z. Esmailnezhad. 2008. Evaluation of serum haptoglobin in clinically healthy cattle and cattle with inflammatory diseases in Shiraz, a tropical area in southern Iran. Bul. J. Vet. Med. 11:95-101.

Shapiro, S. S., and M. B. Wilk. 1965. An analysis of variance test for normality. Biometrika. 52:591-611.

Skinner, J. G., R. A. LI. Brown, and L. Roberts. 1991. Bovine haptoglobin response in clinically defined field conditions. Vet. Rec. 128:147-149.

Yasui, T., K. McCann, R. O. Gilbert, D. V. Nydam, and T. R. Overton. 2014. Associations of cytological endometritis with energy metabolism and inflammation during the periparturient period and early lactation in dairy cows. J. Dairy Sci. 97:2763-2770.

Table 3.1. Analysis of haptoglobin concentrations across blood tubes using colorimetric and ELISA assays.

	Tube Type					
Assay	Serum[1]	Lithium[2]	Sodium[3]	K_2-EDTA[4]	SE	*P*-value
Colorimetric[5]	0.86[a]	1.71[b]	1.67[b]	1.72[b]	0.20	<0.01
ELISA[6]	0.62	0.60	0.64	0.60	0.11	0.16

[1]Serum sepatator tube (BD, Franklin Lakes, NJ).
[2]Lithium herpanized plasma tube, 158 USP spray-coated (BD, Franklin Lakes, NJ).
[3]Sodium heparinized plasma tube, 158 USP spray-coated (BD, Franklin Lakes, NJ).
[4]K_2-EDTA plasma tube, 18 mg spray-coated (BD, Franklin Lakes, NJ).
[5]PHASE haptoglobin assay, cat. no. TP-801; Tri-Delta Development Ltd; Maynooth, Ireland.
[6]Cow haptoglobin ELISA (cat. no. HAPT-11); Life Diagnostics, Inc., West Chester, PA.
[ab] $P \leq 0.05$.

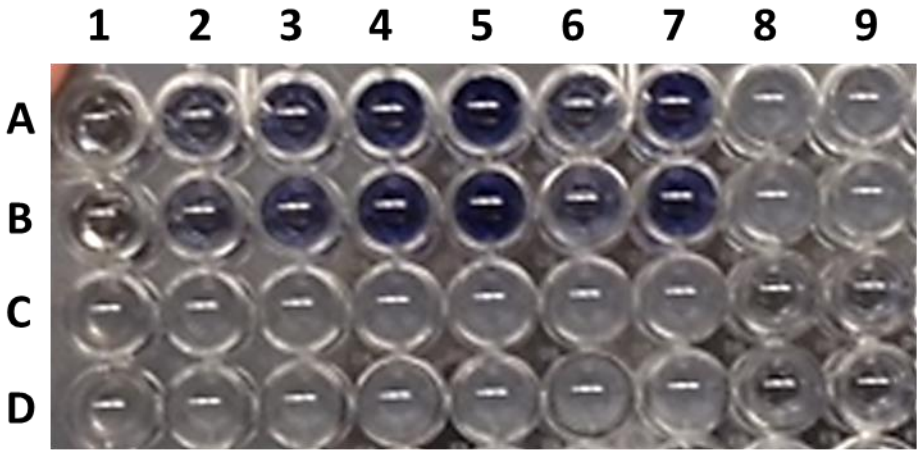

Figure 3.1. Visual assessment across blood tube types using Tri-Delta colorimetric haptoglobin assay. Samples plated in vertical duplicates: A1-A5, standard curve; A6-A7, low and high haptoglobin controls, respectively; A8-A9, lithium heparinized plasma; C1-C3, sodium heparinized plasma; C4-C7, K_2-EDTA plasma; and C8-C9, serum separator tube.

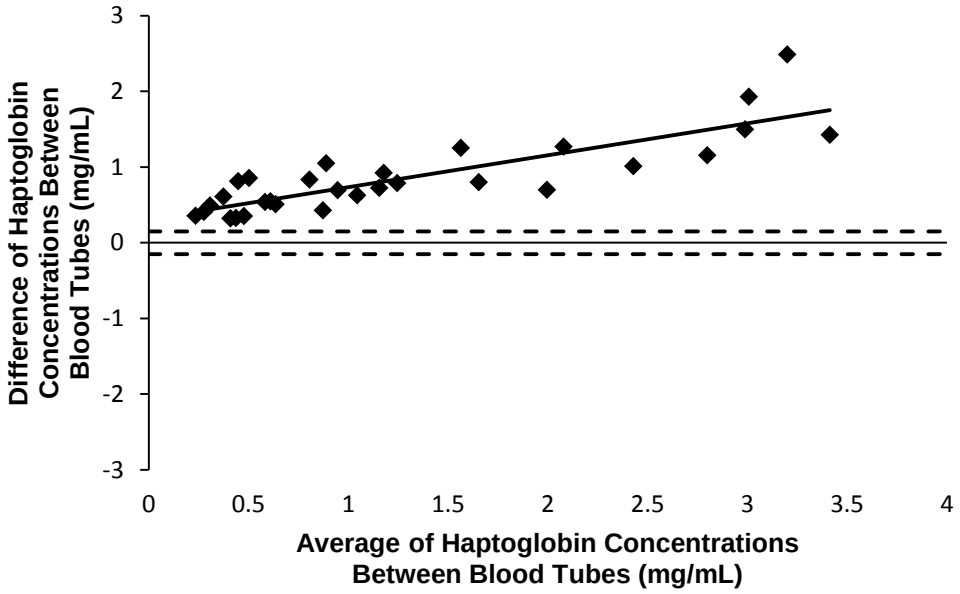

Figure 3.2. Analysis of agreement between blood tube types with Tri-Delta PHASE haptoglobin assay (cat. no. TP-801; Tri-Delta© Development Ltd; Maynooth, Ireland) for serum separator and lithium heparinized plasma blood tubes; $y = 0.42x + 0.31$; $R^2 = 0.71$; mean bias of 0.86 mg/mL; slope bias $P < 0.01$. Maximum allowable differences (dotted lines) set at ± 0.15 mg/mL; largest variation (intra-assay) reported by manufacturer.

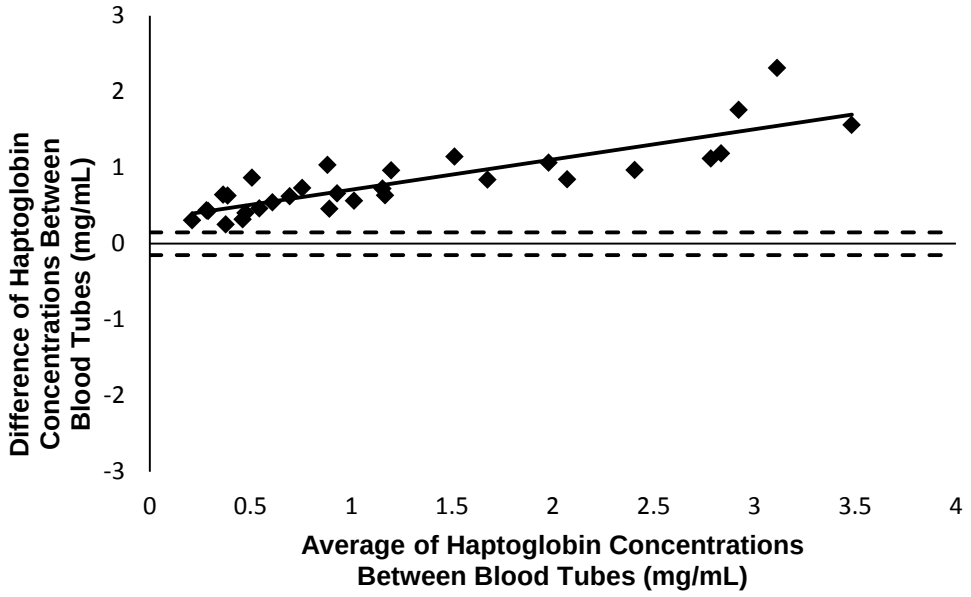

Figure 3.3. Analysis of agreement between blood tube types with Tri-Delta PHASE haptoglobin assay (cat. no. TP-801; Tri-Delta© Development Ltd; Maynooth, Ireland) for serum separator and sodium heparinized plasma blood tubes; $y = 0.40x + 0.31$; $R^2 = 0.73$; mean bias of 0.82 mg/mL; slope bias $P < 0.01$. Maximum allowable differences (dotted lines) set at ± 0.15 mg/mL; largest variation (intra-assay) reported by manufacturer.

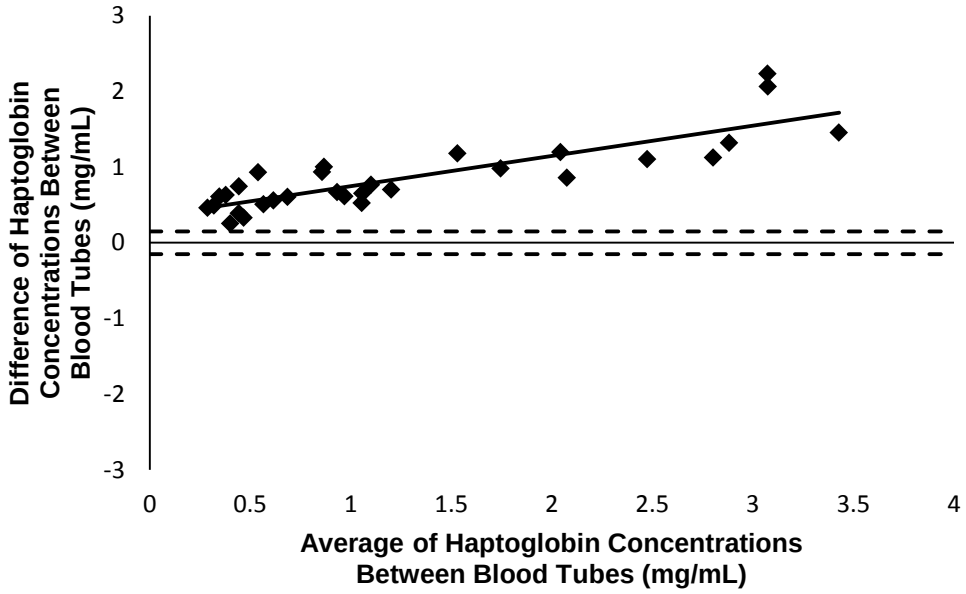

Figure 3.4. Analysis of agreement between blood tube types with Tri-Delta PHASE haptoglobin assay (cat. no. TP-801; Tri-Delta© Development Ltd; Maynooth, Ireland) for serum separator and K2-EDTA blood tubes; $y = 0.40x + 0.35$; $R^2 = 0.72$; mean bias of 0.86 mg/mL; slope bias $P < 0.01$. Maximum allowable differences (dotted lines) set at ± 0.15 mg/mL; largest variation (intra-assay) reported by manufacturer.

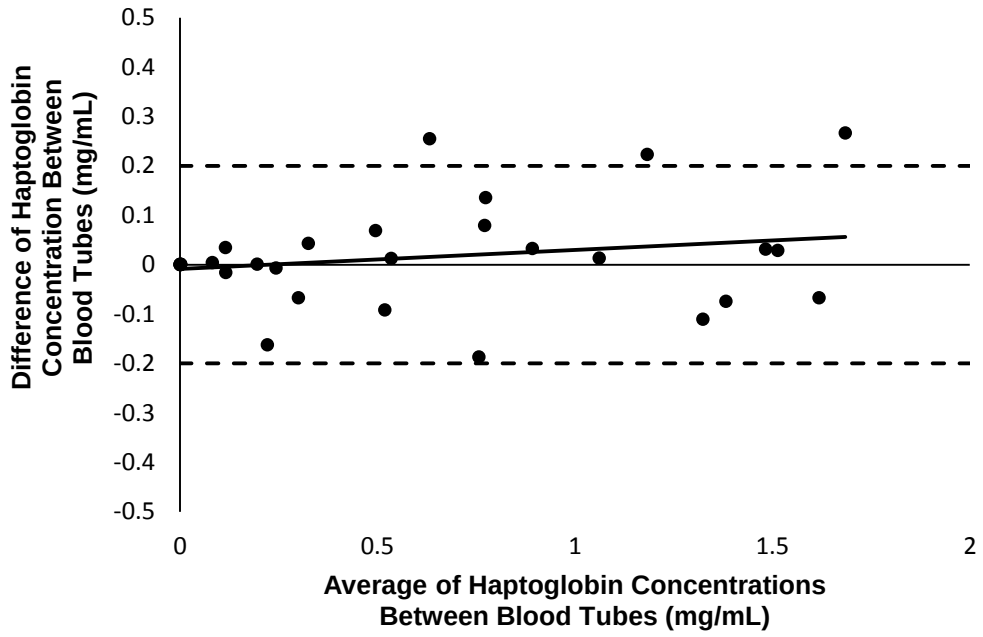

Figure 3.5. Analysis of agreement between blood tubes types with Life Diagnostics Cow Haptoglobin ELISA assay (cat. no. HAPT-11; Life Diagnostics, Inc., West Chester, PA) for serum separator and lithium heparinized plasma blood tubes; $y = 0.039x - 0.009$; $R^2 = 0.04$; mean bias of 0.02 mg/mL; slope bias $P = 0.27$. Maximum allowable differences (dotted lines) set at ± 0.20 mg/mL; largest variation (intra-assay) reported by manufacturer.

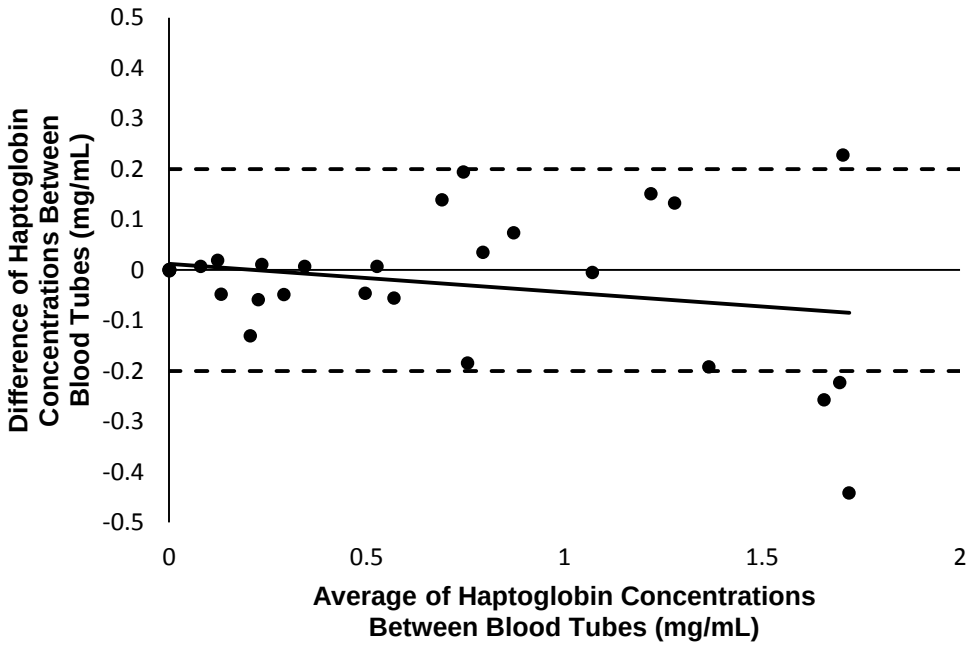

Figure 3.6. Analysis of agreement between blood tubes types with Life Diagnostics Cow Haptoglobin ELISA assay (cat. no. HAPT-11; Life Diagnostics, Inc., West Chester, PA) for serum separator and sodium heparinized plasma blood tubes; $y = -0.057x + 0.012$; $R^2 = 0.06$; mean bias of -0.02 mg/mL; slope bias $P = 0.20$. Maximum allowable differences (dotted lines) set at ± 0.20 mg/mL; largest variation (intra-assay) reported by manufactuer.

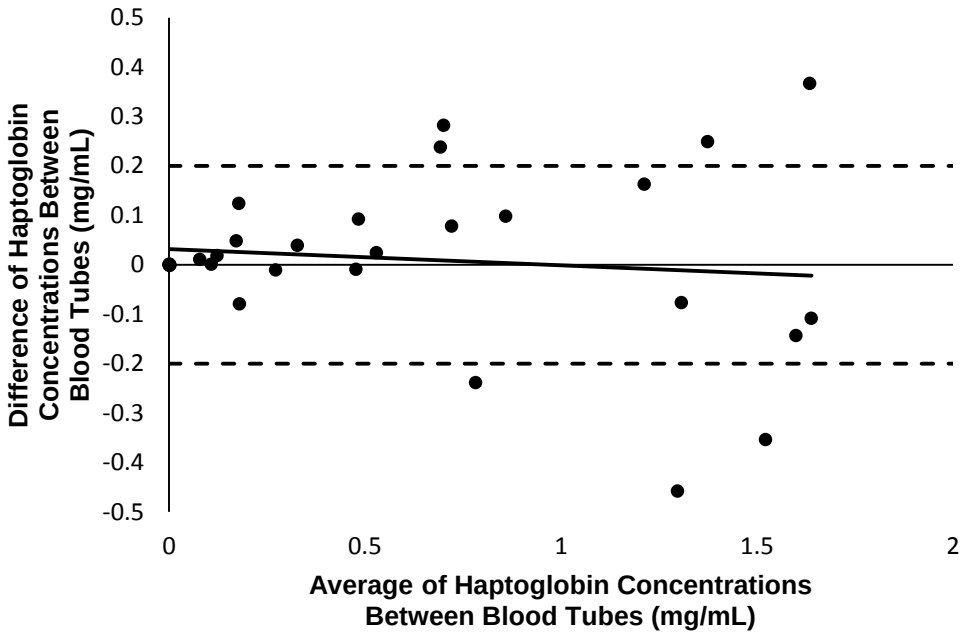

Figure 3.7. Analysis of agreement between blood tubes types with Life Diagnostics Cow Haptoglobin ELISA assay (cat. no. HAPT-11; Life Diagnostics, Inc., West Chester, PA) for serum separator and K2-EDTA blood tubes; $y = -0.034x + 0.032$; $R^2 = 0.01$; mean bias of 0.01 mg/mL; slope bias $P = 0.56$. Maximum allowable differences (dotted lines) set at ± 0.20 mg/mL; largest variation (intra-assay) reported by manufacturer.

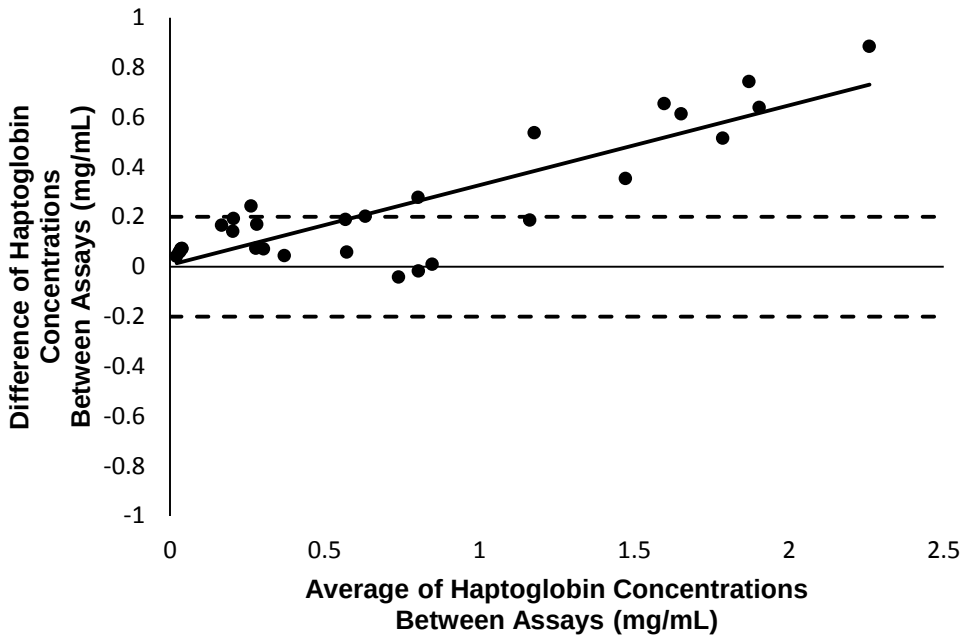

Figure 3.8. Analysis of agreement of serum separator haptoglobin concentrations between Tri-Delta PHASE haptoglobin assay (cat. no. TP-801; Tri-Delta© Development Ltd; Maynooth, Ireland) and Life Diagnostics Cow Haptoglobin ELISA assay (cat. no. HAPT-11; Life Diagnostics, Inc., West Chester, PA); $y = 0.32x + 0.01$; $R^2 = 0.74$; mean bias of 0.24 mg/mL; slope bias $P < 0.01$. Maximum allowable differences (dotted lines) set at ± 0.20 mg/mL based on the largest variation (intra-assay; Life Diagnostics) reported by both manufacturers.

CHAPTER 4: EFFECT OF STOCKING DENSITY AND SOURCE OF FORAGE FIBER ON SHORT-TERM RUMINAL FERMENTATION, BEHAVIORAL, PRODUCTION, AND STRESS RESPONSES OF HOLSTEIN DAIRY COWS

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ABSTRACT

Understanding the interaction of stocking density and diet is vital for the improvement of dairy cow well-being and productivity. The objective of this study was to determine the effect of stocking density and source of forage fiber on short-term ruminal fermentation, behavior, production, and stress responses of Holstein dairy cows. Multiparous (n = 48) and primiparous (n = 20) cows were assigned to 1 of 4 pens (n = 17 cows/pen). A focal group of multiparous (n = 12) and primiparous (n = 4), ruminally fistulated cows (n = 4 cows/pen) was used to evaluate ruminal fermentation. Pens were assigned to treatments in a 4 x 4 Latin square design with 14-d periods using a 2 x 2 factorial arrangement of treatments. Two stocking densities (STKD; 100 or 142% of stalls and headlocks) and two diets (straw; S and no straw; NS) resulted in 4 treatments: 1) 100NS, 2) 100S, 3) 142NS, and 4) 142S. Dietary forage content consisted of 39.7% corn silage and 6.9% haycrop silage versus 39.7% corn silage, 2.3% haycrop silage, and 3.5% chopped straw (dry matter; DM basis) for NS and S, respectively. Alterations in forage fiber source resulted in physically effective neutral detergent fiber (peNDF) values of 23.9% and 25.9% and undigested fiber (uNDFom240) values of 8.5% and 9.7% of DM for NS and S, respectively. Data were analyzed using a mixed model in JMP with pen (n = 4 per treatment) as the experimental unit. Dry matter intake did not differ among treatments, but S increased peNDF and neutral detergent fiber (NDF) intake. Milk, protein, and fat yields decreased with S, but were unaffected by STKD. Daily feeding and rumination times were unaffected by treatment, although 142% STKD decreased rumination within the freestall. Increased STKD decreased lying time, but increased

efficiency of stall use for resting. Feeding upon return from the parlor decreased while lying upon return from the parlor increased with 142% STKD. Serum amyloid-A tended to increase with 142% STKD. Cows experiencing higher STKD tended to have a lower mean and maximum pH and significantly less time spent below pH 5.8. Area under the curve (AUC) and time spent below pH 5.8 also were reduced with S diet. Ruminal volatile fatty acids and ammonia-nitrogen concentrations did not differ among treatments. Increasing STKD negatively impacted ruminal pH and effects tended to be exacerbated when combined with reduced dietary peNDF. Higher peNDF diets may help mitigate sub-acute ruminal acidosis caused by increased stocking density and thereby improve cow well-being.

Key words: overcrowding, physically effective fiber, ruminal fermentation, stress

INTRODUCTION

Overstocking is a commonly used management practice, prevalent with upwards of 78% and 60% of feed bunks and freestalls, respectively, in a sample of northeast dairy herds (USDA, 2010; von Keyserlingk et al., 2012). Defined as providing less than one stall or 0.6 m linear space at the feedbunk per cow, or both (Grant and Albright, 2001), high stocking densities can lead to alterations in cattle behavior as cows compete for resources within the pen. Several studies have demonstrated significant impacts of overstocking on cattle behavior, with decreased lying time (Fregonesi, 2007; Hill et al., 2009; Krawczel et al., 2012b), increased feedbunk aggression (Collings et al., 2011; Krawczel et al., 2012b), and altered rumination time and location (Batchelder, 2000;

Krawczel et al., 2012b). Furthermore, overstocking adversely affects cow health, with increased prevalence of injury and lameness (Barrientos et al., 2013; King et al., 2016), increased stress responses (Friend, 1979; González et al., 2003), and increased risk of culling (Bach et al., 2008). Finally, freestall availability is one of the most important non-nutritional factors influencing efficiency of production (Bach et al., 2008) and herd-level studies have associated increases in overstocking with decreased milk production and components (Deming et al., 2013; Sova et al., 2013).

Despite negative consequences on behavior, health, and production, many producers find economic incentive to overstock pens. Optimal stall stocking densities for highest economic return were greater than 100% and 120% stocking density in 67% and 42% of modeled economic scenarios characteristic of the US, respectively (De Vries et al., 2016).

However, rarely does one management practice occur on a farm in isolation; rather, combinations of these practices are used simultaneously. Due to the lack of production responses in the reported literature, but consistent changes in behavior, overstocking may be defined as a subclinical stressor. Subclinical stress is a reduction in a cow's biological reserve without affecting normal biological function or evidence of clinical symptoms (Moberg, 2000). However, when combined with another stressor, the cow may experience distress not previously observed with either stressor. When used simultaneously with other management strategies, dairy cows may experience greater alterations in behavior and biological function during overstocked conditions.

Balancing energy-dense diets for maximizing milk production, while maintaining optimum rumen health, has become a large challenge for the dairy industry (Zebeli et al., 2011). Therefore, diets are often formulated with higher grain and lower fiber levels, particularly physically effective NDF (peNDF). Defined as the product between the NDF concentration and the physical effectiveness factor (pef) of a feed or diet (Mertens, 1997), peNDF is positively associated with linear increases in feeding, rumination, and total chewing time (Beauchemin and Yang, 2005, Yang and Beauchemin, 2006). Furthermore, peNDF is associated with risk of sub-acute ruminal acidosis (SARA), with greater risk when peNDF is below 21% and lower risk when it is above 23% of diet dry matter (DM; Stone, 2004). Increased SARA, due to insufficient peNDF, can lead to adverse effects on feed digestibility (Grant and Mertens, 1992; Krajcarski-Hunt et al., 2002; Zebeli et al., 2010), milk production (Stone, 1999; Krause and Oetzal, 2005), and inflammatory responses (Nocek, 1997; Gozho et al., 2005; Plaizier et al., 2008).

To date, no research has investigated the effects of stocking density as a sub-clinical stressor and the outcomes when an overcrowded cow is presented with an additional stressor. We hypothesized that the cumulative effects of stocking density and lower peNDF would alter ruminal pH, feeding and resting behavior, milk production, and stress responses greater than either stressor in isolation. Therefore, the objective of this study was to determine the effects of the interaction between stocking density and source of forage fiber on short-term responses in ruminal fermentation, behavior, production, and stress of lactating Holstein dairy cows.

MATERIALS AND METHODS

Animal Housing and Management

Forty-eight multiparous and 20 primiparous, lactating Holstein cows were assigned to 1 of 4 pens (n = 17 cows per pen) in a naturally ventilated, saw-dust bedded 4-row freestall barn at the William H. Miner Agricultural Research Institute (Chazy, NY) from November 12, 2014 to January 7, 2015. Pens were balanced for parity (2.2 ± 1.1; mean ± standard deviation), days in milk (DIM; 190 ± 103), and milk production (45.8 ± 8.2 kg/d) prior to the start of the study. Each pen contained 17 head-to-head freestalls with similar facility specifications as described by Krawczel et al. (2012b). Cows were milked 3 times daily (approximately 1300 h, 2100 h, and 0500 h) in a double-12 parallel parlor (Xpressway Parallel Stall System; Bou-Matic, Madison, WI). Ambient temperature and humidity was measured within the freestalls using Hobo data loggers (Onset, Bourne, MA). Animal care and handling protocols were approved by the William H. Miner Agricultural Research Institute Animal Care and Use Committee.

Experimental Design and Treatments

Pens were assigned randomly to treatments in a 4 x 4 Latin square with 14-d periods using a 2 x 2 factorial arrangement of treatments. The first 7 d served as an adaptation period to the treatment. Two stocking densities (STKD; 100 or 142%) and 2 diets (straw; S and no straw; NS) resulted in 4 treatments: 1) 100NS, 2) 100S, 3) 142NS,

and 4) 142S. Stocking density was achieved through denial of access to both headlocks and freestalls (100%, 17 freestalls and headlocks per pen; 142%, 12 freestalls and headlocks per pen) as described by Krawczel et al. (2012a) as an effective model to assess short-term (ie., 14-d) cow responses to variable stocking densities. Diets were similar except that S diet replaced a portion of haycrop silage with 3.5% chopped wheat straw and 1.1% soybean meal in order to maintain dietary MP (DM basis, Table 4.1). Each diet was formulated for 46 kg milk/d using NDS Professional© based on the Cornell Net Carbohydrate and Protein System model (v. 6.1; RUM&N Sas, Reggio Emilia, Italy) and met both ME and MP requirements. Diets were mixed and delivered once daily at approximately 0600 h with a Keenan mixing truck (Richard Keenan & Co Ltd, Warwickshire, UK) and pushed up approximately 6 times daily.

Environmental Conditions

Temperature and relative humidity was monitored within the freestalls continuously for the duration of the study at 15-min intervals using Hobo data loggers (Onset, Bourne, MA).

Dry Matter Intake and Feed Efficiency

Dry matter intake (DMI) and feed efficiency (kilogram/kilogram milk yield) were measured for each pen on d 8 to 14 of each period. Samples of diets and orts were collected three times per week and dried in a forced-air oven at 105°C for 24 h for DM determination.

Feed Analyses

Diets, orts, and individual feed ingredients were collected 3 times during d 8 to 14 of each period. Samples of feed ingredients and diets were frozen at -20°C until samples were composited and analyzed for chemical composition (CPM Plus; Cumberland Valley Analytical Services, Inc., Hagerstown, MD). The analyzed chemical composition of diets, forages, and other feed ingredients is shown in Tables 4.1, 4.2, and 4.3, respectively. Samples of diets and corn silage were also analyzed for 7-h *in vitro* starch digestibility (Cumberland Valley Analytical Services, Inc., Hagerstown, MD; Tables 4.1 and 4.2).

Particle Size Distribution, Physical Effectiveness, and In Vitro Fermentation

Sub-samples of diets, forages, and orts were used for particle size determination using the Penn State Particle Separator (PSPS; as-fed basis; Lammers et al., 1996) with a 4-mm screen modification (Cotanch et al., 2010) and by dry (forced-air oven at 55°C for 48-h) vertical sieving (Ro-Tap testing sieve shaker model B; W. S. Tyler Combustion Engineering, Inc., Mentor, OH; Mertens, 1997) using a 1.18-mm sieve. The physical characterizations of diets and forages are shown in Table 4.4. Sorting activity was measured as the actual intake as a percentage of predicted intake for each particle fraction of the PSPS as described by Leonardi and Armentano (2003). Diet particles that passed through the 1.18-mm sieve were ground (2-mm grind; Cyclone Sample Mill; UDY

Corporation, Fort Collins, CO) and analyzed for NDF (ash corrected) using the ANKOM A200 Fiber Analyzer filter bag technique (ANKOM Technology Corp., Fairport, NY; Van Soest et al., 1991) with α-amylase and sodium sulfite. The resultant values were used with the $pef_{1.18}$ value to determine peNDF for each diet (Mertens, 2002; Table 4.1). Sub-samples of diets and forages were analyzed for undigested NDF (uNDFom with α-amylase and sodium sulfite) at 30 h, 120 h and 240 h using an *in vitro* fermentation system (Tilley and Terry, 1963) modified with buffered media containing ruminal fluid (Goering and Van Soest, 1970). *In vitro* fermentation data for diets and forages are shown in Tables 4.1 and 4.2, respectively.

Milk Yield and Composition

Milk yield was recorded electronically (ProVantage Information Management System; Bou-Matic, Madison, WI) on d 8 to 14 of each period. Milk samples were collected across six consecutive milkings for each cow on d 13 and 14 of each period and refrigerated at 4°C until analysis. Milk samples were analyzed at Cornell University (Ithaca, NY) using a mid-infrared (MIR) milk analyzer (Delta Instruments; Drachten, Netherlands). Anhydrous lactose and true protein were predicted using traditional virtual MIR filter models with optimized wavelengths and inter-correction factors as described by Kaylegian et al., 2009. A partial least squares (PLS) chemometric MIR prediction model (Delta Instruments parameter number 9600) was used to estimate total fatty acids (Woolpert et al., 2016) and that value was divided by 0.945 to add glycerol to the estimation of total milk fat. Milk urea nitrogen (MUN) was determined using a PLS

model (Delta Instruments, parameter number 0502). Mid-IR estimates for lactose, protein, fat, and MUN were then slope- and intercept-adjusted using a set of 14 modified milk calibration samples as described by Kaylegian et al. (2006a; 2006b). The reference chemistry for the modified milk calibration samples was: fat (AOAC, 2000; method 989.05; 33.2.26), total protein (AOAC, 2000; method 991.20; 33.2.11), nonprotein nitrogen (AOAC, 2000; method 991.21; 33.2.12), and anhydrous lactose (Lynch et al., 2007) with all lab mean reference chemistry reference values as described by Wojciechowski et al. (2016). Milk urea nitrogen was determined using an enzymatic assay (Megazyme, K-UMAMR kit, Wicklow, Ireland) following the operational method detail (done by weight with path length correction) used for the lactose enzymatic assay (Lynch et al., 2007), but using the enzymes and reagents for MUN measurement. Milk somatic cell count was determined with a SomaScope (Delta Instruments, Drachten, Netherlands) using fluorometeric flow cytometry stained with 4', 6-diamidino-2-phenylindole, dilactate, and calibrated with milks that had reference values determined by direct microscopic somatic cell count (Fitts and Laird, 2004). Somatic cell count (SCC) was transformed and analyzed as somatic cell score (SCS) using the equation: SCS = log2(SCC/100) + 3 where SCC is in units of 1,000 cells/mL as described in the methods of Shook (1993).

Body Weight, Body Condition Score, and Lameness

All cows were assessed for body weight, body condition score, and lameness score prior to the start of the study and at the end of each period. An Allweigh

computerized scale (Allweigh Scale System Inc., Red Deer, AB, Canada) was used to measure body weight. Body condition score were assessed using 0.25-unit increments on a 1 to 5 scale (Ferguson et al., 1994) by one trained scorer. Lameness scores were assessed using a 1 to 4 scale (Nordlund et al., 2004). Cows were assessed by one trained scorer on a flat surface upon return from the milking parlor.

Behavioral Analyses

Behavior assessments were performed on all cows using 72-h direct observation, scan-sampling at 10-min intervals (Mitlöhner et al., 2001) on d 8, 9, and 10 of each period. Cows were assessed for ingestive, rumination, and lying behaviors as well as the location of each behavior. Bouts of feeding, rumination, and lying behavior were determined with a 20 min inter-bout criterion. New bouts were established when the cow spent greater than 20 min performing another behavior before returning to the same behavior (Black et al., 2016).

Blood Measurements

Serum amyloid-A (SAA) was chosen as an indicator of acute inflammation to minimize carry-over effects among periods (Horadagoda et al., 1999). Blood samples were taken from a subset of cows (n = 12/pen; balanced for parity, DIM, and milk production) on d 7 and 14 of each period. Samples were collected from the coccygeal vein at approximately 0900 h and drawn into 10-mL BD vacutainer tubes spray coated with sodium heparin (158 USP; BD Diagnostics, Franklin Lake, NJ). Samples were

placed on ice until centrifugation at 1200 x *g* for 20 min at 4°C. Plasma was transferred into 2-mL cryogenic vials (Fisher Scientific, Pittsburgh, PA) and stored at -20°C until analysis. Serum amyloid A was determined using ELISA Tridelta Phase range kits (Tridelta Diagnostics Inc., Maynooth, County Kildare, Ireland; cat. no. TP-802) and absorbances read in a microplate reader (BioTek Synergy 2; Winooski, VT) at 450 nm.

Focal Cows

Twelve multiparous and 4 primiparous, ruminally fistulated (Bar Diamond, Parma, ID) cows were used to form 4 focal groups (n = 4/pen) for ruminal fermentation sample collection. Each focal group was balanced for DIM, milk yield, and parity.

Ruminal pH

Ruminal pH was measured using an indwelling ruminal pH/ORP/REDOX measurement system (Penner et al., 2006; LRCpH; Dascor, Escondido, CA) at 1-min intervals for 72 h on days 12, 13, and 14 of each period. Daily ruminal pH measurements were averaged over 10-min intervals. Measurements were then averaged across days of each period and among cows into a pen average. Ruminal pH data were summarized as mean pH, minimum pH, maximum pH, time spent below pH 5.8 (h/d), as well as the area under the curve (AUC) below pH 5.8 (Bauer et al., 1995).

Ruminal Volatile Fatty Acids and Ammonia Nitrogen

Samples of rumen fluid (approximately 250 mL) were collected from beneath the ruminal digesta mat at 4-h intervals for 24 h on d 13 (0600, 1000, 1400, 1800, 2200 h) and d 14 (0200 h) of each period. Samples were strained through 4 layers of cheesecloth. A portion of each sample of ruminal fluid (approximately 40 mL) was frozen and stored at −20°C until analysis for volatile fatty acids (VFA; Bulletin 856B; Supelco Inc., Bellefonte, PA). The concentrations of VFA (mol/100 mol) were determined by gas chromatography using a Varian CP-3800 gas chromatograph (Varian Inc., Palo Alto, CA) equipped with a flame-ionization detector and a 80/120 Carbopack B-DA/4% Carbowax 20M column (Supelco Inc., Bellefonte, PA). A 10-mL sample of rumen fluid was mixed with 100 µL of 12.1 *N* hydrochloric acid and stored at −20°C for ammonia nitrogen analysis using the procedures described by Chaney et al. (1962).

Statistical Analyses

Data were analyzed using a mixed model in JMP (ver. 12, SAS Institute Inc., NC) for a 2 x 2 factorial arrangement of treatments within a 4 x 4 Latin Square design according to the following model:

$$Y_{ijkl} = \mu + S_i + D_j + SD_{ij} + P_k + R_l + E_{ijkl}$$

where Y_{ijkl} was the dependent variable, μ was the overall mean, S_i was the fixed effect of stocking density, D_j was the fixed effect of diet, SD_{ij} was the fixed effect of the interaction between stocking density and diet, P_k was the fixed effect of period, R_l was the random effect of pen, and E_{ijkl} was the residual error. Preplanned contrasts were

included to compare 100% STKD and 142% STKD, NS and S diets, and the interaction between stocking density and diet. Significance was declared at $P \leq 0.05$ and trends at $0.05 < P \leq 0.10$.

RESULTS AND DISCUSSION

Two multiparous cows were removed from the study (one for severe mastitis and one for bovine leucosis), and one cannulated, primiparous cow was removed due to an infection. While not directly related to the treatments, it is unknown whether treatments may have exacerbated the severity of these responses. All data from these cows were removed from the analyzed data set. Data from two additional cannulated cows were removed from the ruminal fermentation data set due to equipment malfunction.

Environmental Conditions

Daily temperatures within the pens ranged from 2.7°C to 14.6 °C, with an average across periods of 5.9°C. Relative humidity ranged from 57.8% to 90.9% with an average of 76.0% across periods.

Intake and Sorting Activity

Daily DMI was unaffected by both stocking density ($P = 0.78$) and diet ($P = 0.69$). These responses were similar to those observed by Collings et al. (2011) and Krawczel et al. (2012b) at increased stocking densities of 200% and 142%, respectively.

However, S diet increased NDF, peNDF, and uNDFom240 (kg/d) intake (Table 4.6). Sorting was minimal (means within 10% difference; Miller-Cushon and DeVries, 2017) between refused and offered TMR for both NS and S diets and both STKD levels.

Sorting for long particles (actual intake as a percent of predicted intake; > 19.0 mm PSPS particle fraction) increased for 142% STKD ($P = 0.02$) and NS ($P < 0.01$), indicating cows actively sought longer particles when overstocked and fed lower peNDF diets (Table 4.5). Though not statistically different ($P = 0.11$), similar numerical differences occurred using Ro-Tap particle fractions. However, the > 19.0 mm fraction is the most likely PSPS fraction to be sorted (Kononoff and Heinrichs, 2003). Other fractions did not differ among treatments. Sorting behavior with the current study was similar to that reported by DeVries et al. (2008) who observed increased sorting against fine particles and for longer particles with cows at higher risk of SARA when fed 45% forage diets opposed to lower risk of SARA when fed 60% forage diets. However, fractions remained within 10% difference (Miller-Cushon and DeVries, 2017) of offered feed, indicating minimal sorting occurred with both diets and levels of STKD.

Milk Production and Composition

Short term responses in daily milk yield, solids-corrected milk (SCM) yield, component yield and percentages, MUN concentration, and SCS were all unaffected by STKD (Table 4.6), similar to responses reported by Krawczel et al., (2012b) with comparable stocking densities of 100% and 142% and 14-d treatment periods. However, in contrast to daily yield, milk yield and SCM yield from the 3rd milking post-feed

delivery decreased ($P = 0.01$ and $P = 0.05$, respectively) at 142% STKD, likely due to behavioral changes during the 8-h interval prior to milking. Futhermore, SCM yield significantly decreased ($P = 0.01$) from the 1st milking post-feeding, indicating intra-day shifts in production due to behavioral changes with overstocking, though total daily yield remained unaffected. However, it is important to note that these are short-term production responses (2-wk treatment periods) and further research should be done to characterize any longer-term effects of increased stocking density on feed intake and milk production measures.

Daily milk yield tended ($P = 0.06$) to decrease and SCM decreased ($P = 0.03$) with the S diet, driven by a decrease in milk ($P = 0.01$) and SCM ($P < 0.01$) from the first milking post-feed delivery. Feed efficiency and SCM efficiency decreased ($P = 0.02$ and $P = 0.05$, respectively) with S diet. Furthermore, S diet decreased ($P = 0.05$) daily yields (kg/d) of fat, protein, and lactose due to the decreased milk yield. Stone (2004) reported that diets should be formulated for an optimal balance of peNDF at approximately 21 to 23%, as excessive peNDF may constrain DMI and lower overall feed efficiency. The S and NS diets contained 25.9% and 23.9% peNDF, respectively. Although DMI was unaffected by diet, the level of peNDF in the S diet appeared to constrain milk production and lower overall feed efficiency.

Feeding Behavior

Feeding behavior results are summarized in Table 4.7. Daily feeding time (min/d) did not differ among treatments ($P > 0.13$), consistent with responses previously reported

with similar ranges in stocking density up to 142% (Hill et al., 2009; Krawczel et al., 2012b; Wang et al., 2016). In contrast to previously reported decreases in feeding time with stocking densities of 200% (~11 min, Collings et al., 2011), 300% (~19 min, Crossley et al., 2017), and 400% (~45 min, Olofsson, 1999), a break point likely occurs between 142% and 200% stocking density before consistent decreases in feeding time are observed. Furthermore, these findings suggest that cows housed at 142% STKD do not necessarily increase their feeding rates (slug feeding behavior) due to competition at the feedbunk, as evidenced by similar feeding times and daily DMI in contrast to previous literature at stocking densities greater than 200% (12.5 g DM/min, Collings et al., 2011; 40 g DM/min, Crossley et al., 2017; 25 g DM/min, Olofsson, 1999). Feeding time (min/8-h interval) increased for 142% STKD at 17-24 h post-feed delivery ($P < 0.01$), demonstrating a shift in feeding behavior to maintain daily DMI in the face of higher levels of feedbunk competition. Feeding time, both daily and within each 8-h interval, was not affected by diet ($P > 0.20$). While previous studies reported linear increases in eating time with increasing peNDF (Beauchemin and Yang, 2005, Yang and Beauchemin, 2006), the difference in peNDF content in these previous trials altered through chop length of corn silage or barley silage (which made up 41.9 and 46.6 % of DM, respecivtely) opposed to adding wheat straw in at only 3.5% of DM. Further, these previous studies had peNDF values of less than 13.8 % of DM whereas the current study varied from 23.9 to 25.9% of DM, indicating little effects of altered peNDF values over 23.9% of DM on chewing time during ingestion.

Daily feeding bouts (bouts/d) tended ($P = 0.10$) to increase at 142% STKD. However, daily feeding bout length (min/bout) was unaffected by STKD ($P = 0.85$). These results were similar to those observed by Black et al. (2016), who reported no differences in meal length, frequency of feedbunk visits, and time between visits across comparable stocking densities ranging from 100% to 142%, indicating limited effects of stocking density on meal characteristics within this range.

Daily feeding bout length tended ($P = 0.09$) to increase with S diet. Feeding bout length for each interval post-feeding was unaffected, suggesting cumulative influence from each interval on the daily average of feeding bout length. This outcome was also evidenced by an increase ($P = 0.02$) in bout length of first meal following fresh feed delivery with S diet. Likely due to increased rumen fill following fresh feed delivery, feeding bouts tended ($P = 0.06$) to decrease with S diet at 9-16 h post-feed delivery.

Rumination Behavior

Rumination behavior results are summarized in Table 4.8. Total daily rumination time (min/d) did not differ among treatments ($P > 0.72$). These results are similar to previous studies between stocking densities of 100% and 142% (Krawczel et al., 2012b; Wang et al., 2016). Rumination (min/8-h interval) tended ($P = 0.07$) to decrease at 142% STKD 0-8 h post-feed delivery, likely due to the increase in feeding behavior during this interval. Rumination bout number and bout length, both daily and each 8-h interval, were unaffected by treatments. Rumination within a freestall (% of total rumination) decreased ($P < 0.01$) at 142% STKD, indicating a shift in the location of rumination from the

freestall to the alley. This 5.3% difference in the location of rumination between stocking densities was similarly reported by Krawczel et al. (2012b) who observed a 7.8% difference between 100% and 142% stocking density. Furthermore, rumination while lying (% of total rumination) decreased ($P = 0.02$) at 142% STKD, implying a shift in posture while ruminating in addition to the shift in location.

Lying Behavior

Lying behavior results are summarized in Table 4.9. Total daily lying time (min/d) decreased ($P < 0.01$) while time spent in the alley (min/d) increased ($P < 0.01$) at 142% STKD. The approximate 40 min difference between 100 and 142% STKD was consistent with previous studies with similar levels of stocking density (54 min, Hill et al., 2009; 30 min, Krawczel et al., 2012b). Daily distribution of lying time was also affected with higher STKD. Lying time (min/8-h interval) decreased at 142% STKD at 0-8 h post-feed delivery ($P = 0.02$) and 17-24 h post-feed delivery ($P < 0.01$) and tended ($P = 0.06$) to decrease at 9-16 h post-feed delivery. Grant (2007) previously observed a positive relationship with resting time and milk production. This observation was consistent with results in the current study, as milk production decreased following the 17-24 h post-feed delivery interval which had the greatest reduction in lying time at 142% STKD.

Lying bouts, both daily (bouts/d) and for each 8-h interval (bouts/8-h interval), were unaffected by treatments. Lying bout length (daily average) tended ($P = 0.07$) to decrease at 142% STKD, driven by a decrease ($P = 0.04$) in lying bout length at 0-8 h

post-feed delivery due to increased feeding time during that interval. Minimal changes in lying bout number or bout length is consistent with previous literature (Krawczel et al., 2012b, Solano et al., 2015; Wang et al., 2016) at similar levels of stocking density. This demonstrates the inelasticity of lying behavior of dairy cows under various stocking conditions and the inability to make up for reduced lying times by altering bout characteristics, driven by the cow's large lying time requirement of 12-14 h (Grant and Albright, 2001). Lying within a stall (% of stall use) increased at 142% STKD. Previous studies also reported decreased variation in stall use in overstocked conditions (Fregonesi et al., 2007; Ito et al., 2014) and increased cow comfort index (CCI) during peak lying hours (Wang et al., 2016). Driven by the high priority placed on lying time (Metz, 1985; Munksgaard et al., 2005), results in the current study indicate increased efficiency of stall use when overstocked cows finally gain access to the freestall.

Feeding and Lying Latency

Feeding upon immediate return from parlor (% of pen) decreased at 142% STKD with the first milking post-feed delivery ($P < 0.01$) as well as upon return to fresh feed ($P = 0.01$) and tended ($P = 0.06$) to decrease with the second milking post-feed delivery (Table 4.10). In contrast, lying immediately upon return from parlor (% of pen) increased at 142% STKD for the first milking following feed delivery ($P = 0.03$) and upon return to fresh feed ($P < 0.01$). The increased percentage of cows immediately lying down upon return from the milking parlor aligns with reductions in lying time for the 8-h intervals prior to both of these milking. While DeVries et al. (2003) observed greater feeding

activity upon return from the milking parlor and fresh feed delivery, overstocking conditions, which create deprivations in lying time, appear to inhibit this behavioral response. The contrast between feeding and lying immediately upon return from the milking parlor further emphasizes the importance that cows place on lying behavior over other behaviors when resources limit the cow's ability to meet daily behavioral requirements, such as in overstocked situations (Munksgaard et al., 2005).

Blood Measurements

Serum amyloid-A results are summarized in Table 4.11. There were no treatment differences in SAA on d 7 of the period, but 142% STKD tended ($P = 0.08$) to increase SAA on d 14. These data may indicate that increased exposure to high levels of STKD is needed before inflammatory responses occur. In response to SARA challenges, cows increased SAA production upwards of 85 μg/mL or greater (Gozho et al., 2005; Gozho et al., 2007; Khafipour et al., 2006). While differences between means were smaller in the current trial, treatment means represent pen averages, with both non-risk and high-risk of SARA cows contributing to the mean, lessening the magnitude of the difference. Furthermore, Zebeli et al. (2012) identified a positive linear relationship with plasma SAA concentrations and time spent below pH 6.0. It is likely that the greater contribution of SARA from STKD resulted in a trend for increased systemic inflammation, while SAA levels from diet remained unaffected.

Ruminal Fermentation

Ruminal fermentation results are summarized in Tables 4.12 to 4.14. This study was the first to investigate the effects of STKD as well as the interaction of STKD and diet on ruminal fermentation. Time spent below pH 5.8 (h/d) increased for both NS (P = 0.01) and 142% STKD (P < 0.01) treatments. A trend for an interaction (P = 0.10) was found between stocking density and diet on time spent below pH 5.8. Due to the removal of three cannulated cows from the data set, it is possible that the ruminal pH data became underpowered, and a greater number of cows would result in a significant interaction. Area under the curve below pH 5.8 (units x pH) was greater for the NS diet (P = 0.03) and tended (P = 0.06) to be greater at 142% STKD. Higher stocking density also tended (P = 0.07) to lower daily mean pH and maximum pH.

Daily distribution of SARA (Table 4.12) indicated increased time spent below pH 5.8 for each of the three post-feeding intervals at 142% STKD. This suggests that the effects of increased STKD on SARA risk are constant throughout the day. Area under the curve tended (P = 0.06) to increase at 142% STKD in the 17-24 h post-feeding interval but was unaffected during the other time periods. Furthermore, the percentage of each hour spent below pH 5.8 differed with stocking density for the hour before feed delivery as well as an interaction with diet during this interval 21 h post-feed delivery (Figure 4.3). The severity during this time period was likely driven by the reduced lying time and shift in rumination location at 142% STKD. The NS diet increased time below pH 5.8 and AUC for the 9-16 h and 17-24 h post-feeding intervals, but the 0-8 h post-feeding interval was unaffected by diet. As a percent of each hour spent below pH 5.8, significant

differences between diets were observed at 15 h post-feed delivery and between stocking densities at 10 h and 24 h post-feed delivery (Figure 4.3). Furthermore, an interaction between diet and stocking density was observed at 21 h post-feed delivery with the NS, 142% STKD treatment having the greatest percentage of the hour spent below pH 5.8. As seen in Figure 4.3, SARA increased throughout the day for all treatments. Future research should investigate dietary changes or altering feeding management to reduce SARA during the 9-24 h time period.

Despite consistent reports in the literature that chewing increases with dietary peNDF (Beauchemin and Yang, 2005; Yang and Beauchemin, 2006), eating and rumination times were not affected by diet in the current study. Ruminal pH differences between diets are likely explained by increased buffer volume produced during eating and rumination for the S diet, as evidenced by Maekawa et al. (2002a) where increases in the fiber-to-concentrate ratio from 40:60 to 60:40 resulted in increased ensalivation of feed.

As daily feeding and rumination time were not affected by stocking density, it is likely that reduced chewing time, leading to reduced buffer production (Cassida and Stokes, 1986), did not account for the differences in ruminal pH. However, location of rumination was shifted from the freestall to the alley with 142% STKD. As resting and rumination are significant contributors to buffer production (resting, 27.7 % total daily saliva production, rumination, 50.1% total daily saliva production; Maekawa et al., 2002b), it is possible that this shift in the location of rumination may affect the volume or rate of buffer production, partially explaining the increased risk of SARA at higher

stocking densities. As observed in Figure 4.4, there was no correlation between time spent below pH 5.8 and rumination in the freestall (as a % of total rumination) at 100% STKD. However, 44% of the variation in SARA was explained by the location of rumination at 142% STKD, suggesting changes in buffer production dependent upon the location where rumination is performed.

The difference between STKD levels resulted in a 1.4 h difference in SARA, compared to a 0.9 h difference between diets, indicating a greater contribution to SARA occurred with STKD than wth 0 the dietary treatment. Furthermore, addition of straw to the diet at 100% STKD resulted in a 0.4 h difference compared to a 1.4 h difference at 142% STKD. These results suggest a reduction in SARA at both levels of STKD by increasing peNDF or uNDFom in the diet, but the cow experiences a greater benefit from dietary changes at higher STKD.

There were no differences in the daily average of ruminal ammonia nitrogen (mg/dL) or total VFA (m*M*) among treatments. Valerate, as a molar percentage, increased with NS diet while isovalerate tended ($P = 0.06$) to decrease at 142% STKD. Other ruminal VFA were not affected. The effects of SARA on ruminal VFA in literature are mixed. Danscher et al. (2015) observed increases in acetate, but no differences in other VFA or total VFA during grain-induced SARA challenges. In contrast, Stefańska et al. (2016) reported increases in acetate, proprionate, butyrate, valerate, total VFA, and ammonia-N with SARA-positive associated herds. It is likely that mild severity of SARA in the current study, with the most severe treatment averaging just 4.12 h per day (Table 4.12), or limited power, due to the removal of three cannulated cows from the data set,

could explain the lack of differences between treatments. Further, due to the short-term study periods, ruminal VFA responses could be limited, compared to the herd-level associated studies, where cows experienced longer exposure to SARA conditions.

CONCLUSIONS

Increased stocking density resulted in negative effects on ruminal pH, lying time, and location of rumination thereby increasing the risk for SARA. The presence of an additional stressor, such as reduced peNDF, with stocking density tended to exacerbate the negative effects on ruminal pH. Manipulation of the feeding environment can help mitigate the negative effects of high stocking density, such as increasing the peNDF or uNDFom240 content of the diet.

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REFERENCES

AOAC International. 2000. Official Methods of Analysis. 17th ed. AOAC International, Gaithersburg, MD.

Bach, A., N. Valls, A. Solans, and T. Torrent. 2008. Associations between nondietary factors and dairy herd performance. J. Dairy Sci. 91:3259-3267.

Barrientos, A. K., N. Chapinal, D. M. Weary, E. Galo, and M. A. G. von Keyserlingk. 2013. Herd-level risk factors for hock injuires in freestall-housed dairy cows in the northeastern United States and California. J. Dairy Sci. 96:3758-3765.

Batchelder, T. L. 2000. The impact of head gates and overcrowding on production and behavior patterns of lactating dairy cows. Pages 325-330 in Dairy Housing and Equipment Systems. Managing and Planning for Profitability. Publ. 129. Nat. Resour., Agric., and Eng. Serv., Camp Hill, PA.

Bauer, M. L., D. W. Herold, R. A. Britton, R. A. Stock, T. J. Klopfenstein, and D. A. Yates. 1995. Efficacy of laidlomycin propionate to reduce ruminal acidosis in cattle. J. Anim. Sci. 73:3445-3454.

Beauchemin, K. A., and W. Z. Yang. 2005. Effects of physically effective fiber on intake, chewing activity, and ruminal acidosis for dairy cows fed diets based on corn silage. J. Dairy Sci. 88:2117-2129.

Black, R. A., R. J. Grant, and P. D. Krawczel. 2016. Short communication: Short-term changes in stocking density did not alter meal characteristics of lactating Holstein dairy cattle. J. Dairy Sci. 99:6572-6577.

Cassida, P. E., and M. R. Stokes. 1986. Eating and resting salivation in early lactation dairy cows. J. Dairy Sci. 69:1282-1292.

Chaney, A. L., and E. P. Marbach. 1962. Modified reagents for determination of urea and ammonia. Clin. Chem. 8:130-132.

Collings, L. K. M., D. M. Weary, N. Chapinal, and M. A. G. von Keyserlingk. 2011. Temporal feed restriction and overstocking increase competition for feed by dairy cattle. J. Dairy Sci. 94:5480-5486.

Cotanch, K. W., J. D. Darrah, C. S. Ballard, and R. J. Grant. 2010. Modification of the Penn State Particle Separator with 3.18- or 4.76-mm perforated steel sieves to measure physically effective fiber. J. Dairy Sci. 88(E-Suppl. 1):147. (Abstr.).

Crossley, R. E., A. Harlander-Matauschek, and T. J. DeVries. 2017. Variability in behavior and production among dairy cows fed under differing levels of competition. J. Dairy Sci. 100:1-14.

Danscher, A. M., S. Li, P. H. Andersen, E. Khafipour, N. B. Kristensen, and J. C. Plaizier. 2015. Indicators of induced subacute ruminal acidosis (SARA) in Danish Holstein cows. Acta Vet. Scand. 57:39.

Deming, J. A., R. Bergeron, K. E. Leslie, and T. J. DeVries. 2013. Associations of housing, management, milking activity, and standing and lying behavior of dairy cows milked in automatic systems. J. Dairy Sci. 96:344-351.

DeVries, T. J., F. Dohme, and K. A. Beauchemin. 2008. Repeated ruminal acidosis challenges in lactating dairy cows at high and low risk for developing acidosis: Feed sorting. J. Dairy Sci. 91:3958-3967.

DeVries, T. J., M. A. G. von Keyserlingk, and K. A. Beauchemin. 2003. Short communication: Diurnal feeding pattern of lactating dairy cows. J. Dairy Sci. 86:4079-4082.

De Vries, A., H. Dechassa, and H. Hogeveen. 2016. Economic evaluation of stall stocking density of lactating dairy cows. J. Dairy Sci. 99:3848-3857.

Ferguson, J. D., D. T. Galligan, and N. Thomsen. 1994. Principal descriptors of body condition score in Holstein cows. J. Dairy Sci. 77:2695-2703.

Fitts, J. E., and D. Laird. 2004. Direct microscopic methods for bacteria or somatic cells. Pages 269-280 in Standard Methods for the Examination of Dairy Products. 17th ed. H. M. Wehr and J. F. Frank, ed. Am. Public Health Assoc., Washington, DC.

Fregonesi, J. A., C. B. Tucker, and D. M. Weary. 2007. Overstocking reduces lying time in dairy cows. J. Dairy Sci. 90:3349-3354.

Friend, T. H., F. C. Gwazdauskas, and C. E. Polan. 1979. Change in adrenal response from free stall competition. J. Dairy Sci. 62:768-771.

Goering, H. K., and P. J. Van Soest. 1970. Forage Fiber Analysis (Apparatus, Reagents, Procedures, and Some Applications). Agriculture Handbook No. 379. USDA Agricultural Research Service, Washington, DC.

González, M., A. K. Yabuta, and F. Galindo. 2003. Behaviour and adrenal activity of first parturition and multiparous cows under a competitive situation. Appl. Anim. Behav. Sci. 82:259-266.

Gozho, G. N., D. O. Krause, and J. C. Plaizier. 2007. Ruminal lipopolysaccharide concentration and inflammatory response during grain-induced subacute ruminal acidosis in dairy cows. J. Dairy Sci. 90:856-866.

Gozho, G. N., J. C. Plaizier, D. O. Krause, A. D. Kennedy, and K. M. Wittenberg. 2005. Subacute ruminal acidosis induces ruminal lipopolysaccharide endotoxin release and triggers and inflammatory response. J. Dairy Sci. 88:1399-1403.

Grant, R. J. 2007. Taking advantage of natural behavior improves dairy cow performance. Pages 225-236 in Proc. Western Dairy Management Conf., Reno, NV.

Grant, R. J., and D. R. Mertens. 1992. Influence of buffer pH and raw corn starch addition on in vitro fiber digestion kinetics. J. Dairy Sci. 75:2762-2768.

Grant, R. J., and J. L. Albright. 2001. Effect of animal grouping on feeding behavior and intake of cattle. J. Dairy Sci. 84(E-Suppl.):E156-E163.

Hill, C. T., P. D. Krawczel, H. M. Dann, C. S. Ballard, R. C. Hovey, W. A. Falls, and R. J. Grant. 2009. Effect of stocking density on the behavior of dairy cows with differing parity and lameness status. Appl. Anim. Behav. Sci. 117:144-149.

Horadagoda, N. U., K. M. G. Knox, H. A. Gibbs, S. W. J. Reid, A. Horadagoda, S. E. R. Edwards, P. D. Eckersall. 1999. Acute phase proteins in cattle: discrimination between acute and chronic inflammation. Vet. Rec. 144:437-441.

Huzzey, J. M., T. J. DeVries, P. Valois, and M. A. G. von Keyserlingk. 2006. Stocking density and feed barrier design affect the feeding and social behavior of dairy cattle. J. Dairy Sci. 89:126-133.

Ito, K., N. Chapinal, D. M. Weary, and M. A. G. von Keyserlingk. 2014. Associations between herd-level factors and lying behavior of freestall-housed dairy cows. J. Dairy Sci. 97:2081-2089.

Kaylegian, K. E., G. E. Houghton, J. M. Lynch, J. R. Fleming, and D. M. Barbano. 2006a. Calibration of infrared milk analyzers: Modified milk versus producer milk. J. Dairy Sci. 89:2817-2832.

Kaylegian, K. E., J. M. Lynch, G. E. Houghton, J. R. Fleming, and D. M. Barbano. 2006b. Modified versus producer milk calibration: Mid-Infrared analyzer performance validation. J. Dairy Sci. 89:2833-2845.

Kaylegian, K. E., J. M. Lynch, J. R. Fleming, and D. M. Barbano. 2009. Influence of fatty acid chain length and unsaturation on Mid-Infrared milk analysis. J. Dairy Sci. 92:2485-2501.

Khafipour, E., D. O. Krause, and J. C. Plaizier. 2006. Influence of grain induced sub-acute ruminal acidosis (SARA) on lipopolysaccharide endotoxin (LPS) and acute phase proteins. Can. J. Anim. Sci. 86:577. (Abstr.).

King, M. T. M., E. A. Pajor, S. J. LeBlanc, and T. J. DeVries. 2016. Associations of herd-level housing, management, and lameness prevalence with productivity and cow behavior in herds with automated milking systems. J. Dairy Sci. 99:9069-9079.

Kononoff, P. J., and A. J. Heinrichs. 2003. The effect of reducing alfalfa haylage particle size on cows in early lactation. J. Dairy Sci. 86:1445-1457.

Krajcarski-Hunt, H., J. C. Plaizier, J. P. Walton, R. Spratt, and B. W. McBride. 2002. Short communication: Effect of subacute ruminal acidosis on in situ fiber digestion in lactating dairy cows. J. Dairy Sci. 85:570-573.

Krause, K. M., and G. R. Oetzel. 2005. Inducing subacute ruminal acidosis in lactating dairy cows. J. Dairy Sci. 88:3633-3639.

Krawczel, P. D., C. S. Mooney, H. M. Dann, M. P. Carter, R. E. Butzler, C. S. Ballard, and R. J. Grant. 2012a. Effect of alternative models for increasing stocking density on the short-term behavior and hygiene of Holstein dairy cows. J. Dairy Sci. 95:2467-2475.

Krawczel, P. D., L. B. Klaiber, R. E. Butzler, L. M. Klaiber, H. M. Dann, C. S. Mooney, and R. J. Grant. 2012b. Short-term increases in stocking density affect the lying and social behavior, but not the productivity, of lactating Holstein dairy cows. J. Dairy Sci. 95:4298-4308.

Lammers, B. P., D. R. Buckmaster, and A. J. Heinrichs. 1996. A simple method for the analysis of particle sizes of forage and total mixed rations. J. Dairy Sci. 79:922-928.

Leonardi, C., and L. E. Armentano. 2003. Effect of quantity, quality, and length of alfalfa hay on selective consumption by dairy cows. J. Dairy Sci. 86:557-564.

Lynch, J. M., D. M. Barbano, and J. R. Fleming. 2007. Determination of the lactose content of fluid milk by spectrophotometric enzymatic analysis using weight additions and path length adjustment: Collaborative Study. J. AOAC Int. 90:196-216.

Maekawa, M., K. A. Beauchemin, and D. A. Christensen. 2002a. Effect of concentrate level and feeding management on chewing activities, saliva production, and ruminal pH of lactating dairy cows. J. Dairy Sci. 85:1165-1175.

Maekawa, M., K. A. Beauchemin, and D. A. Christensen. 2002b. Chewing activity, saliva production, and ruminal pH of primiparous and multiparous lactating dairy cows. J. Dairy Sci. 85:1176-1182.

Mertens, D. R. 1997. Creating a system for meeting the fiber requirements of dairy cows. J. Dairy Sci. 80:1463-1481.

Mertens, D. R. 2002. Physical and chemical characteristics of fiber affecting dairy cow performance. Pages 125-144 in Cornell Nut. Conf., Proc. 2002., Dept. Anim. Sci., Cornell Univ., Ithaca, NY.

Metz, J. H. M. 1985. The reaction of cows to a short-term deprivation of lying. Appl. Anim. Behav. Sci. 13:301-307.

Miller-Cushon, E. K., and T. J. DeVries. 2017. Feed sorting in dairy cattle: Causes, consequences, and management. J. Dairy Sci. 100:4172-4183.

Mitlöhner, F. M., J. L. Morrow-Tesch, S. C. Wilson, J. W. Dailey, and J. J. McGlone. 2001. Behavioral sampling techniques for feedlot cattle. J. Anim. Sci. 79:1189-1193.

Moberg, G. P. 2000. Biological response to stress: implications for animal welfare. In: Moberg, G. P. and J. A. Mench (eds) The Biology of Animal Stress. CAB. International, Wallingford, UK, pp. 1-21.

Munksgaard, L., M. B. Jensen, L. J. Pedersen, S. W. Hansen, and L. Matthews. 2005. Quantifying behavioural priorities - Effects of time constraints on behavior of dairy cows, *Bos Taurus*. Appl. Anim. Behav. Sci. 92:3-14.

Nocek, J. E. 1997. Bovine acidosis: implications on laminitis. J. Dairy Sci. 80:1005-1028.

Nordlund, K. V., N. B. Cook, and G. R. Oetzel. 2004. Investigation strategies for laminitis problem herds. J. Dairy Sci. 87:(E. Suppl.):E27-E35.

Olofsson, J. 1999. Competition for total mixed diets fed for ad libitum intake using one or four cows per feeding station. J. Dairy Sci. 82:69-79.

Penner, G. B., K. A. Beauchemin, and T. Mutsvangwa. 2006. An evaluation of the accuracy and precision of a stand-alone submersible continuous ruminal pH measurement system. J. Dairy Sci. 89:2132-2140.

Plaizier, J. C., D. O. Krause, G. N. Gozho, and B. W. McBride. 2008. Subacute ruminal acidosis in dairy cows: the physiological causes, incidence, and consequences. Vet. J. 176:21-31.

Shook, G. E. 1993. Genetic improvement of mastitis through selection on somatic cell count. Veterinary Clinics of North America - Food Animal Practice 9:563-581.

Solano, L., H. W. Barkema, E. A. Pajor, S. Mason, S. J. LeBlanc, C. G. R. Nash, D. B. Haley, D. Pellerin, J. Rushen, A. M. de Passillé, E. Vasseur, and K. Orsel. 2015. Associations between lying behavior and lameness in Canadian Holstein-Friesian cows housed in freestall barns. J. Dairy Sci. 99:2086-2101.

Sova, A. D., S. J. LeBlanc, B. W. McBride, and T. J. DeVries. 2013. Associations between herd- level feeding management practices, feed sorting, and milk production in freestall dairy farms. J. Dairy Sci. 96:4759-4770.

Stefańsk, B., W. Nowak, J. Komisarek, M. Taciak, M. Barszcz, and J. Skomial. 2016. Prevalence and consequence of subacute ruminal acidosis in Polish dairy herds. J. Anim. Physiol. Anim. Nutr. Pre-published DOI: 10.1111/jpn.12592.

Stone, W. C. 1999. The effect of subclinical rumen acidosis on milk components. Pages 40-46 in Cornell Nutr. Conf. Feed Manuf., Proc. 1999, Cornell Univ., Ithaca, NY.

Stone, W. C. 2004. Nutritional approaches to minimize subacute rumen acidosis and laminitis in dairy cattle. J. Dairy Sci. 87(E-Suppl.):E13-E26.

Tilley, J. M. A., and R. A. Terry. 1963. A two-stage technique for the in vitro digestion of forage crops. Grass and Forage Sci. 18:104-111.

USDA. 2010. Facility characteristics and cow comfort on U.S. dairy operations, 2007. US Dept. Agric.-Anim. Plant Health Insp. Serv.-Vet. Serv., Cent. Epidemiol. Anim. Health, Fort Collins, CO.

Van Soest, P. J., J. B. Robertson, and B. A. Lewis. 1991. Methods for dietary fiber, neutral detergent fiber, and nonstarch polysaccharides in relation to animal nutrition. J. Dairy Sci. 74:3583-3597.

von Keyserlingk, M. A. G., A. Barrientos, K. Ito, E. Galo, and D. M. Weary. 2012. Benchmarking cow comfort on North American freestall dairies: lameness, leg injuries, lying time, facility design, and management for high-producing Holstein dairy cows. J. Dairy Sci. 95:7399-7408.

Wang, F. X., D. F. Shao, S. L. Li, Y. J. Wang, A. Azarfar, and Z. J. Cao. 2016. Effects of stocking density on behavior, productivity, and comfort indices of lactating dairy cows. J. Dairy Sci. 99:3709-3717.

Wojciechowski, K. L., C. Melilli, and D. M. Barbano. 2016. A proficiency test system to improve performance of milk analysis methods and produce reference values for component calibration samples for infrared milk analysis. J. Dairy Sci. 99:6808-6827.

Woolpert, M. E., H. M. Dann, K. W. Cotanch, C. Melilli, L. E. Chase, R. J. Grant, and D. M. Barbano. 2016. Management, nutrition, and lactation performance are related to bulk tank milk de novo fatty acid concentration on northeastern US dairy farms. J. Dairy Sci. 99:8486-8497.

Yang, W. Z., and K. A. Beauchemin. 2006. Increasing the physically effective fiber content of dairy cow diets may lower efficiency of feed use. J. Dairy Sci. 89:2694-2704.

Zebeli, Q., B. U. Metzler-Zebeli, and B. N. Ametaj. 2012. Meta-analysis reveals threshold level of rapidly fermentable dietary concentrate that triggers systemic inflammation in cattle. J. Dairy Sci. 95:2662-2672.

Zebeli, Q., D. Mansmann, B. N. Ametaj, H. Steingab, and W. Drochner. 2010. A model to optimise the requirements of lactating dairy cows for physically effective neutral detergent fibre. Arch. Anim. Nut. 64:265-278.

Zebeli, Q., S. M. Dunn, and B. N. Ametaj. 2011. Perturbations of plasma metabolites correlated with the rise of rumen endotoxin in dairy cows fed diets rich in easily degradable carbohydrates. J. Dairy Sci. 94:2374-2382.

Table 4.1. Ingredient composition and analyzed chemical composition (dry matter basis) of TMR samples for no straw (NS) and straw (S) diets.

Item	NS	S
Ingredient, % of dry matter (DM)		
Conventional corn silage	39.7	39.7
Haycrop silage	6.9	2.3
Wheat straw, chopped[1]	—	3.5
Citrus pulp, dry	4.8	4.8
Whole cottonseed, fuzzy	3.5	3.5
Soybean meal, 47.5% solvent	—	1.1
Molasses	3.2	3.2
Concentrate mix[2]	41.9	41.9
Chemical analysis, % DM		
DM, %	45.9 ± 0.4[3]	47.5 ± 0.5
Crude protein (CP)	15.0 ± 0.3	15.1 ± 0.3
Soluble protein, % of CP	32.0 ± 0.8	28.2 ± 1.4
NDICP[4]	1.1 ± 0.0	1.1 ± 0.0
Acid detergent fiber (ADF)	20.0 ± 0.3	20.1 ± 0.3
Neutral detergent fiber (NDF)	28.9 ± 0.5	31.7 ± 0.7
Acid detergent lignin (ADL)	3.8 ± 0.1	3.8 ± 0.1
Non-fibrous carbohydrates (NFC)	43.1 ± 0.4	43.7 ± 0.6
Starch	25.0 ± 0.4	25.3 ± 0.6
Starch digestibility (7-h), % of starch	73.3 ± 1.0	74.3 ± 0.5
Sugar	7.4 ± 0.3	8.1 ± 0.4
Fat	5.9 ± 0.2	5.7 ± 0.2
Ash	6.4 ± 0.2	6.4 ± 0.4
Ca	0.71 ± 0.20	0.72 ± 0.03
P	0.38 ± 0.00	0.38 ± 0.01
Mg	0.41 ± 0.00	0.40 ± 0.00
K	1.22 ± 0.03	1.16 ± 0.02
S	0.26 ± 0.01	0.26 ± 0.01
Na	0.45 ± 0.01	0.44 ± 0.01
Cl ion	0.50 ± 0.02	0.47 ± 0.01
Fe, mg/kg of DM	209 ± 9	212 ± 11
Mn, mg/kg of DM	86 ± 1	83 ± 2
Zn, mg/kg of DM	96 ± 1	94 ± 1
Cu, mg/kg of DM	19 ± 0	18 ±1
Net energy of lactation, Mcal/kg of DM	1.76 ± 0.01	1.75 ± 0.02
Physically effective NDF $_{>1.18\ mm}$, % of DM[5]	23.9	25.9
30-h uNDFom, % of DM[6]	13.1	14.9
120-h uNDFom, % of DM	9.0	10.2
240-h uNDFom, % of DM	8.5	9.7

[1]Hay-busted; hammer-mill chopping technique; mo. #H1100, Duratech Industries Inc., Jamestown, North Dakota.
[2]Concentrate mix was composed of the following (% of DM): corn meal, finely ground (32.31), soybean meal 47.5 solvent (15.90), AminoMax (Afgritech LLC, Watertown, NY; 14.28), flaked corn (12.72), Berga Fat F100 (Berg + Schmidt America LLC, Libertyville, IL; 5.65), wheat red dog (4.77), canola meal solvent (3.98), Amino Enhancer (Poulin Grain Inc., Swanton, VT; 3.88), calcium carbonate (2.39), sodium sesquicarbonate (1.62), salt (0.78), magnesium oxide (0.55), Meta Smart (Adisseo, Alpharetta, GA; 0.35), trace mineral mix (contained Diamune SE concentrate (Diamond V, Cedar Rapids, IA, 58.33%, zinc sulfate, 14.04%, manganese sulfate, 13.64%, calcium carbonate, 5.50%, 30% ferrous sulfate, 5.40%, 58% Intellibond copper (Micronutrients, Indianapolis, IN, 1.17%, mineral oil, 1.00%, 3% selenium, 0.53%, cobalt sulfate, 0.29%, and calcium iodate, 0.11%; 0.20), Urea (0.19), Select GH (Alltech, Inc.,Nicholasville, KY; 0.13), Gen 2-AjiPro-L (Ajinomoto Heartland, Inc., Chicago, IL; 0.10), vitamins A, D and E premix (contained calcium carbonate, 78.77%, vitamin E, 18.00%, vitamin A 1000 kIU and vitamin D 200 kIU, 2.34%, mineral oil, 0.50%, Vitamin D, 0.14%; 0.06), Smartamine M (Adisseo, Alpharetta, GA; 0.06), Zinpro Availa 4 (Zinpro Corporation, Eden Prairie, Minnesota; 0.05), vitamin E premix (contained 88.18 kIU vitamin E, 7.08 mg/kg Cu; 0.02), Probios Precise Concentrate (Chr-Hansen, Milwaukee, WI; 0.02), and Rumensin 90 (Elanco Animal Health, Greenfield, IN; 0.01).
[3]Mean ± standard error.
[4]Neutral detergent insoluble CP.
[5]peNDF determined through methods described by Mertens (2002).
[6]uNDFom determined through methods described by Tilley and Terry (1963) with Goering and Van Soest (1970) buffer modifications.

Table 4.2. Analyzed chemical composition (% of dry matter) and *in vitro* fermentation analysis of forages used in no straw (NS) and straw (S) diets.

Item	Conventional corn silage	Haycrop silage	Wheat straw, chopped
Dry matter (DM), %	29.6 ± 0.2[1]	28.0 ± 1.4	86.8 ± 0.3
Crude protein (CP)	7.2 ± 0.2	18.6 ± 0.7	4.2 ± 0.2
Soluble protein, % of CP	51.7 ± 1.7	62.9 ± 1.5	46.6 ± 1.9
NDICP[2]	1.1 ± 0.0	1.9 ± 0.2	1.3 ± 0.0
Acid detergent fiber (ADF)	25.6 ± 0.4	33.8 ± 0.8	55.4 ± 0.6
Neutral detergent fiber (NDF)	43.6 ± 0.1	49.8 ± 1.6	81.6 ± 0.4
Acid detergent lignin (ADL)	2.8 ± 1.0	4.0 ± 0.1	8.9 ± 0.3
Non-fibrous carbohydrates (NFC)	43.5 ± 0.4	19.9 ± 0.9	9.1 ± 0.5
Starch	31.7 ± 0.5	1.1 ± 0.2	0.7 ± 0.3
Starch digestibility (7-h), % of starch	77.3 ± 1.5	-	-
Sugar	1.0 ± 0.0	2.3 ± 0.3	0.9 ± 0.3
Fat	3.2 ± 0.0	4.2 ± 0.4	1.5 ± 0.1
Ash	3.6 ± 0.3	9.4 ± 0.4	5.1 ± 0.2
Ca	0.32 ± 0.02	0.84 ± 0.05	0.29 ± 0.03
P	0.27 ± 0.01	0.34 ± 0.02	0.05 ± 0.00
Mg	0.18 ± 0.01	0.29 ± 0.02	0.11 ± 0.00
K	0.76 ± 0.04	2.82 ± 0.14	0.89 ± 0.07
S	0.12 ± 0.01	0.25 ± 0.01	0.10 ± 0.01
Na	0.00 ± 0.00	0.05 ± 0.01	0.01 ± 0.00
Cl ion	0.09 ± 0.01	0.57 ± 0.05	0.20 ± 0.02
Fe, mg/kg	240 ± 89	228 ± 12	137 ± 15
Cu, mg/kg	5 ± 0	12 ± 0	4 ± 0
Mn, mg/kg	37 ± 2	58 ± 3	23 ± 9
Zn, mg/kg	24 ± 1	28 ± 0	9 ± 1
Net energy of lactation, Mcal/kg of DM	1.61 ± 0.01	1.52 ± 0.02	0.88 ± 0.02
30-h uNDFom, % of DM[3]	24.7 ± 0.7	28.5 ± 0.5	63.1 ± 0.3
120-h uNDFom, % of DM	14.1 ± 0.2	16.0 ± 0.03	45.0 ± 0.9
240-h uNDFom, % of DM	12.3 ± 0.4	13.4 ± 0.2	38.1 ± 0.4

[1]Mean ± standard error.
[2]Neutral detergent insoluble CP.
[3]uNDFom determined through methods described by Tilley and Terry (1963) with Goering and Van Soest (1970) buffer modifications.

Table 4.3. Analyzed chemical composition (% of dry matter) of by-products and grain mix used in no straw (NS) and straw (S) diets.

Item	Citrus pulp, dry	Whole cottonseed, fuzzy	Soybean meal, 47.5% solvent	Molasses	Concentrate mix
Dry matter (DM), %	88.2 ± 0.1[1]	88.4 ± 0.8	88.3 ± 0.2	61.8[2]	87.7 ± 0.1
Crude protein (CP)	7.1 ± 0.0	19.7 ± 1.0	52.0 ± 0.5	6.3	25.6 ± 0.2
Soluble protein, % CP	41.3 ± 2.9	14.6 ± 3.0	20.5 ± 1.3	100.0	24.5 ± 1.4
NDICP[3]	2.3 ± 0.1	2.5 ± 0.3	0.8 ± 0.0	0.0	1.7 ± 0.1
Acid detergent fiber (ADF)	14.4 ± 0.9	35.3 ± 1.2	6.3 ± 0.1	0.0	4.9 ± 0.4
Neutral detergent fiber (NDF)	22.4 ± 0.4	47.9 ± 1.9	8.4 ± 0.2	0.0	11.7 ± 1.1
Acid detergent lignin (ADL)	1.7 ± 0.1	9.4 ± 0.1	1.7 ± 0.4	0.0	2.3 ± 0.1
Non-fibrous carbohydrates (NFC)	62.9 ± 0.4	10.8 ± 0.5	30.8 ± 0.7	81.7	49.3 ± 0.8
Starch	1.3 ± 0.3	0.4 ± 0.2	3.4 ± 0.3	0.0	35.0 ± 0.6
Sugar	27.3 ± 0.4	4.7 ± 0.2	12.9 ± 1.0	61.5	5.6 ± 0.1
Fat	2.1 ± 0.1	19.9 ± 0.3	2.9 ± 0.1	1.0	6.4 ± 0.4
Ash	7.9 ± 0.1	4.3 ± 0.2	6.8 ± 0.0	11.0	8.8 ± 0.2
Ca	2.20 ± 0.01	0.16 ± 0.01	0.28 ± 0.01	1.00	1.40 ± 0.04
P	0.12 ± 0.01	0.63 ± 0.03	0.80 ± 0.01	0.10	0.53 ± 0.01
Mg	0.15 ± 0.00	0.40 ± 0.02	0.33 ± 0.00	0.42	0.59 ± 0.02
K	1.07 ± 0.01	1.18 ± 0.01	2.50 ± 0.02	4.01	1.03 ± 0.02
S	0.10 ± 0.00	0.24 ± 0.01	0.42 ± 0.01	0.47	0.38 ± 0.01
Na	0.07 ± 0.00	0.02 ± 0.00	0.01 ± 0.00	0.22	0.95 ± 0.04
Cl ion	0.12 ± 0.01	0.06 ± 0.00	0.05 ± 0.01	0.75	0.66 ± 0.02
Fe, mg/kg	75 ± 8	62 ± 2	144 ± 3	191	265 ± 11
Cu, mg/kg	7 ± 0	9 ± 1	19 ± 0	66	34 ± 1
Mn, mg/kg	15 ± 1	16 ± 0	39 ± 2	59	86 ± 2
Zn, mg/kg	22 ± 6	36 ± 2	69 ± 3	14	174 ± 7

[1]Mean ± standard error.

[2] Values based on Cornell Net Carbohydrate and Protein System (CNCPS) feed library (ver. 6.1; Agricultural Modeling and Training Systems, LLC, Groton, NY).

[3]Neutral detergent insoluble CP.

Table 4.4. Physical characterization of no straw (NS) and straw (S) diets and forages used in diets.

Item	NS	S	Conventional corn silage	Haycrop silage	Wheat straw, chopped
Particle size distribution, % as-fed					
>19.0 mm	4.7 ± 0.3[1]	3.8 ± 0.4	3.4 ± 0.4	31.7 ± 6.1	14.5 ± 1.0
8.0 to 19.0 mm	55.5 ± 1.5	53.0 ± 1.0	76.8 ± 0.3	57.0 ± 1.5	42.7 ± 1.0
4.0 to 8.0 mm	11.2 ± 0.3	11.4 ± 0.2	12.4 ± 0.2	10.6 ± 0.7	22.5 ± 0.8
<4.0 mm	31.4 ± 0.5	31.8 ± 1.1	7.4 ± 0.2	6.4 ± 0.6	20.3 ± 1.0
Particle size distribution, % of DM					
>19.00 mm	0.3 ± 0.1	0.1 ± 0.0	0.2 ± 0.1	0.6 ± 0.1	0.1 ± 0.1
13.20 to 19.00 mm	0.6 ± 0.1	0.3 ± 0.1	1.3 ± 0.2	1.2 ± 0.2	0.4 ± 0.1
9.50 to 13.20 mm	2.4 ± 0.2	2.5 ± 0.2	3.8 ± 0.6	2.6 ± 0.4	1.5 ± 0.3
6.70 to 9.50 mm	10.7 ± 0.4	11.4 ± 0.5	15.0 ± 0.4	5.0 ± 0.3	4.1 ± 0.4
4.75 to 6.70 mm	14.6 ± 0.6	14.9 ± 0.3	22.3 ± 0.3	5.0 ± 0.4	4.8 ± 0.5
3.35 to 4.75 mm	12.8 ± 0.3	12.8 ± 0.3	21.7 ± 0.6	10.8 ± 0.3	14.7 ± 0.4
2.36 to 3.35 mm	9.9 ± 0.2	9.8 ± 0.2	14.0 ± 0.5	14.8 ± 0.3	20.1 ± 0.6
1.18 to 2.36 mm	15.6 ± 0.3	15.3 ± 0.3	12.8 ± 0.3	35.0 ± 0.9	37.2 ± 0.7
0.60 to 1.18 mm	16.5 ± 0.4	15.0 ± 0.2	4.8 ± 0.2	17.7 ± 0.2	13.1 ± 0.8
0.30 to 0.60 mm	11.9 ± 0.3	12.4 ± 0.3	2.6 ± 0.3	5.0 ± 0.0	3.1 ± 0.3
<0.30 mm	4.7 ± 0.2	5.5 ± 0.2	1.7 ± 0.2	1.5 ± 0.0	1.1 ± 0.1
pef[2]	0.67 ± 0.01	0.67 ± 0.01	0.91 ± 0.01	0.72 ± 0.04	0.83 ± 0.01

[1]Mean ± standard error.

[2]Physical effectiveness factor.

Table 4.5. Effect of stocking density[1] (STKD) and source of forage fiber (no straw; NS and straw; S) on sorting activity of diets determined with PSPS and Ro-Tap particle fractions (n=4 pens/treatment).

	100%		142%			*P*-value		
Variable	NS	S	NS	S	SEM	STKD	DIET	STKD x DIET
Sorting of particles, %[2,3]								
>19.0 mm	102.2	100.6	102.6	101.8	0.3	0.02	<0.01	0.15
8.0 to 19.0 mm	103.3	103.4	104.1	103.2	1.1	0.79	0.70	0.69
4.0 to 8.0 mm	98.9	99.6	98.9	99.0	0.5	0.57	0.53	0.56
<4.0 mm	97.4	96.4	97.9	96.0	1.0	0.95	0.11	0.61
Sorting of particles, %[4]								
>19.00 mm	100.08	100.04	100.53	100.08	0.13	0.11	0.11	0.16
13.20 to 19.00 mm	99.52	99.83	100.19	100.08	0.35	0.28	0.81	0.61
9.50 to 13.20 mm	100.67	100.14	100.31	100.39	0.46	0.91	0.66	0.57
6.70 to 9.50 mm	100.78	101.41	100.05	101.09	0.80	0.50	0.29	0.78
4.75 to 6.70 mm	97.77	98.84	98.97	100.43	0.77	0.10	0.13	0.80
3.35 to 4.75 mm	99.99	99.66	100.05	99.52	0.42	0.92	0.35	0.82
2.36 to 3.35 mm	99.93	99.73	99.67	99.55	0.29	0.45	0.58	0.90
1.18 to 2.36 mm	99.73	99.62	99.17	99.33	0.33	0.23	0.94	0.68
0.60 to 1.18 mm	99.33	98.35	98.84	97.78	0.79	0.51	0.23	0.96
0.30 to 0.60 mm	101.38	100.98	101.37	100.64	0.60	0.76	0.33	0.77
<0.30 mm	100.82	101.40	100.85	101.13	0.15	0.41	0.02	0.30

[1]100%, 17 freestalls and headlocks per pen; 142%, 12 freestalls and headlocks per pen; 17 cows per pen

[2]Actual intake of particle fraction as a percentage of predicted intake.

[3]Penn State Particle Separator (Cotanch et al., 2010).

[4]Ro-Tap testing sieve shaker (model B, W. S. Tyler Combustion Engineering, Inc., Mentor, OH).

Table 4.6. Effect of stocking density[1] (STKD) and source of forage fiber (no straw; NS and straw; S) on short-term intake and lactational responses (n = 4 pens/treatment).

	100%		142%			*P*-value		
Variable	NS	S	NS	S	SEM	STKD	DIET	STKD x DIET
Intake and efficiencies								
Dry matter intake, kg/cow/d	25.4	25.3	25.3	25.2	0.4	0.78	0.69	0.87
NDF[2] intake, kg/cow/d[3]	7.5	8.3	7.2	8.0	0.3	0.30	0.02	0.95
peNDF[4] intake, kg/cow/d[3]	6.1	6.8	5.9	6.6	0.3	0.46	0.03	0.97
uNDFom240[5] intake, kg/cow/d	2.20	2.47	2.09	2.49	0.08	0.47	<0.01	0.32
Milk/DMI, kg/kg	1.62	1.58	1.63	1.58	0.04	0.75	0.02	0.64
Solids-corrected milk/DMI, kg/kg	1.57	1.53	1.57	1.54	0.05	0.70	0.05	0.80
Milk								
Daily yield, kg/cow/d	41.1	40.3	40.6	40.0	0.6	0.18	0.06	0.60
1st milking post-feeding, kg/cow[6,7]	13.9	13.6	13.7	13.5	0.2	0.13	0.01	0.48
2nd milking post-feeding, kg/cow	13.5	13.1	13.5	13.3	0.2	0.82	0.10	0.51
3rd milking post-feeding, kg/cow	13.7	13.6	13.4	13.2	0.2	0.01	0.25	0.96
Solids-corrected milk								
Daily yield, kg/cow/d	39.6	39.2	39.5	38.6	0.7	0.18	0.03	0.31
1st milking post-feeding, kg/cow[6,7]	13.7	13.2	13.4	13.0	0.3	0.01	<0.01	0.74
2nd milking post-feeding, kg/cow	13.0	13.0	13.3	13.0	0.3	0.59	0.58	0.62
3rd milking post-feeding, kg/cow	12.9	13.0	12.8	12.7	0.3	0.05	0.88	0.37
Milk composition								
Fat, %	4.14	4.21	4.22	4.19	0.03	0.26	0.32	0.07
Fat, kg/d	1.72	1.72	1.73	1.69	0.03	0.25	0.05	0.06
True protein, %	3.32	3.32	3.33	3.34	0.04	0.30	0.76	0.85
True protein, kg/d	1.38	1.35	1.37	1.34	0.02	0.19	0.04	0.92
Anhydrous lactose, %	4.52	4.49	4.51	4.51	0.05	0.43	0.20	0.23
Anhydrous lactose, kg/d	1.90	1.86	1.88	1.85	0.05	0.19	0.05	0.70
Somatic cell score	2.10	2.25	2.24	2.07	0.22	0.70	0.89	0.02
MUN[8], mg/dL	11.50	11.29	11.44	11.67	0.56	0.57	0.96	0.45

Body weight, kg	1655	1661	1659	1661	10	0.55	0.19	0.44
Body condition score	3.1	3.1	3.1	3.2	0.0	1.00	0.05	0.27

[1]100%, 17 freestalls and headlocks per pen; 142%, 12 freestalls and headlocks per pen; 17 cows per pen
[2]Neutral detergent fiber
[3]Orts corrected (dry matter offered * NDF) – (dry matter refused * NDF)
[4]Physically effective NDF
[5]undigested NDF (ash-corrected) determined through methods described by Tilley and Terry (1963) with Goering and Van Soest (1970) buffer modifications.
[6]Diets fed 1x/d at approximately 0600h, with feed pushed-up 6 times daily.
[7]Cows milked 3x/d; 1st milking post-feeding at approximately 1300 h, 2nd milking post-feeding at approximately 2100 h, and 3rd milking post-feeding at approximately and 0500 h.
[8]Milk urea nitrogen

Table 4.7. Effect of stocking density[1] (STKD) and source of forage fiber (no straw; NS and straw; S) on daily distribution of feeding behavior (n = 4 pens/treatment).

	100%		142%			*P*-value		
Variable	NS	S	NS	S	SEM	STKD	DIET	STKD x DIET
Feeding time								
Daily total, min/d	233	237	242	240	4	0.13	0.76	0.48
0-8 h post-feeding[2], min	88	94	91	91	3	0.81	0.20	0.23
9-16 h post-feeding, min	86	86	85	84	3	0.54	0.80	0.77
17-24 h post-feeding, min	60	57	66	65	1	<0.01	0.32	0.55
Feeding bout number								
Daily bouts, bouts/d	7.2	7.0	7.6	7.2	0.1	0.10	0.11	0.55
0-8 h post-feeding, bouts	2.7	2.7	2.8	2.7	0.1	0.25	0.63	0.59
9-16 h post-feeding, bouts	2.5	2.4	2.5	2.4	<0.1	0.92	0.06	0.94
17-24 h post-feeding, bouts	2.0	1.9	2.2	2.0	0.1	0.09	0.26	0.68
Feeding bout length								
Daily average bout length, min/bout	35.5	37.1	35.1	37.1	1.0	0.85	0.09	0.80
0-8 h post-feeding, min/bout	36.0	38.5	35.8	37.9	1.3	0.77	0.13	0.92
9-16 h post-feeding, min/bout	38.0	40.3	39.6	41.7	2.0	0.47	0.28	0.95
17-24 h post-feeding, min/bout	34.2	34.1	35.2	37.0	1.4	0.23	0.57	0.52
Length of first meal, min	39.0	43.1	40.9	44.2	1.8	0.26	0.02	0.77

[1]100%, 17 freestalls and headlocks per pen; 142%, 12 freestalls and headlocks per pen; 17 cows per pen

[2]Diets fed 1x/d at approximately 0600 h, with feed pushed-up 6 times daily.

Table 4.8. Effect of stocking density (STKD) and source of forage fiber (no straw; NS and straw; S) on daily distribution of rumination behavior (n = 4 pens/treatment).

	100%		142%			*P*-value		
Variable	NS	S	NS	S	SEM	STKD	DIET	STKD x DIET
Rumination time								
Daily total, min/d	498	491	489	496	9	0.72	0.96	0.19
0-8 h post-feeding[2], min	151	147	151	156	4	0.07	0.96	0.11
9-16 h post-feeding, min	162	165	160	163	3	0.36	0.11	0.91
17-24 h post-feeding, min	186	179	177	177	5	0.23	0.43	0.43
Rumination bout number								
Daily bouts, bouts/d	14.0	13.9	13.9	13.9	0.2	0.93	0.97	0.77
0-8 h post-feeding, bouts	4.5	4.5	4.5	4.6	0.1	0.71	0.84	0.66
9-16 h post-feeding, bouts	4.5	4.5	4.5	4.6	0.1	0.46	0.51	0.87
17-24 h post-feeding, bouts	4.9	4.9	4.8	4.7	0.1	0.14	0.46	0.95
Rumination bout length								
Daily average bout length, min/bout	37.6	37.3	37.4	37.7	0.9	0.87	0.95	0.70
0-8 h post-feeding, min/bout	35.0	35.3	35.9	36.1	0.9	0.32	0.82	0.95
9-16 h post-feeding, min/bout	38.6	39.4	38.6	38.6	0.8	0.59	0.66	0.59
17-24 h post-feeding, min/bout	40.8	39.0	40.3	41.0	1.4	0.59	0.67	0.35
Rumination location								
Rumination in freestall, % total rumination	86.2	86.0	80.5	81.1	1.6	<0.01	0.96	0.60
Rumination while lying, % total rumination	78.3	77.6	74.4	75.8	1.4	0.02	0.70	0.29

[1]100%, 17 freestalls and headlocks per pen; 142%, 12 freestalls and headlocks per pen; 17 cows per pen
[2]Diets fed 1x/d at approximately 0600 h, with feed pushed-up 6 times daily.

Table 4.9. Effect of stocking density[1] (STKD) and source of forage fiber (no straw; NS and straw; S) on daily distribution of lying behavior (n = 4 pens/treatment).

	100%		142%			*P*-value		
Variable	NS	S	NS	S	SEM	STKD	DIET	STKD x DIET
Lying time								
Daily total, min/d	832	827	779	797	11	<0.01	0.56	0.31
0-8 h post-feeding[2], min	276	269	260	264	4	0.02	0.68	0.13
9-16 h post-feeding, min	265	266	255	262	4	0.06	0.23	0.42
17-24 h post-feeding, min	291	292	264	271	6	<0.01	0.50	0.57
Lying bout number								
Daily bouts, bouts/d	6.5	6.4	6.6	6.5	0.1	0.42	0.60	0.73
0-8 h post-feeding, bouts	3.0	2.9	3.1	3.0	0.1	0.22	0.11	0.90
9-16 h post-feeding, bouts	2.4	2.4	2.5	2.4	0.1	0.94	0.83	0.51
17-24 h post-feeding, bouts	2.5	2.5	2.6	2.5	0.1	0.93	0.35	0.79
Lying bout length								
Daily average bout length, min/bout	113.0	113.7	103.2	109.1	2.9	0.07	0.35	0.46
0-8 h post-feeding, min/bout	101.7	101.7	90.9	95.8	3.0	0.04	0.47	0.47
9-16 h post-feeding, min/bout	122.8	126.4	120.1	121.9	3.4	0.36	0.48	0.82
17-24 h post-feeding, min/bout	141.0	142.2	127.7	136.5	6.8	0.23	0.51	0.61
Lying within stall, % stall use	89.7	89.9	91.7	92.8	<0.01	0.01	0.39	0.50
Time spent in alley, min/d	121	125	192	181	9	<0.01	0.65	0.37
Locomotion score	1.6	1.6	1.6	1.6	0.1	0.90	0.90	0.71

[1]100%, 17 freestalls and headlocks per pen; 142%, 12 freestalls and headlocks per pen; 17 cows per pen
[2]Diets fed 1x/d at approximately 0600 h, with feed pushed-up 6 times daily.

Table 4.10. Effect of stocking density[1] (STKD) and source of forage fiber (no straw; NS and straw; NS) on feeding and lying responses upon return from milking parlor (n = 4 pens/treatment).

	100%		142%			*P*-value		
Variable	NS	S	NS	S	SEM	STKD	DIET	STKD x DIET
Feeding immediately upon return from parlor to pen, % of pen[2,3]								
Daily average	67.9	63.5	54.2	52.4	2.2	<0.01	0.20	0.58
1st milking post-feeding	64.7	57.1	48.0	46.9	3.7	<0.01	0.20	0.32
2nd milking post-feeding	51.9	57.1	47.5	46.5	3.2	0.06	0.52	0.39
3rd milking post-feeding (return to fresh feed)	87.1	76.3	67.2	63.5	4.4	0.01	0.18	0.48
Lying immediately upon return from parlor, % of pen								
Daily average	21.6	26.5	31.0	36.2	2.6	0.01	0.12	0.94
1st milking post-feeding	26.6	27.4	32.9	43.6	3.7	0.03	0.20	0.27
2nd milking post-feeding	26.6	33.8	35.3	35.5	4.2	0.27	0.41	0.43
3rd milking post-feeding	11.7	18.2	24.8	29.7	3.6	<0.01	0.13	0.82

[1]100%, 17 freestalls and headlocks per pen; 142%, 12 freestalls and headlocks per pen; 17 cows per pen

[2]Diets fed 1x/d at approximately 0600 h, with feed pushed-up 6 times daily.

[3]Cows milked 3x/d; 1st milking post-feeding at approximately 1300 h, 2nd milking post-feeding at approximately 2100 h, and 3rd milking post-feeding at approximately and 0500 h, each milking lasting approximately 60 min.

Table 4.11. Effect of stocking density[1] (STKD) and source of forage fiber (no straw; NS and straw; S) on stress responses (n = 4 pens/treatment).

	100%		142%			*P*-value		
Variable	NS	S	NS	S	SEM	STKD	DIET	STKD x DIET
Serum amyloid-A, µg/mL, d 7[2]	46.0	54.8	48.2	36.4	10.7	0.27	0.83	0.18
Serum amyloid-A, µg/mL, d 14	42.4	44.8	47.6	50.3	6.7	0.08	0.37	0.97

[1]100%, 17 freestalls and headlocks per pen; 142%, 12 freestalls and headlocks per pen; 17 cows per pen
[2]Day of period.

Table 4.12. Daily ruminal pH responses of focal cows (n = 4 cows/pen, 4 pens/treatment) to diets containing no straw (NS) and straw (S) at 100% and 142% stocking densities[1] (STKD).

	100%		142%			*P*-value		
Variable	NS	S	NS	S	SEM	STKD	DIET	STKD x DIET
Ruminal pH								
Mean pH	6.17	6.13	6.09	6.10	0.03	0.07	0.62	0.39
Minimum pH	5.70	5.67	5.62	5.59	0.05	0.11	0.53	0.95
Maximum pH	6.63	6.58	6.56	6.53	0.04	0.07	0.22	0.68
Time pH < 5.8, h/d	2.29	1.90	4.12	2.77	0.41	<0.01	0.01	0.10
AUC < 5.8 pH, units x pH[2]	0.38	0.19	0.58	0.34	0.10	0.06	0.03	0.75

[1]100%, 17 freestalls and headlocks per pen; 142%, 12 freestalls and headlocks per pen; 17 cows per pen
[2]AUC, area under the curve below pH 5.8.

Table 4.13. Daily distribution of ruminal pH responses of focal cows (n = 4 cows/pen, 4 pens/treatment) to diets containing straw (S) or no straw (NS) at 100% and 142% stocking densities[1] (STKD).

	100%		142%			*P*-value		
Variable	NS	S	NS	S	SEM	STKD	DIET	STKD x DIET
0-8 h post-feeding[2,3]								
Mean pH	6.22	6.19	6.13	6.14	0.03	0.09	0.64	0.57
Time pH < 5.8, h/8-h interval	0.50	0.30	0.97	0.72	0.19	0.03	0.21	0.88
AUC < 5.8 pH, units x pH[4]	0.07	0.04	0.15	0.19	0.05	0.18	0.31	0.77
7-16 h post-feeding								
Mean pH	6.14	6.08	6.03	6.07	0.03	0.03	0.77	0.07
Time pH < 5.8, h/8-h interval	0.85	0.63	1.82	0.98	0.24	<0.01	0.02	0.12
AUC < 5.8 pH, units x pH	0.16	0.05	0.26	0.09	0.06	0.21	0.03	0.62
17-24 h post-feeding								
Mean pH	6.12	6.13	6.08	6.07	0.04	0.16	0.97	0.74
Time pH < 5.8, h/8-h interval	1.20	0.72	1.77	1.08	0.16	0.03	0.01	0.55
AUC < 5.8 pH, units x pH	0.21	0.06	0.27	0.16	0.03	0.06	<0.01	0.55

[1]100%, 17 freestalls and headlocks per pen; 142%, 12 freestalls and headlocks per pen; 17 cows per pen

[2]Diets fed 1x/d at approximately 0600 h, with feed pushed-up 6 times daily.

[3]Cows milked 3x/d; 1st milking post-feeding at approximately 1300 h, 2nd milking post-feeding at approximately 2100 h, and 3rd milking post-feeding at approximately and 0500 h, each milking lasting approximately 60 min.

[4]AUC, area under the curve below pH 5.8.

Table 4.14. Daily ruminal fermentation responses of focal cows (n = 4 cows/pen, 4 pens/treatment) to diets containing straw (S) or no straw (NS) at 100% and 142% stocking densities[1] (STKD).

	100%		142%			*P*-value		
Variable	NS	S	NS	S	SEM	STKD	DIET	STKD x DIET
Volatile fatty acids, mol/100 mol[2]								
Acetate	63.91	64.59	64.33	64.07	0.60	0.90	0.60	0.26
Propionate	22.03	21.86	21.85	22.27	0.66	0.81	0.80	0.54
Butyrate	11.01	10.66	10.86	10.86	0.30	0.92	0.50	0.50
Isobutyrate	0.68	0.64	0.64	0.64	0.02	0.10	0.14	0.22
Valerate	1.91	1.83	1.92	1.75	0.09	0.18	<0.01	0.08
Isovalerate	0.46	0.43	0.41	0.42	0.01	0.06	0.32	0.20
Total volatile fatty acids, m*M*	155.4	155.4	154.1	155.8	0.8	0.57	0.28	0.31
NH_3-N[3], mg/dL	7.03	6.40	6.76	6.53	0.71	0.42	0.89	0.70

[1]100%, 17 freestalls and headlocks per pen; 142%, 12 freestalls and headlocks per pen; 17 cows per pen
[2]Diets fed 1x/d at approximately 0600 h, with feed pushed-up 6 times daily.
[3]Ammonia nitrogen

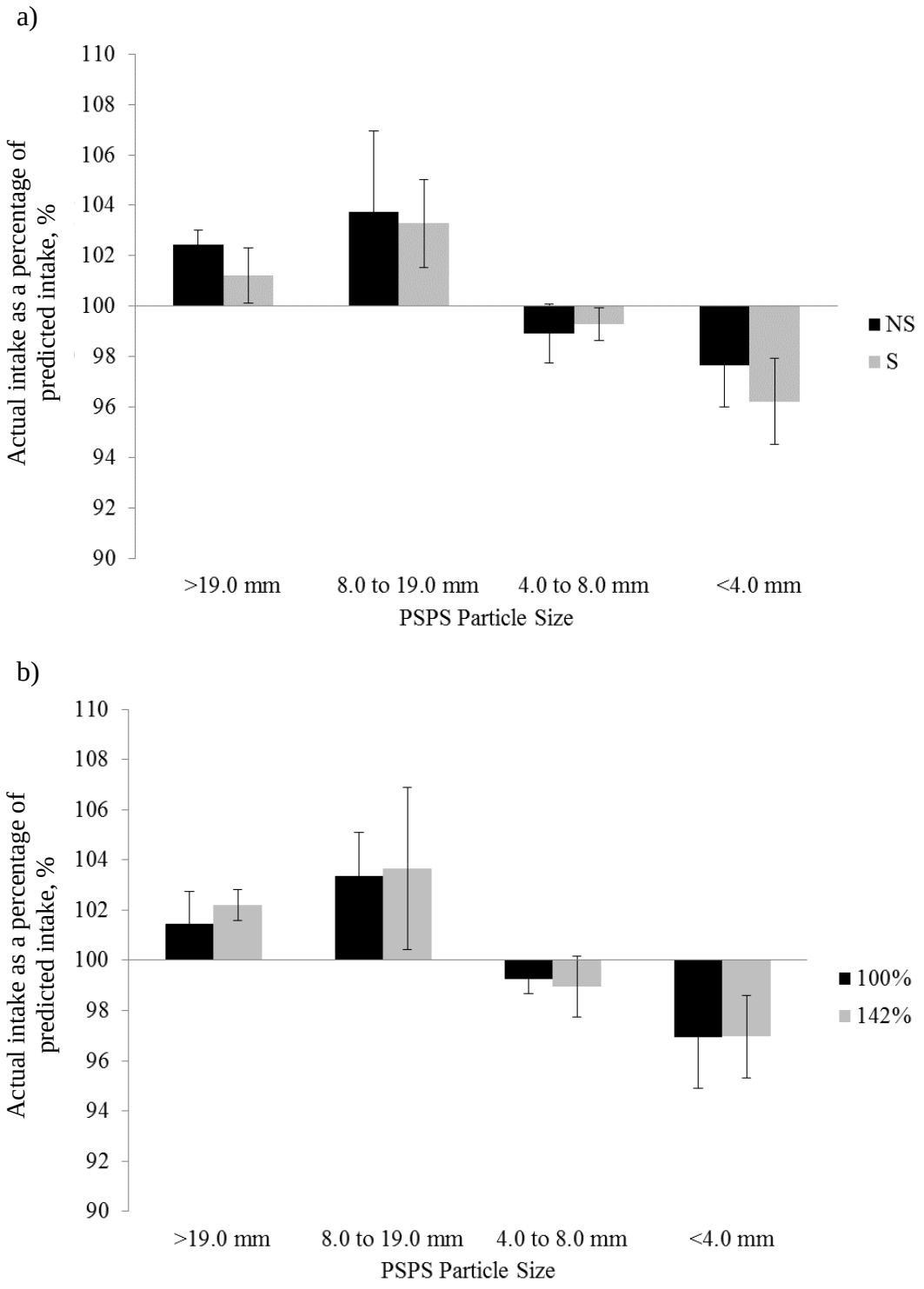


Figure 4.1. Mean ± standard error of the actual intake of total mixed ration as a percentage of predicted intake amongst Penn State Particle Separator (PSPS) particle fractions between (a) no straw (NS) and straw (S) diets and (b) 100% and 142% stocking

density (100%, 17 freestalls and headlocks per pen; 142%, 12 freestalls and headlocks per pen; 17 cows per pen).

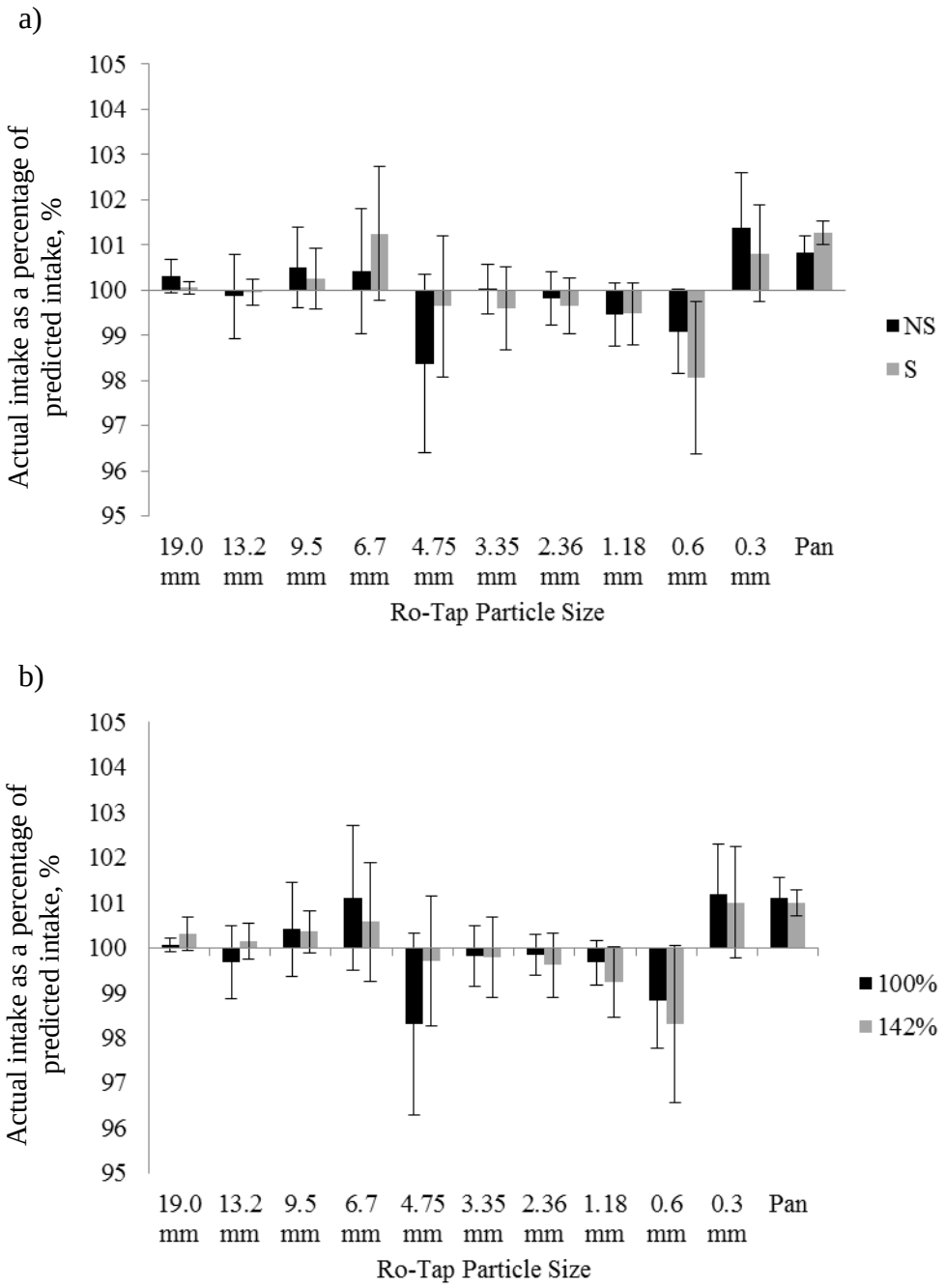


Figure 4.2. Mean ± standard error of the actual intake of total mixed ration as a percentage of predicted intake amongst Ro-Tap particle fractions between (a) no straw (NS) and straw (S) diets and (b) 100% and 142% stocking density (100%, 17 freestalls and headlocks per pen; 142%, 12 freestalls and headlocks per pen; 17 cows per pen).

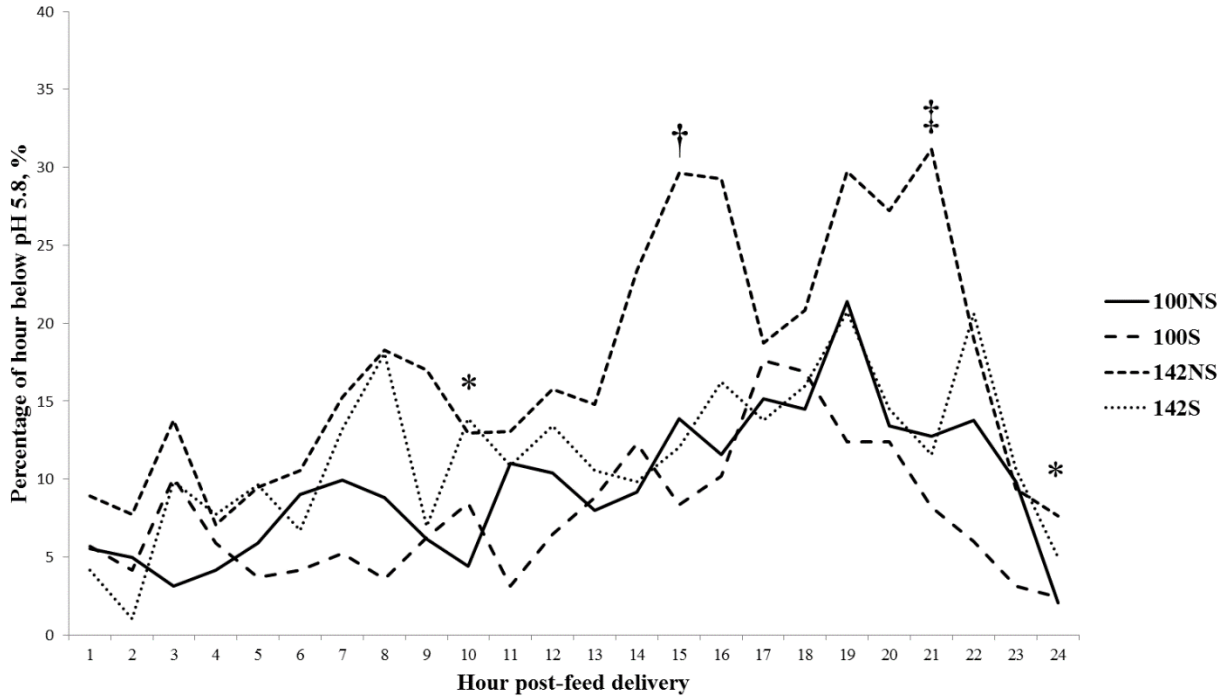
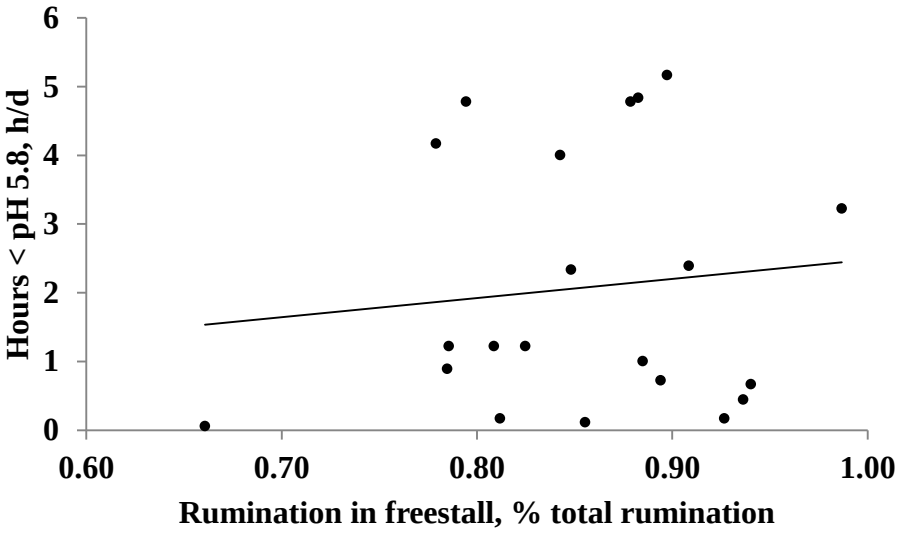


Figure 4.3. Least square means of the percentage of hour spent below pH 5.8 of focal cows across hours post-feed delivery by treatment (n = 4/treatment).*STKD main effect ($P \leq 0.05$), †DIET main effect ($P \leq 0.05$), ‡Interaction between STKD and DIET ($P \leq 0.05$). Least square means for treatments across the entire day were 9.54, 7.73, 17.11, and 11.54 for 100NS, 100S, 142NS, and 142S, respectively (SE = 3.80). (100%, 17 freestalls and headlocks per pen; 142%, 12 freestalls and headlocks per pen; 17 cows per pen; NS, no straw; S, straw).

a)



b)

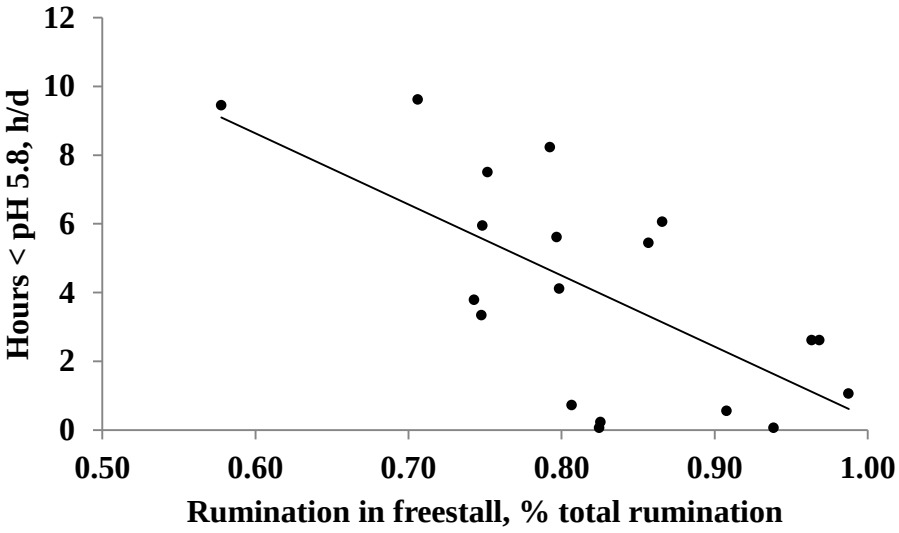


Figure 4.4. Relationship between rumination in freestall (% total rumination) and hours below pH 5.8 (h/d) of focal cows responsive to sub-acute ruminal acidosis (n=12) at a) 100% STKD; y = -2.79x – 0.31; R^2 = 0.01; *P* = 0.63 and b) 142% STKD; y = -20.70x + 21.06; R^2 = 0.44; *P* < 0.01. (100%, 17 freestalls and headlocks per pen; 142%, 12 freestalls and headlocks per pen; 17 cows per pen).

CHAPTER 5: EFFECT OF STOCKING DENSITY AND REDUCED FEED ACCESS ON SHORT-TERM RUMINAL FERMENTATION, BEHAVIORAL, PRODUCTION, AND STRESS RESPONSES OF HOLSTEIN DAIRY COWS

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ABSTRACT

Evaluating the interaction of stocking density and the feeding environment is an important step in furthering dairy cow well-being and ruminal health. The objective of this study was to determine the effect of stocking density and reduced feed access on short-term ruminal fermentation, behavior, production, and stress responses of Holstein dairy cows. Multiparous (n = 48) and primiparous (n = 20) cows were assigned to 1 of 4 pens (n = 17 cows/pen). A focal group of multiparous (n = 16) ruminally fistulated cows were used to evaluate ruminal fermentation. Pens were assigned to treatments in a 4 x 4 Latin square with 14-d periods using a 2 x 2 factorial arrangement of treatments. Two stocking densities (STKD; 100 or 142% of stalls and headlocks) and two levels of feed access (FA): 5 h reduced feed access prior to next feeding; R, and no reduced feed access; NR) resulted in 4 treatments: 1) 100NR, 2) 100R, 3) 142NR, and 4) 142R. Data were analyzed using a MIXED model in JMP with pen (n =4 pens/treatment) as the experiment unit. Dry matter intake and milk production did not differ between treatments. Daily feeding time decreased with R. While daily rumination time was unaffected by treatments, increased STKD decreased rumination within the freestall. In response to reduced feed access, cows altered their feeding and rumination patterns to maintain total rumination, increasing feeding and decreasing rumination 0 to 8 h post-feed delivery while decreasing feeding and increasing rumination 17 to 24 h post-feed delivery. Increased STKD reduced lying time, but increased efficiency of stall-use for resting. Higher STKD decreased latency to lie, indicating a shift in priority towards lying behavior over feeding behavior. There were no observed differences in stress responses

amongst treatments. Treatments had minimal impact on ruminal VFA, though R tended to decrease ruminal ammonia-N. An interaction was found between STKD and FA with time below pH 5.8, indicating that higher STKD negatively impacts ruminal pH and R exacerbates this effect.

Key words: overcrowding, feed access, ruminal pH

INTRODUCTION

Economics play an important role in driving the use of various management practices within the dairy industry. The use of overcrowding has continually grown, with average feedbunk stocking density at 142% and stall stocking densities ranging from 71 to 197% in northeastern dairy farms (von Keyserlingk et al., 2012). While economic return is maximized with stocking rates around 120% (De Vries et al., 2016), overstocking can have significant negative impacts on the cow's time-bu dget (Batchelder, 2000; Fregonesi et al., 2007; Krawczel et al., 2012b), increase agonistic interactions (Collings et al., 2011; Krawczel et al., 2012b), increase health disorders and stress (Friend, 1979; Barrientos et al., 2013; King et al., 2016), and reduce milk production (Deming et al., 2013; Sova et al., 2013; Woolpert et al., 2016). Overstocking can be classified as a sub-clinical stressor, draining the cow of biological reserves with limited observable impacts (Moberg, 2000). However, rarely is overstocking the only stressor present on the farm; rather, combinations of various environmental and management stressors exist.

Due to its widespread use, the majority of dairy cows in the US are fed within overstocked conditions and producers are increasingly feeding for lower amounts of daily feed refusals in an effort to minimize wastage of expensive feed (USDA-ERS, 2014). A survey of western US dairy farms suggests a growing number of producers are targeting 0% feed refusals, commonly referred to as feeding to a "slick or clean bunk" (Silva-del-Rio et al., 2010). With feed costs comprising 55% of total operating costs (USDA-ERS, 2014) and the continued increase in feed costs, producers have moved away from feeding for refusals. However, misjudging dry matter or changes in intake can leave periods of the day with no access to feed, typically late in the night prior to the next feed delivery. Reducing access to feed can result in decreased feed intake (Erdman et al., 1989; Collings et al., 2011), increased feeding rates (Munksgaard et al., 2005; Collings et al., 2011), and greater variation in ruminal pH (Erickson et al., 2003). Furthermore, access to feed can have large impacts on milk production, with routine feed push-up and feeding to ensure feed availability associated with a 4 kg/d increase in milk production (Bach et al., 2008).

Consequently, further understanding is needed about the interaction of stocking density and reduced feed access. We hypothesized that the cumulative effects of stocking density and reduced feed access would alter rumen pH, feeding and resting behavior, milk production, and stress responses to a greater extent than either stressor in isolation. Therefore, the objective of this study was to determine the effect of stocking density and reduced feed access on short-term ruminal fermentation, behavior, production, and stress responses of Holstein dairy cows.

MATERIALS AND METHODS

Animal Housing, Management, and Diet

Forty-eight multiparous and 20 primiparous, lactating Holstein cows were assigned to 1 of 4 pens (n = 17 cows/pen) in a 4-row freestall barn (saw-dust bedded, naturally ventilated) located at the William H. Miner Agricultural Research Institute (Chazy, NY) from February 17, 2016 to April 13, 2016. Pens were balanced for DIM (121 ± 38; mean ± standard deviation), parity (2.3 ± 1.1), and milk production (46.7 ± 8.2 kg/d) at the start of the study. Each of the 4 pens contained 17 freestalls (head-to-head). Facility specifications were similarly described by Krawczel et al. (2012b). Cows were milked at approximately 1300h, 2100h, and 0500h (3x/day) in a double-12 parallel parlor (Xpressway Parallel Stall System; Bou-Matic, Madison, WI). Ambient temperature and humidity were recorded using Hobo data loggers (Onset, Bourne, MA). A TMR was formulated for 52 kg/d milk production using AMTS nutrition software based on the Cornell Net Carbohydrate and Protein System model (ver. 6.1; Agricultural Modeling and Training Systems, LLC, Groton, NY) and the TMR met both ME and MP requirements. TMR was mixed and delivered once daily at approximately 0600 h with a Kuhn Knight RC 270 reel mixer (Kuhn North America, Inc., Brodhead, WI) and pushed up approximately 6 times daily. Animal care and handling protocols were approved by the Animal Care and Use Committee at the William H. Miner Agricultural Research Institute.

Experimental Design and Treatments

Pens were assigned randomly to treatments using a 2 x 2 factorial arrangement of treatments in a 4 x 4 Latin square design. Treatment periodswith 14-d periods and the first 7 d served as treatment adaptation for each period. Two stocking densities (STKD; 100 or 142%) and two levels of feed access (FA): 5 h reduced feed access prior to next feeding; R and no reduced feed access; NR) resulted in 4 treatments: 1) 100NR, 2) 100R, 3) 142NR, and 4) 142R. As described by Krawczel et al. (2012a) as an effective model to assess short-term (i.e., 14-d) cow responses to variable stocking densities, differences in stocking density were achieved through the denial of access to both headlocks and freestalls (100%, 17 freestalls and headlocks per pen; 142%, 12 freestalls and headlocks per pen). Reduced feed access was achieved through removal of feed approximately 5 h prior to the next TMR delivery as an effective model to simulate slick-bunk feeding management (French et al., 2005). Feed was pulled away from headlocks approximately 2.5 m using a New Holland skid-steer (mo.# Ly565; New Holland North America, Inc., New Holland, PA) at approximately 0100 h each day of the study for R treatments.

Environmental Conditions

Temperature and relative humidity were measured at at 15-min intervals using Hobo data loggers (Onset, Bourne, MA) within the freestalls during the study.

Dry Matter Intake and Feed Efficiency

Dry matter intake (DMI) and feed efficiency (kilogram/kilogram milk yield) were measured on d 8 to 14 of each period for each pen. Samples of TMR and orts were collected thrice weekly. Samples were dried in a forced-air oven at 105°C for 24 h for DM determination.

Feed Analysis and Particle Size Distribution

Total mixed ration (TMR), orts, and individual feed ingredients were collected 3 times during d 8 to 14 of each period. Samples of TMR and feed ingredients were frozen at -20°C until samples were composited and analyzed for chemical composition (CPM Plus; Cumberland Valley Analytical Services, Inc., Hagerstown, MD). The analyzed chemical composition of TMR and feed ingredients is shown in Tables 5.1 and 5.2, respectively. Samples of TMR, corn silages, and grains were analyzed for 7-h *in vitro* starch digestibility (Cumberland Valley Analytical Services, Inc., Hagerstown, MD; Tables 5.1 and 5.2). Sub-samples of TMR, forages, and orts were used for particle size determination using the Penn State Particle Separator (PSPS; as-fed basis; Lammers et al., 1996) with a 4-mm screen modification (Cotanch et al., 2010). The physical characterization of TMR and forages is shown in Table 5.3. Using PSPS fractions, sorting activity was measured as the actual intake as a percentage of predicted intake as described by Leonardi and Armentano (2003).

Milk Yield, Composition, and Fatty Acid Analysis

Milk yield was measured on d 8 to 14 of each period and recorded electronically (ProVantage Information Management System; Bou-Matic, Madison, WI). Milk samples were collected for each cow across six consecutive milkings on d 13 and 14 of each period. Samples were refrigerated at 4°C until analysis. Milk samples were analyzed at The William H. Miner Agricultural Institute (Chazy, NY) using a mid-infrared (MIR) milk analyzer (Delta Instruments; Drachten, Netherlands). Ttraditional virtual MIR filter models (with optimized wavelengths and inter-correction factors as described by Kaylegian et al. (2009)) were used to predict true protein and anhydrous lactose. Total milk fat was estimated using a partial least squares (PLS) chemometric MIR prediction model (Delta Instruments parameter number 9600). The model estimated total fatty acids (Woolpert et al, 2016), with the resulting value divided by 0.945 (to add glycerol). A PLS model (Delta Instruments, parameter number 0502) was also used to determine milk urea nitrogen (MUN). Mid-IR estimates for milk components were slope- and intercept-adjusted using a set of 14 modified milk calibration samples as described by Kaylegian et al. (2006a; 2006b). The reference chemistry for the modified milk calibration samples was: fat (AOAC, 2000; method 989.05; 33.2.26), total protein (AOAC, 2000; method 991.20; 33.2.11), nonprotein nitrogen (AOAC, 2000; method 991.21; 33.2.12), and anhydrous lactose (Lynch et al., 2007) with all lab mean reference chemistry reference values as described by Wojciechowski et al. (2016). Milk urea nitrogen was measured using an enzymatic assay (Megazyme, K-UMAMR kit, Wicklow, Ireland). Procedures followed the operational method detail (done by weight with path length correction) used

for the lactose enzymatic assay (Lynch et al., 2007), except the procedure used the enzymes and reagents for MUN measurement. Milk somatic cell count (SCC) was determined with a SomaScope (Delta Instruments, Drachten, Netherlands) utilizing fluorometeric flow cytometry stained with 4', 6-diamidino-2-phenylindole, dilactate. The machine was calibrated with milks that had reference values determined by direct microscopic somatic cell count (Fitts and Laird, 2004). Somatic cell count was transformed and analyzed as somatic cell score (SCS) as described by Shook et al. (1993). using the equation: SCS = log2(SCC/100) + 3 where SCC is in units of 1,000 cells/mL.

Body Weight, Body Condition Score, and Lameness

Body weight, body condition score, and lameness score were assessed on all cows prior to the start of the study and at the end of each period. Body weight was measured using an Allweigh computerized scale (Allweigh Scale System Inc., Red Deer, AB, Canada). Body condition score was assessed by one trained scorer using 0.25-unit increments on a 1 to 5 scale (Ferguson et al., 1994). Lameness was assessed by one trained scorer on a flat surface upon return from the milking parlor using a 1 to 4 scale (Nordlund et al., 2004).

Behavioral Analysis

Ingestive, rumination, and lying behaviors as well as the location of these performed behaviors were assessed on all cows using 72-h direct-observation, scan-

sampling at 10-min intervals (Mitlöhner et al., 2001) on d 8, 9, and 10 of each period. Feeding, rumination, and lying bouts were determined with a 20 min inter-bout criterion, with new bouts established if the cow spent greater than 20 min performing another behavior before performing the same behavior (Black et al., 2016).

Resting posture was based on the four resting positions previously defined as natural postures by Krohn and Munksgaard (1993): 1) lateral, flat on their side, 2) sternal, head back on flank, 3) sternal, head flat on the ground, and 4) sternal, head up right. Resting posture was calculated as a percentage of total, non-ruminating resting time.

Blood Measurements

Blood samples were taken from each cow on d 7 and 14 of each period. Two samples were collected from the coccygeal vein at approximately 0900 h. Samples were drawn into a 10-mL BD vacutainer tube spray-coated with lithium heparin (158 USP) and a 10-mL BD vacutainer tube spray-coated with sodium heparin (158 USP; BD Diagnostics, Franklin Lake, NJ). Samples were placed on ice until centrifugation at 1200 x *g* for 20 min at 4°C. Plasma was aliquoted into 2-mL cryogenic vials (Fisher Scientific, Pittsburgh, PA) and stored at -20°C until analysis.

Free cortisol indices (FCI) were evaluated for each cow in order to evaluate biologically available cortisol (Roberts et al., 2003). Total cortisol concentration (ng/mL) was determined from lithium heparinized plasma using a commercially available radioimmunoassay (MP Biomedicals, Solon, OH) with a sensitivity of 5 ng/mL (Hulbert et al., 2013). Intra- and inter-assay coefficients of variation were 9.42% and 23.76% for

low (7.85 ng/mL) and 11.9% and 16.5% for high (18.10 ng/mL) cortisol standards. Plasma corticosteroid binding globulin (CBG) concentrations (mg/L) were measured by ELISA following the isolation and purification of bovine CBG and antiserum development (Kattesh et. al., 2014) as described previously for porcine CBG (Roberts et al., 2003). Free cortisol indices were calculated using the total cortisol concentration to CBG concentration ratio.

In addition, cows were evaluated for changes in positive acute phase proteins from blood samples taken on d 14. To minimize carry-over effects from period to period, serum amyloid-A (SAA) from sodium heparinized plasma was chosen as an indicator of acute inflammation (Horadagoda et al., 1999) and measured using ELISA Tridelta Phase range kits (Tridelta Diagnostics Inc., Maynooth, County Kildare, Ireland; cat. no. TP-802). Absorbances were read in a microplate reader (BioTek Synergy 2; Winooksi, VT) at 450 nm.

Focal Cows

Four multiparous, ruminally fistulated (Bar Diamond, Parma, ID) cows were used in each pen (n = 16; 4/pen) as a focal group for rumen fermentation data collection. Focal groups were balanced for DIM, parity, and milk yield.

Ruminal pH

Ruminal pH was measured using an indwelling ruminal pH/ORP/REDOX measurement system (Penner et al., 2006; LRCpH; Dascor, Escondido, CA) Reading

were collected at 1-min intervals for 72 h on days 12, 13, and 14 of each period and averaged over 10-min intervals. For each cow, measurements were averaged across the 3 days of each period. Period averages were then averaged among cows into a pen average. Ruminal pH data were summarized as described in Chapter 4 (Bauer et al., 1995).

Ruminal Volatile Fatty Acids and Ammonia Nitrogen

Approximately 250 mL of rumen fluid was collected from beneath the ruminal digesta mat at 4-h intervals for 24 h on d 13 (0600, 1000, 1400, 1800, 2200 h) and d 14 (0200 h) of each period. Following collection, rumen fluid was strained through 4 layers of cheesecloth. Approximately 40 mL of rumen fluid was frozen and stored at −20°C for VFA determination (Bulletin 856B; Supelco Inc., Bellefonte, PA). The concentration of VFA (mol/100 mol) were determined by gas chromatography (Varian CP-3800 gas chromatograph; Varian Inc., Palo Alto, CA). The gas chromatograph was equipped with a flame-ionization detector as well as 80/120 Carbopack B-DA/4% Carbowax 20M column (Supelco Inc., Bellefonte, PA). A sub-sample of rumen fluid (10 mL) was mixed with 100 µL of 12.1 *N* hydrochloric acid. Sub-samples were stored at −20°C until ammonia nitrogen analysis using the procedures described by Chaney et al. (1962).

Statistical Analysis

Data were analyzed using JMP (ver. 12, SAS Institute Inc., NC) for a 2 x 2 factorial arrangement of treatments within a 4 x 4 Latin Square design according to the following mixed model:

$$Y_{ijkl} = \mu + S_i + F_j + SF_{ij} + P_k + R_l + E_{ijkl}$$

where Y_{ijkl} was the dependent variable, μ was the overall mean, S_i was the fixed effect of stocking density, F_j was the fixed effect of feed access, SF_{ij} was the fixed effect of the interaction between stocking density and feed access, P_k was the fixed effect of period, R_l was the random effect of pen, and E_{ijkl} was the residual error. Preplanned contrasts were included to compare stocking density, feed access, and the interaction between stocking density and feed access. Significance was declared at $P \leq 0.05$ and trends at $0.05 < P \leq 0.10$.

RESULTS AND DISCUSSION

Two cows (one multiparous and one primiparous) were removed from the study due to severe mastitis and a leg injury, respectively. While not directly related to the treatments, it is unknown whether treatments may have exacerbated severity of these responses. Data from these cows were removed from the analyzed data set. Due to variability in physical characterization and chemical composition of TMR among pens due to inadequate mixing length (< 1 min following the addition of the last ingredient) during period 1, this period was removed for ruminal fermentation data from the analyzed data set.

Environmental Conditions

Daily temperatures within the pens ranged from 3.3°C to 16.2 °C, with an average across periods of 8.1°C. Relative humidity ranged from 59.4% to 84.1% with an average of 74.0% across periods.

Intake and Production Measures

Daily DMI was unaffected by both STKD and FA ($P > 0.60$, Table 5.4). The response for stocking density was similar to those observed by Krawczel et al. (2012b; 142%) and Collings et al. (2011; 200%). While Erdman et al. (1989) and Collings et al. (2011) observed decreases in DMI with reductions in feed access, both studies reduced feed access by twice the amount of time compared to the current study. This indicates a break point in time without feed before observed reductions in DMI above 5 h per day. Sorting was minimal (within 10% difference; Miller-Cushon and DeVries, 2017) between refused and offered TMR for both NR and R, as well as both STKD levels (Figure 5.1). Sorting for particles (actual intake as a percentage of predicted intake) did not differ among treatments (Table 5.4).

Milk yield and solids-corrected milk (SCM) yield did not differ among treatments, similar to Krawczel et al. (2012b) at similar stocking density and Munksgaard et al. (2005) and Collings et al. (2011) with 10-h feed restrictions. Milk fat percentage and milk fat yield (kg/d) were unaffected by treatment. An interaction was found with milk true protein percentage being lowest for 100R though milk true protein yield was unaffected ($P = 0.42$). Milk urea nitrogen (MUN) tended ($P = 0.06$) to decrease with R,

similar to milk protein percentage with the greatest numerical difference at 100R indicating reduced nitrogen availability. Lactose percentage increased ($P < 0.01$) with higher STKD and decreased ($P = 0.01$) with R although lactose yields (kg/d) were unaffected by either treatment ($P > 0.12$). An interaction was found between stocking density and feed access for feed efficiency with R reducing efficiency at 100% STKD but increasing efficiency at 142% STKD, though this interaction was not found with SCM efficiency. Munksgaard et al. (2005) observed no differences in milk yield and DMI with 10-h reduced feed access and therefore likely observed no differences in feed efficiency. However, Collings et al. (2011) observed a trend toward decreased DMI (27 kg/d, 24-h access; 25.8 kg/d, 14-h access) with similar milk yield responses under a 12-h feed restriction. This indicates there may have been greater feed efficiency with reduced feed access, though these data are not reported. However, it is important to note that these are short-term production responses (2-wk treatment periods). Future research should be done to identify longer term effects of increased stocking density and reduced feed access on feed intake and milk production measures.

Feeding Behavior

Feeding behavior results are summarized in Table 5.5. Daily feeding time (min/d) tended ($P = 0.08$) to decrease with higher STKD, although treatment differences were approximately 3 min and feeding time with each 8-h interval post-feed delivery were not significantly different ($P > 0.18$). Hill et al. (2009) and Krawczel et al. (2012b) observed no effect of stocking density on feeding time at similar stocking densities. In contrast,

Collings et al. (2011) and Crossley et al. (2017) observed decreases in feeding time associated with higher stocking density, although these variations are likely explained by the extent of overstocking (200% and 300%, respectively) compared to the current study. This further indicates a break point relationship around 142% with increased stocking density resulting in decreased feeing times. Higher STKD increased daily feeding bouts ($P < 0.01$) and decreased daily feeding bout length ($P < 0.01$). In contrast to Black et al. (2016) who observed no differences in feeding bouts or meal length at similar stocking density, the current study indicates cows are able to shift their feeding behaviors while maintaining overall daily feeding time. Feeding bouts (# bouts/8-h interval) increased ($P = 0.04$) at 0-8 h post-feed delivery and tended ($P = 0.07$) to increase at 17-24 h post-feed delivery, but were not affected at 9-16 h post-feed delivery. Krawczel et al. (2012b) observed linear increases in feedbunk displacements with increasing stocking density (>2 fold increase from 100% to 142%), indicating the increases in feeding bouts during the 0-8 h interval are likely a result of increased displacements from the feed bunk post-feed delivery. Further, DeVries et al. (2003) observed increased feeding motivation following fresh feed delivery, suggesting increased motivation to feed during this period and likely greater aggression to access the resource. In contrast to feeding motivation, the trend toward increased feeding bouts during the 17-24 h period is likely a result of resource access. This indicates shifts in feeding behavior while stall access is limited during this time period in order to maintain daily feeding time. Feeding bout length (min/bout) decreased ($P = 0.03$) at 9-16 h post-feed delivery and tended ($P = 0.07$) to decrease at 0-8

h post-feed delivery, likely due to increased agonistic interactions during the 0-8 h interval which likely subsided following this time period.

Feeding time (min/d) decreased for R ($P < 0.01$). Feeding time and number of feeding bouts (# bouts/8-h interval) decreased for R at 17-24 h post-feed delivery ($P < 0.01$) due to limited access to feed 5 h prior to feed delivery. Length of first meal following fresh feed delivery (min/meal) was not affected by FA. However, feeding time and feeding bout length (min/bout) increased for R at 0-8 h post-feeding ($P < 0.01$). This indicates that cows with reduced feed access were able to maintain DMI through increased feeding time with each subsequent feeding bout in the 0-8 h time period following feed delivery. Collings et al. (2011) reported similar behavioral adaptations to 12 h of reduced feed access, with an increase in DMI, feeding time, and feeding rate 2 h post-feeding. Non-ingestive time at the feed bunk (as a % of total time at feed bunk) increased for R ($P < 0.01$), demonstrating searching behavior and continued motivation to feed despite the lack of feed present.

Rumination Behavior

Rumination behavior results are summarized in Table 5.6. Total daily rumination time (min/d) and daily distribution of rumination (min/8-h interval) was unaffected by STKD ($P > 0.73$), similar to previous studies with similar levels of stocking density (Krawczel et al., 2012b; Wang et al., 2016). While total time was unaffected, location of rumination differed between stocking density levels. Rumination within a freestall (% of total rumination) decreased ($P < 0.01$) with higher STKD, indicating a shift in location of

rumination from the freestall to the alley, consistent with previous reports by Krawczel et al. (2012b).

While total daily rumination was unaffected by FA, R decreased ($P < 0.01$) rumination time at 0-8 h post-feed delivery but increased ($P = 0.02$) rumination time at 17-24 h post-feed delivery. The increased rumination time observed at 17-24 h post-feed delivery was also evidenced by a trend for increased ($P = 0.06$) rumination bout length. Under a reduced feed access environment, cows inversely alter feeding and rumination behavior throughout the day. While rumination time decreased and feeding time increased 0-8 h post-feed delivery, rumination time increased and feeding time decreased 17-24 h post-feed delivery, meeting daily rumination needs.

Lying Behavior

Lying behavior results are summarized in Table 5.7. Total daily lying time (min/d) decreased ($P = 0.02$) and time spent in the alley (min/d) increased ($P < 0.01$) with higher STKD. The decrease in lying time (approximately 30 min between 100 and 142% stocking densities) within the current study was consistent with previous research (Krawczel et al., 2012b) that restricted access at both headlocks and freestalls. The decrease in total daily lying time with higher STKD was driven through a decrease ($P < 0.01$) in lying time at 17-24 h post-feed delivery, as lying time was unaffected during the 0-16 h post-feed delivery. Daily lying bouts (bouts/d) increased ($P < 0.01$) and bout length (min/bout) decreased ($P < 0.01$) with higher STKD, largely driven by the 0-8 h post-feed delivery in relation to the increase in feeding bouts during this time period.

While number of lying bouts were unaffected with higher STKD during the 17-24 h period, lying bout length decreased ($P = 0.01$), accounting for the decrease in total daily lying time. In contrast to previous work which reported no differences in lying bouts at similar stocking densities (Krawczel et al., 2012b), the current research identified some changes in lying behavior. However, the current study resulted in decreased lying bouts (~8 bouts/d) and increased bout length (~101 min) compared to Krawczel et al. (2012b) who observed approximately 11.2 bouts/d and bout lengths of approximately 68 min, regardless of treatments when comparing similar stocking density levels. The increased lying bouts and reduced lying bout lengths in Krawczel et al. (2012b) may have indicated greater freestall displacements or environmental stressors that reduce the ability for the cow to alter her lying bout characteristics, regardless of changes in stocking density. However, due to differences in total lying time, the current study supports the inelasticity of lying behavior as alterations in bouts or bout length during overstocked conditions are unable to counteract losses in lying time.

Total daily lying time and daily distribution of lying time was not affected by FA ($P > 0.21$). Lying bouts decreased ($P = 0.02$) with R 0-8 h post-feed delivery, as cows increased feeding bout length but were unaffected during other periods. An interaction ($P = 0.05$) was found between STKD and R, with the greatest lying bout length 17-24 h post-feed delivery for 100R. Due to limited feed access during this time period, cows shifted priority from the feedbunk to the freestall. However, due to increased competition for freestalls at 142% STKD, cows were unable to increase lying bout length during

times of reduced access to feed on 142R. Despite changes in lying time and bout characteristics, resting posture was unaffected by treatments.

Feeding and Lying Latency

Feeding upon immediate return from parlor (% of pen) decreased with higher STKD ($P = 0.02$) upon return from all three milkings (Table 5.8). In contrast, lying immediately upon return from parlor (% of pen) increased ($P = 0.04$) with higher STKD for the first milking following feed delivery and tended ($P = 0.09$) to increase for the second milking following feed delivery. This increase in immediately lying with higher STKD directly contrasts with the decrease in feeding immediately upon return to the pen at each milking. This supports the emphasis cows place on lying over feeding behavior when placed in situations where they have to choose between resources such as overstocking (Munksgaard et al., 2005). This percentage had its greatest increase ($P < 0.01$) upon return from milking to fresh feed, highlighting the motivation to lay despite typically high motivation to eat following feed delivery (DeVries et al., 2003). This motivation is likely driven following the 17-24 h interval where lying time was significantly lowered compared to other intervals at 142% STKD.

Feeding immediately upon return from parlor increased ($P = 0.02$) for R due to the 5 h of reduced feed access experienced by cows before fresh feed delivery. In contrast, lying immediately upon return from parlor decreased ($P = 0.03$) upon return to fresh feed for R, indicating increased motivation for fresh feed following 5 h feed restriction, regardless of stocking density.

Stress Responses

Blood measurements are summarized in Table 5.9. There were no differences in FCI amongst treatments on d 7 or d 14. Total cortisol levels were elevated, regardless of treatment, consistent with concentrations found during overstocking of prepartum cows (Fustini et al., 2017; ~4 ng/mL during overstocked treatment) and with early lactation cows prior to ACTH challenges (Trevisi et al., 2013, ~5 to 10 ng/mL). This indicated that all cows experienced greater stress responses, likely due to non-treatment factors during the study. Further, there were no differences in SAA concentrations between treatments. In comparison to SAA concentrations during the previous study (Chapter 4), all treatments appeared elevated during the d 14 sampling (46.3 µg/mL vs. 64.9 µg/mL; Chapter 4 and 5, respectively). This suggests increased contributions from environmental stressors not accounted for by the treatments. Cows experienced 2.5x greater SARA during this study compared to those on the previous study, regardless of diet. With a positive linear relationship with plasma SAA concentration and SARA (Zebeli et al., 2012), it is likely that SARA experienced by cows, regardless of treatment, outweighed the impact of treatments during this study and resulted in the consistently high cortisol levels.

Ruminal Fermentation

Ruminal fermentation results are summarized in Tables 5.10 to 5.13. Ruminal pH measurements were unaffected by reduced feed access. Similar to results reported by

Erickson et al. (2003), there may be increased buffering potential due to the increased rumination time (17-24 h time period) prior to increasing feeding rate during fresh-feed delivery, preventing drops in ruminal pH post-feeding. Time spent below pH 5.8 (h/d) increased ($P = 0.02$) and area under the curve below pH 5.8 (AUC, units x pH) tended ($P = 0.09$) to increase for higher STKD. As described in Chapter 4, a negative linear relationship exists between time below pH 5.8 and rumination within the freestall (% of total rumination). Therefore, cows likely experience reduced saliva production due to decreases in lying time as well as during rumination outside of the freestall which are large contributors to saliva production (Maekawa et al., 2002b). An interaction ($P = 0.02$) was found between overstocking and reduced feed access on time below pH 5.8, indicating an exacerbated response of SARA when cows were subjected to both overstocking and reduced feed access. Due to reduced access to feed during the 17-24 h period, cows under 142R were unable to make up feeding time during this period compared to cows housed at 142NR. Further, under R conditions, cows increase rumination time during the 17-24 h period due to the lack of access to feed. With increased STKD and lack of stall resources, the increase in rumination time is unable to overcome the reduction in saliva production due to the shift in the location of rumination, leading to exacerbated responses in time below pH 5.8. Daily mean, minimum, and maximum pH responses were not affected by treatments.

Distribution of SARA throughout the day (Table 5.11) indicated a trend for increased SARA at 9-16 h post-feeding for STKD ($P = 0.08$). Other time periods were

unaffected by treatment, indicating that the risk for SARA from increased STKD is constant throughout the day and not indicative of a singular time period.

Daily averages of VFA (molar %) and total VFA (m*M*) were unaffected by treatments. Ruminal ammonia nitrogen (mg/dL) increased with higher STKD 4 h post-feeding ($P = 0.05$) due to increased feed intake during the 0-8 h time period and decreased with reduced feed access 20 h post-feeding ($P < 0.01$) due to reduced access to feed during the 17-24 h time period under R conditions. Restricted treatment resulted in a trend for reduced daily average of ruminal ammonia nitrogen, likely contributing to the decreases in milk protein percentage due to restricted microbial protein production (Owens et al., 2014).

CONCLUSIONS

Restricted feed access resulted in negative effects on feeding behavior, although total feed intake remained unaffected. However, cows inversely altered feeding and rumination behavior to maintain daily rumination time, with late night increases in rumination likely buffering increased feeding rates upon fresh feed delivery, resulting in no differences in ruminal pH. However, time spent below pH 5.8 was exacerbated with reduced feed access under overstocked conditions. Due to alterations in feeding behavior and exacerbated responses on ruminal pH, it is not recommended to limit feed access during overstocked conditions.

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REFERENCES

AOAC International. 2000. Official Methods of Analysis. 17th ed. AOAC International, Gaithersburg, MD.

Bach, A., N. Valls, A. Solans, and T. Torrent. 2008. Associations between nondietary factors and dairy herd performance. J. Dairy Sci. 91:3259-3267.

Barrientos, A. K., N. Chapinal, D. M. Weary, E. Galo, and M. A. G. von Keyserlingk. 2013. Herd-level risk factors for hock injuires in freestall-housed dairy cows in the northeastern United States and California. J. Dairy Sci. 96:3758-3765.

Bauer, M. L., D. W. Herold, R. A. Britton, R. A. Stock, T. J. Klopfenstein, and D. A. Yates. 1995. Efficacy of laidlomycin propionate to reduce ruminal acidosis in cattle. J. Anim. Sci. 73:3445-3454.

Black, R. A., R. J. Grant, and P. D. Krawczel. 2016. Short communication: Short-term changes in stocking density did not alter meal characteristics of lactating Holstein dairy cattle. J. Dairy Sci. 99:6572-6577.

Chaney, A. L., and E. P. Marbach. 1962. Modified reagents for determination of urea and ammonia. Clin. Chem. 8:130-132.

Collings, L .K. M., D. M. Weary, N. Chapinal, and M. A. G. von Keyserlingk. 2011. Temporal feed restriction and overstocking increase competition for feed by dairy cattle. J. Dairy Sci. 94:5480-5486.

Cotanch, K. W., J. D. Darrah, C. S. Ballard, and R. J. Grant. 2010. Modification of the Penn State Particle Separator with 3.18- or 4.76-mm perforated steel sieves to measure physically effective fiber. J. Dairy Sci. 88(E Suppl. 1):147. (Abstr.).

Crossley, R. E., A. Harlander-Matauschek, and T. J. DeVries. 2017. Variability in behavior and production among dairy cows fed under differing levels of competition. J. Dairy Sci. 100:1-14.

Deming, J. A., R. Bergeron, K. E. Leslie, and T. J. DeVries. 2013. Associations of housing, management, milking activity, and standing and lying behavior of dairy cows milked in automatic systems. J. Dairy Sci. 96:344-351.

DeVries, T. J., M. A. G. von Keyserlingk, and K. A. Beauchemin. 2003. Short communication: Diurnal feeding pattern of lactating dairy cows. J. Dairy Sci. 86:4079-4082.

De Vries, A., H. Dechassa, and H. Hogeveen. 2016. Economic evaluation of stall stocking density of lactating dairy cows. J. Dairy Sci. 99:3848-3857.

Erdman, R. A., T. W. Moreland, and W. R. Stricklin. 1989. Effect of time of feed access on intake and production in lactating dairy cows. J. Dairy Sci. 72:1210-1216.

Erickson, G. E., C. T. Milton, K. C. Fanning, R. J. Cooper, R. S. Swingle, J. C. Parrott, G. Vogel, and T. J. Klopfenstein. 2003. Interaction between bunk management and monensin concentration on finishing performance, feeding behavior, and ruminal metabolism during an acidosis challenge with feedlot cattle J. Anim. Sci. 81:2869-2879.

Ferguson, J. D., D. T. Galligan, and N. Thomsen. 1994. Principal descriptors of body condition score in Holstein cows. J. Dairy Sci. 77:2695-2703.

Fitts, J. E., and D. Laird. 2004. Direct microscopic methods for bacteria or somatic cells. Pages 269-280 in Standard Methods for the Examination of Dairy Products. 17th ed. H. M. Wehr and J. F. Frank, ed. Am. Public Health Assoc., Washington, DC.

Fregonesi, J. A., C. B. Tucker, and D. M. Weary. 2007. Overstocking reduces lying time in dairy cows. J. Dairy Sci. 90:3349-3354.

French, P., J. Chamberlain, and J. Warntjes. 2005. Effect of feed refusal amount on feeding behavior and production in Holstein cows. J. Anim. Sci. 83(E-Suppl.1):175 (Abstr.).

Friend, T. H., F. C. Gwazdauskas, and C. E. Polan. 1979. Change in adrenal response from free stall competition. J. Dairy Sci. 62:768-771.

Fustini, M., G. Galeati, G. Gabai, L. E. Mammi, D. Bucci, P. A. Accorsi, and A. Formigoni. 2017. Overstocking dairy cows during the dry period affects dehydroepiandrosterone and cortisol secretion. J. Dairy Sci. 100:620-628.

Hill, C. T., P. D. Krawczel, H. M. Dann, C. S. Ballard, R. C. Hovey, W. A. Falls, and R. J. Grant. 2009. Effect of stocking density on the behavior of dairy cows with differing parity and lameness status. Appl. Anim. Behav. Sci. 117:144-149.

Horadagoda, N. U., K. M. G. Knox, H. A. Gibbs, S. W. J. Reid, A. Horadagoda, S. E. R. Edwards, P. D. Eckersall. 1999. Acute phase proteins in cattle: discrimination between acute and chronic inflammation. Vet. Rec. 144:437-441.

Hulbert, L. E., J. A. Carroll, M. A. Ballou, N. C. Burdick, J. W. Dailey, L. C. Caldwell, A. N. Loyd, R. C. Vann, T. H. Welsh Jr., and R. D. Randel. 2013. Sexually dimorphic stress and pro-inflammatory cytokine responses to an intravenous corticotropin-releasing hormone challenge of Brahman cattle following transportation. Innate Immun. 19:378-387.

Kattesh, H. G., C. A. Kurman, B. E. Gillespie, P. D. Krawczel, and A. M. Saxton. 2014. Bedding surface does not alter circulating patterns of cortisol, corticosteroid-binding globulin, or free cortisol index in preweaned Jersey calves. J. Anim. Sci. 92(E-Suppl. 2):1383. (Abstr.).

Kaylegian, K. E., G. E. Houghton, J. M. Lynch, J. R. Fleming, and D. M. Barbano. 2006a. Calibration of infrared milk analyzers: Modified milk versus producer milk. J. Dairy Sci. 89:2817-2832.

Kaylegian, K. E., J. M. Lynch, G. E. Houghton, J. R. Fleming, and D. M. Barbano. 2006b. Modified versus producer milk calibration: Mid-Infrared analyzer performance validation. J. Dairy Sci. 89:2833-2845.

Kaylegian, K. E., J. M. Lynch, J. R. Fleming, and D. M. Barbano. 2009. Influence of fatty acid chain length and unsaturation on Mid-Infrared milk analysis. J Dairy Sci. 92:2485-2501.

King, M. T. M., E. A. Pajor, S. J. LeBlanc, and T. J. DeVries. 2016. Associations of herd-level housing, management, and lameness prevalence with productivity and cow behavior in herds with automated milking systems. J. Dairy Sci. 99:9069-9079.

Krawczel, P. D., C. S. Mooney, H. M. Dann, M. P. Carter, R. E. Butzler, C. S. Ballard, and R. J. Grant. 2012a. Effect of alternative models for increasing stocking density on the short-term behavior and hygiene of Holstein dairy cows. J. Dairy Sci. 95:2467-2475.

Krawczel, P. D., L. B. Klaiber, R. E. Butzler, L. M. Klaiber, H. M. Dann, C. S. Mooney, and R. J. Grant. 2012b. Short-term increases in stocking density affect the lying and social behavior, but not the productivity, of lactating Holstein dairy cows. J. Dairy Sci. 95:4298-4308.

Krohn, C. C., and L. Munksgaard. 1993. Behaviour of dairy cows kept in extensive (loose housing/pasture) or intensive (tie stall) environments II. Lying and lying-down behaviour. Appl. Anim. Behav. Sci. 37:1-16.

Lammers, B. P., D. R. Buckmaster, and A. J. Heinrichs. 1996. A simple method for the analysis of particle sizes of forage and total mixed rations. J. Dairy Sci. 79:922-928.

Leonardi, C., and L. E. Armentano. 2003. Effect of quantity, quality, and length of alfalfa hay on selective consumption by dairy cows. J. Dairy Sci. 86:557-564.

Lynch, J. M., D. M. Barbano, and J. R. Fleming. 2007. Determination of the lactose content of fluid milk by spectrophotometric enzymatic analysis using weight additions and path length adjustment: Collaborative study. J. AOAC Int. 90:196-216.

Maekawa, M., K. A. Beauchemin, and D. A. Christensen. 2002b. Chewing activity, saliva production, and ruminal pH of primiparous and multiparous lactating dairy cows. J. Dairy Sci. 85:1176-1182.

Miller-Cushon, E. K., and T. J. DeVries. 2017. Feed sorting in dairy cattle: Causes, consequences, and management. J. Dairy Sci. 100:4172-4183.

Mitlöhner, F. M., J. L. Morrow-Tesch, S. C. Wilson, J. W. Dailey, and J. J. McGlone. 2001. Behavioral sampling techniques for feedlot cattle. J. Anim. Sci. 79:1189-1193.

Moberg, G. P. 2000. Biological response to stress: Implications for animal welfare. In: Moberg, G. P. and J. A. Mench (eds) The Biology of Animal Stress. CAB. International, Wallingford, UK, pp. 1-21.

Munksgaard, L., M. B. Jensen, L. J. Pedersen, S. W. Hansen, and L. Matthews. 2005. Quantifying behavioural priorities - Effects of time constraints on behavior of dairy cows, *Bos Taurus*. Appl. Anim. Behav. Sci. 92:3-14.

Nordlund, K. V., N. B. Cook, and G. R. Oetzel. 2004. Investigation strategies for laminitis problem herds. J. Dairy Sci. 87:(E. Suppl.):E27-E35.

Owens, F. N., S. Qi, and D. A. Sapienza. 2014. Invited review: Applied protein nutrition of ruminants – Current status and future directions. Prof. Anim. Sci. 30:208-211.

Penner, G. B., K. A. Beauchemin, and T. Mutsvangwa. 2006. An evaluation of the accuracy and precision of a stand-alone submersible continuous ruminal pH measurement system. J. Dairy Sci. 89:2132-2140.

Roberts, M. P., H. G. Kattesh, G. A. Baumbach, B. E. Gillespie, J. D. Godkin, J. F. Schneider, and A. M. Saxton. 2003. Age-related changes in porcine corticosteroid-binding globulin (pCBG) as determined by an enzyme-linked immunosorbent assay. Domest. Anim. Endocrinol. 24:323-339.

Shook, G. E. 1993. Genetic improvement of mastitis through selection on somatic cell count. Veterinary Clinics of North America - Food Animal Practice 9:563-581.

Silva-del-Rio, N., J. M. Heguy, and A. Lago. 2010. Feed management practices on California dairies. J. Dairy Sci. 93(E-Suppl. 1):773 (Abstr.).

Sova, A. D., S. J. LeBlanc, B. W. McBride, and T. J. DeVries. 2013. Associations between herd-level feeding management practices, feed sorting, and milk production in freestall dairy farms. J. Dairy Sci. 96:4759-4770.

Trevisi, E., G. Bertoni, R. Lombardelli, and A. Minuti. 2013. Relation of inflammation and liver function with the plasma cortisol response to adrenocroticotropin in early lactating dairy cows. J. Dairy Sci. 96:5712-5722.

USDA-ERS. 2014. Monthly cost of production estimates. USDA-Economic Research Service, Washington, DC. Accessed April 14, 2014. http://www.ers.usda.gov/data-products/milk-cost-of-productionestimates.aspx.

von Keyserlingk, M. A. G., A. Barrientos, K. Ito, E. Galo, and D. M. Weary. 2012. Benchmarking cow comfort on North American freestall dairies: lameness, leg injuries, lying time, facility design, and management for high-producing Holstein dairy cows. J. Dairy Sci. 95:7399-7408.

Wang, F. X., D. F. Shao, S. L. Li, Y. J. Wang, A. Azarfar, and Z. J. Cao. 2016. Effects of stocking density on behavior, productivity, and comfort indices of lactating dairy cows. J. Dairy Sci. 99:3709-3717.

Wojciechowski, K. L., C. Melilli, and D. M. Barbano. 2016. A proficiency test system to improve performance of milk analysis methods and produce reference values for component calibration samples for infrared milk analysis. J. Dairy Sci. 99:6808-6827.

Woolpert, M. E., H. M. Dann, K. W. Cotanch, C. Melilli, L. E. Chase, R. J. Grant, and D. M. Barbano. 2016. Management, nutrition, and lactation performance are related to bulk tank milk de novo fatty acid concentration on northeastern US dairy farms. J. Dairy Sci. 99:8486-8497.

Zebeli, Q., B. U. Metzler-Zebeli, and B. N. Ametaj. 2012. Meta-analysis reveals threshold level of rapidly fermentable dietary concentrate that triggers systemic inflammation in cattle. J. Dairy Sci. 95:2662-2672.

Table 5.1. Ingredient composition and analyzed chemical composition (dry matter basis) of diet.

Item	TMR
Ingredient, % of dry matter (DM)	
Conventional corn silage	34.5
Haycrop silage	10.7
Whole cottonseed, fuzzy	2.3
Molasses	3.1
Concentrate mix[1]	49.4
Chemical Composition, % of DM	
DM, %	45.4 ± 0.6[2]
Crude protein (CP)	15.9 ± 0.2
Soluble protein, % of CP	35.4 ± 0.8
NDICP[3]	1.3 ± 0.1
Acid detergent fiber (ADF)	18.8 ± 0.3
Neutral detergent fiber (NDF)	29.5 ± 0.4
Acid detergent lignin (ADL)	3.1 ± 0.1
Sugar	5.4 ± 0.3
Starch	22.7 ± 0.5
Starch digestibility (7-h), % of starch	70.5 ± 1.5
Fat	5.8 ± 0.1
Non-fibrous carbohydrates (NFC)	43.1 ± 0.4
Ash	7.02 ± 0.10
Ca	0.82 ± 0.02
P	0.39 ± 0.01
Mg	0.44 ± 0.01
K	1.38 ± 0.02
S	0.28 ± 0.01
Na	0.54 ± 0.02
Cl ion	0.62 ± 0.01
Fe, mg/kg	279 ± 13
Cu, mg/kg	16 ± 0
Mn, mg/kg	64 ± 1
Zn, mg/kg	62 ± 1
Net energy of lactation, Mcal/kg of DM	1.77 ± 0.01

[1]Concentrate mix was composed of the following (% of DM): corn meal, finely ground (27.77), soybean meal, 47.5% solvent (15.40), AminoMax (Afgritech LLC, Watertown, NY; 14.95), whole beet pulp (8.42), steam flaked corn (7.66), bakery meal (6.13), Berga Fat F100 (Berg + Schmidt America LLC, Libertyville, IL; 4.62), Amino Enhancer (Poulin Grain Inc., Swanton, VT; 3.12), calcium carbonate (1.92), sodium sesquicarbonate (1.53), canola meal, solvent (1.53), Megalac (Arm & Hammer Animal Nutrition, Princeton, NJ; 1.52), cane molasses (1.52), sugar, 99% (1.48), salt (0.80), magnesium oxide (0.58), urea (0.31), calcium phosphate dicalcium (0.22), vitamin and trace mineral mix (contained 5,732 kIU/kg vitamin A, 29.77 kIU/kg vitamin E, 1,589

kIU/kg vitamin D3, 21.7% Ca, 0.91% Cl, 0.72% Mg, 0.17% P, 0.16% S, 0.01% K, 25,438 mg/kg Zn, 21,802 mg/kg Mn, 6,427 mg/kg Cu, 500 mg/kg Fe, 428 mg/kg I, 269 mg/kg Se (50% organic), 154 mg/kg Co; 0.19), Meta Smart (Adisseo, Alpharetta, GA; 0.15), Smartamine M (Adisseo, Alpharetta, GA; 0.09), XPC yeast culture (Diamond V, Cedar Rapids, IA; 0.09), Probios Precise concentrate (Chr-Hansen, Milwaukee, WI; 0.01), and Rumensin 90 (Elanco Animal Health, Greenfield, IN; 0.01).
[2]Mean ± standard error.
[3]Neutral detergent insoluble CP.

Table 5.2. Analyzed chemical composition (% of dry matter) of feed ingredients used in diet.

Item	Conventional corn silage	Haycrop silage	Whole cottonseed, fuzzy	Molasses[2]	Concentrate mix
Dry matter (DM), %	30.2 ± 0.3[1]	32.0 ± 0.1	88.6 ± 0.3	61.8	87.7 ± 0.1
Crude protein (CP)	7.1 ± 0.2	14.2 ± 0.9	23.8 ± 0.3	6.3	24.1 ± 0.2
Soluble protein, % of CP	62.5 ± 0.9	55.5 ± 1.9	34.3 ± 0.5	100.0	17.1 ± 1.4
NDICP[3]	0.9 ± 0.0	2.5 ± 0.2	2.0 ± 0.1	0.0	2.1 ± 0.1
Acid detergent fiber (ADF)	25.3 ± 0.4	39.5 ± 0.4	35.9 ± 1.1	0.0	9.7 ± 0.4
Neutral detergent fiber (NDF)	40.4 ± 0.2	57.1 ± 1.6	45.8 ± 0.2	0.0	15.0 ± 0.6
Acid detergent fiber (ADL)	3.1 ± 0.1	6.4 ± 0.4	10.4 ± 0.4	0.0	2.9 ± 0.2
Sugar	0.7 ± 0.0	1.4 ± 0.5	2.2 ± 0.2	61.5	7.9 ± 0.4
Starch	34.0 ± 0.2	1.5 ± 0.1	0.9 ± 0.1	0.0	24.9 ± 0.9
Starch digestibility (7-h), % of starch	78.5 ± 0.9	-	-	-	62.0 ± 2.3
Fat	3.5 ± 0.0	5.0 ± 0.1	18.5 ± 1.2	1.0	5.7 ± 0.2
Non-fibrous carbohydrates (NFC)	46.1 ± 0.5	17.6 ± 1.0	9.4 ± 1.1	81.7	48.6 ± 0.5
Ash	3.8 ± 0.1	8.7 ± 0.0	4.3 ± 0.1	11.0	8.8 ± 0.2
Ca	0.22 ± 0.02	0.88 ± 0.06	0.18 ± 0.00	1.00	1.39 ± 0.03
P	0.21 ± 0.00	0.29 ± 0.02	0.63 ± 0.01	0.10	0.51 ± 0.02
Mg	0.14 ± 0.00	0.36 ± 0.02	0.42 ± 0.00	0.42	0.54 ± 0.01
K	1.15 ± 0.04	1.74 ± 0.18	1.25 ± 0.01	4.01	1.08 ± 0.03
S	0.13 ± 0.00	0.29 ± 0.01	0.26 ± 0.00	0.47	0.38 ± 0.01
Na	0.02 ± 0.00	0.06 ± 0.01	0.02 ± 0.00	0.22	0.66 ± 0.03
Cl ion	0.22 ± 0.00	0.47 ± 0.14	0.06 ± 0.00	0.75	0.66 ± 0.03
Fe, mg/kg	114 ± 3	311 ± 42	72 ± 3	191	365 ± 16
Cu, mg/kg	6 ± 0	11 ± 1	11 ± 1	66	36 ± 16
Mn, mg/kg	24 ± 1	105 ± 16	22 ± 1	59	81 ± 18
Zn, mg/kg	26 ± 1	35 ± 1	43 ± 1	14	118 ± 37

[1]Mean ± standard error.

[2]Values based on Cornell Net Carbohydrate and Protein System (CNCPS) feed library (ver. 6.1; Agricultural Modeling and Training Systems, LLC, Groton, NY).

[3]Neutral detergent insoluble CP.

Table 5.3. Physical characterization of diet and forage ingredients.

Item	Total mixed ration	Conventional corn silage	Haycrop silage
Particle size distribution, % as-fed			
>19.0 mm	4.5 ± 0.3	2.9 ± 0.4	31.5 ± 4.0
8.0 to 19.0 mm	50.5 ± 0.7	76.9 ± 0.7	47.8 ± 2.4
4.0 to 8.0 mm	12.3 ± 0.2	13.6 ± 0.3	13.8 ± 1.4
<4.0 mm	32.7 ± 0.7	6.7 ± 0.4	7.0 ± 0.2
pef[2]	0.67 ± 0.01	0.93 ± 0.00	0.93 ± 0.00

[1]Mean ± standard error.
[2]Physical effectiveness factor.

Table 5.4. Effect of stocking density[1] (STKD) and feed access (FA; no restriction; NR and 5-h restriction; R) on feed intake, sorting activity, and production responses (n = 4 pens/treatment).

	100%		142%			*P*-value		
Variable	NR	R	NR	R	SEM	STKD	FA	STKD x FA
Intake and efficiency measures								
Dry matter intake (DMI), kg/cow/d	25.7	25.9	26.1	25.6	0.3	0.78	0.64	0.34
Milk/DMI, kg/kg	1.72	1.69	1.69	1.73	0.02	0.41	0.68	0.02
Solids-corrected milk/DMI, kg/kg	1.70	1.68	1.69	1.70	0.02	0.90	0.97	0.31
Sorting of particles (% difference)[2]								
>19.0 mm	102.4	101.2	101.2	102.1	0.6	0.84	0.82	0.17
8.0 to 19.0 mm	101.7	100.7	98.8	100.9	2.3	0.36	0.71	0.32
4.0 to 8.0 mm	99.4	99.1	100.0	99.5	0.5	0.43	0.59	0.88
<4.0 mm	96.1	99.1	100.0	97.7	2.4	0.45	0.84	0.16
Milk yield								
Daily yield, kg/cow/d	44.0	43.6	44.2	44.4	0.5	0.30	0.77	0.49
1st milking post-feeding, kg/cow[3,4]	14.8	14.8	14.7	15.2	0.2	0.38	0.11	0.14
2nd milking post-feeding, kg/cow	14.8	14.6	14.9	14.7	0.2	0.42	0.19	0.87
3rd milking post-feeding, kg/cow	14.4	14.2	14.6	14.5	0.2	0.28	0.31	0.84
Solids-corrected milk								
Daily yield, kg/cow/d	43.6	43.5	44.0	43.6	0.5	0.34	0.29	0.55
1st milking post-feeding, kg/cow[3,4]	14.7	14.6	14.9	14.7	0.2	0.09	0.18	0.49
2nd milking post-feeding, kg/cow	14.6	14.5	14.7	14.5	0.2	0.69	0.43	0.68
3rd milking post-feeding, kg/cow	14.4	14.3	14.5	14.3	0.3	0.70	0.58	0.69
Milk composition								
Fat, %	3.95	3.99	4.01	3.95	0.04	0.73	0.52	0.09
Fat, kg/d	1.72	1.71	1.74	1.72	0.02	0.28	0.36	0.47
True protein, %	3.19	3.16	3.18	3.18	0.01	0.61	0.01	0.02
True protein, kg/d	1.38	1.36	1.38	1.39	0.02	0.32	0.42	0.14
Anhydrous lactose, %	4.63	4.62	4.64	4.63	<0.01	<0.01	0.01	0.65

Anhydrous lactose, kg/d	2.02	2.00	2.03	2.03	0.02	0.12	0.68	0.34
MUN[5], mg/dL	12.25	11.69	12.04	11.94	0.34	0.88	0.06	0.16
Body weight, kg	1538	1542	1537	1555	20	0.61	0.38	0.53
Body condition score	3.1	3.2	3.1	3.2	0.1	0.87	0.12	0.87

[1]100%, 17 freestalls and headlocks per pen; 142%, 12 freestalls and headlocks per pen; 17 cows per pen
[2]Actual intake as a percentage of predicted intake.
[3]Diet fed 1x/d at approximately 0600 h, with feed pushed-up 6 times daily.
[4]Cows milked 3x/d; 1st milking post-feeding at approximately 1300 h, 2nd milking post-feeding at approximately 2100 h, and 3rd milking post-feeding at approximately and 0500 h, each milking lasting approximately 60 min.
[5]Milk urea nitrogen

Table 5.5. Effect of stocking density[1] (STKD) and feed access (FA; no restriction; NR and 5-h restriction; R) on daily distribution of feeding behavior (n = 4 pens/treatment).

	100%		142%			*P*-value		
Variable	NR	R	NR	R	SEM	STKD	FA	STKD x FA
Feeding time								
Daily total, min/d	230	222	227	219	6	0.08	<0.01	0.96
0-8 h post-feeding[1], min	87	98	83	95	3	0.18	<0.01	0.85
9-16 h post-feeding, min	84	85	83	83	4	0.43	0.92	0.61
17-24 h post-feeding, min	60	39	61	41	2	0.41	<0.01	0.73
Feeding bout number								
Daily bouts, bouts/d	7.6	7.0	8.0	7.2	0.1	<0.01	<0.01	0.40
0-8 h post-feeding[1], bouts	2.7	2.9	3.0	3.0	0.1	0.04	0.21	0.42
9-16 h post-feeding, bouts	2.6	2.9	2.7	3.0	0.1	0.54	0.11	0.95
17-24 h post-feeding, bouts	2.2	1.4	2.3	1.4	<0.1	0.07	<0.01	0.60
Feeding bout length								
Daily bout length, min/bout	33.8	35.1	31.9	33.4	1.0	<0.01	0.02	0.76
0-8 h post-feeding, min/bout	34.4	37.4	32.5	36.1	0.9	0.07	<0.01	0.71
9-16 h post-feeding, min/bout	37.4	37.4	36.1	34.1	1.8	0.03	0.26	0.24
17-24 h post-feeding, min/bout	31.8	30.3	30.7	32.2	1.4	0.65	0.98	0.15
Length of first meal, min[2]	38.2	36.4	36.8	34.5	1.9	0.30	0.21	0.86
Non-ingestive time at bunk, % time spent at bunk	3.4	14.9	5.6	15.2	1.8	0.47	<0.01	0.57

[1]100%, 17 freestalls and headlocks per pen; 142%, 12 freestalls and headlocks per pen; 17 cows per pen
[2]Diet fed 1x/d at approximately 0600 h, with feed pushed-up 6 times daily.

Table 5.6. Effect of stocking density[1] (STKD) and feed access (FA; no restriction; NR and 5-h restriction; R) on daily distribution of rumination behavior (n = 4 pens/treatment).

	100%		142%			*P*-value		
Variable	NR	R	NR	R	SEM	STKD	FA	STKD x FA
Rumination time								
Daily total, min/d	510	524	519	517	9	0.90	0.43	0.31
0-8 h post-feeding[2], min	162	155	163	152	3	0.73	<0.01	0.33
9-16 h post-feeding, min	164	169	167	167	3	0.87	0.48	0.53
17-24 h post-feeding, min	185	200	189	199	5	0.77	0.02	0.50
Rumination bout number								
Daily bouts, bouts/d	14.2	14.2	14.4	14.4	0.2	0.14	0.95	0.68
0-8 h post-feeding, bouts	4.7	4.6	4.7	4.6	0.1	0.54	0.23	0.97
9-16 h post-feeding, bouts	4.6	4.6	4.7	4.8	0.1	0.32	0.93	0.94
17-24 h post-feeding, bouts	4.9	5.0	5.0	5.0	0.1	0.44	0.27	0.40
Rumination bout length								
Daily bout length, min/bout	38.1	39.2	38.1	38.0	0.8	0.22	0.28	0.23
0-8 h post-feeding, min/bout	36.9	37.2	37.3	36.2	1.0	0.66	0.49	0.29
9-16 h post-feeding, min/bout	38.6	39.6	38.0	37.7	0.7	<0.01	0.22	0.07
17-24 h post-feeding, min/bout	40.9	43.3	40.8	42.5	1.2	0.60	0.06	0.70
Rumination location								
Rumination in freestall, % of total rumination	85.0	84.4	80.0	78.2	1.4	<0.01	0.18	0.53

[1]100%, 17 freestalls and headlocks per pen; 142%, 12 freestalls and headlocks per pen; 17 cows per pen
[2]Diet fed 1x/d at approximately 0600 h, with feed pushed-up 6 times daily.

Table 5.7. Effect of stocking density[1] (STKD) and feed access (FA; no restriction; NR and restriction; R) on daily distribution of lying behavior (n = 4 pens/treatment).

	100%		142%			*P*-value		
Variable	NR	R	NR	R	SEM	STKD	FA	STKD x FA
Lying time								
Daily total, min/d	784	786	761	752	13	0.02	0.68	0.55
0-8 h post-feeding[2], min	264	257	257	250	5	0.21	0.21	0.92
9-16 h post-feeding, min	243	244	241	236	6	0.24	0.57	0.46
17-24 h post-feeding, min	277	286	264	266	6	0.01	0.29	0.54
Lying bout number								
Daily bouts, bouts/d	8.0	7.8	8.4	8.4	0.1	<0.01	0.28	0.22
0-8 h post-feeding[1], bouts	2.7	2.6	3.0	2.9	<0.1	<0.01	0.02	0.82
9-16 h post-feeding, bouts	2.5	2.5	2.6	2.6	<0.1	0.05	0.98	0.40
17-24 h post-feeding, bouts	2.8	2.7	2.8	3.0	<0.1	0.26	0.75	0.13
Lying bout length								
Daily bout length, min/bout	103.9	108.6	97.2	95.8	1.8	<0.01	0.32	0.08
0-8 h post-feeding, min/bout	107.7	107.8	94.0	95.3	2.7	<0.01	0.73	0.77
9-16 h post-feeding, min/bout	113.2	109.3	106.8	107.5	3.9	0.23	0.62	0.48
17-24 h post-feeding, min/bout	114.1	129.6	108.6	104.2	5.0	0.01	0.23	0.05
Resting Posture[3], %								
Sternal, head up	77.6	76.7	77.0	78.8	1.8	0.50	0.69	0.26
Sternal, head back	15.7	15.5	15.8	15.3	1.1	0.94	0.42	0.59
Sternal, head down	4.1	4.7	4.5	3.6	0.9	0.68	0.81	0.42
Lateral	2.6	3.1	2.7	2.3	0.5	0.21	0.78	0.19
Time spent in alley, min/d	151	155	203	219	6	<0.01	0.10	0.28
Locomotion score	1.6	1.6	1.7	1.8	0.1	0.03	0.21	0.29

[1]100%, 17 freestalls and headlocks per pen; 142%, 12 freestalls and headlocks per pen; 17 cows per pen
[2]Diet fed 1x/d at approximately 0600 h, with feed pushed-up 6 times daily.
[3]Excluding time spent ruminating while lying.

Table 5.8. Effect of stocking density[1] (STKD) and feed access (FA; no restriction; NR and 5-h restriction; R) on feeding and lying responses upon return from milking parlor (n = 4 pens/treatment).

	100%		142%			*P*-value		
Variable	NR	R	NR	R	SEM	STKD	FA	STKD x FA
Feeding immediately upon return from parlor to pen, % of pen[2,3]								
Daily average	68.8	68.7	55.2	60.6	2.0	<0.01	0.25	0.24
1st milking post-feeding	61.1	59.7	51.6	53.6	3.2	0.02	0.93	0.54
2nd milking post-feeding	61.2	56.6	46.5	51.2	3.0	0.01	0.99	0.15
3rd milking post-feeding (return to fresh feed)	83.9	89.9	67.6	77.2	2.4	<0.01	0.02	0.49
Laying immediately upon return from parlor, % of pen								
Daily average	19.5	19.9	31.0	26.7	1.8	<0.01	0.27	0.20
1st milking post-feeding	24.3	28.4	35.3	34.5	3.6	0.04	0.63	0.47
2nd milking post-feeding	25.2	25.7	33.9	30.9	3.1	0.09	0.75	0.63
3rd milking post-feeding	9.1	5.6	23.8	14.7	2.3	<0.01	0.03	0.25

[1]100%, 17 freestalls and headlocks per pen; 142%, 12 freestalls and headlocks per pen; 17 cows per pen

[2]Diet fed 1x/d at approximately 0600h, with feed pushed-up 6 times daily.

[3]Cows milked 3x/d; 1st milking post-feeding at approximately 1300 h, 2nd milking post-feeding at approximately 2100 h, and 3rd milking post-feeding at approximately and 0500 h, each milking lasting approximately 60 min.

Table 5.9. Effect of stocking density[1] (STKD) and feed access (FA; no restriction; NR and 5-h restriction; R) on stress responses (n = 4 pens/treatment).

	100%		142%			*P*-value		
Variable	NR	R	NR	R	SEM	STKD	FA	STKD x FA
Total cortisol, ng/mL								
d 7	4.03	4.96	3.95	4.43	0.83	0.74	0.45	0.80
d 14	4.38	4.13	4.04	5.10	0.65	0.66	0.57	0.37
FCI[2]								
d 7	9.36	11.78	9.40	6.91	1.55	0.19	0.98	0.18
d 14	12.21	10.87	11.18	14.51	2.43	0.62	0.70	0.38
Serum amyloid-A, µg/mL								
d 14	69.2	75.8	57.6	57.0	12.7	0.29	0.83	0.79

[1]100%, 17 freestalls and headlocks per pen; 142%, 12 freestalls and headlocks per pen; 17 cows per pen

[2]Free cortisol index; total cortisol (nmol/L) / corticosteroid binding globulin (mg/L)

Table 5.10. Daily ruminal pH responses of focal cows (n = 4 cows/pen, 4 pens/treatment) to feed access (FA; no restriction; NR and 5-h restriction; R) at 100% and 142% stocking densities[1] (STKD).

	100%		142%			*P*-value		
Variable	NR	R	NR	R	SEM	STKD	FA	STKD x FA
Ruminal pH								
Mean pH	5.96	6.03	5.98	5.89	0.06	0.14	0.80	0.08
Minimum pH	5.42	5.50	5.51	5.39	0.07	0.81	0.78	0.12
Maximum pH	6.49	6.61	6.48	6.53	0.04	0.25	0.06	0.29
Time pH < 5.8, h/d	6.62	5.23	6.78	8.77	1.27	0.02	0.49	0.02
AUC < 5.8 pH, units x pH[2]	1.66	1.24	1.73	2.55	0.63	0.09	0.52	0.11

[1]100%, 17 freestalls and headlocks per pen; 142%, 12 freestalls and headlocks per pen; 17 cows per pen

[2]AUC, area under the curve below pH 5.8.

Table 5.11. Daily distribution of ruminal pH responses of focal cows (n = 4 cows/pen, 4 pens/treatment) to feed access (FA; no restriction; NR and 5-h restriction; R) at 100% and 142% stocking densities[1] (STKD).

	100%		142%			*P*-value		
Variable	NR	R	NR	R	SEM	STKD	FA	STKD x FA
0-8 h post-feeding[2, 3]								
Mean pH	6.01	6.09	6.07	5.97	0.04	0.07	0.67	<0.01
Time pH < 5.8, h/8-h interval	1.79	1.31	1.56	2.12	0.31	0.32	0.88	0.12
AUC < 5.8 pH, units x pH[4]	0.50	0.27	0.44	0.61	0.18	0.41	0.83	0.25
9-16 h post-feeding								
Mean pH	5.93	5.97	5.92	5.84	0.07	0.17	0.65	0.25
Time pH < 5.8, h/8-h interval	2.28	1.75	2.56	3.25	0.59	0.08	0.82	0.17
AUC < 5.8 pH, units x pH	0.52	0.37	0.66	0.88	0.26	0.12	0.83	0.29
17-24 h post-feeding								
Mean pH	5.93	5.97	5.94	5.90	0.07	0.52	0.99	0.41
Time pH < 5.8, h/8-h interval	2.55	2.44	2.67	3.11	0.56	0.16	0.49	0.27
AUC < 5.8 pH, units x pH	0.63	0.71	0.65	0.95	0.28	0.47	0.33	0.54

[1]100%, 17 freestalls and headlocks per pen; 142%, 12 freestalls and headlocks per pen; 17 cows per pen

[2]Diet fed 1x/d at approximately 0600 h, with feed pushed-up 6 times daily.

[3]Cows milked 3x/d; 1st milking post-feeding at approximately 1300 h, 2nd milking post-feeding at approximately 2100 h, and 3rd milking post-feeding at approximately and 0500 h, each milking lasting approximately 60 min.

[4]AUC, area under the curve below pH 5.8.

Table 5.12. Daily ruminal fermentation responses of focal cows (n = 4 cows/pen, 4 pens/treatment) to feed access (FA; no restriction; NR and 5-h restriction; R) at 100% and 142% stocking densities[1] (STKD).

	100%		142%			*P*-value		
Variable	NR	R	NR	R	SEM	STKD	FA	STKD x FA
Volatile fatty acids, mol/100 mol[2]								
Acetate	60.36	62.46	61.84	60.42	0.76	0.38	0.29	<0.01
Propionate	25.78	23.73	24.52	26.18	0.63	0.06	0.41	<0.01
Butyrate	10.57	10.45	10.61	10.23	0.33	0.69	0.31	0.57
Isobutyrate	0.54	0.58	0.54	0.54	0.02	0.37	0.33	0.35
Valerate	2.20	2.22	1.94	2.13	0.15	0.14	0.32	0.42
Isovalerate	0.53	0.55	0.54	0.55	0.02	0.94	0.69	0.90
Total volatile fatty acids, m*M*	129.8	128.1	129.4	127.1	2.3	0.58	0.16	0.82

[1]100%, 17 freestalls and headlocks per pen; 142%, 12 freestalls and headlocks per pen; 17 cows per pen

[2]Diet fed 1x/d at approximately 0600 h, with feed pushed-up 6 times daily.

Table 5.13. Ruminal ammonia-N responses of focal cows (n = 4 cows/pen, 4 pens/treatment) to feed access (FA; no restriction; NR and 5-h restriction; R) at 100% and 142% stocking densities[1] (STKD).

	100%		142%			*P*-value		
Variable	NR	R	NR	R	SEM	STKD	FA	STKD x FA
NH_3-N, mg/dL[2,3]								
0 h	5.32	6.51	5.43	5.60	0.58	0.30	0.10	0.20
4 h	5.73	6.93	8.26	7.03	0.88	0.05	0.98	0.07
8 h	7.62	6.41	7.35	7.62	1.09	0.54	0.54	0.35
12 h	9.70	7.24	9.13	8.00	1.15	0.94	0.19	0.61
16 h	8.57	7.89	7.64	8.42	1.11	0.77	0.94	0.29
20 h	7.56	4.67	7.35	5.52	0.80	0.42	<0.01	0.19
Daily average	7.41	6.60	7.52	7.03	0.62	0.37	0.06	0.59

[1]100%, 17 freestalls and headlocks per pen; 142%, 12 freestalls and headlocks per pen; 17 cows per pen

[2]Ammonia nitrogen

[3]Diet fed 1x/d at approximately 0600 h, with feed pushed-up 6 times daily.

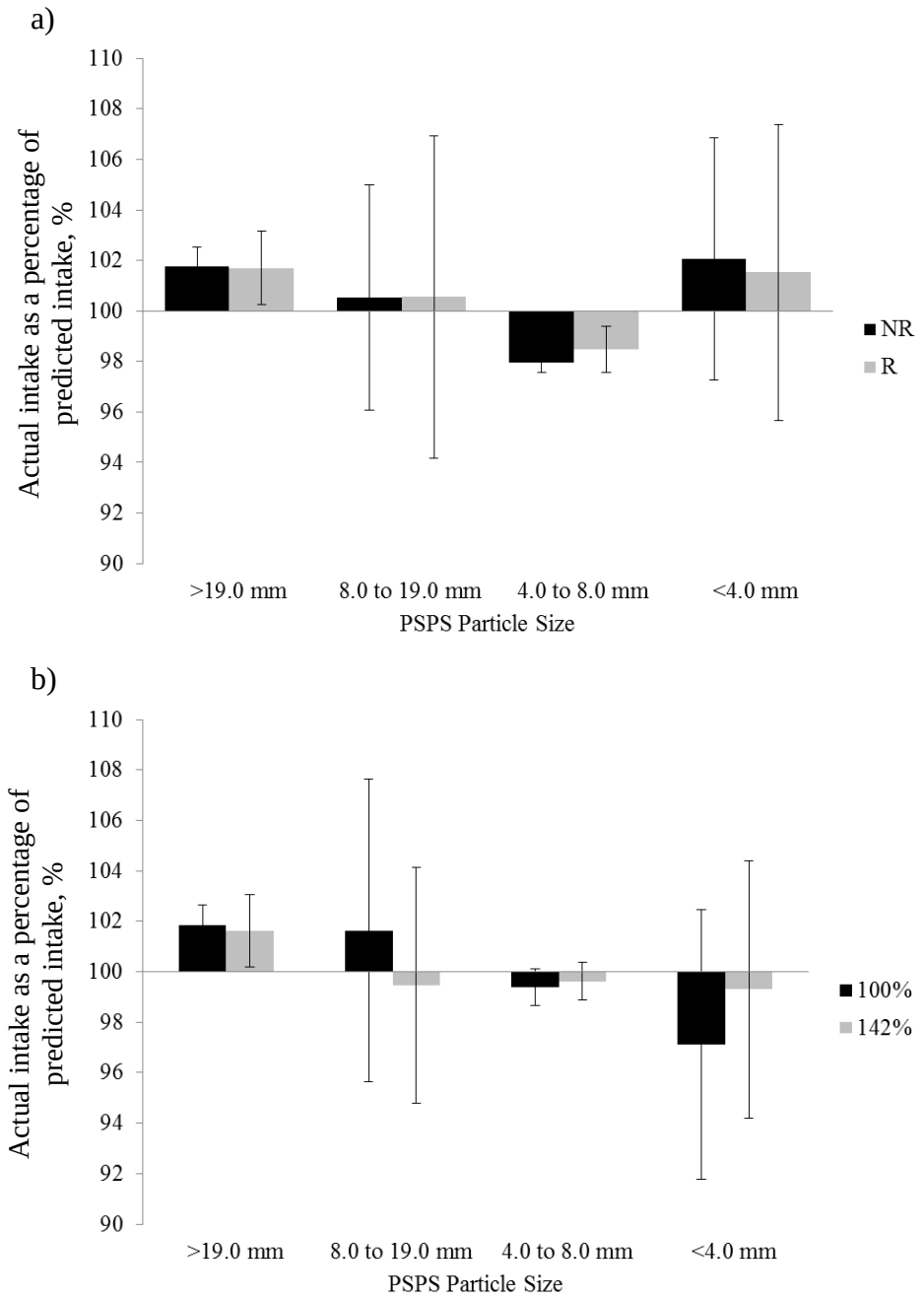

Figure 5.1. Mean ± standard error of the actual intake of total mixed ration as a percentage of predicted intake amongst Penn State Particle Separator (PSPS) particle fractions between a) no restricted feed access (NR) and restricted feed access (R) and b) 100% and 142% stocking density (100%, 17 freestalls and headlocks per pen; 142%, 12 freestalls and headlocks per pen; 17 cows per pen).

CHAPTER 6: GENERAL DISCUSSION

Although overstocking alters natural dairy cow behavior, increases stress, and negatively impacts the cow's affective state, there have been minimal documented impacts on the third pillar of dairy welfare: biological functioning. Based on findings from previous studies, stocking density can be classified as a sub-clinical stressor that compromises biological reserves without visible changes in function (Moberg, 2000). However, overstocking is not the sole stressor a cow experiences on the farm; rather, multiple stressors are present at any given time. Due to continual dietary changes and variations in how management practices are implemented among farm employees, the feeding environment can serve as a significant stressor for the cow.

For the studies in this dissertation, the focus was on two feeding practices that in previous research accounted for large variations in milk production among farms: feeding highly digestible, marginal physically effective NDF (peNDF) diets and feeding for a slick bunk resulting in reduced feed accessibility. For both studies (Chapters 4 and 5), it was hypothesized that the combination of overstocking and the feeding environment stressor would result in an exacerbated negative response, worse than either stressor in isolation. Importantly, a trend was observed towards an exacerbated response with time below pH 5.8 (sub-acute ruminal acidosis; SARA) with low peNDF diets and a significant response with reduced feed access. Therefore, under conditions with the feeding environment serving as a secondary stressor, overstocking negatively impacted all three pillars of dairy well-being.

Limitations of these trials should be considered when applying results to the field. As with many previous studies concerning stocking density, the studies in Chapters 4 and 5 were focused on identifying short-term effects (ie 14-d periods). The role that stocking density serves as a sub-clinical stressor would suggest greater impacts on cows subjected to longer exposure, resulting in biological reserve depletion and altered functions of the cow. While the reality is that many farms remain overstocked for long durations of time, there is a lack of understanding concerning the long-term impacts that stocking density has on production, health and longevity, and the affective state of the cow. To increase applicability and create realistic commercial settings (entire pen competition opposed to one-on-one competition), pen became the experimental unit due to the inability to collect individual intakes. Due to this reduction in sample size (n = 4/treatment), secondary objectives such as behavior and production may have been underpowered. With a greater sample size, it is possible that behaviors such as feeding time or production parameters may be negatively affected at this level of overstocking. Further, due to the removal of three cannulated cows from the dataset in Chapter 4, it is likely that power became limited and a significant interaction of stocking density and source of forage fiber on SARA would have been observed with a larger sample size. Finally, with the experimental design limiting access at both the headlocks and freestalls, the applications of these results are aimed at 4-row barns. Farms with 6-row barns will often have uneven stocking density within the pen, with greater competition at the feedbunk as headlocks become the limiting resource. In this type of facility design, cows will be more likely to

make up lying time and perform their rumination behavior within the freestall, minimizing the exacerbated responses on SARA. However, cows may experience increased stress in these facilities, due to changes in feeding behavior if the stocking density at the feedbunk increases above 142%. Therefore, producers should work towards minimizing secondary stressors regardless of facility design and identify which resource becomes the limiting factor.

Further investigation and trials are needed to explore the physiology controlling certain study outcomes. Consistent in both studies, increasing stocking density resulted in greater SARA, although the differences in buffering capacity need further understanding. While both stocking density treatments resulted in similar DMI, ruminal fermentation, and daily feeding and rumination times, overstocking shifted the location of rumination and a negative relationship was determined between SARA and rumination within the freestall (% of total rumination), likely accounting for the increased SARA. However, future research is needed to investigate potential differences in saliva production during rumination based on position and location within the pen. Further understanding of the impact stocking density has on saliva production through changes in behavior will lead to implementing best management practices for cows under overstocked condititions to minimize impact on biological function and improve dairy cow well-being.

In addition to further investigating the differences in buffering potential concerning overtocking, further research is needed to increase understanding of the buffering effects of the diet from the study in Chapter 4. Although diets were originally based on differences in physically effective NDF, resulting in a 2%-unit difference

between treatments, chewing did not increase as typically reported in the literature to explain the differences in time spent below pH 5.8. Therefore, alternative causes of this buffering response, such as changes in ruminal passage (increased passage rate resulting in outflow of hydrogen atoms out of the rumen), or changes in rumen epithelial absorption (increased absorption and acid/bicarbonate exchange rates), should be explored in future studies in relation to changes in dietary physically effective fiber. Upon further investigation into diet digestibility, diets differed in undigested NDF (uNDFom) resulting in a 1.8% difference in uNDFom30, a 1.2% difference in uNDFom120, and a 1.2% difference in uNDFom240. Changes in the uNDF content in the diet may result in greater saliva production per chew. Increases in saliva production are likely to be enhanced during ingestion of the diet, as evidenced by trends toward increased feeding bout length and length of first meal (increased chewing necessary to swallow feed) with the higher uNDFom diet. Therefore, further investigation should explore the effects of dietary uNDFom content on salivary production during feeding. Greater understanding of the role that feed digestibility plays on saliva production can further elucidate mitigation techniques to reduce the negative impacts of stocking density on ruminal health through dietary manipulation.

Due to the possible negative affects on all three pillars of dairy welfare, some countries are eliminating or developing strategies to reduce the practice of overstocking (WFAR, 2007; NFACC, 2009). With reduced legislative oversight over the dairy industry, the U.S. can gain greater economic advantage with the use of this practice over other countries. However, self-regulation is needed to prevent future government

intervention (driven by public perception) and minimize the impacts of overstocking on cow welfare. This research was the first to investigate the possible impacts of additional stressors on overstocked cows and identified exacerbated responses when stressors were combined. While diet and feed access represent two of the largest management factors affecting milk production between farms, these are not the only management or environmental stressors to which the cow will be exposed. Further investigation, either controlled research studies or on-farm exploration, should focus on the interactions of stocking density with feeding frequency, feed push-up frequency, time spent out of the pen for milking, time spent in headlocks for herd health exams, ventilation and facility design, stall design and comfort, heat stress, and other factors within the farmers control to reduce or eliminate as possible secondary stressors. As seen with the current research, some management practices such as reduced feed access only exacerbate the negative effects of stocking density and its use should be avoided in overstocked conditions. However, other management practices can be manipulated to minimize the negative effects of stocking density such as increasing the dietary peNDF or uNDFom. Through reducing additional stressors and manipulating the feeding environment to minimize stress from overstocking, the U.S. dairy industry stands greater economic benefit with the allowance of this practice. Better understandings of the role that the feeding environment and other environmental stressors have on overstocked dairy cattle will enhance dairy cattle well-being while optimizing productive efficiency.

COMPREHENSIVE BIBLIOGRAPHY

Allen, M. S., and D. R. Mertens. 1988. Evaluating constraints on fiber digestion by rumen microbes. J. Nutr. 118:261-270.

AOAC International. 2000. Official Methods of Analysis. 17th ed. AOAC International, Gaithersburg, MD.

Bach, A., N. Valls, A. Solans, and T. Torrent. 2008. Associations between nondietary factors and dairy herd performance. J. Dairy Sci. 91:3259-3267.

Barbano, D. M., C. Melilli, and T. R. Overton. 2014. Advanced use of FTIR spectra of milk for feeding and health management. Pages 105-113 in Proc. Cornell Nutrition Conf., Syracuse, NY. Cornell University, Ithaca, NY.

Barrientos, A. K., N. Chapinal, D. M. Weary, E. Galo, and M. A. G. von Keyserlingk. 2013. Herd-level risk factors for hock injuires in freestall-housed dairy cows in the northeastern United States and California. J. Dairy Sci. 96:3758-3765.

Batchelder, T. L. 2000. The impact of head gates and overcrowding on production and behavior patterns of lactating dairy cows. Pages 325-330 in Dairy Housing and Equipment Systems. Managing and Planning for Profitability. Publ. 129. Nat. Resour., Agric., and Eng. Serv., Camp Hill, PA.

Bauer, M. L., D. W. Herold, R. A. Britton, R. A. Stock, T. J. Klopfenstein, and D. A. Yates. 1995. Efficacy of laidlomycin propionate to reduce ruminal acidosis in cattle. J. Anim. Sci. 73:3445-3454.

Bauman, D. E., and J. M. Griinari. 2003. Nutritional regulation of milk fat synthesis. Annu. Rev. Nutr. 23:203-227.

Baumann, H., and J. Gauldie. 1994. The acute phase response. Imm. Today. 15:74-80.

Beauchemin, K. A., and W. Z. Yang. 2005. Effects of physically effective fiber on intake, chewing activity, and ruminal acidosis for dairy cows fed diets based on corn silage. J. Dairy Sci. 88:2117-2129.

Beauchemin, K. A., W. Z. Yang, and L. M. Rode. 2003. Effects of particle size of alfalfa-based dairy cow diets on chewing activity, ruminal fermentation, and milk production. J. Dairy Sci. 86:630-643.

Bewley, J., R. W. Palmer, and D. B. Jackson-Smith. 2001. A comparison of free-stall barns used by modernized Wisconsin dairies. J. Dairy Sci. 84:528-541.

Black, R. A., R. J. Grant, and P. D. Krawczel. 2016. Short communication: Short-term changes in stocking density did not alter meal characteristics of lactating Holstein dairy cattle. J. Dairy Sci. 99:6572-6577.

Bland, J. M., and D. G. Altman. 1986. Statistical methods for assessing agreement between two methods of clinical measurement. Lancet. 327:307-310.

Box, G. E. P., and D. R. Cox. 1964. An analysis of transformations. J. Royal Statistical Society, Series B, 26:211-252.

Caraviello, D. Z., K. A. Weigel, M. Craven, D. Gianola, N. B. Cook, K. V. Nordlund, P. M. Fricke, and M. C. Wiltbank. 2006. Analysis of reproductive performance of lactating cows on large dairy farms using machine learning algorithms. J. Dairy Sci. 89:4703-4722.

Cassida, P. E., and M. R. Stokes. 1986. Eating and resting salivation in early lactation dairy cows. J. Dairy Sci. 69:1282-1292.

Chaney, A. L., and E. P. Marbach. 1962. Modified reagents for determination of urea and ammonia. Clin. Chem. 8:130-132.

Chapinal, N., D. M. Weary, J. Rushen, A. M. de Passille, and M. A. G. von Keyserlingk. 2010a. Short communication: Effects of temporal restriction in availability of the total mixed ration on feeding and competitive behavior in lactating dairy cows. Livest. Sci. 137:282-286.

Charlton, G. L., D. B. Haley, J. Rushen, and A. M. de Passillé. 2014. Stocking density, milking duration, and lying times of lactating cows on Canadian freestall dairy farms. J. Dairy Sci. 97:2694-2700.

Coleman, E., E. Khafipour, B. Vlaeminck, B. De Baets, J. C. Plaizier, and V. Fievez. 2013. Grain-based versus alfalfa-based subacute ruminal acidosis induction experiments: Similarities and differences between changes in milk fatty acids. J. Dairy Sci. 96:4100-4111.

Collings, L. K. M., D. M. Weary, N. Chapinal, and M. A. G. von Keyserlingk. 2011. Temporal feed restriction and overstocking increase competition for feed by dairy cattle. J. Dairy Sci. 94:5480-5486.

Cook, N. B., and K. V. Nordlund. 2004. Behavioral needs of the transition cow and considerations for special needs facility design. Vet. Clin. Food Anim. 20:495-520.

Cook, N. B., T. B. Bennett, and K. V. Nordlund. 2005. Monitoring indices of cow comfort in free-stall-housed dairy herds. J. Dairy Sci. 88:3876-3885.

Cooper, M. D., D. R. Arney, and C. J. C. Phillips. 2007. Two- or four-hour lying deprivation on the lying behavior of lactating dairy cows. J. Dairy Sci. 90:1149-1158.

Cotanch, K. W., J. D. Darrah, C. S. Ballard, and R. J. Grant. 2010. Modification of the Penn State Particle Separator with 3.18- or 4.76-mm perforated steel sieves to measure physically effective fiber. J. Dairy Sci. 88(E Suppl. 1):147. (Abstr.).

Crossley, R. E., A. Harlander-Matauschek, and T. J. DeVries. 2017. Variability in behavior and production among dairy cows fed under differing levels of competition. J. Dairy Sci. 100:1-14.

Danscher, A. M., S. Li, P. H. Andersen, E. Khafipour, N. B. Kristensen, and J. C. Plaizier. 2015. Indicators of induced subacute ruminal acidosis (SARA) in Danish Holstein cows. Acta Vet. Scand. 57:39.

Deming, J. A., R. Bergeron, K. E. Leslie, and T. J. DeVries. 2013. Associations of housing, management, milking activity, and standing and lying behavior of dairy cows milked in automatic systems. J. Dairy Sci. 96:344-351.

DeVries, T. J., F. Dohme, and K. A. Beauchemin. 2008. Repeated ruminal acidosis challenges in lactating dairy cows at high and low risk for developing acidosis: Feed sorting. J. Dairy Sci. 91:3958-3967.

DeVries, T. J., M. A. G. von Keyserlingk, and D. M. Weary. 2004. Effects of feeding space on the inter-cow distance, aggression, and feeding behavior of free-stall housed lactating dairy cows. J. Dairy Sci. 87:1432-1438.

DeVries, T. J., M. A. G. von Keyserlingk, and K. A. Beauchemin. 2003. Short communication: Diurnal feeding pattern of lactating dairy cows. J. Dairy Sci. 86:4079-4082.

De Vries, A., H. Dechassa, and H. Hogeveen. 2016. Economic evaluation of stall stocking density of lactating dairy cows. J. Dairy Sci. 99:3848-3857.

Erdman, R. A., T. W. Moreland, and W. R. Stricklin. 1989. Effect of time of feed access on intake and production in lactating dairy cows. J. Dairy Sci. 72:1210-1216.

Erickson, G. E., C. T. Milton, K. C. Fanning, R. J. Cooper, R. S. Swingle, J. C. Parrott, G. Vogel, and T. J. Klopfenstein. 2003. Interaction between bunk management and monensin concentration on finishing performance, feeding behavior, and ruminal metabolism during an acidosis challenge with feedlot cattle J. Anim. Sci. 81:2869-2879.

Espejo, L. A., and M. I. Endres. 2007. Herd-level risk factors for lameness in high-producing Holstein cows housed in freestall barns. J. Dairy Sci. 90:306-314.

Espejo, L. A., M. I. Endres, and J. A. Salfer. 2006. Prevalence of lameness in high-producing Holstein cows housed in freestall barns in Minnesota. J. Dairy Sci. 89:3052-3058.

Falk, A. C., D. M. Weary, C. Winckler, M. A. G. von Keyserlingk. 2012. Preference for pasture versus freestall housing by dairy cattle when stall availability indoors is reduced. J. Dairy Sci. 95:6409-6415.

Ferguson, J. D., D. T. Galligan, and N. Thomsen. 1994. Principal descriptors of body condition score in Holstein cows. J. Dairy Sci. 77:2695-2703.

Fisher, A. D., G. A. Verkerk, C. J. Morrow, and L. R. Matthews. 2002. The effects of feed restriction and lying deprivation on pituitary-adrenal axis regulations in lactating cows. Livest. Prod. Sci. 73:255-263.

Fitts, J. E., and D. Laird. 2004. Direct microscopic methods for bacteria or somatic cells. Pages 269-280 in Standard Methods for the Examination of Dairy Products. 17th ed. H. M. Wehr and J. F. Frank, ed. Am. Public Health Assoc., Washington, DC.

Fraser, D., D. M. Weary, E. A. Pajor, and B. N. Milligan. 1997. A scientific conception of animal welfare that reflects ethical concerns. Anim. Welfare 6:187-205.

Fregonesi, J. A., C. B. Tucker, and D. M. Weary. 2007. Overstocking reduces lying time in dairy cows. J. Dairy Sci. 90:3349-3354.

French, P., J. Chamberlain, and J. Warntjes. 2005. Effect of feed refusal amount on feeding behavior and production in Holstein cows. J. Anim. Sci. 83(E-Suppl.1):175 (Abstr.).

Friend, T. H., and C. E. Polan. 1974. Social rank, feeding behavior, and freestall utilization by dairy cattle. J. Dairy Sci. 57:1214-1220.

Friend, T. H., F. C. Gwazdauskas, and C. E. Polan. 1979. Change in adrenal response from free stall competition. J. Dairy Sci. 62:768-771.

Fustini, M., G. Galeati, G. Gabai, L. E. Mammi, D. Bucci, P. A. Accorsi, and A. Formigoni. 2017. Overstocking dairy cows during the dry period affects dehydroepiandrosterone and cortisol secretion. J. Dairy Sci. 100:620-628.

Gao, X., and M. Oba. 2014. Relationship of severity of subacute ruminal acidosis to rumen fermentation, chewing activities, sorting behavior, and milk production in lactating dairy cows fed a high-grain diet. J. Dairy Sci. 97:3006-3016.

Garrett, E. F., K. V. Norlund, W. J. Goodger, and G. R. Oetzel. 1997. A cross-sectional field study investigating the effect of periparturient dietary management on ruminal pH in early lactation dairy cows. J. Dairy Sci. 80(Suppl. 1):169. (Abstr.).

Goering, H. K., and P. J. Van Soest. 1970. Forage Fiber Analysis (Apparatus, Reagents, Procedures, and Some Applications). Agriculture Handbook No. 379. USDA Agricultural Research Service, Washington, DC.

González, M., A. K. Yabuta, and F. Galindo. 2003. Behaviour and adrenal activity of first parturition and multiparous cows under a competitive situation. Appl. Anim. Behav. Sci. 82:259-266.

Gozho, G. N., D. O. Krause, and J. C. Plaizier. 2007. Ruminal lipopolysaccharide concentration and inflammatory response during grain-induced subacute ruminal acidosis in dairy cows. J. Dairy Sci. 90:856-866.

Gozho, G. N., J. C. Plaizier, D. O. Krause, A. D. Kennedy, and K. M. Wittenberg. 2005. Subacute ruminal acidosis induces ruminal lipopolysaccharide endotoxin release and triggers and inflammatory response. J. Dairy Sci. 88:1399-1403.

Grant, R. J. 2007. Taking advantage of natural behavior improves dairy cow performance. Pages 225-236 in Proc. Western Dairy Management Conf., Reno, NV.

Grant, R. J., and D. R. Mertens. 1992. Influence of buffer pH and raw corn starch addition on in vitro fiber digestion kinetics. J. Dairy Sci. 75:2762-2768.

Grant, R. J., and J. L. Albright. 2001. Effect of animal grouping on feeding behavior and intake of cattle. J. Dairy Sci. 84(E-Suppl.):E156-E163.

Green, M. J., A. J. Bradley, G. F. Medley, and W. J. Brown. 2008. Cow, farm, and herd management factors in the dry period associated with raised somatic cell counts in early lactation. J. Dairy Sci. 91:1403-1415.

Greenough, P.R., and J. J. Vermunt. 1991. Evaluation of subclinical laminitis in a dairy herd and observations on associated nutritional and management factors. Vet Rec 128:11-17.

Hill, C. T., P. D. Krawczel, H. M. Dann, C. S. Ballard, R. C. Hovey, and R. J. Grant. 2007. Effect of stocking density on the short-term behavior of dairy cows. J. Dairy Sci. 90(Suppl. 1):244. (Abstr.).

Hill, C. T., P. D. Krawczel, H. M. Dann, C. S. Ballard, R. C. Hovey, W. A. Falls, and R. J. Grant. 2009. Effect of stocking density on the behavior of dairy cows with differing parity and lameness status. Appl. Anim. Behav. Sci. 117:144-149.

Hoffman, P. C., C. R. Simson, and M. Wattiaux. 2007. Limit feeding of gravid Holstein heifers: Effect on growth, manure nutrient excretion, and subsequent early lactation performance. J. Dairy Sci. 90:946-954.

Hook, S. E., M. A. Steele, K. S. Northwood, A-D. G. Wright, and B. W. McBride. 2011a. Impact of high-concentrate feeding and low ruminal pH on methanogens and protozoa in the rumen of dairy cows. Environ. Microb. 62:94-105.

Hook, S. E., M. A. Steele, K. S. Northwood, J. Dijkstra. J. France, A-D. G. Wright, and B. W. McBride. 2011b. Impact of subacute ruminal acidosis (SARA) adaptation and recovery on the density and diversity of bacteria in the rumen of dairy cows. Microb. Ecol. 78:275-284.

Horadagoda, N. U., K. M. G. Knox, H. A. Gibbs, S. W. J. Reid, A. Horadagoda, S. E. R. Edwards, P. D. Eckersall. 1999. Acute phase proteins in cattle: discrimination between acute and chronic inflammation. Vet. Rec. 144:437-441.

Hosseinkhani, A., T. J. DeVries, K. L. Proudfoot, R. Valizadeh, D. M. Veira, and M. A. G. von Keyserlingk. 2008. The effects of feed bunk competition on the feed sorting behavior of close-up dry cows. J. Dairy Sci. 91:1115-1121.

Hulbert, L. E., J. A. Carroll, M. A. Ballou, N. C. Burdick, J. W. Dailey, L. C. Caldwell, A. N. Loyd, R. C. Vann, T. H. Welsh Jr., and R. D. Randel. 2013. Sexually dimorphic stress and pro-inflammatory cytokine responses to an intravenous corticotropin-releasing hormone challenge of Brahman cattle following transportation. Innate Immun. 19:378-387.

Huzzey, J. M., D. V. Nydam, R. J. Grant, and T. R. Overton. 2011. Associations of prepartum plasma cortisol, haptoglobin, fecal cortisol metabolites, and nonesterified fatty acids with postpartum health status in Holstein dairy cows. J. Dairy Sci. 94:5878-5889.

Huzzey, J. M., R. J. Grant, and T. R. Overton. 2012. Short communication: Relationship between competitive success during displacements at an overstocked feed bunk and measures of physiology and behavior in Holstein dairy cattle. J. Dairy Sci. 95:4434-4441.

Huzzey, J. M., T. J. DeVries, P. Valois, and M. A. G. von Keyserlingk. 2006. Stocking density and feed barrier design affect the feeding and social behavior of dairy cattle. J. Dairy Sci. 89:126-133.

Ito, K., N. Chapinal, D. M. Weary, and M. A. G. von Keyserlingk. 2014. Associations between herd-level factors and lying behavior of freestall-housed dairy cows. J. Dairy Sci. 97:2081-2089.

Jensen, M. B., L. J. Pedersen, and L. Munksgaard. 2005. The effect of reward duration on demand functions for rest in dairy heifers and lying requirements as measured by demand functions. Appl. Anim. Behav. Sci. 90:207-217.

Kattesh, H. G., C. A. Kurman, B. E. Gillespie, P. D. Krawczel, and A. M. Saxton. 2014. Bedding surface does not alter circulating patterns of cortisol, corticosteroid-binding globulin, or free cortisol index in preweaned Jersey calves. J. Anim. Sci. 92(E-Suppl. 2):1383. (Abstr.).

Kaylegian, K. E., G. E. Houghton, J. M. Lynch, J. R. Fleming, and D. M. Barbano. 2006a. Calibration of infrared milk analyzers: Modified milk versus producer milk. J. Dairy Sci. 89:2817-2832.

Kaylegian, K. E., J. M. Lynch, G. E. Houghton, J. R. Fleming, and D. M. Barbano. 2006b. Modified versus producer milk calibration: Mid-Infrared analyzer performance validation. J. Dairy Sci. 89:2833-2845.

Kaylegian, K. E., J. M. Lynch, J. R. Fleming, and D. M. Barbano. 2009. Influence of fatty acid chain length and unsaturation on Mid-Infrared milk analysis. J. Dairy Sci. 92:2485-2501.

Khafipour, E., D. O. Krause, and J. C. Plaizier. 2006. Influence of grain induced sub-acute ruminal acidosis (SARA) on lipopolysaccharide endotoxin (LPS) and acute phase proteins. Can. J. Anim. Sci. 86:577. (Abstr.).

Khafipour, E., S. Li, J. C. Plaizier, and D. O. Krause. 2009. Rumen microbiome composition determined using two nutritional models of subacute ruminal acidosis. Appl. Env. Micro. 75:7115-7124.

King, M. T. M., E. A. Pajor, S. J. LeBlanc, and T. J. DeVries. 2016. Associations of herd-level housing, management, and lameness prevalence with productivity and cow behavior in herds with automated milking systems. J. Dairy Sci. 99:9069-9079.

Kononoff, P. J., and A. J. Heinrichs. 2003. The effect of reducing alfalfa haylage particle size on cows in early lactation. J. Dairy Sci. 86:1445-1457.

Krajcarski-Hunt, H., J. C. Plaizier, J. P. Walton, R. Spratt, and B. W. McBride. 2002. Short communication: Effect of subacute ruminal acidosis on in situ fiber digestion in lactating dairy cows. J. Dairy Sci. 85:570-573.

Krause, K. M., D. K. Combs, and K. A. Beauchemin. 2002b. Effects of forage particle size and grain fermentability in midlactation cows II: Ruminal pH and chewing activity. J. Dairy Sci. 85:1947-1957.

Krause, K. M., D. V. Dhuyvetter, and G. R. Oetzel. 2009. Effect of a low-moisture buffer block on ruminal pH in lactating dairy cattle induced with subacute ruminal acidosis. J. Dairy Sci. 92:352-364.

Krause, K. M., and G. R. Oetzel. 2005. Inducing subacute ruminal acidosis in lactating dairy cows. J. Dairy Sci. 88:3633-3639.

Krawczel, P. D., C. S. Mooney, H. M. Dann, M. P. Carter, R. E. Butzler, C. S. Ballard, and R. J. Grant. 2012a. Effect of alternative models for increasing stocking density on the short-term behavior and hygiene of Holstein dairy cows. J. Dairy Sci. 95:2467-2475.

Krawczel, P. D., C. T. Hill, H. M. Dann and R. J. Grant. 2008. Effect of stocking density on indices of cow comfort. J. Dairy Sci. 91:1903-1907.

Krawczel, P. D., L. B. Klaiber, R. E. Butzler, L. M. Klaiber, H. M. Dann, C. S. Mooney, and R. J. Grant. 2012b. Short-term increases in stocking density affect the lying and social behavior, but not the productivity, of lactating Holstein dairy cows. J. Dairy Sci. 95:4298-4308.

Lammers, B. P., D. R. Buckmaster, and A. J. Heinrichs. 1996. A simple method for the analysis of particle sizes of forage and total mixed rations. J. Dairy Sci. 79:922-928.

Legendre P., and M. J. Anderson. 1999. Distance-based redundancy analysis: testing multispecies responses in multifactorial ecological experiments. Ecol. Monogr. 69:1-24.

Leonard F. C., J. M. O'Connell, and K. J. O'Farrell. 1996. Effect of overcrowding on claw health in firstcalved friesian heifers. Br. Vet. J. 152:459-472.

Leonardi, C., and L. E. Armentano. 2003. Effect of quantity, quality, and length of alfalfa hay on selective consumption by dairy cows. J. Dairy Sci. 86:557-564.

Li, S., E. Khafipour, D. O. Krause, A. Kroeker, J. C. Rodreiguez-Lecompte, G. N. Gozho, and J. C. Plaizier. 2012. Effects of subacute ruminal acidosis challenges on fermentation and endotoxins in the rumen and hindgut of dairy cows. J. Dairy Sci. 95:294-303.

Lobeck-Luchterhand, K. M., P. R. B. Silva, R. C. Chebel, and M. I. Endres. 2015. Effect of stocking density on social, feeding, and lying behavior of prepartum dairy animals. J. Dairy Sci. 98:240-249.

Lynch, J. M., D. M. Barbano, and J. R. Fleming. 2007. Determination of the lactose content of fluid milk by spectrophotometric enzymatic analysis using weight additions and path length adjustment: Collaborative study. J. AOAC Int. 90:196-216.

Maekawa, M., K. A. Beauchemin, and D. A. Christensen. 2002a. Effect of concentrate level and feeding management on chewing activities, saliva production, and ruminal pH of lactating dairy cows. J. Dairy Sci. 85:1165-1175.

Maekawa, M., K. A. Beauchemin, and D. A. Christensen. 2002b. Chewing activity, saliva production, and ruminal pH of primiparous and multiparous lactating dairy cows. J. Dairy Sci. 85:1176-1182.

McCarthy, M. M., T. Yasui, M. J. B. Felippe, and T. R. Overton. 2016. Associations between the degree of early lactation inflammation and performance, metabolism, and immune function in dairy cows. J. Dairy Sci. 99:680-700.

Mentink, R. L., and N. B. Cook. 2006. Short communication: Feed bunk utilization in dairy cows housed in pens with either two or three rows of free stalls. J. Dairy Sci. 89:134-138.

Mertens, D. R. 1997. Creating a system for meeting the fiber requirements of dairy cows. J. Dairy Sci. 80:1463-1481.

Mertens, D. R. 2002. Physical and chemical characteristics of fiber affecting dairy cow performance. Pages 125-144 in Cornell Nut. Conf., Proc. 2002., Dept. Anim. Sci., Cornell Univ., Ithaca, NY.

Metz, J. H. M. 1985. The reaction of cows to a short-term deprivation of lying. Appl. Anim. Behav. Sci. 13:301-307.

Miller-Cushon, E. K., and T. J. DeVries. 2017. Feed sorting in dairy cattle: Causes, consequences, and management. J. Dairy Sci. 100:4172-4183.

Mitlöhner, F. M., J. L. Morrow-Tesch, S. C. Wilson, J. W. Dailey, and J. J. McGlone. 2001. Behavioral sampling techniques for feedlot cattle. J. Anim. Sci. 79:1189-1193.

Moberg, G. P. 2000. Biological response to stress: Implications for animal welfare. In: Moberg, G. P. and J. A. Mench (eds) The Biology of Animal Stress. CAB. International, Wallingford, UK, pp. 1-21.

Munksgaard, L., and H. B. Simonsen. 1996. Behavioral and pituitary adrenal-axis responses of dairy cows to social isolation and deprivation of lying down. J. Anim. Sci. 74:769-778.

Munksgaard, L., M. B. Jensen, L. J. Pedersen, S. W. Hansen, and L. Matthews. 2005. Quantifying behavioural priorities - Effects of time constraints on behavior of dairy cows, Bos Taurus. Appl. Anim. Behav. Sci. 92:3-14.

Munksgaard, L., and P. Lovendahl. 1993. Effects of social and physical stressors on growth hormone levels in dairy cows. Can. J. Anim. Sci. 73:847-853.

Nagaraja, T. G., and E. C. Titgemeyer. 2007. Ruminal acidosis in beef cattle: The current microbiological and nutritional outlook. J. Dairy Sci. 90(E. Suppl.):E17-E38.

National Milk Producers Federation (NMPF). 2017. National Dairy FARM Animal Care Manual. Available at http://www.nationaldairyfarm.com/animal-care-resources.html.

National Research Council. 2001. Nutrient Requirements of Dairy Cattle. 7th rev. ed. National Academy Press. Washington, DC.

Nazifi, S., A. Rezakhani, M. Koohimoghadam, M. Ansari-Lari, and Z. Esmailnezhad. 2008. Evaluation of serum haptoglobin in clinically healthy cattle and cattle with inflammatory diseases in Shiraz, a tropical area in southern Iran. Bul. J. Vet. Med. 11:95-101.

Nelson, A. J. 1996. On-farm nutrition diagnostics. Pages 76-85 in Proc. 29th Annu. Conf. Am. Bovine Pract., San. Diego, CA. Am. Assoc. Bovine Pract., Rome, GA.

NFACC. 2009. Code of practice for the care and handling of dairy cattle. Dairy Farmers of Canada and the National Farm Animal Care Council, Ottawa, Ontario, Canada.

Nocek, J. E. 1997. Bovine acidosis: implications on laminitis. J. Dairy Sci. 80:1005-1028.

Nordlund, K. V., N. B. Cook, and G. R. Oetzel. 2004. Investigation strategies for laminitis problem herds. J. Dairy Sci. 87:(E. Suppl.):E27-E35.

Olofsson, J. 1999. Competition for total mixed diets fed for ad libitum intake using one or four cows per feeding station. J. Dairy Sci. 82:69-79.

Overton, M. W., W. M. Sischo, G. D. Temple, and D. A. Moore. 2002. Using time-lapse video photography to assess dairy cattle lying behavior in a free-stall barn. J. Dairy Sci. 85:2407-2413.

Owens, F. N., S. Qi, and D. A. Sapienza. 2014. Invited review: Applied protein nutrition of ruminants – Current status and future directions. Prof. Anim. Sci. 30:208-211.

Penner, G. B., K. A. Beauchemin, and T. Mutsvangwa. 2006. An evaluation of the accuracy and precision of a stand-alone submersible continuous ruminal pH measurement system. J. Dairy Sci. 89:2132-2140.

Plaizier, J. C., D. O. Krause, G. N. Gozho, and B. W. McBride. 2008. Subacute ruminal acidosis in dairy cows: The physiological causes, incidence, and consequences. Vet. J. 176:21-31.

Proudfoot, K. L., D. M. Veira, D. M. Weary, and M. A. G. von Keyserlingk. 2009. Competition at the feed bunk changes the feeding, standing, and social behavior of transition dairy cows. J. Dairy Sci. 92:3116-3123.

Roberts, M. P., H. G. Kattesh, G. A. Baumbach, B. E. Gillespie, J. D. Godkin, J. F. Schneider, and A. M. Saxton. 2003. Age-related changes in porcine corticosteroid-binding globulin (pCBG) as determined by an enzyme-linked immunosorbent assay. Domest. Anim. Endocrinol. 24:323-339.

Rossi, R., E. Del Prete, and E. Scharrer. 1998. Effects of histamine H1 receptors on the feeding and drinking patterns in pygmy goats. J. Dairy Sci. 81:2369-2375.

Schefers, J. M., K. A. Weigel, C. L. Rawson, N. R. Zwald, and N. B. Cook. 2010. Management practices associated with conception rate and service rate of lactating Holstein cows in large, commercial herds. J. Dairy Sci. 93:1459-1467.

Schirmann, K., M. A. G. von Keyseylingk, D. M. Weary, D. M. Veira, and W. Heuwieser. 2009. Technical note: Validation of a system for monitoring rumination in dairy cows. J. Dairy Sci. 92:6052-6055.

Schutz, K., D. Davison, and L. Matthews. 2006. Do different levels of moderate feed deprivation in dairy cows affect feeding motivation? Appl. Anim. Behav. Sci. 101:253-263.

Shapiro, S. S., and M. B. Wilk. 1965. An analysis of variance test for normality. Biometrika. 52:591-611.

Shook, G. E. 1993. Genetic improvement of mastitis through selection on somatic cell count. Veterinary Clinics of North America - Food Animal Practice 9:563-581.

Silva, P. R. B., A. R. Dresch, K. S. Machado, J. G. N. Moraes, K. Lobeck-Luchterhand, T. K. Nishimura, M. A. Ferreira, M. I. Endres, and R. C. Chebel. 2014. Prepartum stocking density: Effects on metabolic health, reproductive, and productive responses. J. Dairy Sci. 97:5521-5532.

Silva-del-Rio, N., J. M. Heguy, and A. Lago. 2010. Feed management practices on California dairies. J. Dairy Sci. 93(E-Suppl. 1):773 (Abstr.).

Skinner, J. G., R. A. LI. Brown, and L. Roberts. 1991. Bovine haptoglobin response in clinically defined field conditions. Vet. Rec. 128:147-149.

Solano, L., H. W. Barkema, E. A. Pajor, S. Mason, S. J. LeBlanc, C. G. R. Nash, D. B. Haley, D. Pellerin, J. Rushen, A. M. de Passillé, E. Vasseur, and K. Orsel. 2015. Associations between lying behavior and lameness in Canadian Holstein-Friesian cows housed in freestall barns. J. Dairy Sci. 99:2086-2101.

Sova, A. D., S. J. LeBlanc, B. W. McBride, and T. J. DeVries. 2013. Associations between herd-level feeding management practices, feed sorting, and milk production in freestall dairy farms. J. Dairy Sci. 96:4759-4770.

Stefańsk, B., W. Nowak, J. Komisarek, M. Taciak, M. Barszcz, and J. Skomial. 2016. Prevalence and consequence of subacute ruminal acidosis in Polish dairy herds. J. Anim. Physiol. Anim. Nutr. Pre-published DOI: 10.1111/jpn.12592.

Stone, W. C. 1999. The effect of subclinical rumen acidosis on milk components. Pages 40-46 in Cornell Nutr. Conf. Feed Manuf., Proc. 1999, Cornell Univ., Ithaca, NY.

Stone, W. C. 2004. Nutritional approaches to minimize subacute rumen acidosis and laminitis in dairy cattle. J. Dairy Sci. 87(E-Suppl.):E13-E26.

Talebi, A., M. A. G. von Keyserlingk, E. Telezhenko, and D. M. Weary. 2014. Reduced stocking density mitigates the negative effects of regrouping in dairy cattle. J. Dairy Sci. 97:1358-1363.

Telezhenko, E., M. A. G. von Keyserlingk, A. Talebi, and D. M. Weary. 2012. Effect of pen size, group size, and stocking density on activity in freestall-housed dairy cows. J. Dairy Sci. 95:3064-3069.

Teimouri Yansari, A., R. Valizadeh, A. Naserian, D. A. Christensen, P. Yu, and F. Eftekhari Shahroodi. 2004. Effects of alfalfa particle size and specific gravity on chewing activity, digestibility, and performance of Holstein dairy cows. J. Dairy Sci. 87:3912-3924.

Tilley, J. M. A., and R. A. Terry. 1963. A two-stage technique for the in vitro digestion of forage crops. Grass and Forage Sci. 18:104-111.

Trevisi, E., G. Bertoni, R. Lombardelli, and A. Minuti. 2013. Relation of inflammation and liver function with the plasma cortisol response to adrenocroticotropin in early lactating dairy cows. J. Dairy Sci. 96:5712-5722.

Underwood, W. J. 1992. Rumen lactic acidosis. Part II. Clinical signs, diagnosis, treatment, and prevention. Compendium for continuing education for the practicing veterinarian. Food Anim. 14:1265-1270.

USDA. 2010. Facility characteristics and cow comfort on U.S. dairy operations, 2007. US Dept. Agric.-Anim. Plant Health Insp. Serv.-Vet. Serv., Cent. Epidemiol. Anim. Health, Fort Collins, CO.

USDA-ERS. 2014. Monthly cost of production estimates. USDA-Economic Research Service, Washington, DC. Accessed April 14, 2014. http://www.ers.usda.gov/data-products/milk-cost-of-productionestimates.aspx.

USDA-NAL. 2017. Animal Welfare Act and Regulations. Accessed March 11, 2017. https://www.nal.usda.gov/awic/animal-welfare-act

Val-Laillet, D., D. M. Veira, and M. A. G. von Keyserlingk. 2008. Short communication: Dominance in free-stall-housed dairy cattle is dependent upon resource. J. Dairy Sci. 91:3922-3926.

Van Soest, P. J., J. B. Robertson, and B. A. Lewis. 1991. Methods for dietary fiber, neutral detergent fiber, and nonstarch polysaccharides in relation to animal nutrition. J. Dairy Sci. 74:3583-3597.

von Borell, E., and J. Ladewig. 1989. Altered adrenocortical response to acute stressors or ACTH(l-24) in intensively housed pigs. Domest. Anim. Endocrinol. 6:299-309.

von Keyserlingk, M. A. G., A. Barrientos, K. Ito, E. Galo, and D. M. Weary. 2012. Benchmarking cow comfort on North American freestall dairies: lameness, leg injuries, lying time, facility design, and management for high-producing Holstein dairy cows. J. Dairy Sci. 95:7399-7408.

von Keyserlingk, M. A. G., J. Rushen, A. M. de Passillé, and D. M. Weary. 2009. The welfare of dairy cattle – Key concepts and the role of science. J. Dairy Sci. 92:4101-4111.

Walker, S. L., R. F. Smith, J. E. Routly, D. N. Jones, M. J. Morris, and H. Dobson. 2008. Lameness, activity time-budgets, and estrus expression in dairy cattle. J. Dairy Sci. 91:4552-4559.

Wang, F. X., D. F. Shao, S. L. Li, Y. J. Wang, A. Azarfar, and Z. J. Cao. 2016. Effects of stocking density on behavior, productivity, and comfort indices of lactating dairy cows. J. Dairy Sci. 99:3709-3717.

Watters, M. E. A., H. W. Barkema, K. E. Leslie, M. A. G. von Keyserlingk, and T. J. DeVries. 2014. Relationship between postmilking standing duration and risk of intramammary infection in freestall-housed dairy cows milked 3 times per day. J. Dairy Sci. 97:3456-3471.

Webster, A. J. 2001. Farm animal welfare: the five freedoms and the free market. Vet. J. 161:229-237.

Wechsler, B. 2007. Normal behavior as a basis for animal welfare assessment. Anim. Welfare 16:107-110.

WFAR. 2007. The Welfare of Farmed Animals (England) Regulations 2007. Accessed July 24, 2017. http://www.legislation.gov.uk/uksi/2007/2078/made.

Wierenga, H.K., and H. Hopster. 1990. The significance of cubicles for the behaviour of dairy cows. Appl. Anim. Behav. Sci. 26:309-337.

Wojciechowski, K. L., C. Melilli, and D. M. Barbano. 2016. A proficiency test system to improve performance of milk analysis methods and produce reference values for component calibration samples for infrared milk analysis. J. Dairy Sci. 99:6808-6827.

Woolpert, M. E., H. M. Dann, K. W. Cotanch, C. Melilli, L. E. Chase, R. J. Grant, and D. M. Barbano. 2016. Management, nutrition, and lactation performance are related to bulk tank milk de novo fatty acid concentration on northeastern US dairy farms. J. Dairy Sci. 99:8486-8497.

Yang, W. Z., and K. A. Beauchemin. 2006. Increasing the physically effective fiber content of dairy cow diets may lower efficiency of feed use. J. Dairy Sci. 89:2694-2704.

Yasui, T., K. McCann, R. O. Gilbert, D. V. Nydam, T. R. Overton. 2014. Associations of cytological endometritis with energy metabolism and inflammation during the periparturient period and early lactation in dairy cows. J. Dairy Sci. 97:2763-2770.

Zebeli, Q., B. U. Metzler-Zebeli, and B. N. Ametaj. 2012. Meta-analysis reveals threshold level of rapidly fermentable dietary concentrate that triggers systemic inflammation in cattle. J. Dairy Sci. 95:2662-2672.

Zebeli, Q., D. Mansmann, B. N. Ametaj, H. Steingab, and W. Drochner. 2010. A model to optimise the requirements of lactating dairy cows for physically effective neutral detergent fibre. Arch. Anim. Nut. 64:265-278.

Zebeli, Q., J. Dijkstra, M. Tafaj, H. Steingass, B. N. Ametaj, and W. Drochner. 2008. Modeling the adequacy of dietary fiber in dairy cows based on the responses of ruminal pH and milk fat production to composition of the diet. J. Dairy Sci. 91:2046-2066.

Zebeli, Q., M. Tafaj, H. Steingass, B. Metzler, and W. Drochner. 2006. Effects of physically effective fiber on digestive processes and milk fat content in early lactating dairy cows fed total mixed rations. J. Dairy Sci. 89:651-668.

Zebeli, Q., S. M. Dunn, and B. N. Ametaj. 2011. Perturbations of plasma metabolites correlated with the rise of rumen endotoxin in dairy cows fed diets rich in easily degradable carbohydrates. J. Dairy Sci. 94:2374-2382.

Zebeli, Q., V. Ölschläger, M. Tafaj, W. Vahjen, B. Junck, O. Simon, and W. Drochner. 2007a. Evaluation of counts of ruminal fibrolytic bacteria and enzyme activities in response to corn silage particle size in high-yielding dairy cows. J. Dairy Sci. 90(Suppl. 1):618-619. (Abstr.).