Implications of resource competition on behavior, feeding patterns, and feed efficiency in lactating dairy cows and social interactions in dairy heifers

By

Faith Susan Reyes

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The dissertation is approved by the following members of the Final Oral Committee:
Jennifer M. C. Van Os, Assistant Professor, Animal and Dairy Sciences
Heather M. White, Professor, Animal and Dairy Sciences
Kent A. Weigel, Professor, Animal and Dairy Sciences
Joao R. R. Dorea, Assistant Professor, Animal and Dairy Sciences
Ricki J. Colman, Associate Professor, Cell and Regenerative Biology

This dissertation is dedicated to

My husband Arquimides Reyes,
and
My faithful companions, Milo James and Ruger

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ABSTRACT

Understanding the implications of resource competition and grouping strategies in dairy cattle is an important topic for improving animal welfare and production efficiency. To evaluate these aspects, four experiments were conducted with lactating cows (3) and weaned heifers (1). Each experiment was conducted with objectives to evaluate the following: Chapter 2. impact of grouping strategies [based on parity and group composition (same- vs. mixed-parity)] on competition behavior, feeding patterns, and feed efficiency in lactating cows, Chapter 3. preference of social dynamics (same- vs. mixed-parity) at the feed bunk and to assess the impact of parity and bin type on competition behavior, feeding patterns, and feed efficiency, Chapter 4. impact of stocking density on competition behavior and feeding patterns, as well as individual cow consistency across feed bunk stocking densities and associations with feed efficiency, and Chapter 5. impact of brush quantity on brush use and competition in weaned heifers. For lactating dairy cows, Chapter 2 showed that cows in a mixed-parity group (primiparous and multiparous cows) at the feed bunk experienced greater competition and eating time and tended to be less feed efficient vs. same-parity groups. When provided a choice in Chapter 3, cows visited both bin types equally but primiparous cows tended to be involved in more competition, ate faster, and tended to be less feed efficient at same-parity bins. At various stocking densities (1:1 vs. 2:1 vs. 4:1 cows/feeding space) in Chapter 4, cows were involved in the most competition at the intermediate stocking density and modulated their feeding patterns to partially compensate for less opportunity to gain feed access. Less feed efficient cows were less consistent in competitive behavior but more consistent in eating rate across stocking densities. For weaned dairy heifers, Chapter 5 illustrated that brush quantity did not impact brush use and competition, but the provision of four vs. two brushes per eight heifers resulted in longer bouts of brush use, likely due to better opportunity for uninterrupted use. The continued brush use over time supports the importance of providing appropriate outlets for natural behaviors to promote animal welfare. Overall, these findings highlight important animal welfare and feed efficiency considerations for producers making grouping strategy and resource provision decisions on-farm.

CHAPTER 1. LITERATURE REVIEW

1.1 Introduction

Today’s dairy industry continues to strive for a balance between productivity and health, efficiency, positive animal welfare practices and sustainability for the future. Animal care and good husbandry practices are at the heart of producers’ passion and determination for a successful dairy business. Dairy cattle, a gregarious species, are often housed in groups, especially during weaned heifer and adult cow stages. With the use of this type of housing, management practices are usually conducted on a group basis. Group-housed dynamics provide animals the opportunity to interact with conspecifics in a social environment, which allows for the facilitation of motivated behaviors. Cattle social hierarchy is based upon dynamics within the group and other individual factors, such as parity and body weight. Social dynamics can impact many aspects of animals’ experiences in their housing group, especially in a competitive environment, for access to important resources, such as fresh feed and brushes. Resource access is an important topic to investigate due to the important intersection between animal welfare, efficiency, and the productivity potential of each individual. Provision and the opportunity to access important resources can impact behavior, feeding patterns, and potentially feed efficiency. Two important instances where resource competition occurs are that of 1. accessibility of fresh feed at a feed bunk for lactating dairy cows and 2. provision of brushes to dairy heifers. Continuously improving our knowledge about management practices to ensure health, positive animal welfare and access to important resources, while keeping efficiency and feasibility considerations in mind, is important to continue advancing the dairy industry.

1.2 Feed efficiency

Feed efficiency is generally defined as a measure of product output in comparison to the feed input from which the output was produced. In dairy cattle, various metrics have been developed to quantify feed efficiency, including gross feed efficiency. Gross feed efficiency is calculated as a representation of the energy captured in products divided by the gross energy an individual consumed (VandeHaar et al., 2016); however, this particular metric does not account for the source of feed energy. Gross energy consumed is partially lost as output related to digestion and metabolism of feed, which results in a net energy of feed that remains. Net energy is then partially lost due to maintenance and the remaining energy is utilized as energy in milk or body tissue. Generally, net energy intake comprises four main fractions: 1. Milk energy, 2. Maintenance, 3. Body reserve, and 4. Residual (Fischer et al., 2018). The remaining residual is the portion of energy that is unaccounted for from known energy uses and serves as the basis for our calculation of residual feed intake.

Residual feed intake (RFI) is an estimation of feed efficiency commonly used in dairy cattle that indicates a comparison between the actual intake of feed that an individual consumes relative to the predicted intake based on accounting for known energy sinks of body size, change in BW, and milk production (VandeHaar et al., 2016). The residual between the predicted vs. expected intake is representative of RFI. A negative RFI value is indicative of an animal being more feed efficient, whereas a positive RFI value is indicative of an animal being less feed efficient.

1.2.1 Improving RFI

Residual feed intake is a relevant metric for dairy producers to consider for evaluating potential areas to improve feed efficiency and reducing feed costs. Given that feed is the highest dairy farm expenditure, estimated to account for 50% or more of total annual milk production costs (Alqaisi et al., 2019), increasing the feed efficiency of cows can improve dairy farm profitability and sustainability. Therefore, utilizing RFI to assist with selection decisions toward those individuals that are more feed efficient has the potential to increase profitability by reducing the amount of feed required for each unit of milk produced.

Our understanding of feed efficiency can be improved by identifying additional energy sinks that contribute to the variation in RFI. Physiological mechanisms contributing to the variation in RFI were identified as follows: 37% protein turnover, tissue metabolism, and stress; 10% digestibility; 10% activity; 9% heat increment of fermentation; 5% body composition, 2% feeding patterns, and 27% other (as shown in Figure 1; Herd & Arthur, 2009). Social dynamics and competition for resources, such as feed, may contribute to this variation and merit further evaluation. The topics of social dynamics and competition provide interesting overlap within the categories of stress, activity, feeding patterns, and possibly other, which demonstrate the potential for knowledge advancement. Several studies have evaluated relationships between RFI and social dynamics, as well as feeding behaviors in dairy cattle. On a numerical basis, primiparous cows in same-parity groups were more feed efficient than those in mixed-parity groups (Bach et al., 2006). Lactating dairy cows with faster eating rates and longer daily eating times were also less feed efficient. (Connor et al., 2013; Xi et al., 2016; Brown et al., 2022). Another study also speculated that more metabolic heat loss may be associated with less feed efficient cows (Ben Meir et al., 2018). These findings highlight interesting relationships for novel interrogation to disentangle the variation in this measure of feed efficiency. With further improvement of RFI, we can strive for comprehensive understanding of feed energy utilization and improved selection for feed efficient individuals.

1.3 Access to the feed bunk

1.3.1 Social dynamics

Social dynamics in group-housed animals can have important impacts on their welfare and production potential. As a gregarious, herd-oriented species, cattle are known to operate under a dynamic social hierarchy (Arave and Albright, 1981). Social interactions, specifically competition behavior, are commonly measured to evaluate social dynamics and hierarchy in dairy cattle. Competition behavior at the feed bunk has been shown to increase with higher stocking density (Olofsson, 1999; Proudfoot et al., 2009a; Lobeck-Luchterhand et al., 2015), greater feed deprivation time (Collings et al., 2011), re-grouping or mixing events (von Keyserlingk et al., 2008; Schirmann et al., 2011; Smid et al., 2019; Foris et al., 2021a), and larger group size (Bak Jensen and Proudfoot, 2017), as well as depend on feed bunk design (Huzzey et al., 2006).

The feed bunk can be a competitive environment where social dynamics play a role for cows to gain access to feed, yet the mechanisms underlying dominance are somewhat unknown. Parity (primiparous: first lactation vs. multiparous: 2+ lactations) has been used as a proxy for social hierarchy and dominance. For example, individuals of low dominance rank or younger animals are often displaced from the feed bunk by higher-ranking dairy cattle (Huzzey et al., 2006). In a pasture setting, primiparous cows showed less aggressive behavior (threatening, bunting, pushing, or fighting) than multiparous cows (Phillips and Rind, 2001). In addition, composition of the group has been shown to interact with parity for competition behavior in dairy cows. Primiparous cows received more aggressive interactions and were displaced from the feed bunk more frequently when housed with multiparous cows compared to primiparous-only housing (Gibbons et al., 2009). Heavier dairy heifers initiated more head butting and pushing in large mixed body weight groups compared to smaller, similar body weight groups (Hindhede et al., 2010).

Social dynamics and competition can also have further impact on feeding patterns. Parity has a well-established impact on feeding patterns of dairy cows. Multiparous cows have been shown to consume greater daily dry matter intake (DMI) (Beauchemin et al., 2002; DeVries et al., 2011; Neave et al., 2017; Crossley et al., 2018), as influenced by greater DMI/meal and faster eating rates (Azizi et al., 2009; Crossley et al., 2018), but in longer inter-meal intervals (Crossley et al., 2018) and fewer daily meals (Azizi et al., 2009; Crossley et al., 2018). However, depending on social dynamics and study design, parity may not impact meal patterns or eating rate (Beauchemin et al., 2002; Neave et al., 2017), number of feed bunk visits (Neave et al., 2017), or size of meals (Beauchemin et al., 2002). Similar to competition behavior, interactions between parity and group composition have also been reported for feeding patterns. Primiparous lactating cows in same-parity groups had greater feed intake and longer eating times vs. conspecifics in mixed-parity groups (Krohn and Konggaard, 1979). In growing heifers, heavy animals spent more time eating in a large, mixed-body weight group as compared to a small, similar body weight group (Hindhede et al., 2010).

Unfortunately, many of these studies are unable to perform a direct comparison between both parities in different group compositions. Certain studies have focused on primiparous cows specifically, which has resulted in a gap of knowledge on how parity and group composition interact between all combinations. Further consideration for the impact of these social dynamics, such as the interaction between parity and group composition, on competition behavior and feeding patterns is warranted to optimize grouping strategies that minimize the negative impact of excessive competition and enhance beneficial effects of social housing.

1.3.1.1 Social dynamic preference

Measuring preference serves as a method to allow an individual to "choose with their feet" when presented with different options and can provide evidence of potentially improved management practices suited to the animal. Dairy cattle have shown preferences for certain environmental conditions (Von Keyserlingk et al., 2017; Smid et al., 2018), housing designs (Fregonesi et al., 2007; Telezhenko et al., 2007), feedstuffs (Miller-Cushon et al., 2014), and milking parlor locations (Hopster et al., 1998). Yet little is known about cows' preferences for social dynamics when interacting in a competitive environment at the feed bunk. Given the multifaceted implications of social dynamics, there is a need to evaluate preferences and the factors influencing preferences at the feed bunk in dairy cattle to inform beneficial grouping strategies.

1.3.2 Feed bunk stocking density

Overcrowding dairy cattle at the feed bunk by increasing stocking density to greater than 1 cow per feeding space or bin is a common practice in the industry. Industry recommendations state that overcrowding is defined as more than 100% stocking density and/or less than 0.6 m of bunk space per cow. In the United States, dairy farms utilize an average of 142% feed bunk stocking density in freestall housing systems (Von Keyserlingk et al., 2012) and 67.9% utilize less than 0.6 m/cow at maximum cow numbers (USDA, 2010). Stocking density at the feed bunk and the related implications remain important discussion topics in today's dairy industry (Buza, 2017; Bohnert, 2022).

Increased stocking densities at the feed bunk have behavior and feeding pattern implications. As stocking density increased, cows spent less time feeding (Huzzey et al., 2006), while eating at a faster rate (Olofsson, 1999), as well as were involved in more competition (DeVries et al., 2004; Krawczel et al., 2012; Lobeck-Luchterhand et al., 2015) and spent more time standing idle in the feeding area (Hill et al., 2009). Prolonged periods of standing, particularly on hard surfaces like concrete, can also lead to lameness and negative welfare implications (Singh et al., 1993).

1.3.2.1 Behavioral consistency across contexts

On average dairy cattle behavior and feeding patterns change across varying stocking densities. However, individuals also vary in the specific response as shown by large ranges in values for competitive behaviors (Proudfoot et al., 2009b; Huzzey et al., 2012). This individual variation may be associated with success or lack thereof based on specific strategies and adaptations used to gain access to resources. The change in response across different contexts is known as behavioral plasticity (Dingemanse et al., 2010). Individual behavioral differences reflect the capacity of individuals to adapt in the environmental conditions in which they live, which is associated with the animal's welfare status (Mellor, 2016). The relationship between individual behavior consistency across different stocking densities and other variables is not well defined. Identifying consistent individual behavior differences may provide insight into the varied outcomes related to production (feeding behavior and feed efficiency) and social dynamics (competition at the feed bunk, individual social roles within the herd) of animals with one behavior response or strategy compared to another. Furthering our understanding of individual variation in strategies used to access the feed bunk can assist producers when making management and grouping decisions that help to facilitate a beneficial, positive welfare status that yields productivity and efficiency for animals on an individualized and group basis.

1.4 Access to brushes

Facilitating environments that allow individuals the freedom to express natural or motivated behaviors is also an important consideration for dairy cattle. Grooming is a motivated behavioral need for cattle that can be facilitated by placing objects in the environment, such as brushes (McConnachie et al., 2018). Grooming has been shown to have multifaceted benefits for cattle of all ages including preweaned calves (Zobel et al., 2017) to lactating cows (DeVries et al., 2007; Val-Laillet et al., 2009). Benefits have included satisfying a natural behavior (Ewing et al., 1999), improving animal cleanliness (Brownlee, 1950; Simonsen, 1979), assisting with the removal of parasites (Fraser and Broom, 1997), and reducing stress, boredom, and frustration (Wood-Gush and Beilharz, 1983). Additionally, cattle have shown sustained motivation to use brushes over time (across 13 d in group-housed heifers, and 22 and 64 d in beef steers; Park et al., 2020; Van Os et al., 2021; Wilson et al., 2002), which illustrates the importance of providing the opportunity and appropriate accessibility to perform grooming.

The decision to implement a management practice on-farm requires the balance between improved animal welfare and health, practicality, and economic considerations. Stationary brushes are an economical option as compared to mechanical brushes. Mechanical, rotating brushes allow cattle to groom multiple areas of the body (DeVries et al., 2007), yet young dairy cattle in particular groom their head and neck areas a majority of the time (Toaff-Rosenstein et al., 2017; Zobel et al., 2017; Horvath and Miller-Cushon, 2019). In addition, less complex environments and/or lack of engagement opportunities with appropriate items can lead to these natural behaviors directed toward non-preferred outlets, such as rubbing on walls and waterers

(DeVries et al., 2007). Young dairy heifers are also often more likely to be housed in more less complex group-housed environments. Abnormal behaviors have been hypothesized to be linked to the lack of opportunities to perform exploration (i.e. sniffing and licking behaviors; Wood-Gush & Vestergaard, 1989). Stationary brushes can serve as an appropriate outlet for oral manipulation and for grooming behavior (Horvath et al., 2020a; Van Os et al., 2021).

Social dynamics also play a critical role in the competition that may occur when individuals have to compete for access to a brush in a group-housed environment. Previous studies have evaluated brush use, competition behavior, and motivation to use brushes under varying stocking density, including group sizes ranging from four preweaned calves up to 20 lactating cows provided 1 brush (Foris, et al., 2021; Horvath & Miller-Cushon, 2019). One previous study provided four brushes to groups of four and two weaned heifers in separate phases (Van Os et al., 2021), but the effects of stationary brush quantity on brush use and competition was not directly evaluated. Therefore, a gap in our knowledge exists for the implications of brush stocking density on brush use and competition. Advancing our understanding of how brush quantity impacts resource competition has the potential to inform on-farm management decisions about brush provision to dairy cattle in a group-housed setting.

1.5 Measuring competition behavior

Competition behavior usually takes place as a sequence of events (as shown in Figure 2). Each interaction involves an actor (initiating the competitive behavior) vs. a receiver (receiving the competitive behavior). When competing for a resource, direct competition is defined as an initial physical contact between two individuals. The physical contact may result in the receiver being removed from the resource (successful displacement) or the receiver continues to access the resource and is not removed (unsuccessful displacement). If a successful displacement occurs, then the actor who initiated the competitive contact may take the place of the receiver and successfully access the resource (successful replacement) or the actor may not access the resource in place of the receiver (unsuccessful replacement). Studies have utilized one or more of these event categorizations, most commonly as displacement or replacement, to measure resource competition behavior in dairy cattle (Olofsson, 1999; Val-Laillet et al., 2008; Neave et al., 2017). The unsuccessful events of displacements and replacements are not often evaluated directly. Several authors have not recorded unsuccessful attempts since not all physical contacts may be performed with the intent to displace (Huzzey et al., 2012). Even so, intent generally cannot be assumed during behavioral observation. Accurate evaluation of all types of competitive contacts, successful and unsuccessful, provides a more comprehensive account of social dynamics at the feed bunk.

A large component of measuring competition behavior is quantifying magnitude of success in gaining access to the resource (i.e. fresh feed at the feed bunk). Raw event counts are beneficial at providing information about interactions and subsequent results of certain behaviors. Further calculation of event magnitudes into proportions allows for evaluating how often an individual succeeded at gaining access to a resource.

Previous literature has characterized social dynamics or dominance using proportions based on how often a cow serves as an actor vs. a receiver to measure success. An early dominance value was calculated based on the ratio of wins (dominant individual, often involving bunting) to losses (submissive, retreating individual showing avoidance behavior) in competitive events (Beilharz and Mylrea, 1963), with later studies reporting higher dominance values (i.e., greater success at winning competitive events) for multiparous vs. primiparous cows on pasture (Phillips and Rind, 2001). Other indexes use either the number of displacement events (i.e., competition index: number of times a cow initiates a successful displacement/total number of successful displacements initiated and received by that cow; Galindo and Broom, 2000) or the number of conspecifics involved in displacements (i.e., Mendl success index: number of conspecifics an individual displaces/sum of the number of conspecifics an individual displaces and the number of conspecifics displacing that individual; Mendl et al., 1992). Additionally, Gibbons et al. (2009) calculated an aggressive index (number of times an individual was an aggressor/total number of interactions the individual was involved in). A limitation of these metrics is the lack of defining the result of unsuccessful competitive attempts. An event in which a competitive attempt is unsuccessful at removing the individual from the resource still represents another type of success – for the receiver to stand their ground and continue to have access to the resource. No studies have evaluated the magnitude of unsuccessful attempts, which presents an opportunity to provide a novel approach to measuring competition behavior.

When evaluating competitive interactions among group members, the dynamics between specific pairs (dyads) of cows can vary within a single group. Interactions between individuals are complex and bidirectional (Val-Laillet et al., 2008), where individuals may only initiate, only receive, or initiate and receive interactions from certain individuals. Social network analysis (SNA) has been commonly used to investigate individual relationships and patterns in groups of dairy cattle involving disease transmission (de Freslon et al., 2019), the impact of regrouping animals (Rocha et al., 2020; Foris et al., 2021a), and affiliative (positive interaction, i.e. grooming) and agonistic (aggressive, competitive) behavioral patterns (Gygax et al., 2010; Boyland et al., 2016; Foris et al., 2019). Several studies have used SNA to infer social relationships among dairy cattle based on proximity location data (Gygax et al., 2010; Chopra et al., 2020; Rocha et al., 2020), but fewer studies have used competitive behavior (physical displacements from the feed bunk; Foris et al., 2019, 2021). Using observed competition behavior to construct and perform SNA could provide more information about complex, dyad-level relationships.

1.6 Summary and Conclusions

Dairy cattle are often group-housed in environments that involve competition for resources. Social dynamics play a critical role in the interactions between individuals with important animal welfare, behavior, and productivity implications. Resource competition is also influenced by stocking density at the feed bunk or for the provision of brushes. Previous literature showed a lack of brush quantity comparison for cattle of all ages.

For adult cows, factors such as parity can impact how individuals interact when competing for resources and resulting feeding patterns. Dairy cattle have also shown preferences for certain environments and feedstuffs that are used to aid in our understanding of facilitating a beneficial, positive environment. There has been limited inquiry into elucidating the impact of certain grouping strategies on behavior, individual preferences and potential impact on feed efficiency. The evaluation of different grouping strategies and individual preferences for certain dynamics is valuable for the dairy industry to gain knowledge about on-farm management strategies. Furthermore, individual cow strategies used to gain access to a resource may vary between individuals and across contexts. Behavioral consistency and plasticity across contexts, such as feed bunk stocking densities, may serve as identifying variables that could be used to differentiate between successful and/or feed efficient individuals compared to those less successful and/or less feed efficient counterparts. Advancing our understanding of feed efficiency, specifically residual feed intake, provides an opportunity to identify areas of variation that can improve calculation of this metric and assist in future selection decisions.

Overall, further interrogation of these concepts can assist producers with management decisions on-farm to improve animal welfare at various life stages, efficiency, and industry sustainability for the future.

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FIGURES

Figure 1. Biological mechanisms contributing to variation in residual feed intake. (Adapted from Herd and Arthur, 2009).

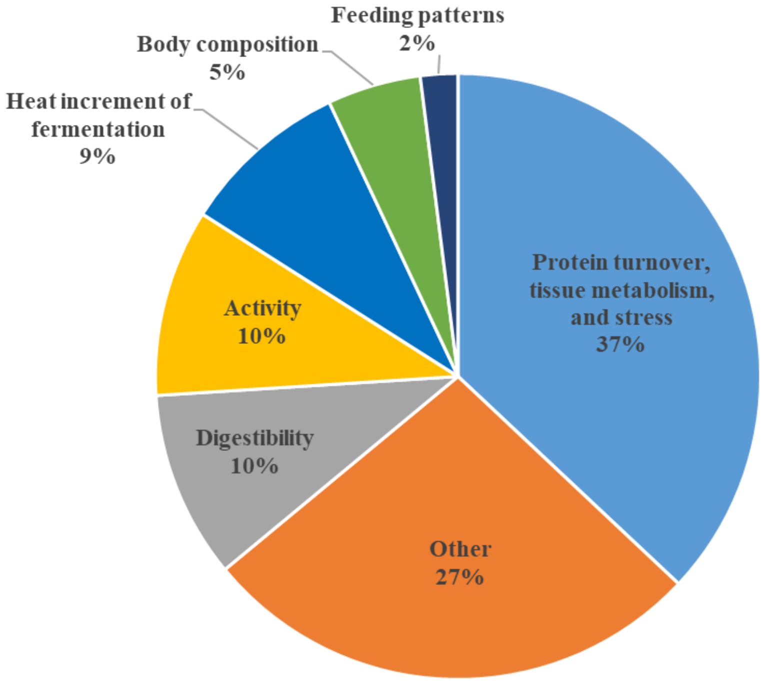
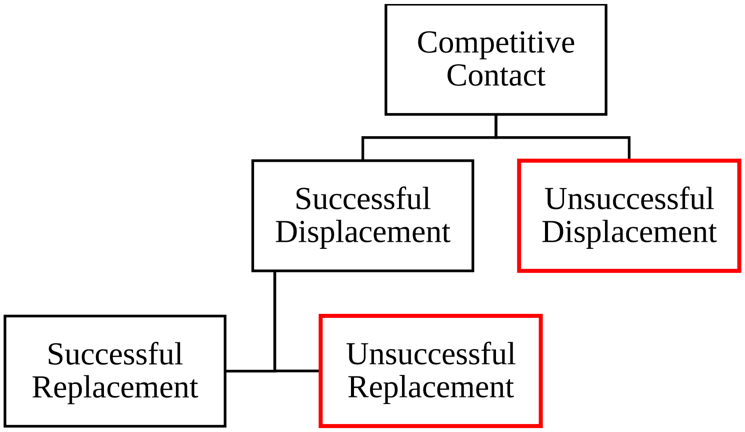


Figure 2. Flowchart of the behavior sequence used for behavior observation of competitive interactions at the feed bunk between dairy cattle. For each behavior, an actor (cow initiating the event) and receiver (individual receiving the event) were recorded. Rectangles outlined in black represent behaviors reported in previous literature (typically not within in a single study). Rectangles outlined in red represent behaviors that serve as areas of limited knowledge that represent important areas for future investigation.



CHAPTER 2. SOCIAL INTERACTIONS, FEEDING PATTERNS, AND FEED EFFICIENCY OF SAME- AND MIXED-PARITY GROUPS OF LACTATING COWS

ABSTRACT

Social dynamics in group-housed animals can have important impacts on their welfare, feed efficiency, and production potential. Our objectives were to: 1) evaluate the effects of parity and social grouping on competition behavior, feeding patterns, and feed efficiency, and 2) investigate cow-level relationships between competition and feeding behavior, production, and feed efficiency. Fifty-nine Holstein cows (144.5 ± 21.8 starting DIM, mean ± SD) were housed in a freestall pen with 30 Roughage Intake Control (RIC) bins. We evaluated the effects of parity [primiparous (PR, n = 29) vs. multiparous (MU, n =30)] and group composition at the feed bunk [same-parity (SM, n = 39) vs. mixed-parity (MX, n = 20, 50% of each parity)] with a 2×2 factorial design (SM-MU: n = 20; SM-PR: n = 19; MX-MU: n = 10; MX-PR: n = 10) on competition behavior, feeding patterns, and feed efficiency. Within the pen, groups of 9 to 10 cows were considered subgroups and assigned to treatments defined by sets of 5 assigned bins (2:1 stocking density). Feed bunk competition and feeding patterns were recorded via continuous video in the first hour after morning feed delivery and 24-h RIC data, respectively. Residual feed intake (RFI) was calculated as the difference between predicted and observed dry matter intake (DMI) after accounting for known energy sinks. Linear models were used to evaluate the effects and interactions of parity and group composition on competition, feeding behavior, and feed efficiency. Within-cow correlations were performed between competition, feeding behavior, and RFI. Cows in MX, compared to SM, were involved in more competitive interactions [mean (95% CI): competitive contacts: 11.5 (8.1,16.3) vs. 7.2 (5.5,9.3) events; displacements: 4.0 (3.0,5.3) vs. 2.1 (1.7,2.7) events, and replacements: 3.5 (2.6,4.7) vs. 1.9 (1.5,2.5) events]. Cows in MX vs. those in

SM had more bunk visits/meal [4.3 (3.9, 4.8) vs. 3.7 (3.4, 3.9) visits/meal] and longer meals (31.2 vs. 27.4 ± 0.9 min/meal) and tended to have higher RFI (0.41 ± 0.3 vs. -0.21 ± 0.2) and were therefore less feed efficient. Multiparous vs. PR cows had greater DMI per day (29.3 ± 0.6 vs. 25.5 ± 0.4 kg/d) and per meal [4.2 (4.0, 4.4) vs. 3.4 (3.2, 3.6) kg/meal], faster eating rates [0.14 (0.13, 0.15) vs. 0.12 (0.11, 0.13) kg/min], and fewer bunk visits/d [26.6 (24.0, 29.4) vs. 32.8 (29.7, 35.9)]. Regardless of grouping or parity, cows with shorter latencies to first visit the bunk after feed delivery were involved in more competition and tended to be less feed efficient. Overall, individual cow- and group-level relationships among competition, feeding behavior, and feed efficiency play an important role in feed bunk social dynamics. At a competitive 2:1 stocking density, mixed-parity groups for lactating cows may have potentially negative animal welfare and feed efficiency implications that should be considered when selecting grouping strategies on the farm.

Key words: competition, grouping strategies, residual feed intake, social dynamics

INTRODUCTION

Social dynamics of group-housed dairy cattle can have important impacts on their welfare, feed efficiency, and production potential. The feed bunk can be a competitive environment, yet much remains unknown about the dynamics of how cows gain access to feed and how to optimize group composition.

Parity and group composition play important roles in understanding competition and feeding behavior. In dairy cattle, individuals of low dominance rank or younger animals are often displaced from the feed bunk by higher-ranking animals (Huzzey et al., 2006). Primiparous cows received more aggressive interactions and were displaced from the feed bunk more frequently when housed with multiparous cows, compared with only other primiparous cows (Gibbons et al., 2009). Furthermore, the dynamics between specific pairs (dyads) of cows can vary within a single group. Social network analysis (SNA) has been used to investigate dyadic relationships and patterns in groups of dairy cattle (Gygax et al., 2010; Boyland et al., 2016; Foris et al., 2019). Using observed competition behavior at the feed bunk to construct networks provides an understudied opportunity to better understand dyad-level relationships.

Social dynamics at the feed bunk may also play a role in feeding patterns and feed efficiency. Given that feed is the greatest dairy farm expenditure (Alqaisi et al., 2019), increasing feed efficiency can improve dairy farm profitability and sustainability. Residual feed intake (RFI), a representation of the unexplained variation in feed intake after considering body size, change in BW, and milk production, can be used to estimate feed efficiency (VandeHaar et al., 2016). Our understanding of feed efficiency can be improved by identifying additional energy sinks that contribute to the variation in RFI, such as competition for resources. Although some studies have evaluated RFI and feeding behaviors, such as daily time spent eating (Connor et al., 2013; Xi et al., 2016; Brown et al., 2022), our understanding of how RFI relates to competition behavior at the feed bunk is limited.

Certain grouping strategies may minimize the negative impact of excessive competition, improve feed efficiency, and enhance the welfare benefits of social housing. Additionally, improving our understanding of the interrelationships among competition, feeding behavior and feed efficiency has the potential to add clarity to both the unexplained variation in efficiency and the mechanisms underlying social dynamics in group-housed dairy cattle. Therefore, our objectives were: 1) to evaluate the effects of parity (primiparous vs. multiparous) and group composition (same- vs. mixed-parity groups) on competition behavior at the feed bunk, feeding patterns, and feed efficiency and 2) to investigate cow-level relationships among competition behavior, feeding patterns, and feed efficiency.

MATERIALS AND METHODS

Animals, Housing and Treatments

The study was conducted from July to October 2020 at the University of Wisconsin-Madison (UW-Madison) Emmons Blaine Dairy Cattle Research Center in Arlington, WI. All procedures were approved by the Institutional Animal Care and Use Committee (protocol # 005658-R01-A01).

Thirty primiparous and 30 multiparous lactating Holstein dairy cows (138.3 ± 21.2 vs. 150.4 ± 21.1 DIM, respectively; Table 1) were initially enrolled. All cows were housed in the same pen with 64 freestalls and 30 roughage intake control (RIC) system bins (Hokofarm Group, Marknesse, the Netherlands), which recorded individual cow feed intake continuously. Cows were milked twice daily at 0300 and 1500 h and fed thrice daily at 0900, 1500, and 2100 h. The third daily feeding at 2100 h was added at the beginning of wk 1 of the experimental period to ensure all cows were fed to approximately ≥1% refusals for each bin. Fresh feed was delivered during the morning feeding; additional feed mixed in morning was added to the bins in the afternoon feed deliveries. The same TMR diet was fed to all cows, regardless of treatment assignment. Diet composition and nutrient analysis are presented in Table 2. Refusals were manually recorded daily and feeding amounts were adjusted by treatment to ensure all cows were fed ad libitum (0900: 67.2 ± 3.6 kg/bin, 1500: 33.4 ± 12.5 kg/bin, 2100: 34.1 ± 16.6 kg/bin; as fed, mean ± SD). Water was provided ad libitum via 3 automatic water troughs.

Treatments were applied to cows based on bin assignments at the feed bunk. The study used a 2×2 factorial design, with factors of parity [primiparous (PR) or multiparous (MU)] and group composition [same-parity (SM; n = 40 enrolled, 39 retained) or mixed-parity group (MX; n = 20) with 50% of each parity] at the feed bunk, with the latter factor randomly assigned. The sample size within each level was: SM-PR: n = 20 (enrolled, 19 retained), SM-MU: n = 20, MX-PR: n = 10, MX-MU: n = 10. We applied SNA to subgroups of 9-10 cows, representing 2 networks of each group composition and parity combination (i.e., SM-PR, SM-MU, and MX; 6 networks total); the MX subgroups included cows analyzed as MX-MU and MX-PR. Each network shared a set of 5 bins (2:1 stocking density) with equidistant spacing along the feed bunk. After bin assignment, the distribution of BW and DIM were checked to ensure equal variation across networks of each type. Cow demographics by parity and group composition are summarized in Table 1. Twenty-two MU cows had previous experience with the RIC system. All cows were trained to their assigned bins during a 4-wk period and were considered trained once ≤20% of daily attempted bin visits were directed to non-assigned bins (mean ± SD: 8.6 ± 5.9%; range: 0.0-20.5%). Due to health issues unrelated to the study, 1 SM-MU and 1 SM-PR cow were removed; the former was replaced before training, but the latter could not be replaced due to timing in the bin training process, resulting in 19 cows in SM-PR for the remainder of the study. Once training was complete, the experimental period lasted 45 d.

Measures

Competition Behavior. Continuous video was recorded from 10 cameras (Platinum 4.0 MP Network Matrix IR Bullet Camera, CMIP9342W-28M; LT Security Inc., Washington, NY) mounted at 3.7 m high, which were set to record with 2688 × 1520 resolution at 10 frames/s through a network video recorder (Platinum Enterprise Level 64 Channel NVR, LTN8964-8; LT

Security Inc.). Video was recorded for 2 d consecutively in experimental wk 1 and 6. Four h/d were observed (3 h from 0900 h, after feed delivery, starting when all cows were released from the stall area and had access to the feed bunk; 1 h from 1500 h, after milking and feed top-up, starting when all cows had returned from the parlor); these times were based on peak daily feed bunk visits. Each cow was marked with spray paint (Tell Tail, FIL Industries Limited, Mount Maunganui, New Zealand) for individual identification. Three trained observers coded the video recordings, observed using VSPlayer (Hikvision Digital Technology, Hangzhou, China), for competitive interactions based on a sequence of possible events (defined in Table 3, sequence shown in Figure 1). Inter-observer reliability was determined on a subsample of video that included all focal behaviors; Cohen's kappa ranged from 0.64 to 0.95, indicating 'substantial' to 'almost perfect' agreement (Landis and Koch, 1977).

Initial inspection of the data revealed that 65.8% (2697/4102) of competitive behaviors at the feed bunk occurred within 1 h after AM feed delivery [h 2 = 10.2% (417/4102), h 3 = 7.3% (300/4102); h 1 after PM feed delivery/milking = 16.8% (688/4102)]. Because small magnitudes of events in the subsequent observation hours could skew interpretation when averaged on a per-hour basis, we only retained the first hour after morning feed delivery for analysis. Only interactions between cows within a network (i.e., assigned to the same bins) were analyzed to evaluate the impacts of parity and assigned group composition; thus, 34% of the total competitive contacts (SM-PR = 28.2%, SM-MU = 36.9%, MX = 34.9%; proportion for each network indicating the network of the actor) and 19% of the displacements (SM-PR = 22.9%, SM-MU = 45.0%, MX = 32.0%) were excluded. After exclusion, values were summarized as the average among the 4 observation days. Additionally, the event values for each cow (mean of 4 d) were used to calculate 4 behavior "ratios" reflecting proportions of behavioral subsets (replacements, displacements, competitive contacts), and 3 behavior “indexes” reflecting the proportion of events in which a cow served as an actor (defined in Table 3).

Feeding Behavior and Dry Matter Intake. Individual TMR ingredients were collected weekly and dried by forced air oven (Isotemp Oven, Fisher Scientific, Waltham, MA) at 55°C for 48 h (concentrates in triplicate, forages in quadruplicate), composited by week and analyzed by a commercial laboratory (Dairyland Labs, Inc., Arcadia, WI). The weekly ingredient samples were dried at 105°C for 24 h (in duplicate) to convert feed intake to a DM basis.

The feed intake and visit details (time of day, duration, bin location) were recorded automatically by the RIC system. A visit was defined as a single event when a cow entered an assigned bin and associated RIC data were recorded. Other variables derived from RIC data were latency (min) to first visit the feed bunk after AM feed delivery, number of visits/d, DMI/d, eating rate (kg/min), total eating time (min/d, regardless of intake), and the maximum daily non-eating interval (min/d, longest daily period without eating). Latency to the first bunk visit was available for 20 d of the experimental period because video was needed to determine when feed was delivered. For days with first-visit data, DMI and duration during this visit were calculated, along with summed eating time within the first 30 min after AM feed delivery; 30 min was selected based on the average length of a meal (DeVries et al., 2003). Finally, the proportion of first visits to each of the 5 assigned bins was identified and the highest value retained to represent the most-chosen first bin as a reflection of possible location preference.

To evaluate meal characteristics for each cow, meal analysis (DeVries et al., 2003; Horvath and Miller-Cushon, 2019) was performed using visit duration data across the entire experimental period. In brief, interval durations between each cow’s bin visits were summarized and converted to $\log_{10}$-transformed frequency distributions to calculate the inter-bout criteria. The inter-bout criteria were calculated by fitting a mixture of 2 normal distributions to the $\log_{10}$ distributions of inter- and intra-visit intervals using exact maximum likelihood to determine the point at which the distribution curve of within-bout (intra-bout) intervals intersected the distribution curve of between-bout (inter-bout) intervals (R package *mixdist*; Macdonald and Du, 2018). A single inter-bout criterion pooled across all individuals was calculated (20.98 min). Meal characteristics were defined as the number of meals/d, number of visits/meal, average meal time (min/meal), DMI/meal, DMI of largest meal/d, and average inter-meal interval (min). All feed, visit, and meal related variables were summarized for each cow across the experimental period.

Milk Yield and Components. Milk yields were recorded in DairyCOMP 305 (Valley Ag Software, Spencer, MA) and summarized as kg/d for each cow. Milk samples from 4 consecutive milkings/wk were collected and preserved with 2-bromo-2-nitropro- pane-1,3-diol (Advanced Instruments Inc., Norwood, MA) and analyzed at a commercial laboratory (AgSource, Menomonie, WI) for milk composition (fat, protein, lactose, and milk urea nitrogen) and SCC.

Residual Feed Intake. Residual feed intake was calculated as a measure of feed efficiency for each cow (greater value indicates less efficient) by regressing DMI on milk energy output, median DIM, metabolic BW, and change in BW, each nested within parity. All values were summarized as an average across the experimental period for each cow. Milk energy output (kg/d) was calculated as [9.29 × milk fat (kg)] + [5.63 × true protein (kg)] + [3.95 × lactose (kg)] (NRC, 2021). Bodyweight was recorded before morning feed delivery 3 d/wk during wk 1, 4, and 7 of the experimental period using a calibrated stationary scale (EW6, Tru-Test Limited). Metabolic BW (kg) was calculated as $BW^{0.75}$. The daily change in BW was calculated using the LINEST function in Microsoft Excel to create a simple linear regression of all 9 BW values. Body condition score (reported descriptively in Table 2; not used in RFI) was assessed on 1 d in wk 1, 4, and 7 in conjunction with BW by 2 trained observers using the 5-point scale (Dairy Body Condition Score Chart, Elanco Animal Health) at increments of 0.25.

Statistical Analysis

Missing Data. Daily RIC data were missing for 1 d in wk 1 due to a power outage. During the observation periods, some cows were uninvolved in competitive interactions, and thus data were not included for 2 cows (1 SM-MU, 1 SM-PR) for all competitive behaviors, and 3 others (2 SM-MU, 1 MX-MU) for displacement and replacement variables.

Statistical Models. All response variables were analyzed using R software (v. 3.6.3, RStudio) and SAS software (9.4, SAS Institute). Cow was the experimental unit. Residuals were assessed visually using graphs and numerically using the Shapiro-Wilk test for normality. Linear models (R Core Team, 2019) were used to evaluate the effects and interactions of parity and group composition on feeding patterns and feed efficiency. Non-normal continuous variables were $\log_n$ transformed to improve normality and meet model assumptions. Generalized linear models were used to evaluate effects on count-based competition variables and on proportions (competition indexes and ratios) using a negative binomial distribution (PROC GLIMMIX, SAS) and a Tweedie distribution (PROC GENMOD, SAS), respectively. Latency to the first bunk visit was analyzed using a gamma distribution (PROC GLIMMIX, SAS). These models included fixed effects of parity (PR, MU), group composition (SM, MX), and their interactions. Significance was defined at a threshold of $P < 0.05$ and tendencies as $P \leq 0.10$. All values are reported as least-squares means.

Pearson (normal distribution) and Spearman’s rank (non-normal distribution) correlations were performed between RFI and 1) competition behavior and 2) feeding patterns. To evaluate how an individual cow’s first bunk visit after fresh feed delivery in a competitive environment may impact other outcomes, Pearson and Spearman's rank correlations were performed between first-visit variables (latency, DMI, duration, most-chosen first bin proportion), eating time within the first 30 min, total competitive contacts, and RFI.

Social Network Analysis. To evaluate dyadic relationships, networks of cumulative competitive interactions at the feed bunk were constructed for each of the 6 groups of cows sharing sets of bins. Matrices indicating the actor and receiver of each interaction were used to characterize each network (Farine and Whitehead, 2015). In brief, a network consisted of nodes (individuals) connected by edges (interaction/relationship between two nodes). Each network was considered directed (edge direction was indicated between individuals; at most two edges/dyad) and weighted (strength of the edges, number of interactions) to depict the magnitude of competition in each dyad. Because patterns for the competition subsets (displacements, replacements) were similar over time, only initial competitive contacts were included in the networks. Network visuals were created, and metrics (network and node level) were calculated, as described by Makagon et al. (2012) using the *igraph* package in R (Csardi and Nepusz, 2006). Node-level metrics included degree centrality (in: number of incoming edges for a node, number of others from which the focal cow received interactions; out: number of outgoing edges for a node, number of others toward which the focal cow initiated interactions) and strength (in: total incoming edge weight for a node, number of interactions a cow received; out: total outgoing edge weight for a node, number of interactions a cow initiated; Foris et al., 2019). Node-level degree and strength measures can be used to evaluate individual roles within the network, specifically if one individual initiates and/or receives more interactions than others. Network-level metrics included degree centralization (variation in node degree centrality to illustrate the involvement of individuals in the network) and reciprocity (extent to which pairs of nodes make reciprocal connections to each other; Makagon et al., 2012) to add insight into network cohesiveness and stability.

RESULTS

Competition Behavior

Compared to SM groupings, cows in MX groupings had more interactions for all competitive behavior subsets ($P \leq 0.045$, Table 4). Competition behaviors did not differ between parities and across parity and group composition interactions ($P \geq 0.11$).

Within the MX group composition, Table 5 descriptively reports competition categorized by interaction types: PR initiating against another PR (PR-PR), MU initiating against another MU (MU-MU), PR initiating against MU (PR-MU), and MU initiating against PR (MU-PR). On a numerical basis, more competitive interactions, including competitive contacts, displacements, and replacements, occurred between inter-parity actor and receiver dyads (PR-MU or MU-PR) compared to same-parity dyads (PR-PR or MU-MU). Inter-parity dyadic interactions also yielded larger ranges across cows for event count variables compared with same-parity dyads. However, the indexes and ratios did not differ across dyad types, but showed high individual variation.

Social networks are shown in Figure 2 and associated descriptive SNA metrics are summarized in Table 6. Individuals in each network interacted with a majority, if not all other cows, resulting in similar node-level degree values for initiated (out) and received (in) interactions and similarly low network-level degree centralization for all 6 networks. Numerically, cows in MX were involved in more competitive interactions than those in SM, with large variation among cows as shown in the strength values. Reciprocity was greater in both MX and SM-PR compared to SM-MU, indicating that in the former groupings, dyads initiated and received interactions in both directions more often than in the latter.

Feeding Patterns

All feeding patterns did not differ across parity and group composition interactions ($P \geq 0.29$; Table 7). For the main effect of parity, MU vs. PR cows had greater first-visit DMI, DMI/d, largest meal/d, and DMI/meal, faster eating rates, fewer visits/d and meals/d, as well as longer inter-meal and maximum non-eating intervals ($P \leq 0.019$). Additionally, MU cows tended to exhibit fewer visits/meal ($P = 0.050$) and longer first visit durations ($P = 0.060$) than PR cows; all other feeding metrics did not differ ($P \geq 0.15$). For the main effect of group composition, cows in MX vs. SM groupings had more visits/meal, longer meals, and greater largest meal/d ($P \leq 0.019$). Furthermore, cows in MX groupings tended to consume fewer meals/d ($P = 0.060$), greater DMI/meal ($P = 0.062$), and spend less total time eating ($P = 0.054$) than those in SM; all other feeding metrics did not differ ($P \geq 0.12$).

Feed Efficiency

Cows in MX groupings tended to have a higher RFI (less feed efficient) than those in SM ($P = 0.079$), regardless of parity ($P = 0.95$; Table 7). Residual feed intake values did not differ across parity and group composition interactions (Figure 3; $P = 0.45$). Additionally, RFI was not correlated with any competition behavior variables (R_s range: -0.05 to 0.18; $P \geq 0.18$). However, associations with RFI and feeding patterns were present. Cows with a higher RFI consumed more feed ($R = 0.39$, $P = 0.003$) and visited the feed bunk more often ($R = 0.27$, $P = 0.040$), as well as tended to eat faster ($R = 0.23$, $P = 0.078$) and have more meals/d ($R = 0.24$, $P = 0.064$). Cows with higher RFI also had shorter maximum non-eating intervals ($R = -0.40$, $P = 0.002$) and tended to have shorter inter-meal intervals ($R = -0.25$, $P = 0.055$).

Individual Cow-Level Correlations

Correlations among competition behavior at the feed bunk, first-visit feeding patterns, and feed efficiency are summarized in Table 8. Following morning feed delivery, cows with shorter latencies to first visit the feed bunk accessed the same bin for that visit more often across days ($P = 0.024$), were involved with more total competitive contacts ($P < 0.001$), and spent more time eating within the first 30 min ($P < 0.001$). In addition, cows with shorter first-visit latencies tended to have shorter first-visit durations ($P = 0.068$) and tended to have higher RFI (less feed efficient; $P = 0.084$). Cows involved in more total competitive contacts consumed less feed during shorter first bunk visits ($P < 0.001$) but spent more time eating within the first 30 min after feed delivery ($P = 0.004$). Cows with longer first visits to the feed bunk consumed more feed at that time ($P < 0.001$). Finally, cows who chose the same bin more often for their first visit spent more time eating within the first 30 min after feed delivery ($P = 0.009$).

DISCUSSION

The purpose of this study was to evaluate the impacts of parity and group composition on competition behavior at the feed bunk, feeding behavior, and feed efficiency. We also evaluated the cow-level relationships among competition, feeding behavior after fresh feed delivery, and feed efficiency to further our understanding of factors relating to social dynamics. At a 2:1 feed bunk stocking density, we found that assigning lactating cows to feed in mixed- vs. same-parity groups resulted in greater feed bunk competition, altered feeding patterns, and tended to yield less feed efficient cows. These findings suggest same-parity groupings may have practical advantages for animal welfare and feed efficiency.

Competition Behavior

Competitive interactions are commonly measured to characterize social dynamics and hierarchy in dairy cattle. However, few studies have evaluated how parity and group composition interact to affect competition. We found greater competition in MX compared to SM groupings, regardless of parity. Similarly, in a previous study, heavy heifers (BW > 250 kg) exhibited greater agonistic behaviors in a large, heterogenous-BW group than a small, homogenous one (Hindhede et al., 2010). In our study, with a 2:1 feed bunk stocking density, parity did not influence competitive behavior, similar to a previous study quantifying displacements at a feed bunk with headlocks at 80% or 100% stocking densities in lactating Jerseys (Lobeck-Luchterhand et al., 2015). In contrast, pastured primiparous cows in a same-parity group showed less aggressive behavior than those in a mixed-parity group and less than multiparous cows in a same-parity group (Phillips and Rind, 2001). A limitation of the design of our research facility is that all subgroups were housed in the same pen. We report only competitive interactions between cows assigned to the same bins, but some cows initiated contacts at bins to which they were not assigned. Such interactions may have been impacted by factors including parity; numerically, we excluded a greater percentage of out-of-network interactions in groupings involving multiparous cows.

We did not detect interactions between parity and group composition, in contrast with studies done in other contexts. Compared to those in same-parity groups, primiparous cows in a mixed-parity group tended to be more aggressive (Phillips and Rind, 2001) and initiated more competitive behaviors (Boyle et al., 2013), and primiparous heifers received more aggressive interactions and were displaced from the feed bunk more often (Gibbons et al., 2009). These studies differed from ours by evaluating a different management system (i.e., pasture; Phillips and Rind, 2001) or by only focusing on the outcomes for primiparous cows and using a lower proportion of primiparous cows in the mixed-parity groups (Gibbons et al., 2009; Boyle et al., 2013), whereas we evaluated the outcomes for cows of both parities within each group composition. Additionally, these studies were performed at varying stocking densities in a confined setting (0.6 or 0.3 m/head at a diagonal railed feed barrier in Gibbons et al., 2009; 0.62 m/head at an open feed barrier in Boyle et al., 2013) and pasture (4.3 cows/ha, Phillips and Rind, 2001) that may not all be considered competitive conditions. We hypothesize that the competitive stocking density in our study may have enhanced the importance of the social hierarchy within each group of cows assigned to the same bin since not all cows could eat at once, and these within-group dynamics may have masked the impact of parity and overall group composition. The competitive 2:1 stocking density at the feed bunk and facility design (with cows assigned to bins but housed in a single pen) used in our study may have also played a role in the lack of interactive effects between parity and group composition. The use of RIC bins may not translate exactly to cows competing for access to an open feed bunk system, but provides high-resolution individual intake and feeding behavior data. Nonetheless, similar patterns have been reported in both feed bin and feed bunk systems involving increased displacements at higher stocking densities (DeVries et al., 2004; Huzzey et al., 2006; Proudfoot et al., 2009).

Within the mixed-parity groups, ours is the first study to report the interactions by parity. Previous studies evaluating agonistic behavior at the feed bunk by parity (e.g., Neave et al., 2017) did not report within- or between-parity interactions. In our study, on a numerical basis, most competitive interactions occurred between inter-parity dyads (PR-MU or MU-PR) compared to same-parity dyads (PR-PR or MU-MU). This mirrors the pattern of greater competition in MX vs. SM groups and further suggests that cows compete more with different- vs. same-parity individuals.

Social network analysis visualizes dyadic interactions, including inter- vs. intra-parity interactions within mixed-parity groups, and this tool captures the directionality of interactions. Ours is the first social network constructed with all competitive contacts at the feed bunk (i.e., including both unsuccessful and successful displacement attempts), which provides insight into the magnitude of total interactions; the smaller number of events in each subset (displacements, replacements) would provide less contrast among dyads. Previous networks based on bunk displacements showed that in 52% of dyads both individuals displaced each other, while in 42% of dyads only one cow displaced the other, and 6% of dyads never interacted (Val-Laillet et al., 2008). In our study, on a descriptive basis and regardless of group composition, competition was non-transitive and bidirectional, as seen from edges directed between a majority of individuals in a network, and further supported by the low network-level degree centralization and high reciprocity, especially within SM-PR and MX. The low level of degree centralization indicates relatively uniform distribution of involvement from most individuals in the network. Node metrics revealed that cows interacted with an average of 4.4 others out of 8 to 9 conspecifics, with high inter-individual variation as seen in the range for degree (range: 0 to 9, across all networks) and strength (range: 0 to 73, across all networks), similar to Foris et al. (2019). When assigning treatments to subgroups of cows, we balanced for similarity in DIM and BW to compare parity and group composition differences, but intra-group variation still existed that may have contributed to these findings. Other characteristics, such as social roles and personality (Krause et al., 2010), or variation in milk production (which had a relatively narrow range in our study population), may influence social behavior, and future research should investigate these factors.

In addition to reporting the counts of competitive events, previous literature has characterized social dynamics or dominance using indexes reflecting how often a cow serves as an actor vs. receiver (Mendl et al., 1992; Galindo and Broom, 2000; Gibbons et al., 2009). Ours is the first study to characterize indexes across all competitive behavior subtypes, starting from the initial attempts (competitive contacts), to displacements, to replacements. The indexes for all behavior types showed high individual variation, ranging from 0 to 1.0 (initiating 0 to 100% of interactions). On average, regardless of parity or group composition, cows initiated about half of the interactions they were involved in. Another study, which housed lactating cows in mixed-parity groups with 200% stocking density at a post-and-rail feed bunk, likewise found individual variation in displacement index values ranging from 0.1 to 1.0 (Huzzey et al., 2012), whereas pre-partum, multiparous cows housed at 150% feed bunk stocking with RIC bins showed a relatively narrower range (0.35 to 0.60; Proudfoot et al., 2009). This individual variation highlights the need for further research to evaluate characteristics, other than parity, that are associated with initiating competition at the feed bunk with consideration for comparison across stocking densities.

A weakness of the commonly used dominance, competitive, or success indexes is that they mask differences in the magnitude of the events, which underscores the need to also report the counts of events. Such indexes also fail to account for displacement attempts that are unsuccessful. To address this gap, our study is the first to include both unsuccessful displacements and replacements at the feed bunk. Many authors do not record unsuccessful competitive attempts since not all physical contacts may be performed with the intent to displace (Huzzey et al., 2012). Nonetheless, intent generally cannot be assumed during behavioral observation, and the evaluation of all types of initiated contacts, successful and unsuccessful, provides a more comprehensive account of feed bunk social dynamics. Our approach allows not only for characterizing how successful cows are at displacing or replacing others, relative to how often they initiate (successful displacement and replacement ratios), but also captures how well cows resist displacement, relative to how often others attempt to displace them (displacement resistance ratio).

The ratios calculated across all behavior types did not differ among parity and group composition combinations. On average, cows were relatively successful: 67% of initiated competitive contacts resulted in successful displacements (range: 0 to 100%), and 86% (range: 33 to 100%) of those displacements resulted in replacements (and thus, 58% of initial contacts resulted in replacements; range: 0 to 100%). In addition, cows were able to resist displacement (i.e., stand their ground) for an average of 31% of competitive contacts received (range: 0 to 100%). Interestingly, on a numerical basis, PR cows in MX were able to resist displacement more often when receiving contact from an MU vs. another PR cow, which suggests they may adapt to greater competition in MX and respond differently to certain conspecifics. All ratios showed high variation in the degree of success among individual cows, indicating a need for future research to identify individual characteristics associated with competition strategies and success rates.

Feeding Patterns

Our findings align with several other studies evaluating the impact of parity on feeding patterns. Multiparous vs. primiparous cows had greater daily DMI, driven by greater DMI/meal and faster eating rates, but with longer inter-meal intervals and fewer daily meals (DeVries et al., 2011; Neave et al., 2017; Crossley et al., 2018), and no differences in meal duration (Beauchemin et al., 2002; Crossley et al., 2018). Several studies did not agree with our findings of parity differences in the number of feed bunk visits or daily meals, eating rate, or size of meals; these differences could be explained by the stage of life cycle (transition cows in Neave et al., 2017), housing type, or meal definition (stanchion housing, no meal criterion analysis in Beauchemin et al., 2002). Other studies reported multiparous cows spent more, rather than similar time eating each day compared to primiparous cows (Lobeck-Luchterhand et al., 2015; Neave et al., 2017), when only evaluating a mixed-parity group composition. Finally, reporting maximum non-eating intervals and largest meal/d is relatively novel in dairy cows. We found that multiparous cows had longer maximum daily non-eating intervals than primiparous cows, which highlights an interesting parity difference that may be related to varying intake demands, which could be investigated further. Our finding that multiparous cows consumed a greater largest meal/d compared to primiparous cows aligns with numerical values previously reported (Brown et al., 2022).

Group composition impacted daily feeding patterns in alignment with previous studies. Cows in MX tended to have greater total daily eating time, similar to heavy dairy heifers in a large, heterogenous-BW group compared to a small, homogenous-BW one (Hindhede et al., 2010). We hypothesize the longer eating times in our mixed-parity groups may be explained by the greater levels of competition, including displacements, which were accompanied by more visits/meal. Additionally, as feed bunk competition increased, cows in mixed-parity groups consumed less frequent, larger meals (Hosseinkhani et al., 2008; Crossley et al., 2017), which may be related to our findings of tendencies for these meal variables to be greater in MX vs. SM groups. In contrast, an older study reported primiparous cows in a same-parity group had greater feed intake and longer eating times (measured with scan sampling) compared with those in mixed-parity groups (Krohn and Konggaard, 1979), but those authors did not report findings for multiparous cows.

In addition to daily feeding patterns, we evaluated those immediately following the morning feed delivery. Latency to first visit the bunk did not differ among parity and group composition combinations, but with large variation among individual cows, ranging from approximately 5 to 120 min. In previous work on dry cows, displacement success did not impact latency to eat in mixed-parity groups, but latency likewise showed large individual variation, ranging from 0 min to 3 h (Huzzey et al., 2012). To further investigate this individual variation, we used a novel approach of calculating several feeding variables for the first bunk visit. We found MU cows consumed more dry matter in the first bunk visit than PR cows, similar to previous work showing that mature cows (≥ third lactation) consumed more dry matter during the first meal than younger (≤ second lactation) cows (Crossley et al., 2018). However, despite the greater competition we observed in MX vs. SM groups, all first-visit feeding variables did not differ between group compositions.

At the individual level, the magnitude of involvement in competition showed interesting relationships with first-visit feeding variables. Cows with a shorter latency to the first bunk visit were involved with more total competitive contacts, and those involved in more competition consumed less feed during a shorter first visit. Shorter latencies to the first bunk visit were also correlated with choosing the same bin more often for the visit, and the latter variable was correlated with more total time spent eating within the first 30 min after feed delivery. These findings suggest that cows are not only motivated to consume fresh feed, but may also have a preference to consume feed at a particular location. The latter topic is an area of limited research, especially related to grouping strategies, and the potential connections among preference, competition, and feeding patterns merit further evaluation.

Feed Efficiency

Feed efficiency is often measured with RFI, which is the unexplained variance in feed intake after accounting for known energy sinks, specifically metabolic BW, BW change, and secreted milk energy (VandeHaar et al., 2016). Advancing our understanding of factors that may contribute to this residual variation is important for identifying new energy sinks, such as competitive behavior, which could be included in the calculation of RFI. Previous work reported primiparous cows in same-parity groups were more feed efficient than those in mixed-parity groups, but only descriptively and using a simple calculation of efficiency instead of RFI (Bach et al., 2006). Ours is the first study to evaluate RFI between different grouping strategies. We found SM groups tended to be more feed efficient than MX groups under a 2:1 feed bunk stocking density, indicating negative implications for efficiency when lactating dairy cows feed in mixed-parity groups. This finding may be linked to the increased competition and disrupted meal patterns in MX groups compared to SM, despite similar daily DMI and visits, resulting in inefficient use of feed.

In addition, we evaluated relationships between RFI and cow-level behavioral outcomes. Evaluating these cow-level relationships can aid in our understanding of individual strategies at the feed bunk to gain access to feed in a competitive environment, as well as highlight potential variation between more vs. less feed efficient cows for further investigation. Previous work found indirect relationships between behavior and feed efficiency, with slight improvements to predictive models of feed efficiency when sensor-derived measures were included, such as activity, rumination, lying, and time spent in certain areas of the barn (Martin et al., 2021). We did not find direct relationships between competitive behavior and RFI, although lower RFI (greater feed efficiency) tended to be correlated with longer latencies to the first bunk visit after fresh feed delivery and slower eating rates. In previous studies, feed bunk competition has been shown to increase eating rate (Olofsson, 1999), and cows who ate at slower rates were also more feed efficient (Connor et al., 2013; Brown et al., 2022). Furthermore, less feed efficient cows visited the bunk more often with shorter maximum non-eating intervals, as well as tended to have more meals/d and shorter intervals between meals, which may be influenced by the aforementioned competition and disrupted feeding pattern in MX. In previous work with RIC bins, more frequent bunk visits at specific timepoints throughout the day also tended to be associated with less feed efficient cows at a 2:1 stocking density (Brown et al., 2022). Therefore, cows may benefit from waiting to go to the feed bunk after fresh feed delivery to reduce involvement in competition, which may reduce eating rate and increase feed efficiency. However, other cows who access the bunk first may sort the feed, resulting in a negative impact (reduced nutritional value; DeVries et al., 2005; Hosseinkhani et al., 2008) on those who wait; future studies could disentangle the efficiency implications of strategies relating to the timing of feeding. Finally, a previous study speculated that the inefficient cows may produce more metabolic heat than efficient cows (Ben Meir et al., 2018). Quantifying the energy expenditure from competition at the feed bunk could provide insight into RFI variation. Further investigation into factors that affect competition, such as grouping strategies, stocking density, and bunk management, can improve our understanding of RFI and the attributes of a feed-efficient cow.

CONCLUSIONS

Under a competitive 2:1 feed bunk stocking density, cows in mixed-parity, compared to same-parity groups, were involved in more competitive feed bunk interactions, exhibited greater total eating time, and tended to be less feed efficient. Social network analysis further illustrated heightened competition in mixed-parity groups and showed that most interactions were bidirectional. Regardless of grouping, multiparous cows, compared to primiparous ones, ate more dry matter per meal and per day, had faster eating rates, and visited the bunk fewer times each day. At the individual level, cows with shorter latencies to first visit the bunk after fresh feed delivery were involved in more competition and tended to be less feed efficient. Mixed-parity group housing for lactating dairy cows may have potentially negative animal welfare and efficiency implications that should be considered when selecting grouping strategies on farm.

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TABLES AND FIGURES

Table 1. Descriptive statistics[1] for mid-lactation Holstein cows by parity and group composition combinations[2]

	Same-parity groups		Mixed-parity groups	
Variable	PR	MU	PR	MU
Starting DIM	136 ± 24 (99, 170)	151 ± 20 (95, 172)	143 ± 16 (124, 163)	150 ± 24 (106, 172)
BW, kg	654 ± 52 (563, 738)	732 ± 61 (650, 887)	638 ± 42 (587, 733)	720 ± 105 (619, 910)
Daily ΔBW	0.5 ± 0.4 (-0.4, 1.1)	0.4 ± 0.4 (-0.3, 1.4)	0.4 ± 0.2 (0.1, 0.7)	0.4 ± 0.3 (-0.1, 0.8)
BCS	3.4 ± 0.2 (3.2, 4.2)	3.2 ± 0.4 (2.6, 4.3)	3.1 ± 0.1 (2.9, 3.4)	3.1 ± 0.5 (2.6, 4.1)
Total ΔBCS	-0.1 ± 0.2 (-0.5, 0.3)	0.1 ± 0.2 (-0.3, 0.5)	0.1 ± 0.1 (-0.2, 0.3)	0.1 ± 0.4 (-0.8, 0.8)
Lactation	1	2.3 ± 0.6 (2, 4)	1	2.5 ± 0.5 (2, 3)
Milk yield, kg/d	38.0 ± 4.8 (30.5, 48.4)	45.8 ± 5.1 (38.8, 55.8)	40.0 ± 6.9 (31.9, 45.7)	45.7 ± 6.9 (35.5, 59.9)
Milk energy output, Mcal/d	27.4 ± 3.8 (22.0, 37.0)	31.2 ± 3.5 (24.0, 38.5)	27.3 ± 3.2 (22.1, 33.0)	31.6 ± 4.5 (24.1, 40.1)

[1] Mean ± SD with range in parentheses listed for each variable.

[2] Cows were assigned to a 2×2 factorial design by parity (PR: primiparous or MU: multiparous) and group composition (SM: same-parity or MX: mixed-parity); SM-PR = 19 cows, SM-MU = 20 cows, MX-PR = 10 cows, MX-MU = 10 cows.

Table 2. Calculated ingredient composition and nutrient analysis of the diet fed to primiparous and multiparous mid-lactation Holstein cows

Item, % of DM	Mean
Ingredient composition	
Alfalfa haylage	20.36 ± 1.48
Corn silage	32.29 ± 1.28
Distillers grain	2.36 ± 0.05
Cottonseed	4.81 ± 0.16
Ground corn grain	11.26 ± 0.30
Protein mineral mix[1]	28.93 ± 0.73
Nutrient analysis	
DM, % as fed	48.24
OM	92.28
CP	17.46
NDF	28.20
ADF	22.87
Lignin	2.96
NFC	44.07
Starch	25.93
Fat	4.04
NE_L 3x, Mcal/kg of DM[2]	1.64

[1] Protein mineral mix was formulated on an as-fed basis to contain fine ground corn (25.09%), canola meal (28.96%), soy hull pellet (17.60%), 42% CP Exceller meal (12.73%; Quality Roasting Inc.), 46% CP soybean meal (4.99%), calcium carbonate (4.49%), sodium bicarbonate (2.50%), trace mineral salt (1.25%), grease (0.25%), magnesium oxide (0.75%), urea (0.62%), potassium carbonate (0.30%), DynaMate (0.15%, The Mosaic Company), Smartamine M (0.15%, Adisseo), and Fortress LG (0.16%, VitaPlus).

[2] Estimated from the NASEM (2022) equations to calculate NE_L at 3× maintenance.

Table 3. Ethogram used for observing feed bunk interactions and proportions calculated from counts of competition behavior at the feed bunk during the first hour after morning feed delivery

Variable	Definition	Success Ratio	Actor/Receiver Index
Competitive contact	Actor makes physical contact with receiver eating at a bin. The event stops when the actor ceases physical contact.		Competitive (actor) index: Initiated competitive contacts / sum of initiated and received competitive contacts
Successful displacement	The physical contact performed by the actor results in the receiver backing out of the bin completely, so that her head is no longer through the metal bars of the feed bunk and/or the bin's gate closes.	Successful displacement (actor) ratio: Initiated successful displacements / total initiated competitive contacts	Displacement (actor) index: Initiated successful displacements / sum of initiated and received successful displacements
Successful replacement	The successful displacement results in the actor entering the bin and the gate opens to allow for her to begin to eat.	Successful replacement (actor) ratio: Initiated successful replacements / total initiated competitive contacts Successful displacement to replacement (actor) ratio: Initiated successful replacements / total initiated successful displacements	Replacement (actor) index: Initiated successful replacements / total initiated and received successful replacements
Unsuccessful displacement	After the physical contact performed by the actor, the receiver continues to eat at the bin.	Displacement resistance (receiver) ratio: Received unsuccessful displacements / total received competitive contacts	
Unsuccessful replacement	The successful displacement does not result in the actor accessing and eating from the same bin.		

Table 4. Competition behavior[1] recorded in the first hour after morning feeding with a 2:1 stocking density at the feed bunk, reported by parity and group composition combinations[2] for mid-lactation Holstein cows, averaged across 4 observation days

Variable	Same-parity groups		Mixed-parity groups		*P*-values		
	PR	MU	PR	MU	Parity	Group Comp	Parity× GroupComp
Event counts							
Competitive contacts as actor	3.7 (2.6, 5.4)	3.4 (2.4, 5.0)	5.9 (3.7, 9.5)	5.7 (3.5, 9.1)	0.77	0.028	0.92
Competitive contacts as receiver	3.7 (2.5, 5.5)	3.4 (2.3, 5.0)	7.0 (4.3, 11.4)	5.6 (2.7, 7.6)	0.25	0.045	0.43
Total competitive contacts	7.5 (5.2, 10.8)	6.9 (4.8, 9.9)	12.9 (7.9, 21.0)	10.2 (6.2, 16.8)	0.47	0.034	0.73
Successful displacements as actor	2.3 (1.6, 3.2)	2.0 (1.4, 2.9)	4.5 (3.1, 6.6)	3.5 (2.3, 5.3)	0.32	0.002	0.75
Successful displacements as receiver	2.3 (1.6, 3.3)	2.0 (1.4, 3.0)	4.6 (3.0, 7.1)	3.5 (2.2, 5.5)	0.32	0.004	0.71
Unsuccessful displacements as receiver	1.5 (0.9, 2.4)	1.4 (0.9, 2.4)	2.4 (1.3, 4.5)	1.1 (0.5, 2.4)	0.19	0.69	0.21
Successful replacements as actor	2.0 (1.4, 2.8)	1.9 (1.3, 2.7)	4.0 (2.7, 5.9)	3.1 (2.0, 4.8)	0.46	0.003	0.65
Successful replacements as receiver	2.0 (1.3, 2.9)	1.8 (1.3, 2.7)	4.2 (2.8, 6.4)	2.9 (1.8, 4.5)	0.27	0.005	0.44
Ratios							
Successful displacement ratio[3]	0.64 (0.54, 0.76)	0.70 (0.59, 0.82)	0.75 (0.61, 0.93)	0.61 (0.49, 0.77)	0.43	0.23	0.13
Displacement resistance ratio[4]	0.32 (0.22, 0.46)	0.30 (0.20, 0.44)	0.27 (0.16, 0.47)	0.32 (0.19, 0.54)	0.80	0.66	0.64
Successful replacement ratio[5]	0.55 (0.46, 0.66)	0.62 (0.53, 0.74)	0.65 (0.52, 0.82)	0.53 (0.41, 0.68)	0.29	0.22	0.11
Displacement to replacement ratio[6]	0.85 (0.79, 0.92)	0.91 (0.84, 0.98)	0.86 (0.78, 0.95)	0.88 (0.79, 0.98)	0.28	0.92	0.72
Indexes							
Competitive index[7]	0.52 (0.44, 0.61)	0.50 (0.43, 0.59)	0.50 (0.40, 0.62)	0.58 (0.47, 0.72)	0.77	0.82	0.33
Displacement index[8]	0.51 (0.44, 0.58)	0.50 (0.44, 0.57)	0.51 (0.43, 0.62)	0.61 (0.51, 0.73)	0.84	0.79	0.81
Replacement index[9]	0.50 (0.43, 0.57)	0.50 (0.44, 0.58)	0.50 (0.41, 0.60)	0.61 (0.51, 0.74)	0.90	0.97	0.23

[1] Back-transformed means and 95% confidence intervals reported from a $\log_n$-based negative binomial distribution (count values) or Tweedie distribution (proportions).

[2] Cows were assigned to a 2×2 factorial design by parity (PR: primiparous or MU: multiparous) and group composition (SM: same-parity or MX: mixed-parity); SM-PR = 19 cows, SM-MU = 20 cows, MX-PR = 10 cows, MX-MU = 10 cows.

[3] Initiated successful displacements / initiated competitive contacts.

[4] Received unsuccessful displacements / received competitive contacts.

[5] Initiated successful replacements / initiated competitive contacts.

[6] Initiated successful replacements / initiated successful displacements.

[7] Initiated competitive contacts / total competitive contacts initiated and received.

[8] Initiated successful displacements / total successful displacements initiated and received.

[9] Initiated successful replacements / total successful replacements initiated and received.

Table 5. Descriptive statistics[1] of competition behavior recorded in the first hour after morning feeding within the mixed-parity group composition with a 2:1 stocking density at the feed bunk, reported by parities (primiparous or multiparous) of mid-lactation Holstein cows involved in the interactions, averaged across 4 observation days

	Parities of cows involved as actor and receiver[1,2]			
Variable	PR (a) – PR (r)	MU (a) – MU (r)	PR (a) – MU (r)	MU (a) – PR (r)
Event counts				
Competitive contacts (actor)	2.4 ± 1.2 (0.3, 4.3)	1.1 ± 0.9 (0, 2.8)	3.5 ± 2.9 (0.8, 10.8)	4.6 ± 5.9 (0.3, 15.5)
Competitive contacts (receiver)	2.4 ± 1.4 (0.5, 4.5)	1.1 ± 0.8 (0, 2.8)	4.6 ± 5.0 (0.3, 16.5)	3.5 ± 4.4 (0, 12.8)
Competitive contacts (total)	4.8 ± 2.3 (0.5, 4.5)	2.1 ± 1.5 (0, 5.5)	8.1 ± 7.7 (2.0, 27.3)	8.1 ± 10.2 (0.3, 28.3)
Successful displacements (actor)	1.8 ± 0.8 (0.3, 2.8)	0.8 ± 0.6 (0, 2.0)	2.7 ± 2.3 (0.3, 8.3)	2.8 ± 3.2 (0, 8.8)
Successful displacements (receiver)	1.8 ± 0.8 (0.5, 2.8)	0.8 ± 0.7 (0, 2.0)	2.8 ± 3.0 (0, 9.8)	2.7 ± 3.7 (0, 10.8)
Unsuccessful displacements (receiver)	0.6 ± 0.7 (0, 1.8)	0.3 ± 0.4 (0, 1.0)	1.9 ± 2.2 (0, 6.8)	0.8 ± 1.0 (0, 2.5)
Successful replacements (actor)	1.7 ± 0.9 (0.3, 2.8)	0.6 ± 0.6 (0, 2.0)	2.3 ± 2.1 (0.3, 7.5)	2.5 ± 3.0 (0, 8.0)
Successful replacements (receiver)	1.7 ± 0.9 (0.3, 2.8)	0.6 ± 0.6 (0, 1.8)	2.5 ± 2.9 (0, 9.5)	2.3 ± 3.0 (0, 8.3)
Ratios				
Successful displacement ratio[3]	0.81 ± 0.17 (0.56, 1.0)	0.74 ± 0.34 (0, 1.0)	0.78 ± 0.26 (0.11, 1.0)	0.69 ± 0.33 (0, 1.0)
Displacement resistance ratio[4]	0.18 ± 0.18 (0, 0.44)	0.31 ± 0.38 (0, 1.0)	0.36 ± 0.32 (0, 1.0)	0.28 ± 0.35 (0, 1.0)
Successful replacement ratio[5]	0.73 ± 0.20 (0.33, 1.0)	0.51 ± 0.35 (0, 1.0)	0.63 ± 0.25 (0.11, 1.0)	0.66 ± 0.33 (0, 1.0)
Displacement to replacement ratio[6]	0.90 ± 0.14 (0.60, 1.0)	0.71 ± 0.34 (0, 1.0)	0.83 ± 0.16 (0.50, 1.0)	0.94 ± 0.10 (0.71,1.0)
Indexes				
Competitive index[7]	0.50 ± 0.14 (0.25, 0.67)	0.48 ± 0.15 (0.25, 0.75)	0.50 ± 0.20 (0.19, 0.90)	0.60 ± 0.28 (0.17, 1.0)
Displacement index[8]	0.50 ± 0.10 (0.33, 0.67)	0.51 ± 0.14 (0.33, 0.75)	0.56 ± 0.19 (0.38, 1.0)	0.62 ± 0.23 (0.33, 1.0)
Replacement index[9]	0.50 ± 0.10 (0.33, 0.69)	0.49 ± 0.25 (0, 0.67)	0.55 ± 0.20 (0.40, 1.0)	0.63 ± 0.23 (0.33, 1.0)

[1] Mean ± SD with range in parentheses listed for each variable.

[2] Within a mixed-parity group, primiparous (PR) and multiparous (MU) cows could be involved in interactions as actor (a) or receiver (r) in 4 combinations: PR acting on another PR cow, PR acting on MU, MU acting on another MU, and MU acting on PR.

[3] Initiated successful displacements / initiated competitive contacts.

[4] Received unsuccessful displacements / received competitive contacts.

[5] Initiated successful replacements / initiated competitive contacts.

[6] Initiated successful replacements / initiated successful displacements.

[7] Initiated competitive contacts / total competitive contacts initiated and received.

[8] Initiated successful displacements / total successful displacements initiated and received.

[9] Initiated successful replacements / total successful replacements initiated and received.

Table 6. Metrics[1] for social networks constructed using the number of competitive contacts[2] between mid-lactation Holstein cows with a 2:1 stocking density at the feed bunk, reported by parity and group composition combinations

	Node level				Network level	
Network Type[3]	Degree – In (r)[4]	Degree – Out (a)[4]	Strength – In (r)[5]	Strength – Out (a)[5]	Degree Centralization[6]	Reciprocity[7]
SM-PR (9-10 cows each)	4.4 ± 2.0 (0, 7)	4.4 ± 1.8 (0, 7)	14.9 ± 10.9 (0, 36)	14.9 ± 9.7 (0, 36)	0.30 ± 0.10	0.75 ± 0.04
SM-MU (10 cows each)	4.2 ± 2.4 (0, 9)	4.2 ± 2.1 (0, 8)	13.7 ± 11.7 (0, 39)	13.7 ± 13.9 (0, 53)	0.20 ± 0.05	0.49 ± 0.10
MX (10 cows each)	4.6 ± 2.1 (0, 9)	4.6 ± 1.7 (1, 9)	23.2 ± 19.8 (0, 69)	23.2 ± 18.8 (0, 73)	0.33 ± 0.14	0.70 ± 0.004

[1] Mean ± standard deviation with range in parentheses reported.

[2] Sum of contacts initiated or received across all 4 observation periods.

[3] Networks of 9-10 cows were created to evaluate parity (PR: primiparous, MU: multiparous) and group composition (SM: same-parity, MX: mixed-parity) combinations for interactions at the feed bunk; averaged between two networks of each type.

[4] Degree centrality: number of edges for a node; number of other cows from which the focal individual received (r) interactions (in) or initiated interactions (out) as the actor (a).

[5] Total edge weight for a node; number of interactions an individual received (r, in) or initiated (out) as the actor (a).

[6] Variation in node degree centrality illustrating the involvement of individuals in the network.

[7] Extent to which pairs of nodes make reciprocal connections to each other.

Table 7. Feeding patterns and feed efficiency reported by parity and group composition combinations[1] for mid-lactation Holstein cows (starting DIM: 144.5 ± 21.8 d; MY: 42.3 ± 6.2 kg) with a 2:1 stocking density at the feed bunk

	Same-parity groups		Mixed-parity groups		*P*-values		
Variable	PR	MU	PR	MU	Parity	Group Comp	Parity× Group Comp
Daily variables							
DMI, kg/d	25.5 ± 0.6	28.9 ± 0.6	25.5 ± 0.9	29.7 ± 0.9	< 0.001	0.58	0.62
Visits/d[2]	30.9 (27.7, 34.8)	25.0 (22.4, 27.9)	34.5 (29.4, 40.4)	28.2 (23.8, 32.8)	0.002	0.12	0.95
Eating rate, kg/min[2]	0.13 (0.12, 0.14)	0.15 (0.14, 0.16)	0.12 (0.11, 0.13)	0.14 (0.13, 0.16)	< 0.001	0.23	0.46
Visits/meal[2]	3.9 (3.5, 4.4)	3.4 (3.1, 3.8)	4.6 (4.0, 5.3)	4.1 (3.6, 4.8)	0.031	0.010	0.83
Meals/d	8.0 ± 0.2	7.4 ± 0.2	7.6 ± 0.3	6.9 ± 0.3	0.009	0.059	0.86
DMI/meal, kg[2]	3.3 (3.1, 3.5)	4.0 (3.8, 4.3)	3.5 (3.2, 3.8)	4.4 (4.0, 4.8)	< 0.0001	0.062	0.69
DMI of largest meal/d, kg[2]	6.1 (5.8, 6.4)	7.4 (7.0, 7.8)	6.7 (6.2, 7.2)	7.9 (7.3, 8.5)	< 0.0001	0.019	0.62
Inter-meal interval, min	154 ± 4.5	167 ± 4.4	160 ± 6.2	178 ± 6.2	0.004	0.11	0.66
Meal time, min/meal	26.3 ± 1.1	28.4 ± 1.1	30.5 ± 1.5	32.0 ± 1.5	0.15	0.005	0.83
Total eating time, min	202 ± 5.8	203 ± 5.7	222 ± 8.0	210 ± 8.0	0.60	0.055	0.39
Max non-eating interval, min[2]	302 (284, 317)	327 (308, 344)	311 (287, 337)	334 (308, 358)	0.019	0.40	0.84
Variables following AM feed delivery							
Latency to first visit the bunk, min[2]	29.5 (21.1, 41.4)	33.9 (24.4, 47.2)	30.2 (18.9, 48.0)	28.9 (18.2, 46.1)	0.81	0.73	0.66
DMI of first visit, kg	1.2 ± 0.1	1.5 ± 0.1	0.9 ± 0.2	1.5 ± 0.2	0.005	0.54	0.32
First visit duration, min	7.3 ± 0.7	8.1 ± 0.7	6.3 ± 1.0	8.9 ± 1.0	0.10	0.90	0.29
Most chosen first bin, %[2,3]	46.2 (40.3, 53.0)	42.8 (37.5, 49.0)	46.0 (38.1, 55.6)	41.8 (34.6, 50.6)	0.29	0.87	0.91
Eating time within first 30 min, min	10.5 ± 1.4	10.5 ± 1.3	10.1 ± 1.9	11.4 ± 1.9	0.78	0.88	0.67
Residual feed intake[4]	-0.11 ± 0.29	-0.31 ± 0.28	0.24 ± 0.40	0.58 ± 0.40	0.95	0.079	0.45

[1] Cows were assigned to a 2×2 factorial design by parity (PR: primiparous or MU: multiparous) and group composition (SM: same-parity or MX: mixed-parity); SM-PR = 19 cows, SM-MU = 20 cows, MX-PR = 10 cows, MX-MU = 10 cows.

[2] Back-transformed from $\log_n$ values with 95% confidence interval in parentheses.

[3] Highest proportion of first visits to one of the 5 assigned bins.

[4] Estimation of feed efficiency, calculated for each cow by regressing DMI on milk energy output, median DIM, metabolic BW, and change in BW, each nested within parity.

Table 8. Correlation matrix for cow-level relationships between competition and feeding behavior[1] and feed efficiency for mid-lactation Holstein cows

	Total competitive contacts[2]	Latency to first visit the bunk	DMI of first visit	First visit duration	Most chosen first bin[3]	Eating time within first 30 min[4]	Residual feed intake[5]
Total competitive contacts	-	-0.52 **	-0.54 **	-0.58 **	0.10	0.37 **	0.11
Latency to first visit the bunk		-	0.16	0.24 †	-0.29 *	-0.92 **	-0.23 †
DMI of first visit			-	0.90 **[6]	0.18	0.12 [6]	0.08 [6]
First visit duration				-	0.15	0.05 [6]	-0.02 [6]
Most chosen first bin					-	0.34 **	0.05
Eating time within first 30 min						-	0.18 [6]
Residual feed intake							-

[1] Competition in the first hour following AM feed delivery and feeding patterns following that feed delivery.

[2] Sum of competitive contacts at the feed bunk initiated and received by each cow.

[3] Highest proportion of first visits to one of the 5 assigned bins.

[4] Sum of the time spent eating during the first 30 min after AM feed delivery.

[5] Estimation of feed efficiency, calculated for each cow by regressing DMI on milk energy output, median DIM, metabolic BW, and change in BW, each nested within parity.

[6] Pearson correlation based on normally distributed data for both variables compared; otherwise, Spearman rank correlation used for non-normally distributed data.

*$P < 0.05$; **$P \leq 0.01$; †$0.05 \leq P \leq 1.0$

Figure 1. Flowchart of the behavior sequence used for behavior observation of competitive interactions at the feed bunk between mid-lactation Holstein cows. For each behavior, an actor (cow initiating the event) and receiver (individual receiving the event) were recorded.

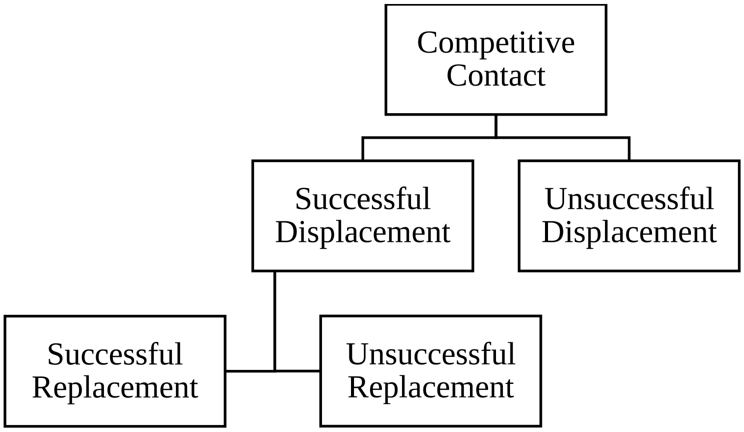


Figure 2. Social networks of mid-lactation Holstein cows for three combinations of parity (PR: primiparous, MU: multiparous) and group composition (SM: same-parity, MX: mixed-parity): A) SM-PR, B) SM-MU, C) MX (9-10 cows per network, 2 networks per combination) were constructed using *igraph* package in R. Each network shows the total frequency of competitive contacts at the feed bunk during the first hour after AM feed delivery, with arrows indicating the direction of the interaction and thickness representing the frequency. Red vs. blue circles represent PR vs. MU cows, respectively; in the MX networks, those colors represent the parity of the cow initiating the interaction. For MX networks, lighter colored arrows indicate interactions between cows of the same parity, while darker colored arrows indicate interactions between cows of different parities; color is based on arrow origin (parity of individual who initiated interaction).

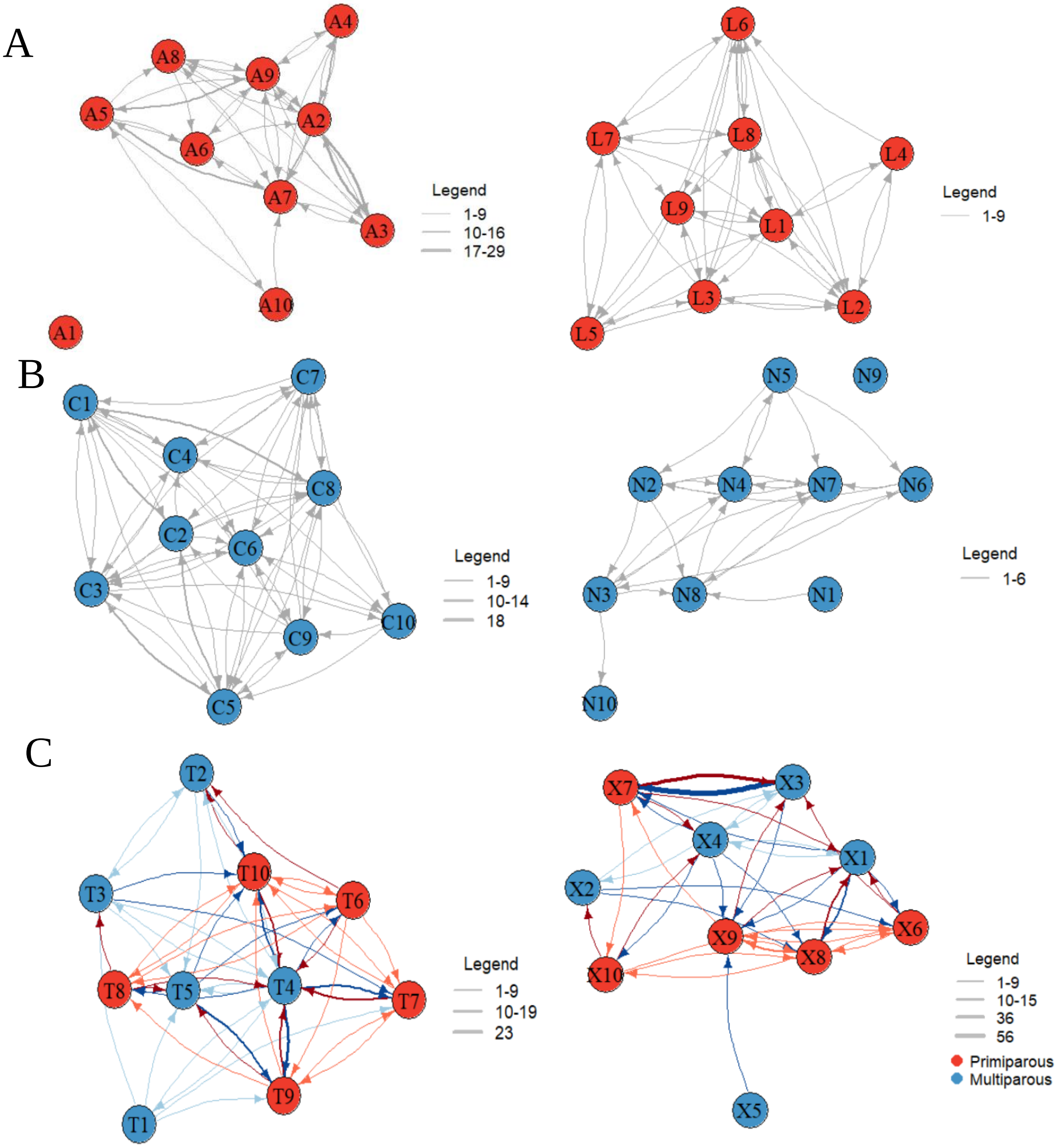
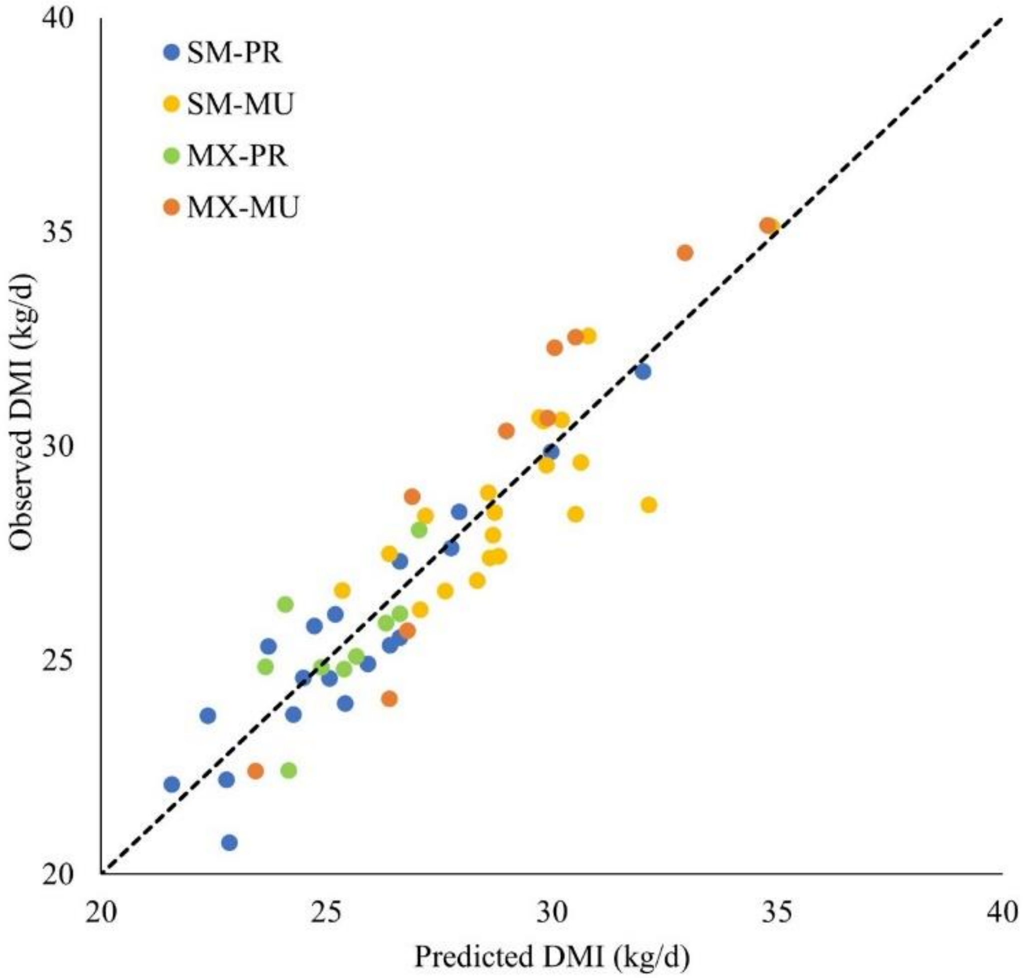


Figure 3. Observed vs. predicted DMI plotted for each parity (PR: primiparous, MU: multiparous) and group composition (SM: same-parity, MX: mixed-parity) combination for mid-lactation Holstein cows. Data points above the line of unity (dashed line) represent cows consuming more feed than predicted, associated with a positive residual feed intake (RFI) value and lesser feed efficiency. Data points below the line of unity represent cows consuming less feed than predicted, associated with a negative RFI and greater feed efficiency.



CHAPTER 3: PREFERENCE FOR COMPETING AGAINST COWS OF THE SAME OR DIFFERENT PARITY AND RELATIONSHIPS WITH FEEDING BEHAVIOR AND FEED EFFICIENCY

ABSTRACT

Our objectives were to: 1) evaluate cows' preferences for visiting feed bins limited to either same- vs. mixed-parity social interactions, depending on their parity; 2) examine the impact of parity and bin type on competition behavior and feeding patterns, and 3) investigate cow-level relationships between feed bunk competition behavior, feeding patterns, and feed efficiency. Twenty-eight primiparous (PR) and 28 multiparous (MU) lactating Holstein cows (127.8 ± 30.1 and 145.3. ± 10.4 DIM, respectively) were housed in a freestall pen with 28 Roughage Intake Control (RIC) bins (2:1 stocking density). Each cow was assigned to 2 bins, including 1 shared with 3 other cows of the same parity (SM) and 1 with 3 cows of mixed parities (MX, 50% of each parity). Feed bunk competition and feeding patterns were recorded via video in the first hour after morning feed delivery and 24-h RIC data, respectively. Residual feed intake (RFI) was calculated as the difference between predicted and observed dry matter intake (DMI) after accounting for known energy sinks. Cows showed no overall preference for bin type based on number of visits (one-sample t-test vs. 0.5, chance), although the individual magnitude of visit preference varied among cows and remained consistent (correlated) over time; individual involvement in competition was not consistent over time. Primiparous cows tended to have shorter latencies to visit the SM bin compared to the MX one after fresh feed delivery; at the former bin type, they also tended to be involved in more total competitive contacts, initiated more successful displacements and replacements than they received, and ate faster (paired t-tests or Wilcoxon signed rank tests). Those primiparous cows who visited the SM bin more often within the first h after morning feed delivery tended to be less feed efficient. Multiparous cows initiated more successful replacements after a displacement at the MX vs. SM bin, with no difference in feeding patterns between bin types (paired t-tests or Wilcoxson signed rank tests). Regardless of parity or bin type, visiting the bunk sooner after feed delivery was correlated with involvement in more competitive interactions and more time eating within the first 30 min. Consuming more feed during a longer first visit to the bunk after fresh feed delivery was correlated with being less feed efficient. Overall, when given the choice of feeding from bins shared with cows of the same or mixed parities at a 2:1 stocking density, primiparous cows showed shifts in behavior at the same-parity bin type, with implications for feed efficiency; these effects are perhaps an unintended consequence of compensatory strategies to avoid direct competition with multiparous cows.

INTRODUCTION

Social dynamics of group-housed dairy cattle, especially when access to the feed bunk is limited, can impact their welfare, feeding patterns, and feed efficiency. The feed bunk is often a competitive environment, with dairy farms sometimes providing fewer than 1 feeding space per cow. Factors affecting competition and feed access include age (or parity) and bodyweight differences. Previous work found that heavy dairy heifers (BW > 250 kg) showed more agonistic behaviors in large, heterogenous BW groups compared with small, homogenous BW groups (Hindhede et al., 2010). Similarly, in a recent study, our group found that lactating cows were involved with more competition when assigned to feed in mixed-parity compared to same-parity groups at the feed bunk (Reyes et al., in review - Chapter 2 – Chapter 2). Within mixed-parity groups, the majority of competitive interactions occurred between inter-parity dyads (primiparous cows initiating against multiparous ones or vice versa) compared to same-parity dyads (primiparous and multiparous cows interacting only within their own parity); together, these results indicated cows competed more with different- vs. same-parity individuals (Reyes et al., in review – Chapter 2). To date, studies have not yet explored cows' preferences and competitive behavior if given a choice between interacting with others of the same vs. different parities at the feed bunk.

Social dynamics at the feed bunk, including competition, also impact feeding patterns and feed efficiency. Previously, our group found that lactating cows tended to be less efficient when feeding in mixed-parity vs. same-parity groups, perhaps as a result of the greater levels of competition observed in the former (Reyes et al., in review - Chapter 2). Furthermore, we observed that cows who waited longer to visit the bunk after fresh feed delivery tended to be more feed efficient, perhaps by avoiding direct competition. An older study reported, on a descriptive basis, that primiparous cows in same-parity groups were more feed efficient than those in mixed-parity groups (Bach et al., 2006). However, it remains unknown how cows' social strategies, such as avoidance or engagement in competition, particularly when able to choose between same- vs. mixed-parity interactions, affects their efficiency.

Residual feed intake (RFI) is often used as an estimate of feed efficiency in dairy cattle, which is the difference between actual and predicted feed intake after accounting for known energy sinks, specifically metabolic BW, BW change, and secreted milk energy (VandeHaar et al., 2016). Advancing our understanding of feed efficiency and ensuring accurate calculation of RFI is important, particularly by identifying other factors that may contribute to the currently unexplained variance. One factor that may play a role in feed efficiency is feed competition. While the mechanism by which feed competition impacts feed efficiency was not delineated in the studies noted above, it is possible that increased engagement in competitive interactions could have an energetic cost that could influence individual cow RFI. Therefore, further evaluation of potential additional energy sinks, such as feed competition, can help to improve our understanding of feed efficiency and the calculation of RFI.

Our objectives in the present study were: 1) to evaluate cows' preferences for visiting feed bins limited to either same- vs. mixed-parity interactions, depending on their parity, 2) to examine the impact of parity and bin type on competition behavior and feeding patterns, and 3) to investigate cow-level relationships between competition behavior at the feed bunk, feeding patterns, and feed efficiency.

MATERIALS AND METHODS

Animals, Housing, and Treatments

The study was conducted from May to July 2021 at the University of Wisconsin – Madison (UW-Madison) Emmons Blaine Dairy Cattle Research Center in Arlington, WI. All procedures were approved by the Institutional Animal Care and Use Committee (protocol # 005658-R01-A02).

Twenty-eight primiparous (PR) and 28 multiparous (MU) lactating Holstein cows (127.8 ± 30.1 and 145.3 ± 10.4 DIM, respectively) were enrolled on the study. Cow demographics by parity are summarized in Table 1. All cows were housed in a pen with 64 freestalls and 28 roughage intake control (RIC) system bins (Hokofarms Insentec BV, Marknesse, the Netherlands; 2:1 stocking density at the feed bunk), which recorded individual cow feed intake continuously. Cows were milked twice daily at 0300 and 1500 h and fed thrice daily at 0900, 1500, and 2100 h. The same TMR diet was fed to all cows. Diet composition and nutrient analysis are presented in Table 2. Refusals were manually recorded daily and feeding amounts were adjusted to ensure all cows were fed ad libitum. Water was provided ad libitum via 3 automatic water troughs.

The group composition of the 4 cows sharing each bin was assigned based on parity. Each cow was randomly assigned to access 2 proximate bins: one shared with cows of the same (SM) parity and one shared with mixed parities (MX, 50% of each parity). A cohort was defined as pairs of same-parity cows who shared the same 2 bins. Bins were assigned SM or MX in a balanced pattern by cohorts (Figure 1). Twenty-two of the MU cows had previous experience with the RIC system. All cows were trained to their assigned bins during a 2-wk period and were considered trained once ≤ 30% of daily attempted bin visits were directed to non-assigned bins (mean ± SD: 16.5 ± 11.3%; range: 3.6 to 27.1%). Due to health issues unrelated to the study, 1 PR cow was removed and replaced before training and 1 MU cow was removed and replaced (by a cow with RIC experience) before the second week of training. Once training was complete, the experimental period lasted 45 d. No cows were removed during the study.

Measures

Competition Behavior. Continuous video was recorded from 10 cameras (Platinum 4.0 MP Network Matrix IR Bullet Camera, CMIP9342W-28M; LT Security Inc., Washington, NY) mounted at 3.7 m high, which were set to record with 2688 × 1520 resolution at 10 frames/s through a connection to a network video recorder (Platinum Enterprise Level 64 Channel NVR, LTN8964-8; LT Security Inc.). Video was recorded on 1 d each in wk 1 and 6 of the experimental period. Four h/d were observed (1 h after AM milking at 0300 h and 3 h post-feed delivery at 0900 h); these times were based on peak daily feed bunk visits. Each cow was marked with spray paint (Tell Tail, FIL Industries Limited, Mount Maunganui, New Zealand) for individual identification. Three trained observers watched the video recordings using VSPlayer

(Hikvision Digital Technology, Hangzhou, China) and coded competitive interactions based on a sequence of possible events (defined in Table 3, sequence shown in Figure 2) in Microsoft Excel. Inter-observer reliability was determined on a subsample of video that included all focal behaviors; Cohen's kappa ranged from 0.75 to 0.89, indicating 'substantial' to 'almost perfect' agreement (Landis and Koch, 1977) for all behaviors except unsuccessful displacement attempts ($\kappa = 0.54$, indicating moderate agreement).

Initial inspection of the data revealed that 70.7% (2502/3540) of competitive behaviors at the feed bunk occurred within 1 h after AM feed delivery [h 2 = 5.9% (208/3540), h 3 = 3.4% (119/3540); h 1 after AM milking = 20.1% (711/3540)]. Therefore, as in a previous study (Reyes et al., in review - Chapter 2), we retained only the first hour after morning feed delivery for analysis to avoid including small magnitudes of events in the subsequent observation hours that could skew interpretation when averaged on a per-h basis. To exclude interactions that could not result in replacements, analysis of the effect of bin type included only interactions in which the actor was assigned to (and could thus access) the bin at which the interaction occurred; thus, 41% and 24% of the total competitive contacts and displacements, respectively, were excluded. After exclusion, values were summarized as the average between the two observation days. Additionally, the event values for each cow were used to calculate four behavior "ratios" reflecting proportions of behavioral subsets (replacements, displacements, competitive contacts), and three behavior "indexes" reflecting the proportion of events in which a cow served as an actor (defined in Table 3).

Feeding Behavior and Dry Matter Intake. Individual TMR ingredients were collected weekly and dried by forced air oven (Isotemp Oven, Fisher Scientific, Waltham, MA) at 55°C for 48 h (concentrates in triplicate, forages in quadruplicate), composited by week, and analyzed by a commercial laboratory (Dairyland Labs, Inc., Arcadia, WI). The weekly ingredient samples were dried at 105°C for 24 h (in duplicate) to convert feed intake to a DM basis.

The feed intake and visit details (time of day, duration, bin location) were recorded automatically by the RIC system. A visit was defined as an event when a cow entered an assigned bin and associated RIC data were recorded. Other variables derived from RIC data were latency (min) to first visit the feed bunk after morning feed delivery, number of visits/d, DMI/d, eating rate (kg/min), total eating time (min/d, regardless of intake), and the maximum daily non-eating interval (min/d; longest daily period without eating). Latency to the first bunk visit was available for 33 d of the experimental period because video was needed to determine when feed was delivered. For days with first-visit data, DMI and duration of this visit were calculated, along with total eating time within the first 30 min after morning feed delivery; 30 min was selected based on the average length of a meal (DeVries et al., 2003b). All RIC variables were also summarized by parity regardless of bin type and by each bin type. Finally, the proportion of first visits to the SM bin was calculated as another indicator of preference.

To evaluate meal characteristics for each cow, meal analysis (DeVries et al., 2003b; Horvath and Miller-Cushon, 2019) was performed using visit data across the entire experimental period. In brief, interval durations between each cow's bin visits were summarized and converted to $\log_{10}$-transformed frequency distributions to calculate the inter-bout criteria. The inter-bout criteria were calculated by fitting a mixture of 2 normal distributions to the $\log_{10}$ distributions of inter- and intra-visit intervals using exact maximum likelihood to determine the point at which the distribution curve of within-bout (intra-bout) intervals intersected the distribution curve of between-bout (inter-bout) intervals (R package *mixdist*; Macdonald and Du, 2018). A single inter-bout criterion pooled across all individuals was calculated (20.98 min). Meal characteristics were defined as the number of meals/d, number of visits/meal, average meal time (min/meal), DMI/meal, DMI of largest meal/d (kg), and average inter-meal interval (min). All feed, visit, and meal related variables were summarized for each cow across the experimental period.

Milk Yield and Components. Milk yields were recorded in DairyComp 305 (Valley Ag Software, Visalia, CA) and summarized as kg/d for each cow. Milk samples from 4 consecutive milkings/wk were collected and preserved with 2-bromo-2-nitropro- pane-1,3-diol (Advanced Instruments Inc., Norwood, MA) and analyzed at a commercial laboratory (AgSource, Menomonie, WI) for milk composition (fat, protein, lactose, and milk urea nitrogen).

Residual Feed Intake. Residual feed intake was calculated as a measure of feed efficiency for each cow (greater value indicates less efficient) by regressing DMI on milk energy output, midpoint DIM, metabolic BW, and change in BW, with each energy sink nested within parity. All values were summarized as an average across the experimental period for each cow. Milk energy output (kg/d) was calculated as [9.29 × milk fat (kg)] + [5.63 × true protein (kg)] + [3.95 × lactose (kg)] (NRC, 2021). Bodyweight was recorded prior to morning feed delivery 3 d/wk during wk 1, 4, and 7 of the experimental period using a calibrated stationary scale (EW6, Tru-Test Limited). Metabolic BW (kg) was calculated as $BW^{0.75}$. The daily change in BW was calculated using the LINEST function in Microsoft Excel to create a simple linear regression of all 9 BW values. Body condition score (reported descriptively in Table 1; not used in RFI) was assessed on 1 d in wk 1, 4, and 7 in conjunction with BW by 2 trained observers using the 5-point scale (Dairy Body Condition Score Chart, Elanco Animal Health) at increments of 0.25.

Statistical Analysis

Missing and Excluded Data. Daily RIC data were missing for 2 d in wk 1 and for 3 d in wk 2 of the experimental period due to a power outage and/or equipment failure. In addition, four cows were uninvolved in competitive interactions during the observation periods, and thus their data were not included when calculating proportions (2 PR cows for all competitive ratios and indexes, 1 MU cow for displacement and replacement ratios and indexes, and 1 PR cow for replacement ratios and indexes).

Statistical Models. All response variables were analyzed using R software (v. 4.2.1, RStudio) and SAS software (9.4, SAS Institute). Cow was the experimental unit. Significant effects were defined using a threshold of $P < 0.05$ and tendencies as $P \leq 0.10$. The proportion of visits to the SM bin over 24 h (on 40 d), as well as the proportion of first visits and visits within the first h after morning feed delivery to the SM bin (on 33 d), were used to evaluate preference, compared to 0.5 (chance, no preference between the 2 bin types) with one-sample t-tests, separately by parity. To evaluate the consistency of the magnitude of individual cow preference for the SM bin across the trial, Pearson correlations were calculated between wk 1 and 7 for the proportion of visits at the SM bin, summarized as: 1) visits over 24 h, 2) within the first hour after morning feed delivery (both averaged across 3 consecutive d in each week), and 3) first visits after morning feed delivery (summarized across the same 3 consecutive d in each week), both separately by parity and regardless of parity. Spearman rank correlations were also calculated between the proportion of total competitive contacts at the SM bin in wk 1 and 6 to further evaluate motivation and bin access attempts, separately by parity and overall. Paired t-tests (normal distribution) and Wilcoxon signed-rank paired tests (non-normal distribution) were used to compare within-cow competition behavior and feeding patterns between bin types (SM vs. MX), separately by parity.

Linear models were used to evaluate the fixed effect of parity on feeding patterns. Residuals were assessed visually using graphs and numerically using the Shapiro-Wilk test for normality. Non-normal continuous variables were $\log_n$ or square root-transformed to improve normality and meet model assumptions. Generalized linear models were used to evaluate the fixed effect of parity on count-based competition variables and proportions (competition indexes and ratios) using a negative binomial distribution and a Tweedie distribution, respectively. Latency to the first bunk visit after AM feed delivery was analyzed with a similar model using a gamma distribution. All values are reported as least-squares means.

Pearson (normal distribution) and Spearman rank (non-normal distribution) correlations were calculated between RFI and the magnitude of preference for SM (separately using proportion of visits to the SM bin over 24 h (on 40 d), as well as within the first h after AM feed delivery and first visits to that bin after feed delivery (on 33 d). Finally, Pearson and Spearman rank correlations were also computed among first-visit variables (latency, DMI, duration), eating time within the first 30 min, total competitive contacts, and RFI, regardless of bin type or parity.

RESULTS

Bin Preference

No overall preference was shown for SM bins by cows of either parity ($P \geq 0.20$; Table 4), whether based on bunk visits over 24 h or immediately after morning feed delivery (first visits and total visits within the first hour). When evaluating regardless of parity, individual cows were consistent in their magnitude of bin preference from wk 1 to 7 based on bunk visits over 24 h (R: 0.30, $P = 0.030$; Figure 3) and tended to remain consistent based on visits within the first hour after morning feed delivery (R: 0.25, $P = 0.068$; Figure 3). Interestingly, PR cows were consistent in their bin preference between wk 1 and 7 based on visits within the first hour (R: 0.38, $P = 0.047$), but not daily visits (R: 0.18, $P = 0.35$). In contrast, MU cows showed the opposite pattern and were consistent in their bin preference across weeks based on daily visits (R: 0.38, $P = 0.046$), but not visits within the first hour (R: 0.18, $P = 0.37$). Individual cows did not show consistency between wk 1 and 7 in which bin they first visited after fresh feed delivery, whether examining cows separately by parity or regardless of parity (R range: 0.05 to 0.19, $P \geq 0.34$).

Competition Behavior

Overall competitive behavior, when combining both bin types, did not differ ($P \geq 0.16$) between parities, with the exception that PR cows were more frequently replaced at the feed bunk compared to MU cows (Table 5, $P = 0.029$). When evaluating parities separately to compare dynamics between bin types, PR cows tended to be involved with more total competitive contacts at SM than MX bins ($P = 0.093$; Table 6). Additionally, the proportion of displacements and replacements PR cows initiated (vs. received) was greater at SM bins compared with MX bins, as shown by higher displacement and replacement index values, ($P \leq 0.017$). All other competitive behaviors involving PR cows did not differ between bin types ($P \geq 0.13$). Multiparous cows had a higher displacement to replacement ratio at the MX compared to SM bins ($P = 0.010$), but all other behaviors involving MU cows did not differ between bin types ($P \geq 0.13$; Table 6). Finally, the proportion of total competitive contacts at the SM bin were not consistent for individual cows from wk 1 to wk 6, regardless of parity (R range: -0.11 to 0.23, $P \geq 0.30$; Figure 3).

Feeding Patterns

Compared to PR cows, MU cows had greater DMI/d, largest meal/d, and DMI/meal, faster eating rates, shorter total eating time, fewer visits/d and visits/meal, as well as longer inter-meal and maximum non-eating intervals, and their first visit to the bunk after AM feed delivery was longer and with greater DMI ($P \leq 0.018$; Table 7). Additionally, MU cows tended to exhibit fewer meals/d ($P = 0.059$) and more visits within the first hour after feed delivery ($P = 0.089$) than PR cows; all other feeding metrics did not differ ($P \geq 0.16$; Table 7).

When evaluating the parities separately and comparing feeding patterns between bin types, PR cows ate at a faster rate at SM vs. MX bins ($P = 0.004$) and tended to have shorter latencies to first visit the former bins after morning feed delivery ($P = 0.081$), while their other feeding patterns did not differ between bin types ($P \geq 0.16$; Table 8). For MU cows, all feeding patterns did not differ between bin types ($P \geq 0.25$; Table 8).

Residual Feed Intake and Related Correlations

Residual feed intake is shown descriptively in Table 1 and Figure 4. Primiparous cows who visited the SM bin type more frequently within the first hour after morning feed delivery tended to be less efficient (higher RFI; R = 0.33, $P = 0.088$). There were no other correlations between measures of preference for the SM bin and RFI, whether evaluating all cows collectively (R range: -0.10 to 0.03; $P \geq 0.47$) or each parity separately (PR: R range: 0.03 to 0.18, $P \geq 0.35$; MU: R range: -0.24 to -0.14, $P \geq 0.22$).

Individual Cow Correlations

Correlations among competition behavior at the feed bunk, first visit feeding patterns, and feed efficiency are summarized in Table 9. Following morning feed delivery, cows with shorter latencies to first visit the feed bunk were involved with more total competitive contacts ($P = 0.027$) and spent more time eating within the first 30 min ($P < 0.001$). Cows who spent more time eating at the feed bunk within the first 30 min after feed delivery tended to be involved in more total competitive contacts ($P = 0.079$), spent more time at the feed bunk during the first visit ($P = 0.007$), and consumed more feed during the first visit ($P = 0.009$); the latter 2 metrics were directly associated ($P < 0.001$). Finally, cows who consumed more feed during the first visit tended to have higher RFI (less feed efficient; $P = 0.073$).

DISCUSSION

The purpose of this study was to evaluate cows' preferences for visiting feed bins that were limited to either same- vs. mixed-parity interactions and to assess the impact of parity and bin type on competition behavior, feeding patterns, and feed efficiency. Residual feed intake is a commonly used estimation of feed efficiency in dairy cattle that considers known energy sinks to calculate the unexplained variance in feed intake. To deepen our understanding of feed efficiency and improve accurate calculations of RFI, it is valuable to identify other factors that may play a role in the currently unexplained variance. We focused on feed competition, which may differentially affect primiparous vs. multiparous cows, as a factor that may have a potential impact on RFI. Regardless of parity, lactating cows showed no overall preference for bin type based on the proportion of visits to each, but individual cows' magnitude of visits to each bin (within the first hour after feed delivery and over 24 h) were consistent from the beginning to the end of the study. Primiparous cows tended to be involved in more competitive contacts and ate faster at same-parity bins compared to mixed-parity bins and had shorter latencies to visit the former bin type after fresh feed delivery. Additionally, primiparous cows tended to be less feed efficient when they visited the same-parity bin more often. Multiparous cows did not show differential competition behavior and feeding patterns between bin types.

Comparison Between Bin Types

Our study is the first to evaluate cows' preferences and feeding patterns when they are given a choice between feeding spaces shared with others of the same vs. mixed parities. We found that lactating cows with a 2:1 stocking density at the feed bunk did not show an overall preference for visiting bins shared with cows of the same vs. mixed parities. However, the magnitude of preference for visiting each bin type varied among cows, and individual cows' preferences remained consistent throughout the study based on visits within the first hour after feed delivery and visits over 24 hours, as shown by low but positive correlations between wk 1 and 7 preference measures. At the individual cow level, it is difficult to disentangle whether the differences in visits between bin types reflected a true preference for same- vs. mixed-parity social dynamics or some other factor such as bin location. Nonetheless, each cow's 2 bins were 1 to 4 bins apart, and cows were assigned to bins by parity in a balanced fashion along the entire feed bunk to control for potential location preferences.

Patterns for visit preference consistency also depended on parity and the temporal resolution with which visits were quantified. Primiparous cows showed preference consistency between weeks when considering visits within the first hour after fresh feed delivery, while multiparous showed consistency between weeks when evaluating visits over 24 hours. The differences in patterns for primiparous and multiparous cows may potentially be explained by individual strategies for either directly competing for access after fresh feed delivery or avoiding intense competition that often occurs after fresh feed delivery by rescheduling bunk visits throughout the day.

Cows are highly motivated to access fresh feed (DeVries et al., 2003a). When feeding bins are used in a 2:1 stocking density, not all cows can simultaneously access the bunk; however, in past studies in a similar environment, there were only 33 min/d, immediately after fresh feed delivery, when > 90% of bins were occupied (Brown et al., 2022a). In the study presented herein, the potential for competition was intentionally increased by allowing each cow access to only two bins, resulting in high levels of direct competition observed both in this study and our group's previous work (Reyes et al., in review - Chapter 2; 5 bins/cow). Our definition of preference based on visits reflects the successful attempts to access a certain bin type, but visits may not fully capture motivation, as cows may have had unsuccessful attempts to gain access. Therefore, evaluating attempts to access and remain at the feed bunk, represented by competitive contacts, may add insight into an individual's intent or motivation to access the bunk.

Evaluating social dynamics and resource access by quantifying competitive interactions is common in dairy cattle, but examining how parity impacts competition when cows are given a choice to interact with others of the same vs. mixed parities has not been investigated previously. Additionally, few studies report the full chain of competitive interactions from initial contacts through replacements. We found primiparous cows tended to be replaced more often at the feed bunk than multiparous cows, regardless of the bin type, which is consistent with previous work on dairy cattle social hierarchy, in which larger, more dominant cows displaced low ranking or younger cows (Huzzey et al., 2006). Furthermore, we found that multiparous cows more frequently replaced others after displacing them at mixed-parity bins than they did at their same-parity bins, as indicated by the displacement to replacement ratio. These parity-based social dynamics are consistent with our group's previous finding of more competitive contacts when cows were assigned to feed in mixed-parity vs. same-parity groups at feed bins with a similar 2:1 stocking density (Reyes et al., in review - Chapter 2). Likewise, in mixed-parity compared to same-parity groups in previous studies, primiparous cows initiated more competitive behaviors at an open rail feed barrier (Boyle et al., 2013) and tended to be more aggressive on pasture

(Phillips and Rind, 2001), and heifers received more interactions and were displaced more often (Gibbons et al., 2009).

In the current study, when comparing bin types within parity, primiparous cows tended to be involved in more total competitive contacts at their same-parity bins vs. the mixed-parity bins, with this pattern remaining numerically consistent over time. Furthermore, primiparous cows initiated more displacements and replacements than they received at their same-parity bin vs. the mixed-parity bin. Initially, these findings seem contrary to our predictions and with previous work, including our own, in studies where cattle were assigned to feed only with same- or mixed-parity groups. These findings may reflect that in this setting, where a choice was provided, competitive contacts are an indicator of primiparous cows' motivation to utilize their same-parity bin. Primiparous cows may have attempted to avoid direct competition with multiparous cows at mixed-parity bins, increasing competition at their same-parity bins. In further support of this theory, primiparous cows tended to visit their same-parity bin sooner after fresh feed delivery than they did the mixed-parity bin, which could reflect an initial preference to avoid competing with multiparous cows at the mixed-parity bin. Primiparous cows also ate faster at their same-parity bin, which may have been a strategy to compensate for increased same-parity competition at this bin; similar strategic behavioral changes have been observed in dairy cows with changes in feed bunk stocking density (DeVries et al., 2004; Proudfoot et al., 2009).

With greater direct competition at the same-parity bins, primiparous cows may have been unable to fully express their preference, as quantified by successful visits. The relatively equal proportion of visits between the bin types could be explained by either a true lack of preference, the inability to express their preference, or the tradeoff between competing motivations for obtaining fresh feed vs. avoiding certain types of competition. With heightened actual competition at their same-parity bin, gaining bunk access and thus obtaining fresh feed may have become more challenging than gaining access to feed at the mixed-parity bin, which may have resulted in the benefits of visiting the mixed-parity bin outweighing the potential costs of facing competition with multiparous cows.

Adding to our theory that competing for the same-parity bin may have become costly for primiparous cows, we found that those who visited their same-parity bin more often in the first hour after feed delivery tended to be less feed efficient. In a previous study with 2:1 stocking density, but where cows had access to all bins in the pen (~32 RIC bins vs. 2 RIC bins/cow in our study), total daily visit duration (similar to total eating time) showed a significant genetic correlation with RFI (0.20), while the number of visits was more weakly correlated (0.15; Cavani et al., 2022). Furthermore, the same study reported significant genetic and phenotypic correlations between the number of visits and total visit duration (0.68 and 0.37, respectively), which may provide insight into the potential connection we found in the present study between the number of visits within the first hour after fresh feed delivery and RFI. The tendency we observed for greater competition and faster eating rates at the same-parity bins may have resulted in negative impacts on nutrient utilization and overall feed efficiency, which may have been compounded for those individual primiparous cows who adopted this strategy to a greater extent. In addition, in our previous work, cows housed in mixed- vs. same-parity groups were involved in more competition and tended to be less feed efficient (Reyes et al., in review - Chapter 2). Perhaps, cows involved in more competition have caloric expenditure implications with potential impact on feed efficiency, which highlights an opportunity for further exploration. In addition, previous studies have shown that cows with faster daily eating rates are less feed efficient (Connor et al., 2013; Brown et al., 2022; Reyes et al., in review - Chapter 2).

For multiparous cows, neither their levels of competition nor their feeding patterns differed between bin types, which is consistent with perhaps a lesser need or benefit for them to strategically adapt to gain bunk access. Overall, our findings illustrate the more nuanced and complete understanding of competition that arises from observing the full chain of events from initial contacts through replacements, which cannot be achieved in studies that observe only a subset of these events (e.g., replacements inferred from RIC data; Huzzey et al., 2014; Crossley et al., 2018).

Feeding Patterns Between Parities

Overall, our findings for feeding patterns when comparing primiparous and multiparous cows, regardless of bin type, were similar to those previously reported in the literature. As predicted, multiparous vs. primiparous cows had greater daily DMI by way of greater DMI/meal and faster eating rates, along with fewer meals/d and longer inter-meal intervals (DeVries et al., 2011; Neave et al., 2017; Crossley et al., 2018), but with similar meal durations (Beauchemin et al., 2002; Reyes et al., in review - Chapter 2). Parity differences in total daily eating time are more variable across the literature. Although we found primiparous cows had longer eating times than multiparous cows, we detected no difference in a previous study (Reyes et al., in review - Chapter 2), and several other studies found the opposite pattern (Lobeck-Luchterhand et al., 2015; Neave et al., 2017; Munksgaard et al., 2020). Finally, our study replicated patterns from previous experiments at this facility (Reyes et al., in review - Chapter 2; Brown et al., 2022), in which multiparous cows exhibited longer maximum daily non-eating intervals and had greater daily largest meal size compared to primiparous cows. These patterns indicate parity differences that are likely driven by both varying intake demands and ability to gain feed access in a competitive feed bunk environment with a 2:1 stocking density.

In addition to day-level feeding patterns, we evaluated those directly following fresh feed delivery, when we predicted motivation for feed access to be the greatest, but ability to gain access to be the most limited and competitive. We replicated both statistical and numerical patterns from our previous work (Reyes et al., in review - Chapter 2) including large individual variation in latencies to first visit the feed bunk (similarly reported by another group for dry cows; Huzzey et al., 2012), along with multiparous cows having longer first bunk visits with greater DMI than primiparous cows. The intake outcome was unsurprising, based on established daily intake differences between parities in the literature (e.g., Beauchemin et al., 2002; Azizi et al., 2009). The difference in first-visit duration and intake, along with overall parity differences in eating rate, may have resulted in multiparous cows reaching satiety sooner. This may explain why multiparous cows tended to visit the bunk less often within the first hour after feed delivery compared to primiparous cows. These findings could also result from differences in social dominance that may allow older cows to eat first while resisting displacement, resulting in a faster rate of satiety.

Individual Cow Variation

We evaluated cow-level relationships among competitive behavior and feeding patterns, regardless of bin type or parity, to attempt to disentangle individual strategies to gain access to feed in a competitive environment. Cows who first accessed the feed bunk faster after fresh feed delivery were involved with more total competitive contacts and spent more time eating at the feed bunk within the first 30 min after feed delivery, replicating the novel findings in our previous study (Reyes et al., in review - Chapter 2); however, cows who consumed more feed during the first bunk visit after fresh feed delivery tended to be less feed efficient. We speculate this relationship may be moderated by subtle changes in eating rate, which could plausibly result from greater direct competition following fresh feed delivery, paired with greater feed intake to compensate. Indeed, cows often eat faster in a competitive environment (Olofsson, 1999), and faster eating rates have been correlated with less feed efficient cows (Connor et al., 2013; Brown et al., 2022; Reyes et al., in review - Chapter 2). Future work to further investigate connections between efficiency and competition could continue to improve our knowledge about the attributes of feed efficient dairy cows and advancements toward increased sustainability.

On average, regardless of parity, cows were moderately successful at direct competition, with 50% of initiated competitive contacts resulting in successful displacements (range: 0 to 100%), and 79% of those displacements resulting in replacements (range: 0 to 100%), translating to 40% of initial competitive contacts resulting in replacements (range: 0 to 83%). In our previous study in the same pen, also using a 2:1 stocking density, we reported higher proportions of success (58% of competitive contacts translating to replacements in Reyes et al., in review - Chapter 2), which may be explained by other differences in experimental design, such as cows being assigned to feed only with others of the same or mixed parities at 5 RIC bins spread across the entire feed bunk vs. 2 proximate bins with different social dynamics in our current study. As in our previous study, we observed high individual variation in the indexes and ratios, which is also consistent with the individual variation reported in displacement index values for mixed-parity lactating cows at 200% post-and-rail feed bunk stocking density (Huzzey et al., 2012). Such variation highlights a need for future work to evaluate individual characteristics related to competitive strategies at the feed bunk.

CONCLUSIONS

After fresh feed delivery, in a competitive 2:1 stocking density, primiparous cows tended to visit their same-parity bin sooner than the mixed-parity bin when given a choice between both options. Primiparous cows also tended to be involved in more competition at the same-parity bin type, where they ate faster. However, these patterns did not translate into a greater proportion of visits (first visits or visits within the first h) after feed delivery or over 24 h to the same- vs. mixed-parity bin type. However, individual cows varied in their magnitude of preference for the two bin types, and those cows who visited the same-parity bin more often tended to be less feed efficient. In contrast, multiparous cows did not show different competition or feeding patterns between bin types, likely based on higher social dominance rank and ability to gain or retain access to feed. Overall, the patterns in primiparous cows may reflect strategies to avoid direct competition with multiparous cows, but with unintended consequences for competitive interactions at the feed bunk, feeding patterns, and feed efficiency.

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TABLES AND FIGURES

Table 1. Descriptive statistics[1] for mid-lactation Holstein cows, by parity (n = 28 of each)

Variable	Parity	
	Primiparous	Multiparous
Starting DIM	127.8 ± 30.1 (69, 168)	145.3 ± 10.4 (127, 164)
BW, kg	638.7 ± 38.4 (562, 710)	760.0 ± 76.5 (582, 961)
Daily ΔBW	0.4 ± 0.3 (-0.2, 1.1)	0.4 ± 0.4 (-0.1, 1.3)
BCS	3.4 ± 0.2 (3.0, 3.7)	3.4 ± 0.3 (2.8, 4.0)
Total[2] ΔBCS	0.04 ± 0.1 (-0.3, 0.3)	0.1 ± 0.2 (-0.3, 0.6)
Lactation	1.0	2.4 ± 0.6 (2, 4)
Milk yield, kg/d	39.5 ± 6.5 (28.8, 54.2)	47.1 ± 7.6 (33.1, 58.5)
Milk energy output, Mcal/d	28.8 ± 3.6 (23.2, 37.0)	32.6 ± 4.7 (21.5, 40.6)
Residual feed intake[3]	0.004 ± 0.8 (-1.6, 1.5)	-0.004 ± 1.2 (-2.2, 2.4)

[1] Mean ± SD with range in parentheses listed for each variable.

[2] Change occurred over 42 d.

[3] Estimation of feed efficiency, calculated for each cow by regressing DMI on milk energy output, median DIM, metabolic BW, and change in BW, each nested within parity.

Table 2. Calculated ingredient composition and nutrient analysis of the diet fed to primiparous and multiparous mid-lactation Holstein cows

Item, % of DM	Mean
Ingredient composition	
Alfalfa haylage	22.08 ± 0.47
Corn silage	30.16 ± 0.41
Distillers grain	2.56 ± 0.05
Cottonseed	4.88 ± 0.22
Ground corn grain	11.83 ± 0.95
Protein mineral mix[1]	28.50 ± 0.61
Nutrient analysis	
DM, % as fed	50.25
OM	92.00
CP	18.00
NDF	23.01
ADF	22.03
Lignin	2.21
NFC	40.91
Starch	22.86
Fat	5.18
NE_L 3x[2]	1.58

[1] Protein mineral mix was formulated on an as-fed basis to contain fine ground corn (25.22%), canola meal (19.88%), soy hull pellet (17.48%), 46% CP soybean meal (15.86%), 42% CP Exceller meal (11.24%; Quality Roasting Inc.), calcium carbonate (4.21%), sodium bicarbonate (2.50%), trace mineral salt (1.25%), magnesium oxide (0.75%), urea (0.62%), potassium carbonate (0.30%), grease (0.25%), DynaMate (0.15%, The Mosaic Company), Smartamine M (0.15%, Adisseo), and Fortress LG (0.16%, VitaPlus).

[2] Estimated from the NASEM (2022) equations to calculate NE_L at 3× maintenance.

Table 3. Ethogram used for feed bunk interactions and proportions calculated from counts of competition behavior at the feed bunk during the first hour after morning feed delivery

Variable	Definition	Success Ratio	Actor/Receiver Index
Competitive contact	Actor makes physical contact with receiver eating at a bin. The event stops when the actor ceases physical contact.		Competitive (actor) index: Initiated competitive contacts / sum of initiated and received competitive contacts
Successful displacement	The physical contact performed by the actor results in the receiver backing out of the bin completely, so that her head is no longer through the metal bars of the feed bunk and/or the bin's gate closes.	Successful displacement (actor) ratio: Initiated successful displacements / total initiated competitive contacts	Displacement (actor) index: Initiated successful displacements / sum of initiated and received successful displacements (similar to dominance indexes, e.g., Galindo and Broom, 2000)
Successful replacement	The successful displacement results in the actor entering the bin and the gate opens to allow for her to begin to eat.	Successful replacement (actor) ratio: Initiated successful replacements / total initiated competitive contacts Successful displacement to replacement (actor) ratio: Initiated successful replacements / total initiated successful displacements	Replacement (actor) index: Initiated successful replacements / total initiated and received successful replacements
Unsuccessful displacement	After the physical contact performed by the actor, the receiver continues to eat at the bin.	Displacement resistance (receiver) ratio: Received unsuccessful displacements / total received competitive contacts	
Unsuccessful replacement	The successful displacement does not result in the actor accessing and eating from the same bin.		

Table 4. Magnitude of the preference[1] for visiting feed bins shared with cows of the same parity vs. with those of mixed parities (50% primiparous and multiparous) for mid-lactation, Holstein cows with 2:1 feed bunk stocking density (4 cows/bin, 2 bins/cow)

Variable	Mean (%)	95% CI	*P*-value
Primiparous (n = 28)			
Daily visits	50.4	(46.1, 54.8)	0.83
First visits after morning fresh feed delivery	50.9	(43.3, 58.4)	0.82
Visits in first hour after morning fresh feed delivery	51.3	(45.4, 57.2)	0.66
Multiparous (n = 28)			
Daily visits	48.5	(42.7, 54.2)	0.59
First visits after morning fresh feed delivery	44.4	(35.7, 53.1)	0.20
Visits in first hour after morning fresh feed delivery	46.1	(39.6, 51.9)	0.20

[1] Means and 95% CI reported from one-sample t-tests performed to compare to 50% (chance, no preference).

Table 5. Competition behavior[1] recorded in the first hour after morning feed delivery, reported by parity (n = 28 each primiparous and multiparous) for mid-lactation Holstein cows with 2:1 feed bunk stocking density

	Parity		
Variable	Primiparous	Multiparous	*P*-value
Event counts[2]			
Competitive contacts (actor)	6.8 (5.1, 9.1)	5.1 (3.7, 6.9)	0.17
Competitive contacts (receiver)	6.1 (4.7, 8.1)	5.7 (4.3, 7.6)	0.73
Total competitive contacts	12.9 (10.0, 16.7)	10.8 (8.3, 14.0)	0.33
Successful displacements (actor)	3.0 (2.3, 3.9)	2.4 (1.8, 3.2)	0.29
Successful displacements (receiver)	3.1 (2.3, 4.0)	2.3 (1.7, 3.1)	0.16
Unsuccessful displacements (receiver)	3.1 (2.1, 5.0)	3.4 (2.4, 5.0)	0.70
Successful replacements (actor)	2.4 (1.8, 3.2)	1.8 (1.3, 2.4)	0.16
Successful replacements (receiver)	2.6 (2.0, 3.4)	1.6 (1.2, 2.2)	0.029
Ratios			
Successful displacement ratio[3]	0.47 (0.38, 0.57)	0.53 (0.43, 0.64)	0.40
Displacement resistance ratio[4]	0.46 (0.37, 0.59)	0.50 (0.40, 0.62)	0.67
Successful replacement ratio[5]	0.39 (0.30, 0.49)	0.41 (0.32, 0.51)	0.77
Displacement to replacement ratio[6]	0.82 (0.72, 0.94)	0.76 (0.67, 0.87)	0.40
Indexes			
Competitive index[7]	0.49 (0.41, 0.59)	0.47 (0.40, 0.56)	0.76
Displacement index[8]	0.45 (0.38, 0.54)	0.51 (0.44, 0.60)	0.29
Replacement index[9]	0.33 (0.26, 0.42)	0.39 (0.32, 0.48)	0.31

[1] Back-transformed means and 95% confidence intervals reported from a natural logarithm-based negative binomial (count values) or Tweedie distribution (proportions).

[2] Averaged between two observations days.

[3] Initiated successful displacements / initiated competitive contacts.

[4] Received unsuccessful displacements / received competitive contacts.

[5] Initiated successful replacements / initiated competitive contacts.

[6] Initiated successful replacements / initiated successful displacements.

[7] Initiated competitive contacts / total competitive contacts initiated and received.

[8] Initiated successful displacements / total successful displacements initiated and received.

[9] Initiated successful replacements / total successful replacements initiated and received.

Table 6. Competition behavior[1] recorded in the first hour after morning feed delivery, reported separately for primiparous and multiparous mid-lactation Holstein cows (n = 28 of each parity), comparing interactions at feed bins (2:1 stocking density) shared with others of the same parity or with mixed parities[2]

	Primiparous cows			Multiparous cows		
Variable	Same-parity bin (PR only)	Mixed-parity bin (PR, MU)	*P*-value	Same-parity bin (MU only)	Mixed-parity bin (MU, PR)	*P*-value
Event counts[3]						
Competitive contacts (actor)	3.82 ± 0.66	2.98 ± 0.54	0.33 [4]	2.46 ± 0.52	2.59 ± 0.34	0.40 [4]
Competitive contacts (receiver)	3.80 ± 0.77	2.32 ± 0.39	0.13	2.48 ± 0.46	3.25 ± 0.65	0.13 [4]
Total competitive contacts	7.63 ± 1.19	5.30 ± 0.79	0.093	4.95 ± 0.85	5.84 ± 0.81	0.41
Successful displacements (actor)	1.77 ± 0.35	1.21 ± 0.31	0.37 [4]	1.14 ± 0.22	1.25 ± 0.20	0.73
Successful displacements (receiver)	1.78 ± 0.36	1.30 ± 0.24	0.32	1.14 ± 0.22	1.16 ± 0.23	0.94
Unsuccessful displacements (receiver)	2.10 ± 0.56	1.00 ± 0.22	0.15 [4]	1.32 ± 0.37	2.11 ± 0.57	0.102 [4]
Successful replacements (actor)	1.48 ± 0.31	0.93 ± 0.23	0.34 [4]	0.75 ± 0.17	1.05 ± 0.18	0.24 [4]
Successful replacements (receiver)	1.48 ± 0.31	1.13 ± 0.23	0.46 [4]	0.75 ± 0.14	0.86 ± 0.15	0.57
Ratios						
Successful displacement ratio[5]	0.50 ± 0.06	0.41 ± 0.06	0.46	0.51 ± 0.07	0.46 ± 0.06	0.57
Displacement resistance ratio[6]	0.42 ± 0.06	0.37 ± 0.05	0.67	0.43 ± 0.07	0.53 ± 0.06	0.21
Successful replacement ratio[7]	0.42 ± 0.05	0.34 ± 0.06	0.48	0.32 ± 0.06	0.41 ± 0.06	0.55
Displacement to replacement ratio[8]	0.84 ± 0.04	0.74 ± 0.07	0.30	0.60 ± 0.07	0.84 ± 0.05	0.010
Indexes						
Competitive index[9]	0.54 ± 0.05	0.51 ± 0.06	0.87	0.49 ± 0.05	0.49 ± 0.05	0.85
Displacement index[10]	0.49 ± 0.04	0.39 ± 0.05	0.017	0.50 ± 0.04	0.50 ± 0.04	0.92
Replacement index[11]	0.54 ± 0.04	0.34 ± 0.05	0.0004	0.44 ± 0.05	0.51 ± 0.05	0.53

[1] Means and standard error reported; all variables analyzed with paired t-test unless denoted otherwise.

[2] Based on parity (primiparous: PR, or multiparous: MU), cows were assigned to 2 feeding bins at the feed bunk (4 cows/bin): 1 shared with 3 other cows of the same parity and 1 shared with 3 other cows of mixed parities (1 of the same parity and 2 of the other parity).

[3] Averaged between two observation days.

[4] Analyzed with a Wilcoxon signed-rank paired test.

[5] Initiated successful displacements / initiated competitive contacts.

[6] Received unsuccessful displacements / received competitive contacts.

[7] Initiated successful replacements / initiated competitive contacts.

[8] Initiated successful replacements / initiated successful displacements.

[9] Initiated competitive contacts / total competitive contacts initiated and received.

[10] Initiated successful displacements / total successful displacements initiated and received.

[11] Initiated successful replacements / total successful replacements initiated and received.

Table 7. Comparison of feeding patterns at feed bins with 2:1 stocking density by parity (n = 28 each primiparous and multiparous) for mid-lactation, Holstein cows

	Parity[1]		
Variable	Primiparous	Multiparous	*P*-value
Daily variables			
DMI, kg/d	24.8 ± 0.5	29.5 ± 0.5	<0.0001
Visits/day[2]	30.3 (26.8, 34.1)	23.1 (20.5, 26.0)	0.003
Eating rate, kg/min	0.12 ± 0.004	0.16 ± 0.004	<0.0001
Visits/meal[2]	4.3 (3.9, 4.8)	3.5 (3.1, 3.9)	0.013
Meals/day	7.2 ± 0.2	6.8 ± 0.2	0.083
DMI/meal, kg[2]	3.6 (3.4, 3.8)	4.5 (4.3, 4.8)	<0.0001
DMI of largest meal/day, kg	6.6 ± 0.2	8.0 ± 0.2	<0.0001
Inter-meal interval, min[2]	152.9 (145.5, 160.8)	170.7 (164.0, 179.5)	0.002
Meal time, min/meal	29.7 (27.4, 31.5)	28.5 (26.3, 30.6)	0.43
Total eating time, min	206 ± 5.1	187 ± 5.1	0.009
Max non-eating interval, min	295 ± 6.6	320 ± 6.6	0.009
Variables following AM feed delivery			
Latency to first bin visit, min[2]	26.1 (20.7, 32.9)	23.9 (18.9, 30.2)	0.60
DMI of first visit, kg	1.2 ± 0.1	1.9 ± 0.1	<0.0001
First visit duration, min	7.3 ± 0.6	9.5 ± 0.6	0.012
Eating time within first 30 min, min	9.8 ± 1.0	12.0 ± 1.0	0.14
Visits within first h[2]	4.9 (4.3, 5.6)	4.1 (3.6, 4.7)	0.081

[1] Based on parity (primiparous or multiparous), cows were assigned to 2 feeding bins at the feed bunk (4 cows/bin): 1 shared with 3 other cows of the same parity and 1 shared with 3 other cows of mixed parities (1 of the same-parity and 2 of the other parity).

[2] Back-transformed means and 95% CI from a natural logarithm-based distribution.

Table 8. Feeding patterns[1] reported separately for primiparous and multiparous mid-lactation Holstein cows (n = 28 of each parity), comparing feed bins (2:1 stocking density) shared with others of the same parity or with mixed parities[2]

Variable	Primiparous cows			Multiparous cows		
	Same-parity bin (PR only)	Mixed-parity bin (PR, MU)	*P*-value	Same-parity bin (MU only)	Mixed-parity bin (MU, PR)	*P*-value
Daily variables						
DMI, kg/d	12.8 ± 0.6	12.0 ± 0.6	0.50	14.5 ± 1.0	15.2 ± 1.0	0.71
Visits/day	15.8 ± 1.0	15.9 ± 1.2	0.90	12.3 ± 1.3	12.2 ± 0.9	0.98
Eating rate, kg/min	0.123 ± 0.003	0.121 ± 0.003	0.013	0.162 ± 0.01	0.162 ± 0.01	0.76
Total eating time, min	105.9 ± 5.4	100.9 ± 5.2	0.61 [3]	93.0 ± 7.2	94.8 ± 5.6	0.87
Max non-eating interval, min	227.3 ± 7.7	229.0 ± 6.9	0.89	241.9 ± 8.2	248.7 ± 11.9	0.89
Variables following AM feed delivery						
Latency to first visit, min	27.8 ± 4.3	29.6 ± 3.5	0.081 [3]	22.6 ± 2.7	23.7 ± 3.4	0.55
DMI of first visit, kg	1.2 ± 0.09	1.1 ± 0.1	0.35	1.6 ± 0.1	1.9 ± 0.2	0.48 [3]
First visit duration, min	7.4 ± 0.6	6.9 ± 0.6	0.51	8.0 ± 0.7	9.3 ± 0.9	0.58 [3]
Eating time within first 30 min, min	5.4 ± 0.7	4.4 ± 0.8	0.41	5.4 ± 0.9	6.6 ± 1.0	0.45
Visits within the first h	3.1 ± 0.3	3.1 ± 0.3	0.93	2.5 ± 0.2	2.8 ± 0.2	0.31

[1] Means and standard error reported; all variables analyzed with paired t-test, unless denoted otherwise.

[2] Based on parity (primiparous: PR, or multiparous: MU), cows were assigned to 2 feeding bins at the feed bunk (4 cows/bin): 1 shared with 3 other cows of the same parity and 1 shared with 3 other cows of mixed parities (1 of the same parity and 2 of the other parity).

[3] Analyzed with a Wilcoxon signed-rank paired test.

Table 9. Correlation matrix for cow-level relationships between competition and feeding behavior[1] and feed efficiency for mid-lactation Holstein cows (n = 56)

	Total competitive contacts[2]	Latency to first visit the bunk	DMI of first visit	First visit duration	Eating time within first 30 min[3]	Residual feed intake[4]
Total competitive contacts	-	-0.30 *	-0.11	-0.17	0.24 †	0.002
Latency to first visit the bunk		-	0.02	-0.03	-0.85 **	-0.10
DMI of first visit			-	0.86 ^{**5}	0.35 ^{**5}	0.24 $^{\dagger 5}$
First visit duration				-	0.36 ^{**5}	0.15 ^{5}
Eating time within first 30 min					-	0.13 ^{5}
Residual feed intake						-

[1] Competition in the first hour following AM feed delivery and feeding patterns following that feed delivery.

[2] Sum of competitive contacts at the feed bunk initiated and received by each cow.

[3] Sum of the time spent eating during the first 30 min after AM feed delivery.

[4] Estimation of feed efficiency, calculated for each cow by regressing DMI on milk energy output, median DIM, metabolic BW, and change in BW, each nested within parity.

[5] Pearson correlation based on normally distributed data for both variables compared; otherwise, Spearman rank correlation used for non-normally distributed data.

$^{*}P < 0.05$; $^{**}P \leq 0.01$; $^{\dagger}0.05 \leq P \leq 1.0$

Figure 1. Sample roughage intake control bin assignment layout with two bins of each group composition (SM: same-parity, MX: mixed-parity dynamic at the feed bunk) based on parity (PR: primiparous, MU: multiparous) assigned to each cohort (2 cows/cohort). Four cohorts are shown as follows: Cohort 1 (Cows A and B; PR) outlined in yellow, Cohort 2 (Cows C and D; PR) in orange, Cohort 3 (Cows E and F; MU) in green, and Cohort 4 (Cows in G and H; MU) in blue.

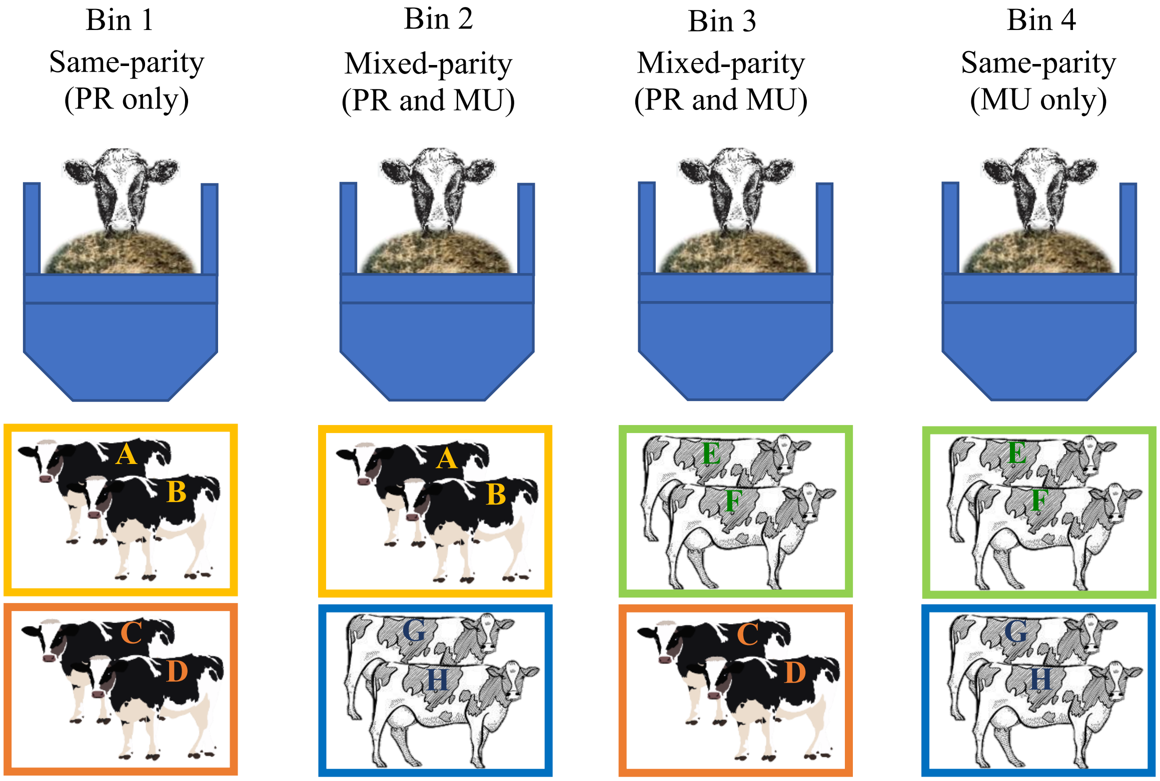


Figure 2. Flowchart of the behavior sequence used for behavior observation of competitive interactions at the feed bunk between mid-lactation Holstein cows. For each behavior, an actor (cow initiating the event) and receiver (individual receiving the event) were recorded.

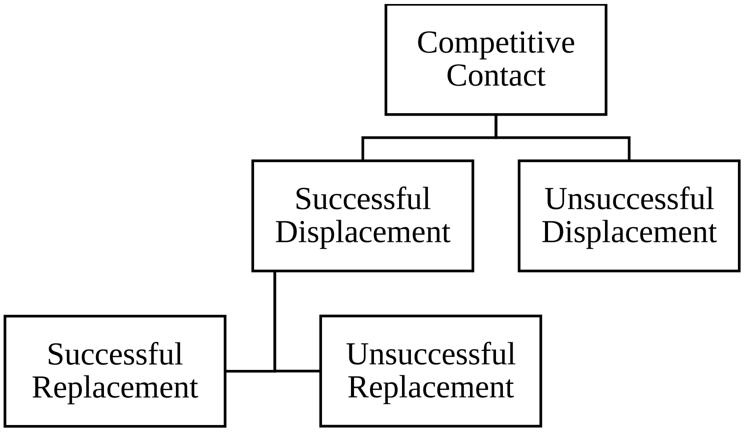


Figure 3. Individual mid-lactation Holstein cows’ correlations between weeks for the proportion of events at their same-parity feed bin (vs. one shared with cows of mixed parities; 2 bins/cow, 4 cows/bin, 2:1 stocking density) for: (A) total competitive contacts (as actor or receiver; between wk 1 and 6), (B) visits within the first hour after morning feed delivery, and (C) visit during 24 h (both between wk 1 and 7).

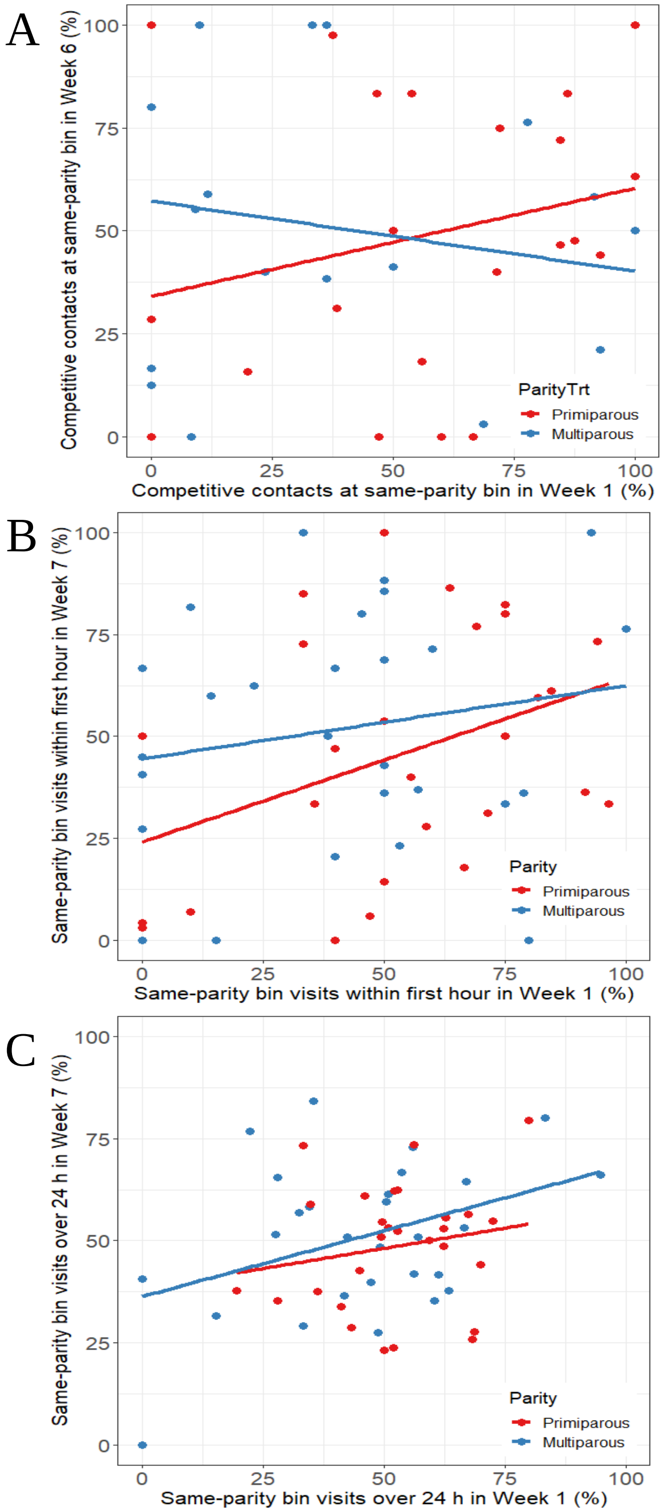
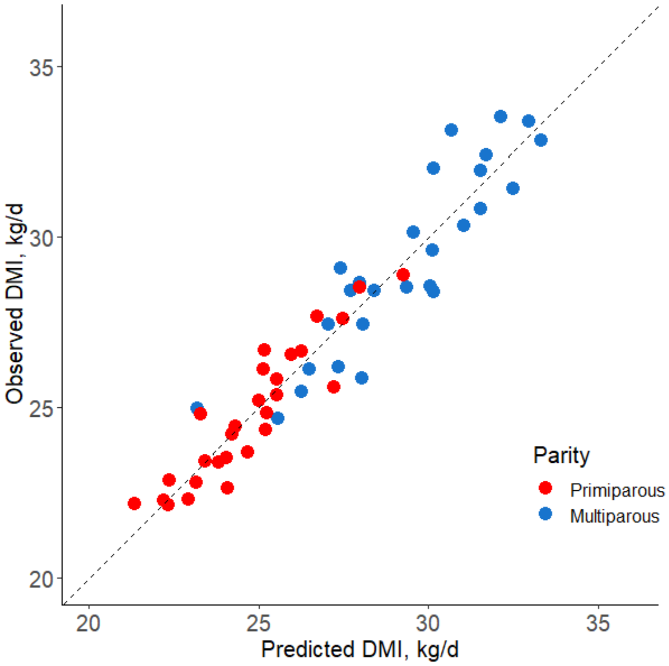


Figure 4. Observed vs. predicted DMI plotted for each parity for mid-lactation Holstein cows. Data points above the line of unity (dashed line) represent cows consuming more feed than predicted, associated with a positive residual feed intake (RFI) value and lesser feed efficiency. Data points below the line of unity represent cows consuming less feed than predicted, associated with a negative RFI and greater feed efficiency.



CHAPTER 4: EVALUATION OF STOCKING DENSITY ON COMPETITIVE BEHAVIORS, FEEDING PATTERNS, AND BEHAVIORAL CONSISTENCY IN LACTATING COWS

ABSTRACT

Dairy cows are often managed at feed bunk stocking densities that result in competition for feed resources, which can impact competitive behaviors, feeding patterns and, potentially, feed efficiency. Our objectives were to assess how varying feed bunk stocking densities in 1-h tests affect competitive behavior and feeding patterns, evaluate intra-individual behavioral consistency across stocking densities, and quantify associations with feed efficiency. Thirty-two primiparous (130.7 ± 29.0 DIM) and 32 multiparous (111.3 ± 38.3 DIM) lactating Holstein cows were housed in a freestall pen equipped with roughage intake control (RIC) bins. Each cow was assigned to share 8 RIC bins with cows of the same parity and similar body weight (16 cows/block; 2:1 stocking density). Competition behaviors and feeding patterns were evaluated via video and RIC data, respectively, at 3 stocking densities (1:1, 2:1, 4:1 cows/bin) during 1-h tests within each block (2 tests/stocking density; 6 tests/cow) following 2 h of feed deprivation. Residual feed intake (RFI) was calculated as the difference between observed and predicted dry matter intake (DMI) after accounting for known energy sinks. Linear mixed models were used to evaluate the impact of stocking density on competition behavior and feeding patterns. Models included fixed effects of stocking density treatment and block, as well as a random effect of cow. To evaluate intra-individual consistency of responses between pairs of stocking densities (1:1 vs. 2:1 and 2:1 vs. 4:1), individual stability statistic (ISS) scores were computed for each variable using normalized z-scores. To further understand consistency across all 3 scenarios for all test variables, Spearman rank correlations were used to assess associations between $ISS_{1:1,2:1}$ vs. $ISS_{2:1,4:1}$ to evaluate consistency when doubling the number of cows per bin. Correlations were also used to investigate relationships between RFI and ISS scores. Cows displayed the most competitive behaviors at the intermediate (2:1) stocking density. Feeding patterns were modulated in a dose-response fashion as stocking density increased, presumably to partially compensate for limited access to the feed bunk; for example, eating rate increased as eating time and DMI decreased. As stocking density doubled (1:1 vs. 2:1 and 2:1 vs. 4:1), cows showed intra-individual in consistency eating rate, but the consistency patterns reversed between stocking densities for competition at the feed bunk. Feed efficiency was differentially associated with behavioral consistency, with less efficient cows showing less consistency in competitive behavior but more consistency in eating rate. Feed bunk stocking density may have behavioral and feed efficiency implications to consider in farm management decisions.

Keywords: behavioral plasticity, overstocking, residual feed intake, social dynamics

INTRODUCTION

Competition for resources, specifically feed, often occurs between dairy cows under current industry management practices. Overcrowding dairy cows in free-stall barns at stocking densities greater than 1 cow per stall or feeding space (i.e., 100% capacity) may be utilized by dairy producers to improve financial returns or when making facility updates (Bewley et al., 2001). A decade-old survey in North America showed that feed bunk stocking densities ranged from 58% to 228%, with an average of 142% in the northeastern United States (Von Keyserlingk et al., 2012). In the United States, on average, 67.9% of producers provided a feed bunk space allowance of less than 0.6 m, the industry recommendation, at maximum cow numbers (USDA, 2010). Stocking density at the feed bunk and the possible implications remain common topics for discussion in today’s industry (Bohnert, 2022).

Increasing feed bunk stocking density has been shown to increase competition behavior and alter feeding patterns of dairy cows. As stocking density at the feed bunk increases, cows are involved in more displacements at the bunk (DeVries et al., 2004; Krawczel et al., 2012; Lobeck-Luchterhand et al., 2015), spend more time standing in the feeding area (Hill et al., 2009), spend less time feeding (Huzzey et al., 2006), and eat at a faster rate (Olofsson, 1999). These changes in feeding patterns may, in turn, have an impact on feed utilization efficiency. Cows with faster eating rates have been implicated as less feed efficient (Connor et al., 2013; Brown et al., 2022; Reyes et al., in review – Chapter 2). In previous studies at a 2:1 stocking density, cows involved in more direct competition at the feed bunk tended to be less feed efficient, dependent upon parity and group composition dynamics (Reyes et al., in review - Chapter 2; Reyes et al., in review - Chapter 3).

Parity is often used as a proxy for dominance, but our previous report of high individual variation within parity highlights that other factors also contribute to social dynamics (Reyes et al., in review - Chapter 2). Investigating individual characteristics that may contribute to this variation could provide insight into strategies cows utilize to obtain feed access in competitive feed bunk environments. Under varying stocking densities, individual cows may either maintain or adjust their strategies, resulting in behavioral consistency or plasticity (Dingemanse et al., 2010), respectively. For example, lactating cows displayed consistency in aggressor (individual initiating the interaction) behaviors but were not consistent in recipient (individual receiving the interaction) behaviors when provided different competitive feeding space allowances (0.6 vs. 0.3 m/cow; Gibbons et al., 2009). Depending on the environment and the individual, either behavioral consistency or plasticity may serve as a potentially advantageous strategy. Behavioral plasticity has been measured using a reaction norm approach to evaluate the behavioral response of an individual over an environmental gradient, which provides insight into how inter-individual variation interacts with the change in environment (Dingemanse et al., 2010). To our knowledge, no other studies have explored the intra-individual consistency or plasticity of competitive behavior and feeding patterns in dairy cattle under varying stocking density levels at the feed bunk, nor the relationship between individual behavioral consistency and feed efficiency.

Overall, our main objectives were to 1) evaluate competitive behavior at the feed bunk and feeding patterns under varying stocking densities and 2) evaluate intra-individual behavioral consistency at the feed bunk under different stocking density levels and the associations with feed efficiency in lactating dairy cows.

MATERIALS AND METHODS

Animals, Housing, and Treatments

The study was conducted from May to July 2022 at the University of Wisconsin – Madison (UW-Madison) Emmons Blaine Dairy Cattle Research Center in Arlington, WI. All procedures were approved by the Institutional Animal Care and Use Committee (protocol # 005658-R02-A01).

Thirty-two primiparous (130.7 ± 29.0 DIM) and 32 multiparous (111.3 ± 38.3 DIM) lactating Holstein dairy cows were housed in a pen (53.3×12.6 m) equipped with 32 roughage intake control (RIC) system bins (Hokofarms Insentec BV, Marknesse, the Netherlands), which recorded individual cow feed intake continuously. Cows were milked twice daily at 0300 and 1500 h and fed thrice daily at 0900, 1500, and 2100 h. Fresh feed was delivered during the morning feeding; additional feed mixed in morning was added to the bins in the afternoon feed deliveries. The same TMR diet was fed to all cows. Diet composition and nutrient analysis are presented in

Supplemental Table S1. Refusals were manually recorded daily and feeding amounts were adjusted by parity to ensure all cows were fed ad libitum. Water was provided ad libitum via 3 automatic water troughs.

Each cow was assigned to share 8 adjacent bins with cows of the same parity and similar body weight (8 bins/block; 16 cows/block, 2:1 stocking density). Each block was based on the combination of parity [primiparous (PR) or multiparous (MU)] and BW [low (LO) or high (HI)], resulting in 4 blocks of n = 16 cows each (PR-LO, PR-HI, MU-LO, and MU-HI). Within each parity, the median BW was used as the threshold for the 2 BW categories. Blocks were formed to control for known variation in social dynamics related to BW and parity (Hindhede et al., 2010; Reyes et al., in review - Chapter 2) and evaluate the responses at the level of the individual animal at varying stocking densities. Due to health issues unrelated to the study, 1 MU-LO cow was removed in wk 3 of the experimental period, resulting in 15 cows in MU-LO for the remainder of the study. Cow demographics by parity and BW are summarized in Table 1.

All MU cows had previous experience with the RIC system. All cows were trained to their assigned bins during a 1-wk period and exposed once to each of 3 competitive testing scenarios (1 cow:1 bin, 2:1, and 4:1) to be used during the experimental period, in a randomized order of exposure. Outside of the training sessions, cows had access to only their 8 assigned bins (standard 2:1 stocking density). Cows were considered trained once ≤30% of daily attempted bin visits were directed to non-assigned bins (mean ± SD: 18.3 ± 7.7%; range: 0.0 to 30.6%). Once training was complete, the experimental period lasted 45 d, which included the competitive testing periods in the first 4 wk.

Competitive Tests

Competitive tests were performed for each block of cows under all three stocking densities (1 cow:1 bin, 2:1, and 4:1; Figure 1). Before each test, the cows were feed deprived for 2 h to standardize minimum time since last feeding and increase feeding motivation; bedding stalls (1 stall/cow) were accessible and water was provided ad libitum during this period.

Two tests were performed with different groups of cows each day, immediately after fresh feed delivery at 0900 and 1500 h. For the 1:1 tests, blocks were randomly divided in half because only 8 bins were available; these subgroups remained consistent throughout training and testing at this stocking density. Test cows were limited to half of the feed bunk (16 bins) during each test. In the 1:1 and 2:1 tests, the 8 bins unassigned to test cows were empty, manually locked closed, and blocked with caution tape. In the 4:1 tests, only the 4 central assigned bins were accessible; the 2 normally assigned bins on each end were empty, manually locked for the test period, and blocked with caution tape to discourage attempts to access those bins.

Once fresh feed was delivered to only the bins accessible during the test, the test cows were moved from the stall area to the open half of the feed bunk. Water access was provided ad libitum throughout the feed deprivation period and competition tests for all cows, whether tested or not. During tests, all non-tested cows were locked to the stall side and did not have access to the feed bunk. The test was conducted for one hour, during which RIC data and continuous video were collected. Once each test was complete, all cows were provided free access to the entire pen.

Each group was tested twice (once each in the morning and afternoon on separate days) at each stocking density, with the order of exposure balanced among groups. For MU-LO, which was missing one cow, both 4:1 tests were performed with only 15 cows, and one 1:1 test was performed with a cow from the other subgroup to achieve 8 cows/8 bins. Data for this particular cow were discarded for this 1:1 test because she had already been tested in the other MU-LO subgroup.

Measures

Competition Behavior. Continuous video for each 1-h test was recorded from 10 cameras (Platinum 4.0 MP Network Matrix IR Bullet Camera, CMIP9342W-28M; LT Security Inc., Washington, NY) mounted at 3.7 m high, which were set to record with 2688 × 1520 resolution at 10 frames/s through a network video recorder (Platinum Enterprise Level 64 Channel NVR, LTN8964-8; LT Security Inc.). Each cow was marked with spray paint (Tell Tail, FIL Industries Limited, Mount Maunganui, New Zealand) for individual identification. Three trained observers coded the video recordings, observed using VSPlayer (Hikvision Digital Technology, Hangzhou, China), for competitive interactions using continuous sampling (defined in Table 2; sequence shown in Figure 2) and for behavioral inventories using instantaneous scan sampling in 5-min intervals [defined in Table 2; 1 body length (~2 m) was visually assessed compared to the width of 2.5 RIC bins for scale]. Inter-observer reliability was determined on a subsample of video that included all focal behaviors. Cohen's kappa ranged from 0.85 to 1.0 for instantaneous scan sampling indicating 'almost perfect' agreement and ranged from 0.61 to 1.0 for continuous sampling, indicating 'substantial' to 'almost perfect' agreement (Landis and Koch, 1977) for all behaviors except unsuccessful displacement attempts ($\kappa = 0.60$, indicating 'moderate' agreement).

Feeding Behavior and Dry Matter Intake. Individual TMR ingredients were collected weekly and dried by forced air oven (Isotemp Oven, Fisher Scientific, Waltham, MA) at 55°C for 48 h (concentrates in triplicate, forages in quadruplicate), composited by week and analyzed by a commercial laboratory (Dairyland Labs, Inc., Arcadia, WI). The weekly ingredient samples were dried at 105°C for 24 h (in duplicate) to convert feed intake to a DM basis.

The feed intake and bin visit details (time of day, duration, bin location) during the tests were recorded automatically by the RIC system, which collected data 24 h/d; day-level DMI data were used for calculating RFI. A visit was defined as a single event when a cow entered an assigned bin and associated RIC data were recorded. Other variables derived from RIC data were latency (min) to first access a feed bin after test start, number of visits/h, DMI/h, eating rate (kg/min), and total eating time (min/h, regardless of intake). Dry matter intake and duration during the first successful visit to the feed bunk were calculated, along with summed eating time within the first 30 min after test start; 30 min was selected based on the average length of a meal (DeVries et al., 2003b). Daily intakes (kg as fed) were evaluated between days that tests were or were not conducted (Supplemental Table S2) to ensure that the test day schedule did not impact daily intake, which was used for calculating RFI.

Milk Yield and Components. Milk yields were recorded in DairyCOMP 305 (Valley Ag Software, Spencer, MA) and summarized as kg/d for each cow. Milk samples from 4 consecutive milkings/wk were collected and preserved with 2-bromo-2-nitropro-pane-1,3-diol (Advanced Instruments Inc., Norwood, MA) and analyzed at a commercial laboratory (AgSource, Menomonie, WI) for milk composition (fat, protein, lactose, and milk urea nitrogen) and SCC.

Residual Feed Intake. Residual feed intake was calculated as a measure of feed efficiency for each cow (greater value indicates less efficient) by regressing DMI on milk energy output, median DIM, metabolic BW, and change in BW, each nested within parity. All values were summarized as an average across the experimental period for each cow. Milk energy output (kg/d) was calculated as [9.29 × milk fat (kg)] + [5.63 × true protein (kg)] + [3.95 × lactose (kg)] (NRC, 2021). Body weight was recorded before morning feed delivery 3 d/wk during wk 1, 4, and 7 of the experimental period using a calibrated stationary scale (EW6, Tru-Test Limited). Metabolic BW (kg) was calculated as $BW^{0.75}$. The daily change in BW was calculated using the LINEST function in Microsoft Excel to create a simple linear regression of all 9 BW values. Body condition score (reported descriptively in Table 1; not used in RFI) was assessed on 1 d in wk 1, 4, and 7 in conjunction with BW by 2 trained observers using the 5-point scale (Dairy Body Condition Score Chart, Elanco Animal Health) at increments of 0.25.

Statistical Analysis

Missing and Excluded Data. A portion (25 min) of one 1:1 test for PR-HI cows was not video-recorded due to equipment malfunction. For competitive behavior (continuous sampling only), two 4:1 tests are not yet included in this dataset, one each for PR-LO and PR-HI cows.

The proportion of time spent eating, as recorded via instantaneous scan sampling, was reported only descriptively to account for the total time cows spent during the tests (besides standing either ≤ or > 1 body length from eating cows); this variable was not analyzed statistically because total eating time was obtained automatically from RIC bin visit data.

Statistical Models. All response variables were analyzed using R programming language (R version 4.2.1) and SAS software (9.4, SAS Institute). Cow was the experimental unit. Residuals were assessed visually using graphs and numerically using the Shapiro-Wilk test for normality. For our first objective to evaluate the impact of stocking density on feeding patterns, linear models (lmer package; R Core Team, 2019) were used. Non-normal continuous variables were $\log_n$ transformed to improve normality and meet model assumptions. Generalized linear mixed models (PROC GLIMMIX, SAS) were used to evaluate effects on count-based competition variables and on proportions (competition indexes and ratios) using a negative binomial or Poisson distribution, based on model fit. Latency to the first bin visit was analyzed using a gamma distribution. These models included fixed effects of stocking density treatment and block, as well as a random effect of cow. All values are reported as least-squares means.

For our second objective to evaluate behavioral consistency, we used individual stability statistic (ISS) scores to evaluate consistency of competition behavior and feeding patterns across stocking densities at the feed bunk. For competition behavior, only competitive contacts (initiated, received, and total) were included in the analysis to represent the start of the sequence of competitive events; all feeding pattern variables were included in the analysis. For each cow, ISS scores were calculated for each variable at each stocking density treatment, as described in Asendorpf, (1990):

$$ISS_{xy} = \frac{1 - (z_x - z_y)^2}{2}$$

where z refers to the z-score of a given variable within the x and y stocking density treatments. Because ISS is calculated pairwise between situations and we were interested in consistency across the 3 tests as stocking densities doubled, we computed ISS between the 1:1 vs. 2:1 and 2:1 vs. 4:1 tests. For variables that were not normally distributed in the linear mixed model, z scores were calculated with natural-log transformed values. The calculated ISS scores were skewed and thus further transformed to achieve approximate normal distributions (Asendorpf, 1990; Sinn et al., 2008). A higher ISS score is indicative of less change (greater consistency) between two stocking density treatments. To evaluate consistency among all 3 testing scenarios, Spearman's rank correlations were performed between pairs of ISS scores. Specifically, we computed correlations between $ISS_{1:1,2:1}$ and $ISS_{2:1,4:1}$ to evaluate consistency as stocking density changed when doubling the number of cows per bin (from 1:1 to 2:1 and 2:1 to 4:1). Additionally, Pearson and Spearman's rank correlations were used to evaluate relationships between RFI and test consistency (separately for $ISS_{1:1,2:1}$ and $ISS_{2:1,4:1}$). Significance was defined at a threshold of $P < 0.05$ and tendencies as $P \leq 0.10$.

RESULTS

Stocking Density Implications

Cows were involved in the greatest number of competitive behavior events (initiated, received, and total) at the intermediate (2:1) stocking density, compared with 1:1 and 4:1, for all events in the sequence ($P < 0.001$; Table 3). Competition ratios and indexes did not differ across stocking densities. During one-hour testing periods, cows spent the lowest proportion of time loitering within 1 body length of cows eating at the 1:1 stocking density compared to 2:1 and 4:1 ($P < 0.001$; Table 3). In addition, cows spent the greatest proportion of time standing more than 1 body length away from cows eating at the 4:1 stocking density, while 1:1 and 2:1 did not differ ($P < 0.001$; Table 3).

As stocking density increased, latency to the first bin visit and eating rate increased, whereas DMI, eating time within the first 30 min, and total eating time decreased ($P < 0.001$; Table 4). At the 4:1 stocking density, compared to the lower stocking densities, cows' first bin visits were the shortest, and with the least feed consumed; they also visited the bunk less frequently during those 1-h tests, while patterns did not differ between 1:1 and 2:1 ($P < 0.001$; Table 4).

Behavioral Consistency and Feed Efficiency

An example of a descriptive behavioral reaction norm plot for total competitive contacts is shown in Figure 3, with an example for interpreting ISS scores for relatively consistent vs.

inconsistent individuals across stocking densities; similar plots for all variables are shown in Supplemental Figure S1.

The intra-individual behavioral consistencies for all variables across different stocking densities (i.e., correlations between $ISS_{1:1,2:1}$ and $ISS_{2:1,4:1}$) are summarized in Table 5. When cows were more consistent in initiated and total competitive contacts between the 1:1 and 2:1 stocking densities, they were also more consistent between the 2:1 and 4:1 tests (R range = 0.40 to 0.49, $P \leq 0.001$). In contrast, when cows were more consistent for eating time within the first 30 min and eating rate (R range = -0.55 to -0.35, $P \leq 0.006$) between the 1:1 vs. 2:1 stocking densities, they were less consistent for those variables between the 2:1 and 4:1 stocking densities. No other variables showed relationships with consistency between the stocking density comparisons (R range = -0.09 to 0.20, $P \geq 0.11$).

ISS and Feed Efficiency. Relationships between individual consistency and feed efficiency are summarized in Table 6. Residual feed intake values based on the comparison between observed and predicted DMI are shown in Figure 4. Less feed efficient (higher RFI) cows remained more consistent between the 1:1 vs. 2:1 stocking densities in the duration of the first bin visit (R = 0.28, $P = 0.024$) and tended to be more consistent in the DMI of the first visit (R = 0.24, $P = 0.061$). Less feed efficient cows also remained more consistent in eating rate between the 2:1 vs. 4:1 stocking densities (R = 0.33, $P = 0.009$; Figure 5). These individuals were also more consistent between the 2:1 vs. 4:1 stocking densities in the number of initiated and total competitive contacts (R range = -0.27 to -0.25, $P \leq 0.045$; Figure 5) and tended to be more consistent between the 2:1 vs. 4:1 stocking densities in the competitive contacts they received (R = -0.22, $P = 0.079$). No other variables showed associations with consistency between stocking density comparisons and feed efficiency (R range = -0.12 to 0.14, $P \geq 0.27$).

DISCUSSION

The purpose of this study was to evaluate competitive behaviors and feeding patterns under varying feed bunk stocking densities, and to evaluate intra-individual behavioral consistency across those testing scenarios and associations with feed efficiency. Cows showed the most involvement in competition at the intermediate 2:1 stocking density. Cows modulated all feeding patterns in a dose-response fashion as stocking density increased, presumably to adjust for the lack of opportunity to access the feed bunk; in particular, eating rate increased as eating time and DMI decreased. As stocking density doubled (1:1 vs. 2:1 and 2:1 vs. 4:1), cows showed intra-individual consistency in competitive behavior. Feed efficiency was variably associated with behavioral consistency, with less feed efficient cows showing less consistency in competitive behavior but more consistency in eating rate.

Stocking Density Implications

Competition Events. Higher stocking densities at the feed bunk have been shown to impact competition, although the direction of the effect is variable across studies. In our study using 1-h tests, we found that cows were involved in the highest level of competition behavior at the intermediate 2:1 stocking density, with slightly less at 4:1 but greater than at 1:1, when all cows could eat simultaneously. This nonlinear pattern could be explained by the reduced amount of space with the lower number of available bins (4 vs. 8 bins) and thus opportunity to attempt to compete for access with another cow eating at the bunk. Additionally, individuals may have changed their strategy at a 4:1 stocking density to avoid direct competition, rather than attempting to compete for access to feed.

Our finding of greater competition at 2:1 vs. 1:1 stocking density is consistent with several other studies that compared stocking densities between 1 and 2 lactating cows per feeding space over longer observation periods. Those studies reported that displacements increased at higher stocking densities (1.0 vs. 0.5 of feeding space, DeVries et al., 2004; 113 vs. 142% stocking density, with 100% representing 1 cow:1 headlock, Krawczel et al., 2012). However, another study found the opposite pattern, where cows exhibited fewer aggressive interactions and displacements when feeding space per cow decreased from 0.6 to 0.4 m (Gibbons et al., 2009), which could be partly explained by differences in feed bunk design. When stocking density increased at both the feed bunk and resting stalls, the number of displacements did not vary (Hill et al., 2009), perhaps due to competing motivations to lie down after milking rather than consume fresh feed when both resources were limited (Munksgaard et al., 2005), which was not the case in the current study.

Fewer studies have evaluated feed bunk stocking densities greater than 2 cows per feeding space. Consistent with our findings, one older study showed that displacements increased with 4:1 vs. 1:1 cows per electronic feeding bin (Olofsson, 1999), but the study did not have an intermediate treatment. However, another study did not detect differences in the number of successful replacements per day (extrapolated from RIC data via Huzzey et al., 2014) as stocking density increased from 1:1 to 3:2 and 3:1 cows per bin (Crossley et al., 2017); they did not evaluate displacement or replacement attempts, which require video analysis. Therefore, more studies are needed to evaluate whether our nonlinear response pattern in direct competition across stocking densities would be replicated in other settings. During our 1-h tests, cows could not access the resting stalls, potentially resulting in more direct interactions due to limited space and resources.

Competition Ratios and Indexes. Competition behavior ratios and indexes in our study were not impacted by stocking density. A previous study likewise found no difference in displacement and aggression indices (similar to our study's displacement and competitive indices, respectively) when cows had 0.6 vs. 0.3 m of feeding space (Gibbons et al., 2009). In addition, all indexes and ratios showed high inter-individual variation, as observed in our previous work (Reyes et al., in review - Chapter 2, Reyes et al., in review - Chapter 3) and by others who have reported displacement indexes (Proudfoot et al., 2009; Huzzey et al., 2012). On a numerical basis, all actor-based ratios were lower than our previous studies in the same pen (both 2:1 stocking density; Reyes et al., in review - Chapter 2, Reyes et al., in review - Chapter 3), meaning cows were less successful when attempting to gain access to feed. Conversely, the displacement resistance ratio was numerically higher than in our previous studies, indicating that cows were "standing their ground" and resisting displacement by not leaving the bunk. These numerical observations could, in part, be explained by cows perhaps learning from repeated exposure to the 4:1 condition (during both the training period and the 2 tests) that bunk access was sometimes scarce in the current study.

Feeding Patterns and Proximity to Cows Currently Eating. When 1 bin was available per cow during the 1-h test, they spent approximately 40 min eating. This was reduced to 28 min in the 2:1 test, which is nearly identical to the average meal durations of 27 to 29 min that we reported previously at the same stocking rate in this pen (Reyes et al., in review - Chapter 2, Reyes et al., in review - Chapter 3). At 4:1 stocking density, the number of available bins was halved, as was eating time (a 64% decrease relative to the 1:1 scenario). These patterns were as predicted and were consistent with the general observations in previous studies that eating time decreased as a function of space per cow or with stocking densities >100%, either at the 24-h level (DeVries et al., 2004; Huzzey et al., 2006) or during peak times after fresh feed delivery (DeVries et al., 2004; Wang et al., 2016).

When cows were not eating, we recorded their proximity to others actively eating from a bin as a possible reflection of their potential or motivation to compete for bunk access, in addition to quantifying direct competition. At 1:1 stocking density, when cows were not eating, they spent about an equal proportion of time either less or greater than 1 body length away from those who were eating. Time spent within 1 body length of eating cows was 2.3- and 2.7-fold greater, respectively, in the 2:1 and 4:1 tests. Time spent more than 1 body length away was similar between the 1:1 and 2:1 tests, when all 8 bins were active, and this proportion nearly doubled in the 4:1 test, when only 4 bins were available. These patterns could suggest cows were attempting to avoid direct competition in the 4:1 test, in which we observed less direct competition than in the 2:1 scenario. However, this could also be a limitation of the space available near eating cows when only 4 could eat at a time, as our behavioral definition was based on proximity to cows eating rather than proximity to the feed bunk. In a previous study, as feeding space decreased from 0.8 to 0.2 m/cow, cows spent 78% more time standing in the feed alleys (Huzzey et al., 2006).

Consistent with previous literature, as stocking density increased and eating time decreased, cows ate more rapidly (Olofsson, 1999; Crossley et al., 2017), likely in attempt to compensate; average eating rate increased by 12.5% between the 1:1 and 2:1 tests and by another 22% in the 4:1 test (a total increase of 37.5% compared to 1:1). This strategy was partially successful; DMI during the test decreased by 19% between the 1:1 and 2:1 tests and by another 41% in the 4:1 test (a total decrease of 52% compared to 1:1) but was not halved as stocking density doubled. A previous study evaluating DMI during peak feeding time likewise found that

DMI decreased as stocking density increased from 89 to 129% (Wang et al., 2016). However, stocking density did not affect eating rate or DMI at the 24-h level in that study or two others that used RIC bins (1 cow:1 bin vs. 2:1, DeVries and von Keyserlingk, 2009; Wang et al., 2016; 1:1 vs. 3:2 vs. 3:1, Crossley et al., 2017). Likewise, in our study, cows seemed able to compensate and maintain daily DMI. We observed, descriptively, that DMI did not differ significantly between days that cows were or were not tested, with the exception of PR-LO cows; nonetheless, this difference (1.7 ± 0.7 kg) was deemed biologically insignificant. However, it is worth noting that the previous studies maintained the assigned stocking density for at least an entire 24-h period, whereas in our study a 2:1 stocking density at the feed bunk was used after the testing periods for that day.

In terms of visit and meal patterns, cows did not differ in the frequency of bunk visits between the 1:1 and 2:1 tests, consistent with previous studies that evaluated these stocking densities (Proudfoot et al., 2009; Collings et al., 2011), but the number of visits was reduced in the 4:1 test. Previous work comparing 1:1 and 2:1 stocking densities also found that cows exhibited less frequent, longer meals in the latter (DeVries and von Keyserlingk, 2009). In our study, we did not characterize meal variables during the 1-h tests, because some meals may have been interrupted by the end of the test, when all cows in the pen were allowed access to the feed bunk area. Instead, we characterized patterns when each cow first successfully gained access to the feed bunk during the test. We found that, as the number of cows per bin doubled between the 2:1 vs. 1:1 tests, the average latency to first access a bin also doubled. However, as the stocking density doubled again to 4:1, the latency increased by 2.8-fold (a total 5.3-fold increase relative to 1:1), which could be due to reduced physical space around the 4 accessible bins. Interestingly, once cows first gained bin access, the duration of the first visit was reduced by more than half in the 2:1 vs. 1:1 test but did not differ in the 4:1 test; DMI during that visit followed the same pattern. In addition to first visit patterns, we also calculated the amount of time each individual spent eating within the first 30 min of the 1-h test as a way to evaluate variation in those individuals show were able to access feed immediately or relatively quickly after test start compared to those that were unable to access or perhaps waited to access the feed bunk until later. Cows spent less time eating within the first 30 min as stocking density increased. Similar magnitudes for eating time within the first 30 min were observed in the 2:1 test, as reported in our previous work at this stocking density after fresh feed delivery (ranging from 10.1 to 11.4 min; Reyes et al., in review - Chapter 2). These patterns provide additional support to suggest cows may have been attempting to avoid direct competition in the 4:1 test, during which cows were involved with less direct competition and spent more time more than 1 body length away from a cow eating than in the 2:1 test.

Individual Behavioral Consistency and Feed Efficiency

To our knowledge, this is the second study to evaluate intra-individual behavioral consistency of dairy cows in competitive behaviors as stocking density increases and the first to evaluate more than two stocking densities. A previous study reported that lactating cows were consistent at the individual level in number of initiated contacts and non-contact behaviors (threats and blocking) as feeding space was halved from the standard 0.6 to 0.3 m per cow (Gibbons et al., 2009). We found cows who were more consistent between the 1:1 and 2:1 tests for initiated and total competitive contacts were also more consistent between the 2:1 and 4:1 tests. This may suggest that cows are consistent in their individual strategies for the involvement level in competition at the feed bunk. Gibbons et al. (2009) reported that cows showed consistency in initiated interactions at two different feeding space stocking densities; however, cows did not show consistency in received interactions. In our study, consistency in received competitive contacts was not statistically significant, but showed a weak, positive correlation. Variation in competition involvement at the feed bunk by role (actor, receiver) represents an area for future research to continue to explore behavioral strategies and consistency.

As noted earlier, our study is the first to evaluate the intra-individual behavioral consistency of feeding patterns across multiple stocking densities. Cows who were more consistent between the 1:1 vs. 2:1 stocking densities for eating time within the first 30 min and eating rate were less consistent between the 2:1 and 4:1 stocking densities. We interpret these findings to suggest increased behavioral plasticity between the intermediate and highest stocking densities. This theory aligns with the linear increase observed in within-group variability (measured via standard deviation units) for feeding time and eating rate across 1:1, 3:2, and 3:1 stocking densities in previous work (Crossley et al., 2017).

Importantly, it should be noted that intra-individual consistency coexists with inter-individual (within-group) variability (Réale et al., 2007; Hirata et al., 2013). That is, individuals may remain consistent for certain behaviors across different environments, but those responses may vary between individuals within and across environments, thus yielding inter-individual variation. These individual differences in biological responses demonstrate the importance of understanding individual-level responses in addition to group-level patterns.

One limitation of the consistency metric we used is that it can only compare two environments at a time. Another is that, because ISS is calculated using z-scores, the magnitude of intra-individual consistency can only be described relative to others in the sample, which limits implications for the larger population. Behavioral plasticity has also been analyzed in wildlife contexts using a behavioral reaction norm approach with random regression

(Dingemanse et al., 2010); however, this method is most applicable when more than two environments vary in even increments. Alternatively, character state models can be applied to discrete environments (Houslay et al., 2018) but require a vastly larger number of subsamples for accurate estimation of intra- and inter-individual variance in each context.

Feed Efficiency. We found novel associations between individual behavioral consistency and feed efficiency. Less efficient cows were, or tended to be, more consistent in the duration and DMI of their first successful bin visit between the 1:1 and 2:1 stocking densities. In our previous work at a 2:1 stocking density, less efficient cows tended to have higher DMI in the first bin visit after fresh feed delivery (Reyes et al., in review - Chapter 3). Less efficient cows were also more consistent with eating rates between the 2:1 vs. 4:1 tests. Increased eating rate has been associated with lower feed efficiency in a previous study (Connor et al., 2013) and in our work in a similar environment with 2:1 stocking density (Brown et al., 2022; Reyes et al., in review - Chapter 2). Faster rates of feed consumption on a meal-basis can reduce rumen pH (Allen, 1997), which may impact digestion and nutrient utilization, and thus feed efficiency.

When evaluating the intermediate 2:1 test against the 4:1 test, the less efficient cows were less consistent in measures of direct and indirect competitive behavior. These less feed efficient cows were less consistent in competitive contacts (initiated, received, and total) between the 2:1 vs. 4:1 stocking densities. Our previous studies at a 2:1 stocking density reported that cows involved in more competition at the feed bunk tended to be less feed efficient (Reyes et al., in review - Chapter 2; Reyes et al., in review - Chapter 3). Nonetheless, the exact link between consistency at different feed bunk stocking densities and RFI is unknown; future research interrogating other factors that may impact intra-individual behavioral consistency is warranted.

It is important to note that our ability to draw inferences about the associations between intra-individual behavioral consistency across stocking densities and feed efficiency is limited because our tests were limited to 1 h. We did this to focus on peak feeding times, when social dynamics and dominance play major roles in gaining access to fresh feed (Huzzey et al., 2006); however, cows have been shown to adjust their strategies and patterns throughout the day (DeVries et al., 2003a). Further evaluation of intra-individual behavioral consistency and plasticity across varying stocking densities for longer periods of time, as well as their relationships with feed efficiency, are potentially interesting topics for future research. Advancing our understanding of individual behavioral strategies has the potential to assist in on-farm decisions regarding grouping strategies, and optimizing these strategies may lead to improved feed efficiency.

CONCLUSIONS

Feed bunk stocking density impacts competition behavior and feeding patterns in lactating dairy cows. In this study, competitive interactions were greatest at the intermediate 2:1 stocking density. Feeding patterns were modulated to adjust to limited access to the feed bunk; eating rate increased to partially compensate for decreased eating time and DMI. Furthermore, intra-individual consistency was associated with feed efficiency. Less efficient cows were also less consistent in their competitive behaviors but were more consistent in eating rate at high stocking density. Overall, our findings reiterate the important behavioral and feed efficiency implications at high stocking densities at the feed bunk, as well as the behavioral complexities of intra-individual consistencies across different stocking densities.

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TABLES AND FIGURES

Table 1. Descriptive statistics[1] for mid-lactation Holstein cows by block[2]

Variable	Primiparous, Low BW	Primiparous, High BW	Multiparous, Low BW	Multiparous, High BW
Starting DIM	117 ± 29 (70, 159)	144 ± 23 (85,175)	93 ± 35 (59,171)	128 ± 34 (75,176)
BW, kg	586 ± 26 (525,624)	662 ± 26 (617,719)	728 ± 34 (652,761)	813 ± 45 (764,919)
Daily ΔBW	0.5 ± 0.3 (0.1,1.0)	0.6 ± 0.2 (0.2,1.0)	0.3 ± 0.4 (-0.3,1.0)	0.7 ± 0.3 (0.02,1.3)
BCS	3.4 ± 0.2 (3.1,3.9)	3.4 ± 0.2 (3.2,3.8)	3.1 ± 0.2 (2.9,3.4)	3.3 ± 0.3 (2.8,3.9)
Total ΔBCS[3]	-0.03 ± 0.1 (-0.4,0.3)	-0.1 ± 0.1 (-0.3,0.1)	0.0 ± 0.1 (-0.3,0.3)	0.01 ± 0.2 (-0.2,0.5)
Lactation	1.0	1.0	3.4 ± 1.5 (2,6)	4.4 ± 1.1 (2,6)
Milk yield, kg/d	39.3 ± 5.2 (31.1,48.3)	39.1 ± 4.7 (30.1,45.6)	56.5 ± 7.6 (43.5,69.6)	51.8 ± 10.9 (31.2,68.2)
Milk energy output, Mcal/d	28.6 ± 2.9 (23.9,34.4)	29.8 ± 3.5 (24.3,34.8)	37.4 ± 4.3 (29.3,42.6)	34.2 ± 5.3 (22.2,40.3)
Residual feed intake[4]	-0.3 ± 1.0 (-2.4,1.2)	0.3 ± 1.0 (-1.4,2.1)	-0.1 ± 1.5 (-3.0,2.0)	0.1 ± 2.2 (-3.8,5.4)

[1] Mean ± SD with range in parentheses listed for each variable.

[2] Cows were assigned to a blocks of 16 cows each by combination of parity (PR: primiparous or MU: multiparous) and BW (LO: low BW or HI: high BW); MU-LO had only 15 cows after removal due to illness unrelated to the study.

[3] Change over 42 d.

[4] Estimation of feed efficiency, calculated for each cow by regressing DMI on milk energy output, median DIM, metabolic BW, and change in BW, each nested within parity.

Table 2. Ethogram used for observing feed bunk interactions and proportions calculated from counts of competition behavior at the feed bunk during 1-h tests at different feed bunk stocking densities

Variable	Definition	Success Ratio	Actor/Receiver Index
Continuous Sampling			
Competitive contact	Actor makes physical contact with receiver eating at a bin. The event stops when the actor ceases physical contact.		Competitive (actor) index: Initiated competitive contacts / sum of initiated and received competitive contacts
Successful displacement	The physical contact performed by the actor results in the receiver backing out of the bin completely, so that her head is no longer through the metal bars of the feed bunk and/or the bin's gate closes.	Successful displacement (actor) ratio: Initiated successful displacements / total initiated competitive contacts	Displacement (actor) index: Initiated successful displacements / sum of initiated and received successful displacements
Successful replacement	The successful displacement results in the actor entering the bin and the gate opens to allow for her to begin to eat.	Successful replacement (actor) ratio: Initiated successful replacements / total initiated competitive contacts Successful displacement to replacement (actor) ratio: Initiated successful replacements / total initiated successful displacements	Replacement (actor) index: Initiated successful replacements / total initiated and received successful replacements
Unsuccessful displacement	After the physical contact performed by the actor, the receiver continues to eat at the bin.	Displacement resistance (receiver) ratio: Received unsuccessful displacements / total received competitive contacts	
Unsuccessful replacement	The successful displacement does not result in the actor accessing and eating from the same bin.		
Instantaneous Scan Sampling			
Eating	Head in the feed bin with the gate open	-	-
Loitering	Standing ≤ 1 body length (~2 m) away from an eating cow	-	-
Not Loitering	Standing > 1 body length away from an eating cow	-	-

Table 3. Competition behavior[1] at the feed bunk recorded during 1-h tests at different stocking densities for mid-lactation Holstein cows, blocked by parity and body weight

Variable	1 cow:1 bin	2 cows:1 bin	4 cows :1 bin	*P*-value
Event counts[2]				
Competitive contacts (actor)	3.9 (3.2, 4.6) ^{c}	26.9 (24.1, 30.0) ^{a}	19.1 (17.0, 21.4) ^{b}	<0.0001
Competitive contacts (receiver)	3.8 (2.9, 4.3) ^{c}	26.5 (23.1, 30.4) ^{a}	19.0 (16.5, 22.0) ^{b}	<0.0001
Total competitive contacts	7.6 (6.7, 8.7) ^{c}	53.5 (48.2, 59.4) ^{a}	38.1 (34.2, 42.4) ^{b}	<0.0001
Successful displacements (actor)	1.1 (0.8, 1.4) ^{c}	5.3 (4.7, 6.1) ^{a}	3.9 (3.4, 4.5) ^{b}	<0.0001
Successful displacements (receiver)	1.1 (0.8, 1.4) ^{c}	5.3 (4.6, 6.1) ^{a}	3.8 (3.3, 4.5) ^{b}	<0.0001
Unsuccessful displacements (receiver)	2.6 (2.1, 3.3) ^{c}	20.6 (17.6, 24.1) ^{a}	15.1 (12.8, 17.7) ^{b}	<0.0001
Successful replacements (actor)	0.8 (0.6, 1.0) ^{c}	3.7 (3.2, 4.2) ^{a}	2.3 (2.0, 2.8) ^{b}	<0.0001
Successful replacements (receiver)	0.8 (0.6, 1.0) ^{c}	3.6 (3.1, 4.1) ^{a}	2.4 (2.0, 2.8) ^{b}	<0.0001
Ratios				
Successful displacement ratio[3]	0.30 (0.19, 0.48)	0.24 (0.14, 0.39)	0.19 (0.11, 0.35)	0.46
Displacement resistance ratio[4]	0.65 (0.35, 1.00)	0.76 (0.46, 1.12)	0.72 (0.40, 1.11)	0.89
Successful replacement ratio[5]	0.24 (0.14, 0.40)	0.17 (0.10, 0.32)	0.13 (0.07, 0.26)	0.36
Displacement to replacement ratio[6]	0.76 (0.55, 1.04)	0.69 (0.51, 0.93)	0.60 (0.42, 0.86)	0.62
Indexes				
Competitive index[7,8]	0.49 (0.26, 0.77)	0.50 (0.27, 0.78)	0.56 (0.33, 0.84)	0.94
Displacement index[9]	0.50 (0.26, 0.78)	0.51 (0.27, 0.78)	0.54 (0.30, 0.82)	0.98
Replacement index[10]	0.52 (0.45, 0.60)	0.48 (0.41, 0.57)	0.52 (0.44, 0.61)	0.73
Behavior Inventory[11]				
Eating[12]	0.68 ± 0.02	0.47 ± 0.02	0.25 ± 0.02	-
Loitering	0.15 ± 0.02 ^{c}	0.34 ± 0.02 ^{b}	0.40 ± 0.02 ^{a}	<0.0001
Not-loitering	0.18 ± 0.02 ^{b}	0.19 ± 0.02 ^{b}	0.35 ± 0.02 ^{a}	<0.0001

[1] Back-transformed means and 95% confidence intervals reported from a natural logarithm-based negative binomial distribution, unless otherwise specified.

[2] Averaged between two 1-h tests at each stocking density.

[3] Initiated successful displacements / initiated competitive contacts.

[4] Received unsuccessful displacements / received competitive contacts.

[5] Initiated successful replacements / initiated competitive contacts.

[6] Initiated successful replacements / initiated successful displacements.

[7] Initiated competitive contacts / total competitive contacts initiated and received.

[8] Back-transformed means and 95% confidence intervals reported from a natural logarithm-based Poisson distribution.

[9] Initiated successful displacements / total successful displacements initiated and received.

[10] Initiated successful replacements / total successful replacements initiated and received.

[11] Proportion of time spent performing each behavior within 1 h; averaged between two 1-h tests for each stocking density. Behaviors included eating (head in the feed bin), loitering (standing within 1 body length of an eating cow), and not loitering (standing more than 1 body length away from a cow eating).

[12] Not included in analysis as eating time is already represented in total eating time from electronic bin data.

Table 4. Comparison of feeding patterns at different stocking densities, averaged between two 1-h tests per stocking density, for mid-lactation Holstein cows, blocked by parity and body weight

Variable	1 cow:1 bin	2 cows:1 bin	4 cows :1 bin	*P*-value
Latency to first visit a bin, min[1]	2.8 (2.1, 3.7) ^{c}	5.3 (4.0, 7.1) ^{b}	14.7 (10.9, 19.7) ^{a}	< 0.001
DMI of first visit, kg[1]	1.6 (1.3, 2.0) ^{a}	0.8 (0.6, 0.9) ^{b}	0.8 (0.7, 1.0) ^{b}	< 0.001
First visit duration, min[1]	8.6 (6.8, 10.7) ^{a}	3.7 (2.9, 4.6) ^{b}	3.5 (2.8, 4.3) ^{b}	< 0.001
Eating time within first 30 min, min	23.3 ± 0.7 ^{a}	13.2 ± 0.7 ^{b}	6.8 ± 0.7 ^{c}	< 0.001
Number of bin visits[1]	5.8 (5.3, 6.4) ^{a}	6.8 (6.2, 7.5) ^{a}	3.9 (3.6, 4.4) ^{b}	< 0.001
DMI, kg	6.3 ± 0.2 ^{a}	5.1 ± 0.2 ^{b}	3.0 ± 0.2 ^{c}	< 0.001
Eating rate, kg/min[1]	0.16 (0.15, 0.16) ^{c}	0.18 (0.17, 0.19) ^{b}	0.22 (0.20, 0.23) ^{a}	< 0.001
Total eating time, min	40.3 ± 1.2 ^{a}	28.2 ± 1.2 ^{b}	14.6 ± 1.2 ^{c}	< 0.001

[1] Back-transformed means and 95% CI from a natural logarithm-based distribution.

^{abc} Superscripts within a row indicate significant treatment differences, $P \leq 0.05$.

Table 5. Spearman's rank correlations between individual stability statistic (ISS) scores[1] of behavioral and feeding patterns calculated between different pairs of stocking densities[2] at the feed bunk, applied during 1-h tests, in mid-lactation Holstein cows

Variable	R-value	*P*-value
Total competitive contacts[3]	0.40	0.001
Competitive contacts (actor)[3]	0.49	<0.0001
Competitive contacts (receiver)[3]	0.20	0.11
Prop. of time spent loitering[4]	-0.09	0.50
Prop. of time spent not loitering[4]	0.11	0.38
Latency to first visit a bin, min	0.03	0.84
DMI of first visit, kg	0.02	0.90
First visit duration, min	-0.05	0.72
Eating time within the first 30 min, min	-0.35	0.006
Number of bin visits	-0.0	0.68
DMI, kg	0.11	0.39
Eating rate, kg/min	-0.55	<0.0001
Total eating time, min	-0.08	0.51

[1] Individual stability statistic (ISS) scores calculated as a measure of behavioral consistency across two different stocking densities; higher values indicate more consistency.

[2] ISS scores were calculated between 1:1 and 2:1 stocking densities (1 vs. 2 cows/bin) and between 2:1 and 4:1 (2 vs. 4 cows/bin). Correlations were computed between $ISS_{1:1,2:1}$ and $ISS_{2:1,4:1}$.

[3] Physical contact between two individuals with one cow initiating the contact (actor) and the other receiving the contact while eating at the feed bunk (receiver); total represents the sum between initiated and received competitive contacts.

[4] Proportion of time spent performing each behavior within 1 h; averaged between two 1-h tests for each stocking density. Behaviors included loitering (standing within 1 body length of an eating cow), and not loitering (standing more than 1 body length away from an eating cow).

Table 6. Correlations between individual stability statistic (ISS) scores[1] for responses during 1-h tests across different stocking densities[2] and residual feed intake[3] at the feed bunk in mid-lactation Holstein cows

Variable[4]	$ISS_{1:1,2:1}$	$ISS_{2:1,4:1}$
Total competitive contacts[5,6]	-0.11 (0.39)	-0.27 (0.028)
Competitive contacts (actor)[5,6]	-0.19 (0.14)	-0.25 (0.045)
Competitive contacts (receiver)[5,6]	-0.08 (0.53)	-0.22 (0.079)
Prop. of time spent loitering[4,6]	0.01 (0.96)	-0.12 (0.36)
Prop. of time spent not loitering[4,7]	0.02 (0.86)	-0.05 (0.71)
Latency to first visit a bin, min[5]	0.01 (0.93)	0.14 (0.27)
DMI of first bin visit, kg	0.24 (0.061)	-0.08 (0.56)
First visit duration, min	0.28 (0.024)	-0.08 (0.54)
Eating time within the first 30 min, min	0.01 (0.93)	0.11 (0.40)
Number of bin visits	0.15 (0.25)	-0.12 (0.34)
DMI, kg	0.02 (0.85)	0.29 (0.022)
Eating rate, kg	-0.18 (0.15)	0.33 (0.009)
Total eating time, min	0.02 (0.88)	0.13 (0.31)

[1] Individual stability statistic (ISS) scores calculated as a measure of behavioral consistency across two different stocking densities; higher values indicate more consistency.

[2] ISS scores were calculated between 1:1 and 2:1 stocking densities (1 vs. 2 cows/bin) and between 2:1 and 4:1 (2 vs. 4 cows/bin). Correlations were computed between $ISS_{1:1,2:1}$ and $ISS_{2:1,4:1}$.

[3] Estimation of feed efficiency, calculated for each cow by regressing DMI on milk energy output, median DIM, metabolic BW, and change in BW, each nested within parity.

[4] Pearson correlation coefficient with *P*-value listed in parentheses, unless otherwise specified.

[5] Spearman's rank correlation coefficient with *P*-value listed in parentheses.

[6] Physical contact between two individuals with one cow initiating the contact (actor) and the other receiving the contact while eating at the feed bunk (receiver); total represents the sum between initiated and received competitive contacts.

[7] Proportion of time spent performing each behavior within 1 h; averaged between two 1-h tests for each stocking density. Behaviors included loitering (standing within 1 body length of an eating cow), and not loitering (standing more than 1 body length away from an eating cow).

Figure 1. Flowchart of the behavior sequence used for observation of competitive interactions at the feed bunk between mid-lactation Holstein cows. For each behavior, an actor (cow initiating the event) and receiver (individual receiving the event) were recorded.

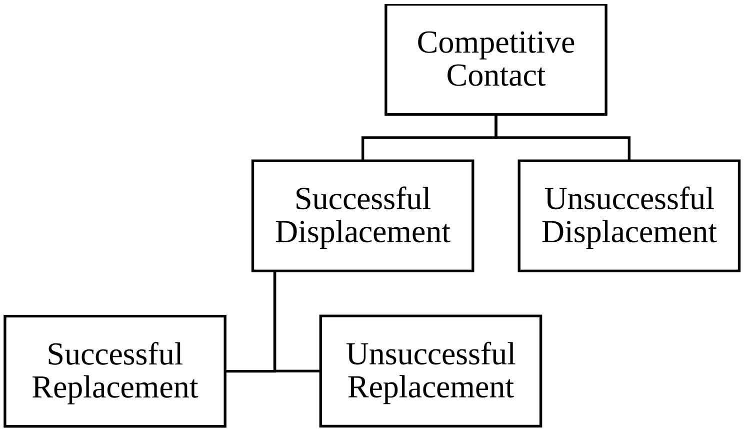


Figure 2. Screenshots from video recordings of 1-h tests of each feed bunk stocking density treatment: (A) 1 cow: 1 bin (8 cows: 8 bins), (B) 2:1 (16 cows: 8 bins), and (C) 4:1 (16 cows: 4 bins).

Figure 3. (A) Descriptive behavioral reaction norm showing individual mid-lactation Holstein cows' total competitive contacts to changes in feed bunk stocking density (1 cow: 1 bin, 2:1, or 4:1) in 1-h tests (average of 2 tests per stocking density). Cows were assigned to blocks of 16 cows each by combination of parity (PR: primiparous or MU: multiparous) and body weight (LO: low bodyweight or HI: high bodyweight). (B) The same plot is shown with 2 specific individuals colored as examples of being relatively consistent (red line) vs. inconsistent (yellow line).

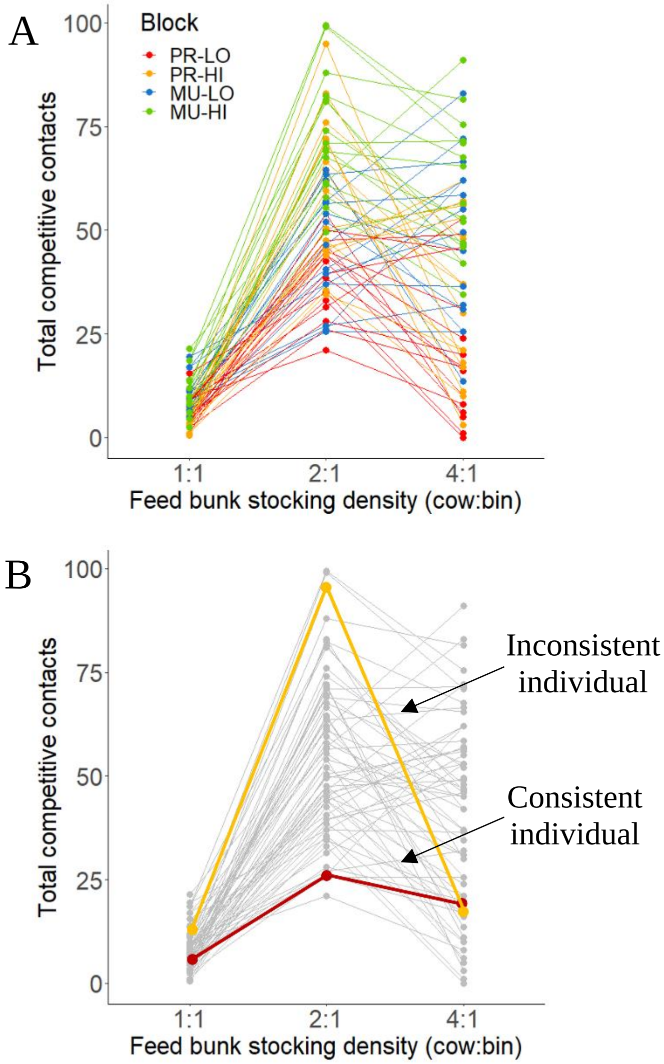


Figure 4. Observed vs. predicted DMI plotted for each block [based on parity (PR: primiparous, MU: multiparous) and body weight (LO: low bodyweight, HI: high bodyweight)] for mid-lactation Holstein cows. Data points above the line of unity represent cows consuming more feed than predicted, associated with a positive residual feed intake (RFI) value and lesser feed efficiency. Data points below the line of unity represent cows consuming less feed than predicted, associated with a negative RFI and greater feed efficiency.

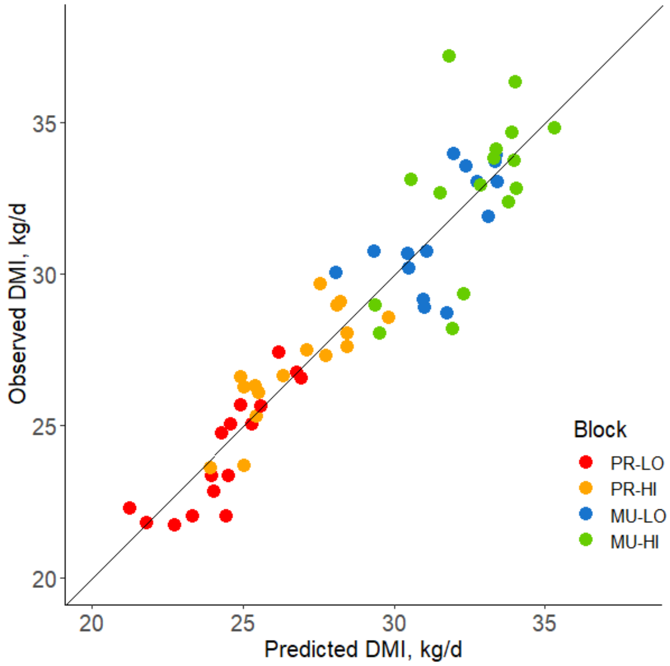
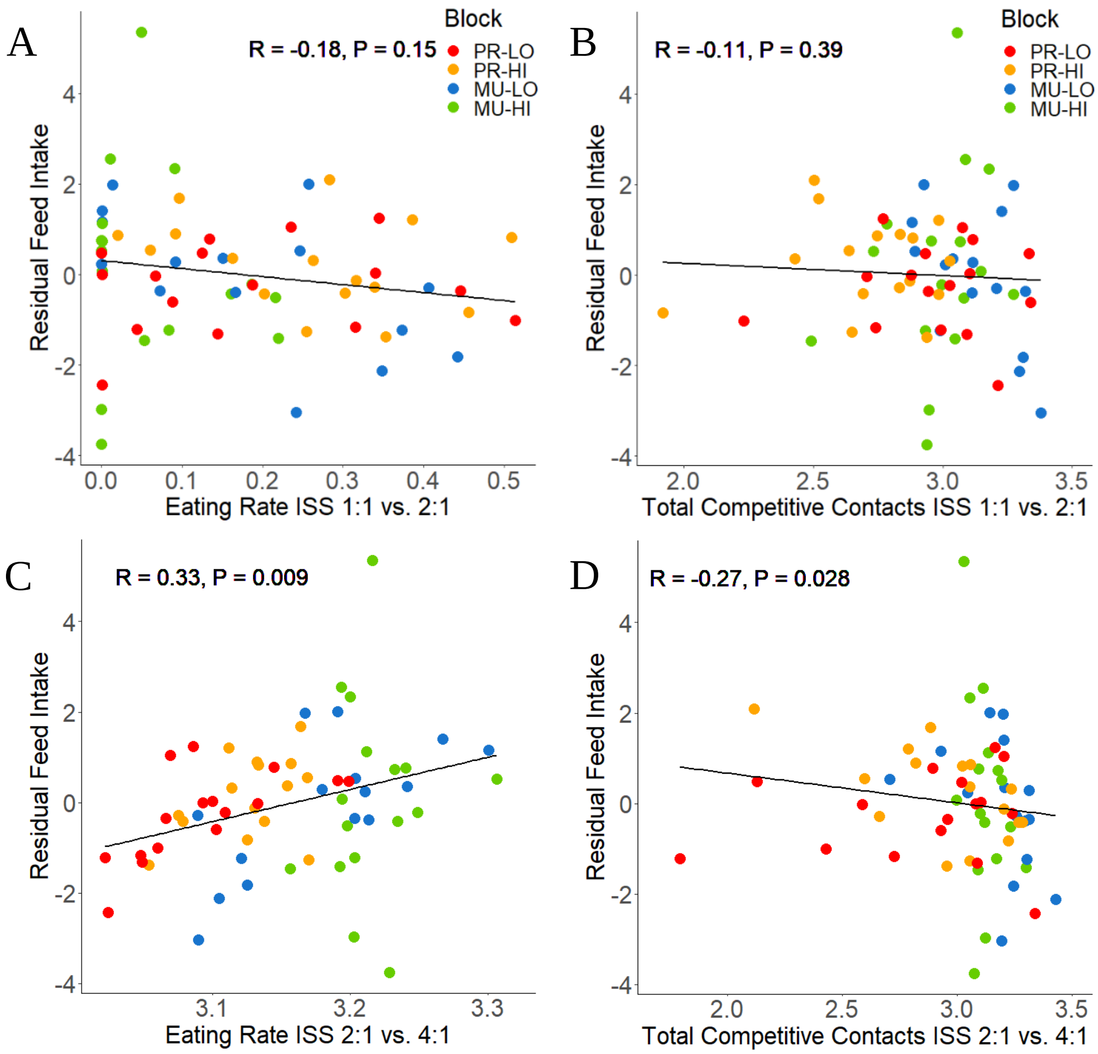


Figure 5. Correlations between feed efficiency (residual feed intake; higher values indicate less efficient) in mid-lactation Holstein cows and consistency in total competitive contacts (B and D) and eating rate (A and C). Consistency was calculated using an individual stability statistic (ISS; higher values indicate more consistent) during 1-h tests at feed bunk stocking density comparisons of: (A,C) 1 cow:1 bin vs. 2:1 and (B,D) 2:1 vs. 4:1. Cows were assigned to blocks of 16 cows each by combination of parity (PR: primiparous or MU: multiparous) and body weight (LO: low bodyweight or HI: high bodyweight).



CHAPTER 5. IMPACT OF STATIONARY BRUSH QUANTITY ON BRUSH USE IN GROUP-HOUSED DAIRY HEIFERS

ABSTRACT

Our objectives were to evaluate the effect of stationary brush quantity on brush use and competition in weaned dairy heifers naïve to brushes. Sixty-three Holstein heifers (95 ± 5.7 days old) were housed in groups of eight (with the exception of 1 group of 7) with two or four stationary brushes (n = 4 groups/treatment). Brush-directed behaviors of grooming, oral manipulation, and displacements were recorded continuously for all heifers 0–6, 18–24, 120–126 and 138–144 h after brush exposure. Linear mixed models were used to evaluate the effects of brush quantity and exposure duration. Total brush use and competition were not affected by brush quantity, but heifers with access to more brushes used them for longer bouts, suggesting greater opportunity for uninterrupted use. Total brush use was greater in the first and final 6 h observation periods, which was driven by the greatest duration of oral manipulation and grooming in those respective periods. The continued use of brushes by all heifers in the final period indicates the importance of providing appropriate outlets for these natural behaviors to promote animal welfare. The effect of brush quantity on bout characteristics suggests that brush use was less restricted with four compared to two brushes per eight heifers.

INTRODUCTION

Grooming is a motivated behavioral need for cattle that can be facilitated with objects in the environment, such as brushes (McConnachie et al., 2018; DeVries et al., 2007). Grooming can improve cleanliness (Brownlee, 1950; Simonsen, 1979), remove parasites (Fraser and

Broom, 1997), satisfy a natural behavior (Ewing et al., 1999), and may reduce boredom, stress, and frustration (Wood-Gush and Beilharz, 1983). The use of either mechanical rotating or stationary brushes has been documented in cattle of several age groups, including pre-weaned calves (Zobel et al., 2017; Horvath and Miller-Cushon, 2019; Horvath et al., 2020a), weaned heifers (Strappini et al., 2021; Velasquex-Munoz et al., 2019; Van Os et al., 2021), periparturient cows (Newby et al., 2013; Mandel and Nicol, 2017), dry cows (Foris et al., 2021), and lactating cows (DeVries et al., 2007; Val-Laillet et al., 2009). The provision of appropriate resources to perform this highly motivated behavior (McConnachie et al., 2018) may thus improve the welfare of cattle, especially weaned dairy heifers, who are often housed in relatively barren confined environments.

Stationary brushes can be a more economical option than mechanical rotating brushes (Miller-Cushon and Van Os, 2021). Although rotating brushes are designed to allow cattle to groom harder to reach areas of the body (i.e., the back) (DeVries et al., 2007; Broom and Fraser, 2007), young cattle primarily choose to brush their head and neck compared to other body areas, regardless of brush type (vertically mounted rotating brushes (Zobel et al., 2017; Toaff-Rosenstein et al., 2017); stationary brushes (Horvath and Miller-Cushon, 2019; Van Os et al., 2021). Furthermore, stationary brushes also provide an outlet for oral manipulation (Horvath et al., 2020a; Van Os et al., 2021), which has not been reported for mechanical brushes. In addition to grooming, this behavior is also important, especially for young cattle, for whom the provision of stationary brushes has been shown to decrease the abnormal oral behaviors of sucking on pen structures (Horvath et al., 2020a) and tongue rolling (Park et al., 2020). Despite the current evidence that the provision of brushes offers appropriate outlets for cattle to perform these important natural behaviors, our understanding of brush use based on the availability of brushes in a group setting is limited. It is unknown how many brushes are needed to provide group-housed heifers with adequate opportunities for use. Competition for brush access (i.e., displacement events) has been reported for mechanical brushes (DeVries et al., 2007; Foris et al., 2021; Val-Laillet et al., 2008), but rarely for stationary brushes (Van Os et al., 2021). The majority of previous studies provided only a single brush to groups of cattle (i.e., 4 pre-weaned dairy calves, Horvath and Miller-Cushon, 2019; 19 to 20 weaned dairy calves, Velasquez-Munoz et al., 2019; 9 beef steers, Park et al., 2020; 4 or 8 beef steers, Horvath et al., 2020b; and 20 lactating dairy cows, Foris et al., 2021). Van Os et al. (2021) provided four brushes to groups of four weaned heifers in an open bedded-pack pen, followed by four brushes per pair of heifers in a freestall pen, but no comparison of brush use was performed depending on the quantity of brushes offered. To our knowledge, no studies have directly evaluated the role of stocking density on brush use or competition. Information about how brush quantity affects resource competition could improve our understanding of brush use and inform management decisions related to providing brushes to cattle.

In addition to group dynamics, temporal patterns of brush use also play a role in adequate access to brushes. Cattle exhibit varying temporal patterns when using brushes, both across days as well as relating to specific events or bouts. For example, weaned dairy heifers exhibited the greatest use of stationary brushes on the first day of observation, with use continuing throughout the 13 day observation period (Van Os et al., 2021). Similar patterns were observed in beef steers, with the greatest stationary brush use on the first day of observation (day 1 of exposure, Wilson et al., 2002; day 2 of exposure, Park et al., 2020) and continued use throughout the observation periods (ending on day 22 and day 64, respectively). Many studies have reported brush use as average durations during an observation period (e.g., across 24 h). In contrast, brush visit or bout characteristics describing the temporal patterns of brush use events have only been reported in pre-weaned calves (Zobel et al., 2017; Horvath and Miller-Cushon, 2019), but not for other age classes of dairy cattle. Similarly to feeding behavior (DeVries et al., 2003), brush use often occurs for short durations separated by short breaks. Calculating bout characteristics to group events into bouts can provide a more informative depiction of brush use duration and frequency (e.g., Horvath and Miller-Cushon, 2019) than reporting raw observations (e.g., Zobel et al., 2017).

Our main objective was to evaluate the effect of the number of stationary brushes on brush use and competition in group-housed, weaned dairy heifers naïve to brushes. This included evaluating the time scale of brush use across hours of exposure, along with the bout characteristics of brush use within observation periods. We predicted that the provision of more brushes would result in less competition along with more frequent and longer bouts based on the greater opportunity for brush access, resulting in greater total brush use. We also predicted that individual heifers who were more successful at displacing other heifers from brushes would exhibit greater brush use. Finally, we predicted that brush use would decrease after the initial 6 h observation period when the brushes were most novel.

MATERIALS AND METHODS

Animals, Housing, and Treatments

The study was conducted from June to August 2019 at the University of Wisconsin – Madison (UW-Madison) Marshfield Agricultural Research Station in Marshfield, WI. All procedures were approved by the Institutional Animal Care and Use Committee of University of Wisconsin – Madison (protocol A006133-A01, approved 10 May 2019).

Sixty-three Holstein heifers were housed in groups of 8, with the exception of one pen with 7 heifers (due to one heifer dying before arrival for reasons unrelated to the study). Each group was determined by birth order and previously housed together in outdoor group hutches before being moved to the treatment pens. Groups of heifers were moved to the treatment pens at approximately the same age. Therefore, heifer age remained relatively consistent among and within groups. Four experimental pens were filled sequentially by arrival date (95 ± 5.7 days old upon arrival, mean ± SD; 118.3 ± 11.9 kg bodyweight, excluding the pen of 7 heifers, for which bodyweight data were unavailable), with each pen used for 2 groups.

Each pen included a 4.9 m × 4.9 m open lying area bedded with straw (0.2 m bedding height; added on an as-needed basis). Two opposite sides of each pen had 1.6 m-tall wooden walls and a feed bunk containing 9 self-locking headlocks, opposite a metal gate. Twice daily, heifers were fed grain (0730 and 1515 h) and unchopped grass hay (0830 and 1600 h). All heifers, except those in the pen of 7, were also enrolled in a separate, larger nutrition trial involving diets that varied in formulation of feed additives (Nolden et al., 2020). We do not expect this factor affected brush use. Water was provided ad libitum from one self-filling water trough per pen.

The experimental treatment was the number of brushes provided in each pen (2 vs. 4/pen; Pro Series Wash Brush, 25.4 cm-long × 6.0 cm-wide with 3.8 cm-long bristles, described as “stiff” by the manufacturer, Camco Manufacturing; Figure 1), with brush treatment alternating between adjacent pens (Figure 2). A total of n = 4 groups/treatment were tested; the 2-brush treatment had 3 groups of 8 heifers and one group of 7 heifers, and the 4-brush treatment had 4 groups of 8 heifers. Treatments were assigned alternately to groups of heifers by filling the next sequential unoccupied pen. Brushes were mounted vertically on the 2 wooden walls in each pen at a height of 0.9 m, measured from the floor beneath the bedding to the bottom of the brush. In the 2-brush pens, the brushes were mounted 1.5 m from the feed bunk, based on previous work showing that dairy cattle use brushes located near the feed bunk (Van Os et al., 2021; Mandel et al., 2019); in the 4-brush pens, the additional 2 brushes were mounted 1.5 m from the gate.

Power Analysis

Sample size justification was performed using data from a previous study (Van Os et al., 2021), which examined brush use in group-housed heifers (n = 13 groups of 4 heifers each). The durations of brush use, reported as oral manipulation (mean range of 7.5 to 14.8 min, SD = 0.5 to 5.1), grooming (mean range of 11 to 27.2 min, SD = 3.4 to 11.4), and total brush use (mean range of 21.0 to 45.9 min, SD = 2.8 to 17.6) translated to very large effect sizes (Cohen's d = 1.4 to 1.5), which resulted in requiring a sample size of n = 7 to 8 groups of heifers/ treatment. However, the present aim was to examine behavior within larger groups (7 to 8 heifers/group), whereas behavioral observation for the previous study was based on 2 focal heifers within each group. As described by St-Pierre (2007), the number of replicates needed to achieve a given power is decreased when larger groups are the experimental unit, as variance among pens is generally less than the variance of animals within pens. In consideration of decreased variability when using larger groups, we enrolled 4 groups of heifers per treatment.

Measures

To characterize heifer behavior, video was recorded using 4 video cameras (3 MP ProHDDome IP Fixed Outdoor Camera, Amcrest, with internal SD cards) mounted 3.5 m high on the barn wall closest to the gates. Recorded files were off-loaded from the cameras to external hard drives for observation. Behavior was coded for each individual heifer from video for four 6 h periods on the first and sixth days relative to entering the pen on day 1, defined as follows, with approximate times of day: period 1: 0–6 h after exposure (day 1, 1230–1830 h); period 2: 18–24 h after exposure (day 1, 0630–1230 h); period 3: 120– 126 h after exposure (day 6, 1230–1830 h); period 4: 138–144 h after exposure (day 6, 0630– 1230 h). For one group assigned to a 4-brush pen, video data on day 6 (120–126 and 138– 144 h of brush exposure) were lost due to hard drive malfunction. Behavior was only observed during daylight hours to ensure accuracy of observations. Two trained observers watched the recordings with a video-supported player (Amcrest Smart Player, 1080 HDCVI) and coded behaviors in Excel spreadsheets for all heifers continuously during those periods. Individual heifers were identified by their coat patterns. Specific brushes were identified by the location in each pen. Brush-directed behavior was recorded as two different types: (1) oral manipulation – contact between the mouth or tongue and a brush (Figure 1A) – and (2) grooming – rubbing the head, neck, or body against a brush (Figure 1B). The duration of each behavior was determined by recording the start and stop times to the nearest second. Displacement events occurring at each brush were also recorded when one heifer's contact with the brush (receiver) ended due to physical contact from another heifer (actor). Inter-observer reliability for all brush-directed behaviors was determined by comparing a subset of data between the two observers using linear regression (PROC REG; SAS software version 9.4; SAS Institute, Cary, N.C., USA). The coefficient of determination (R^2) was ≥ 0.89, and the slope and intercept did not differ from 1 and 0, respectively ($p > 0.05$), which indicated good agreement and lack of systematic bias.

Statistical Analysis

Latency to initially contact any brush was calculated on an individual heifer basis relative to their respective entry time into the pen. Latency-related parameters are reported only descriptively. Total brush use was calculated as the sum of brush grooming and oral manipulation. A competitive index was calculated for each heifer using her frequency of initiated displacements (actor role) divided by the total number of displacements she was involved in (actor and receiver roles), multiplied by 100; one heifer (in the 4-brush treatment) uninvolved in displacements was excluded.

To evaluate the bout characteristics of brush use, bout analysis was performed for total brush use in each of the four 6 h observation periods, pooled among all individual heifers. The analysis was performed as described by Horvath and Miller-Cushon (2019) and DeVries et al. (2003). In brief, interval durations between each brush use event for each heifer were summarized and converted to log10-transformed frequency distributions to calculate the inter-bout criteria. The inter-bout criteria were calculated by fitting a mixture of two normal distributions to the log10 distributions of brush-use intervals using exact maximum likelihood to determine the point at which the distribution curve of within-bout (intrabout) intervals intersected the distribution curve of between-bout (inter-bout) intervals (R package mixdist; in MacDonald and Du, 2018). The calculated inter-bout criterion defines the interval between brush use bouts, accounting for behavioral bouts comprising multiple single events separated by short breaks. This criterion establishes a threshold such that brush use events separated by pauses shorter than the criterion are considered within the same bout, whereas those events separated by breaks longer than the criterion are considered as occurring in separate bouts. An individual inter-bout criterion was calculated for each 6 h period and pooled among all individual heifers. Similar inter-bout criteria were calculated for periods 1 (19.95 s) and 2 (39.81 s), when the brushes were relatively novel to the heifers, compared to periods 3 (125.95 s) and 4 (100 s). Therefore, two criteria were calculated, defined as follows: novel period: 0–6 and 18–24 h of exposure (periods 1 and 2), calculated as 25.11 s (Figure 3A); non-novel period: 120–126 and

138–144 h of exposure (periods 3 and 4), calculated as 63.10 s (Figure 3B). Using the respective bout criteria for the novel and non-novel observation periods, brush use bout characteristics were calculated for each heifer and summarized at the group level for each of the four observation periods. Two heifers (one each in the 2- and 4-brush treatments) in the novel period and 2 heifers (both assigned to the 2-brush treatment, but in different groups) in the non-novel period were excluded from the calculations of total time and bout duration due to either only 1 event recorded within that period or none of the events involving intra-bout intervals (i.e., intervals less than the inter-bout criterion). Bout characteristics were defined as total time (sum of duration of observed brush use and intra-bout intervals shorter than the interbout criterion), bout frequency (number of intervals between bouts, as defined by the inter- bout criterion), and bout duration (total time divided by bout frequency). Total time was used only for calculation of bout frequency and is not reported in the results.

All statistical analysis was performed using R software (v. 3.6.1, RStudio, Boston, MA, USA). Linear mixed models were used to evaluate the effects of brush quantity and time of exposure on brush use (grooming, oral manipulation, and total brush use, along with the bout characteristics for total brush use) and competitive displacement events. These models included fixed effects of treatment (2 vs. 4 brushes/pen), period (0–6, 18–24, 120–126, or 138–144 h of brush exposure), and the interaction between treatment and period, and a random effect of group to account for the repeated measures of period. A Kenward– Roger adjustment was performed for all analyses of variance of linear mixed models. The competitive index had only one value per group (no effects of period), and thus the linear model for this variable included the fixed effect of only treatment. Residuals were assessed for normality and equal variance visually using graphs and numerically using the Shapiro–Wilk test for normality. The experimental unit was defined as a group of heifers (n = 4 groups/treatment) for all variables. For the competitive index, Pearson correlations were performed at the individual heifer level between the duration of total brush use (averaged across the four 6 h periods) and competitive index values; these relationships were evaluated both pooled between treatments and separately by treatment.

All values are reported as least square means. When there were significant ($p < 0.05$) effects or tendencies ($p \leq 0.10$), pairwise comparisons were performed with Tukey–Kramer adjustments.

RESULTS

Latency to use any brush after entering the pen was 4.0 ± 8.4 min (mean ± SD), with individual latency ranging from 0.1 to 31.1 min and 0.1 to 58.4 min in the 2- and 4-brush treatments, respectively. In the 4-brush treatment, brushes located near the feeder were used first 75% of the time.

All brush-use behaviors will be discussed based on main effects related to brush treatment and period, as there were no significant interactions ($p \geq 0.17$).

Brush Quantity

The number of brushes provided to group-housed heifers did not impact the duration of oral manipulation, grooming, or total brush use ($p \geq 0.10$; Figure 4). Heifers provided with more brushes exhibited longer bouts ($p = 0.029$), but there was no treatment difference observed for bout frequency (Table 1).

Brush Use across Time of Exposure

Total brush use in the final observation period (138–144 h of exposure) was greater than after 18–24 and 120–126 h of exposure ($p \leq 0.007$), but it was similar to the first observation period (0–6 h of exposure, $p = 0.28$; Figure 5). Heifers tended to exhibit greater total brush use in the initial 6 h compared to 18–24 h of exposure ($p = 0.052$), but it remained similar during 120–126 h of exposure ($p = 0.20$). The greater duration of total brush use in the initial 6 h was facilitated by greater durations of oral manipulation compared to all other periods ($p \leq 0.027$; Figure 5). Oral manipulation decreased from 18–24 h to 120–126 h of exposure ($p = 0.045$), but then remained consistent in 138–144 h compared with the previous two periods ($p \geq 0.12$). Despite the decrease in oral manipulation from the initial to the final 6 h period, the consistent levels of total brush use between those periods were facilitated by greater durations of grooming in the final observation period (138–144 h of exposure) compared to all other periods ($p \leq 0.026$; Figure 5).

Bouts were more frequent in the initial 6 h of exposure than during 120–126 h of exposure ($p = 0.007$; Table 2). Bout frequency was similar among the other periods ($p \geq 0.21$), although bouts tended to be more frequent during 18–24 h than in 120–126 h of exposure ($p = 0.051$). Bout duration was similar within the novel (0–6 and 18–24 h of exposure) and non-novel periods (120–126 and 138–144 h of exposure), respectively ($p \geq 0.88$); overall, heifers exhibited shorter bouts during the novel compared to the non-novel period ($p < 0.001$; Table 2).

Competition

The quantity of brushes had no impact on the number of displacements ($p = 0.75$, Table 1). Fewer displacements occurred during 120–126 h of exposure compared to the initial 6 h after exposure ($p = 0.003$; Table 2) and 138–144 h of exposure ($p = 0.039$), but it remained similar during 18–24 h of exposure ($p = 0.55$). No correlation was observed between individual competitive index values and brush use within or across treatments ($p \geq 0.58$; Figure 6).

DISCUSSION

Overall Brush Use

Upon first exposure, naïve heifers began using brushes nearly immediately, with average latency similar to the previously reported results for group-housed heifers naïve to stationary brushes (3.4 ± 4.9 min, ranging from 0.1 to 17.8 min; Van Os et al., 2021), but individual maximums were greater in the current study. This difference could indicate that certain heifers may not have had enough voluntary access in the current study, with one brush provided for every two or four heifers, compared to one brush per heifer in Van Os et al. (2021). However, the greatest latency in our study was observed in the four-brush treatment, which could alternatively reflect variation in individual heifer motivation to access the brush upon entrance into the novel pen. In addition to individual variation in latency, heifers in the current study showed variation in which brush they first contacted in the four-brush treatment, using brushes closest to the feeder 75% of the time. This initial use of brushes near the feed bunk was consistent with previous work (Van Os et al., 2021), although those authors reported that initial preference did not continue after the first day of observation.

Providing two vs. four brushes for a group of eight heifers did not impact the duration of brush-directed behaviors (oral manipulation, grooming, or total brush use). Heifers used the brushes for grooming over 73% of the time, with the remainder spent orally manipulating the brushes. This distribution is similar to other studies that reported cattle used brushes for grooming for the majority of the observation time (>60% in weaned heifers, Van Os et al., 2021; >70% in pre-weaned calves, Horvath et al., 2020a). This consistency among studies suggests that stationary brushes provide an appropriate outlet for both of these behaviors, but that grooming potentially has a higher motivation when using brushes.

The current study is the first to evaluate the effects of different brush quantities on brush use in any age of cattle. Van Os et al. (2021) observed brush use in two different scenarios with four brushes per four heifers and four brushes per two heifers; however, no direct evaluation of stocking density was performed. The previous study reported, on average, greater total stationary brush use when four brushes were provided to four heifers compared to our findings using a 1:4 ratio, while remaining similar to our findings using a 1:2 ratio. On the contrary, lesser total brush use was reported when four brushes were provided to two heifers (Van Os et al., 2021), although this was most likely due to limited access with the brushes located inside the freestalls. Studies involving only one brush provided to a group (Strappini et al., 2021) or a 1:1 ratio for individually housed dairy calves (Horvath et al., 2020a; Pempek et al., 2017) reported, on average, lesser total stationary brush use compared to the levels observed in our study, which may relate to the age of the animals or other differences in housing management. One exception was a study in which greater brush use was observed when nine beef steers were provided one large stationary brush structure (Park et al., 2020); however, this may be explained by the massive size of the structure, with 12 brushes combined, compared to only a single stationary brush in the other studies.

Overall, total brush use was greater in the first and final observation periods (0–6 and 138–144 h of exposure). This pattern was driven by the greatest duration of oral manipulation in the first period and the greatest duration of grooming in the last period. We speculate that the greater oral manipulation after initial brush exposure may have reflected heifers’ exploration of their new environment. In previous studies, group-housed heifers (Van Os et al., 2021) and beef steers (Park et al., 2020) used stationary brushes the most within the first 24 h, after which brush use declined and stabilized. In the former study, oral manipulation was stable across 6 d, whereas grooming decreased after the first day of observation (Van Os et al., 2021), which the authors speculated reflected heifers’ initially greater urge to groom themselves due to the lack of appropriate scratching surfaces in their previous housing environment. Our findings of greater grooming in the final observation period may have reflected learning and increased motivation to perform this natural behavior after heifers recognized the brushes as an appropriate outlet for this type of behavior. Regardless of the temporal variation we observed across periods, all heifers continued to use brushes through the final observation period. Likewise, continued use of stationary brushes has been documented in group housed heifers observed across 13 d (Van Os et al., 2021) and in beef steers for 22 and 64 d (Park et al., 2020; Wilson et al., 2002). These patterns among studies suggest that cattle remain motivated to use brushes after they are no longer novel. This supports the importance of providing stationary brushes as appropriate, beneficial outlets for grooming and other behaviors to promote animal welfare.

Bout Characteristics

Patterns of brush use by cattle involve short durations separated by short breaks. Defining a bout criterion improves biological interpretation of brush-use events. Horvath and Miller-Cushon (2019) performed the first calculation of brush bout criteria in cattle (group-housed, pre-weaned calves with a rotating brush), and those authors later applied the method to individually housed pre-weaned calves with stationary brushes (Horvath et al., 2020a) and group-housed beef steers with a mechanical brush (Horvath et al., 2020b). In our study, we used this methodology to calculate criteria for each six-hour period (ranging from 20.0 to 125.9 s), which we pooled into two periods when brushes were more vs. less novel (25.1 vs. 63.1 s, respectively), based on descriptive similarity in their criteria. In previous studies in which bout criteria were calculated, inter-bout criteria were longer (125.9 s in pre-weaned calves using a rotating brush, Horvath and

Miller-Cushon, 2019; 158.5 and 251.1 s in two experiments with weaned beef steers using a mechanical brush, Horvath et al., 2020b; 13.2 min in individually housed calves using a stationary brush, Horvath et al., 2020a). These data do not suggest a clear pattern between inter-bout criteria and the ratio of cattle to brushes, but rather suggest that bout characteristics may vary depending on other management or individual animal variation. The variation across studies may also be associated with behavioral observation methods, such as when only select focal individuals are observed in a group setting or when time sampling is used instead of continuous observation (Horvath et al., 2020b).

Our bout analysis revealed brush use frequencies between 12.2 and 18.8 bouts per 6 h, which lasted 19.9 to 41.6 s per bout. Patterns of brush use previously reported in the literature vary, in part because some studies described visits (raw counts of brush use events) as opposed to bouts defined with a bout criterion. For example, pair-housed dairy calves with one mechanical brush displayed 94 events per 20 h, each event lasting 17.8 s (Zobel et al., 2017). In contrast, when studies have explicitly calculated bout characteristics, fewer but longer bouts of brush use were reported in group-housed dairy calves (10.4 bouts/12 h lasting 154.2 s/bout; Horvath and Miller-Cushon, 2019), beef steers (7 to 8 bouts/d lasting 4 to 6 min/bout in 2 experiments; Horvath et al., 2020b), and individually housed dairy calves (7 to 8 bouts/12 h lasting 6 to 8 min/bout; Horvath et al., 2020a). In addition to selecting appropriate behavioral observation methods for capturing brief brush-use events, calculating bout characteristics should be considered for future studies of brush use to most accurately report and interpret findings.

Within our study, access to more brushes (1:2 vs. 1:4 brush-to-heifer ratio) resulted in longer for simultaneous use, and thus expression of more complete bouts. This could imply that, to promote animal welfare by providing the best opportunities for heifers to perform more complete bouts of brush use, farmers should consider providing a high ratio of brushes to groups when possible (e.g., one brush per 1–2 heifers). However, across other studies, brush stocking density does not appear to explain bout length. In the absence of competition, individually housed dairy calves exhibited much longer bouts (6 to 8 min/bout; Horvath et al., 2020a) than in our study. However, this was also the case in other studies with only one brush per group of four dairy calves (154.2 s/bout; Horvath and Miller-Cushon, 2019) and four or eight beef steers (4.2 and 5.6 min/bout, respectively; Horvath et al., 2020b). The longer bouts in past studies compared to ours may be related to environmental factors, such as pen size and brush type or location relative to other resources, rather than just group size. In our study, two brushes in each treatment were located near the feed bunk, and an additional brush in the four-brush treatment was near the waterer, which may have facilitated short bouts of brush use while heifers used nearby resources. Lactating cows have been shown to be similarly motivated to access a brush and fresh feed (McConnachie et al., 2018). Perhaps because heifers in our study could all occupy the feed bunk at once, they had more opportunity to switch back and forth between resources compared to in a more competitive feeding environment; this hypothesis could be evaluated in future studies. Finally, the heifers in our study were provided unchopped hay, which may have affected bout length due to the interconnectedness of oral behaviors; Horvath et al. (2020a) observed that individually housed dairy calves fed chopped bermudagrass had shorter bouts of brush use than those without hay.

In addition, we evaluated bout characteristics over time because we predicted that bout frequency and duration would change as the novelty of the brushes decreased. Overall, we found that bouts were shorter and more frequent upon initial brush exposure and lengthened and became less frequent as novelty decreased after the first day. These patterns could reflect the transition from short, initial oral exploratory behavior to longer, sustained use of the brushes for grooming. The temporal patterns may also suggest that the provision of multiple brushes provided opportunity for voluntary brush use without constant interruption and competition for use. In a previous study, group-housed preweaned dairy calves similarly performed more bouts during week four of life compared to later weeks, although bout length did not change (Horvath and Miller-Cushon, 2019). Ours is the first study to evaluate changes in bout characteristics beginning with the first exposure to brushes, and further research is needed to understand how and why bout characteristics change after initial use.

Competition

The quantity of brushes had no impact on the number of competition events. Overall, displacements were relatively infrequent, occurring at an average rate of one per hour. In previous work by our group on weaned heifers with access to one brush per heifer (four heifers per bedded-pack pen), fewer than four displacements occurred in the first 24 h of exposure, decreasing to two displacements per day on subsequent observation days (Van Os et al., 2021). During the second phase of the same study, in which two brushes were available per heifer (two heifers per freestall pen), only one displacement event was observed across two days of observation (Van Os et al., 2021). In contrast, many other studies provided only one brush per larger group of cattle (e.g., Horvath and Miller-Cushon, 2019; Strappini et al., 2021; Velasquez-Munoz et al., 2019; Newby et al., 2013), but to date, only three have reported competitive behavior. For groups of lactating cows with one mechanical brush, less than one displacement per two hours was reported for groups of 12 (DeVries et al., 2007; Val-Laillet et al., 2008), and less than one displacement per hour occurred in groups of 20 (Foris et al., 2021). Although the rate of displacements reported in the literature has been relatively low, patterns among studies suggest a possible relationship with the ratio of cattle to brushes. More research is needed on the effects of brush availability on competition and brush use when the ratio of cattle to brushes is greater than in our studies on small groups of heifers, particularly for stationary brushes, which may be more economically feasible for farms to provide in quantities greater than one per group of cattle. Furthermore, displacements are one of many possible measures of competition, which may also involve non-physical interactions, such as subordinate individuals rescheduling use to avoid contact with dominant individuals.

Contrary to our prediction, the calculated competitive index for each heifer showed no correlation with individual brush use. This could be because heifers who displaced another conspecific may not have intended to use the brush, and the contact could have been accidental, especially at brushes located near other resources such as the feed bunk and waterer. Nonetheless, intent cannot be assumed during behavioral observation, and all physical contacts between heifers resulting in cessation of brush use were coded as displacements. In previous work on lactating cows, a low correlation was likewise reported between a competitive success index and brush use (Val-Laillet et al., 2008). Therefore, a competitive index may not always reflect motivation to use a brush. Another approach for characterizing social dynamics is to classify cattle as dominant or subordinate. Foris et al. (2021) did so for lactating cows based on successful replacements at the feed bunk and water troughs. Although the number of displacements from a mechanical brush did not vary between dominant or subordinate cows, the former used the mechanical brush more during peak feeding time and overall (Foris et al., 2021). This suggests that dominance plays a role in brush access and supports the idea that subordinate individuals may choose to avoid physical competition for the resource.

CONCLUSIONS

Our study is the first to evaluate the role of stocking density on brush use and competition in any age class of cattle. Brush quantity did not impact the overall duration of brush use or competition. However, heifers provided with more brushes performed longer bouts, suggesting that the provision of four brushes to a group of eight heifers provided greater opportunity for uninterrupted brush use. Naïve heifers used brushes soon after exposure, with the greatest oral manipulation of brushes in the initial 6 h, perhaps reflecting exploration. In contrast, grooming, which comprised the majority of brush use, was greatest at the end of the sixth day after brush exposure. The continued use of brushes over time by all heifers illustrates the importance of providing appropriate outlets for both of these important natural behaviors to promote animal welfare.

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TABLES AND FIGURES

Table 1. Brush-directed behaviors performed by group-housed heifers (7 to 8 heifers/group) for each treatment (2 vs. 4 brushes/pen, n = 4 groups/treatment), averaged among four 6-h observation periods unless otherwise indicated.

	Treatment				
Variable	2 Brush	4 Brush	SE[1]	*F*-value[2]	*p*-value
Competition events (no.)	5.7	6.2	1.02	0.8 (1,6)	0.75
Competitive index[3]	47.5	48.5	2.07	0.1 (1,6)	0.75
Bout Frequency (no.)[4]	14.6	17.4	1.79	1.2 (1,6)	0.31
Bout Duration (s/bout)[4]	27.5	35.1	1.85	8.5 (1,5.7)	0.029

[1] Pooled standard error.

[2] Degrees of freedom associated with each F statistic are reported in parentheses.

[3] Frequency of initiated displacements (actor role) divided by the total number of displacements the individual heifer was involved in (actor and receiver roles), multiplied by 100; calculated on an individual heifer basis across the entire study.

[4] Bout characteristics based on 2 pooled inter-bout criteria calculated from 0 – 6 and 18 – 24 h of exposure (periods 1 and 2, when the brushes were most novel) vs. 120 – 126 and 138 – 144 h of exposure (periods 3 and 4).

Table 2. Brush-directed behaviors performed by group-housed heifers (7 to 8 heifers/group) for each 6-h observation period, regardless of brush quantity treatment (2 vs. 4 brushes/pen, n = 4 groups/treatment).

	Period[1]						
Behavior	1	2	3	4	SE[2]	*F*-value[3]	*p*-value
Competition events (no.)	8.6^{a}	4.9^{bc}	3.1^{b}	7.1^{ac}	1.08	6.9 (3,16.4)	0.003
Bout Frequency (no.)[4]	18.8^{a}	17.1^{ab}	12.2^{b}	15.8^{ab}	14.50	5.2 (1,16.3)	0.011
Bout Duration (s/bout)[4]	22.5^{b}	19.9^{b}	41.2^{a}	41.6^{a}	2.60	20.2 (1,16.7)	< 0.001

[1] Behavior observations were performed across four 6-h periods, defined as periods 1 – 4 (0 – 6, 18 – 24, 120 – 126, and 138 – 144 h after exposure, respectively).

[2] Pooled standard error.

[3] Degrees of freedom associated with each F statistic are reported in parentheses.

[4] Bout characteristics based on 2 pooled inter-bout criteria calculated from 0 – 6 and 18 – 24 h of exposure (periods 1 and 2, when the brushes were most novel) vs. 120 – 126 and 138 – 144 h of exposure (periods 3 and 4).

[abc] Superscripts that differ within row indicate significant differences ($p < 0.05$).

(a) (b)

Figure 1. Group-housed heifers were provided with either two or four wash brushes per pen, mounted on two opposite wooden plank walls of the pen. Brush-directed behavior was observed for all heifers via continuous video recordings and coded as (a) oral manipulation, defined as contact between the mouth or tongue and the brush and (b) grooming, defined as rubbing the head, neck, or body against the brush.

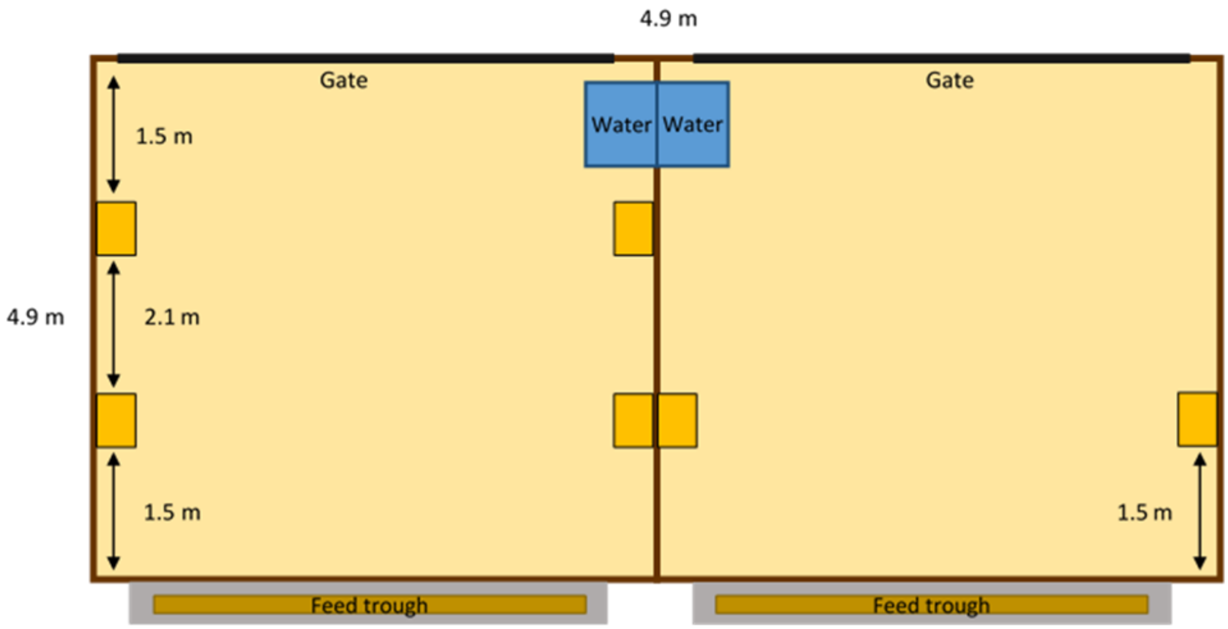


Figure 2. Pen diagram for each of the two brush treatments, providing two or four brushes to group-housed, naïve heifers. The yellow boxes represent an individual brush. Solid wooden walls separated the 4 pens in the barn. Note: This diagram is not drawn to scale.

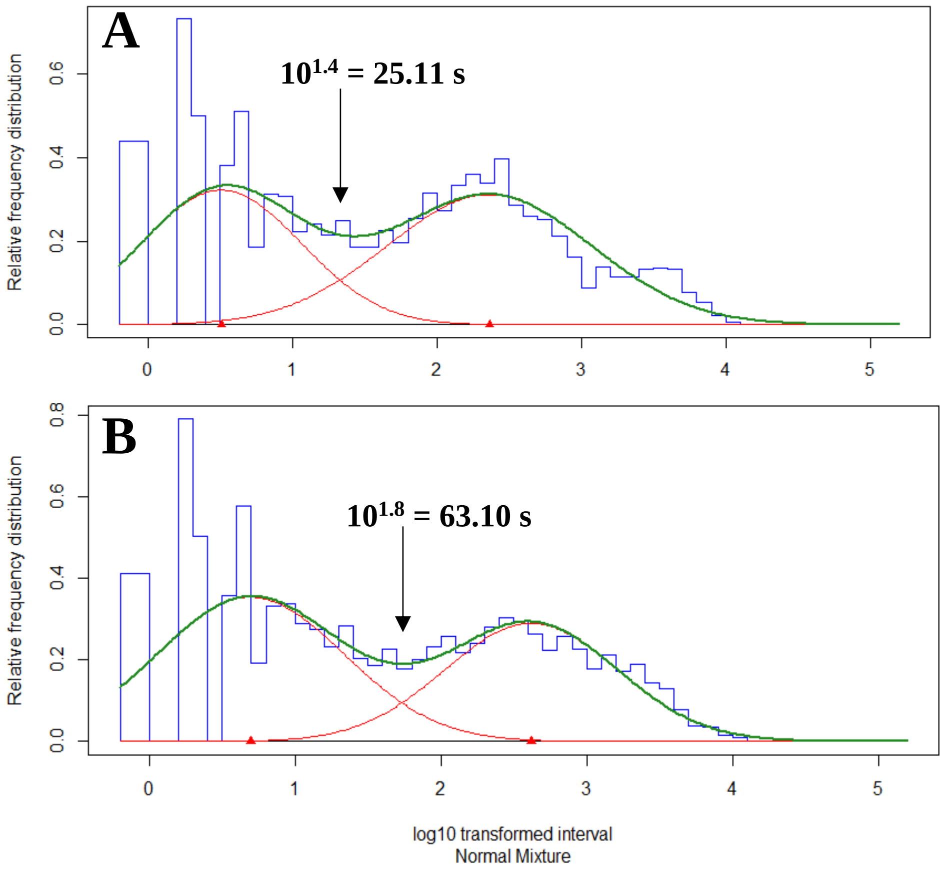


Figure 3. Log_{10}-transformed relative frequency distributions of the intervals between the events of heifers using stationary brushes, fitted with mixture normal distributions. Data presented are summarized for the (A) novel period (0–6 and 18–24 h of exposure, pooled across 63 heifers) and (B) non-novel period (120–126 and 138–144 h of exposure, pooled across 55 heifers). The blue bars represent the frequency of each log_{10}-transformed inter-bout interval. The red lines represent the contribution of individual distributions to the overall probability density (green line).

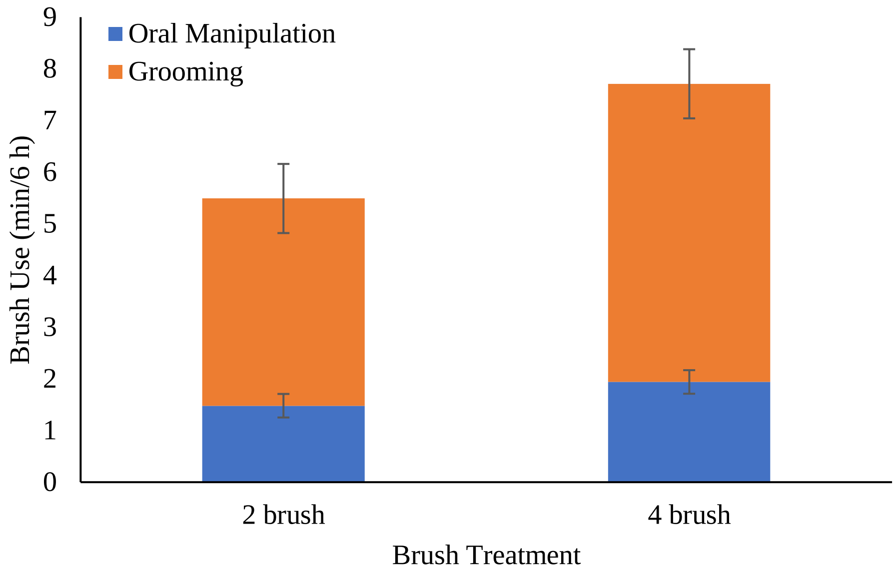


Figure 4. The mean ± SE duration of brush use for oral manipulation and grooming, averaged per 6 h period of observation, for each brush treatment (2 vs. 4 brushes provided to 7 to 8 group-housed heifers). The error bars represent the standard error of the means.

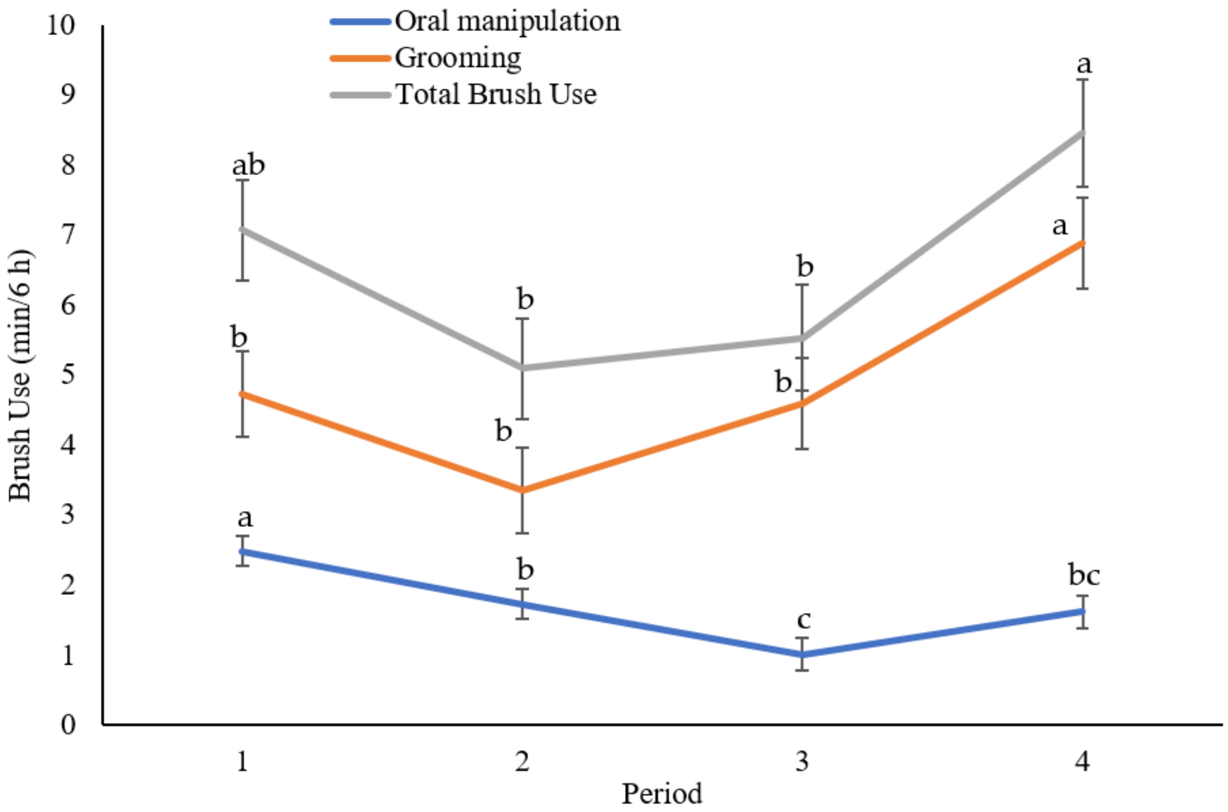


Figure 5. The mean ± SE duration of oral manipulation, grooming, and total brush use (the sum of the two aforementioned subsets) for each observed period (periods 1–4: 0–6, 18–24, 120–126, and 138–144 h after exposure, respectively), regardless of brush treatment. Letters within the same-colored line indicate significant ($p < 0.05$) pairwise differences between periods.

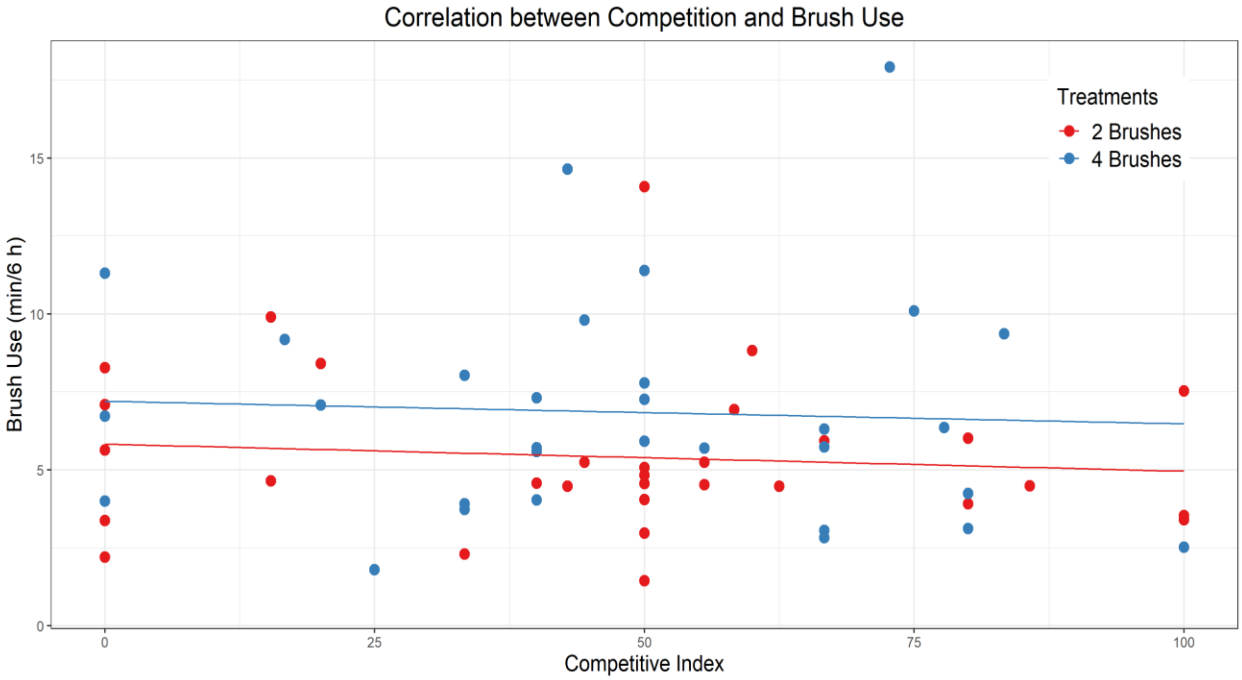


Figure 6. Correlation between individual heifer competitive index value (calculated as frequency of initiated displacements (actor role) divided by the total number of displacements she was involved in (actor and receiver roles), multiplied by 100) and the duration of brush use (average per 6 h period). One heifer in the 4-brush treatment was uninvolved in displacements and was excluded. Pearson correlations were performed regardless of treatment (R = −0.07, p = 0.61) and within each treatment (2-brush: R = −0.10, p = 0.58; 4-brush: R = −0.05, p = 0.78).

CHAPTER 6: GENERAL CONCLUSIONS

Dairy cattle are often housed and managed in a group environment that involves competition for resources. Social dynamics play a complex and important role in the interactions that occur between individuals in a group. Understanding the implications of resource competition and grouping strategies in dairy cattle is an important topic for improving animal welfare and production efficiency.

Research presented in Chapter 2 interrogated the impact of grouping strategies, involving the interaction of parity and group composition, on competition behavior, feeding patterns, and feed efficiency in lactating cows. Cows in mixed-parity groups, compared to those in same-parity ones, were involved in more competition at the feed bunk, exhibited greater total daily eating time, and tended to be less feed efficient. Novel ratios were calculated using successful attempts to show an actor's success at translating a physical contact into a displacement and replacement, as well as with unsuccessful attempts to show a receiver's success at resisting a potential displacement. These ratios provided the opportunity to gain a more comprehensive understanding of all competitive interactions and different rates of success. Parity and group composition combinations did not impact these ratios regarding the success rate but highlighted high individual cow variation in the resulting success for cows to displace one another or resist displacement. Furthermore, competitive interactions were reported by parity within the mixed-parity group, which provided innovative data to descriptively highlight that more interactions occur between inter-parity dyads compared to same-parity dyads, suggesting that competitive strategies may differ depending on the individuals involved. The first social network with competitive contacts between dairy cattle was constructed using both successful and unsuccessful interactions, adding further understanding into individual involvement in a competitive, group environment. Overall, these findings provided insight into potentially negative behavioral and efficiency implications of mixed-parity feed bunk grouping for lactating dairy cows, but further research with separate grouping strategies applied on the pen-level would be needed to best represent management practices used on dairy farms.

Research presented in Chapter 3 provided lactating cows with a choice between social dynamics (same- vs. mixed-parity bin types) at the feed bunk. This novel approach showed that primiparous and multiparous cows visited both bin types equally. Yet, primiparous cows tended to visit their same-parity bin sooner after fresh feed delivery than the mixed-parity bin, as well as tended to be involved in more competition, and ate faster at their same-parity bin compared to the mixed-parity bin. Primiparous cows who visited the same-parity bin more often than the mixed-parity bin also tended to be less feed efficient. This potentially suggested that primiparous cows may have used strategies to avoid direct competition with multiparous cows that led to unintended consequences in the same-parity dynamic at the feed bunk. On the contrary, multiparous cows showed similar competition and feeding patterns between same- and mixed-parity bin dynamics at the feed bunk. An intriguing exception was that multiparous cows resisted displacement (stood their ground) at the feed bunk more often at the mixed-parity bins compared to the same-parity bin. These findings contributed to our understanding of social dynamic preferences and possible parity differences in strategy utilization to gain access to feed.

Correlations were computed in Chapters 2 and 3 to provide information about the associational intersections between competition behavior, feeding patterns, and feed efficiency at the individual cow level. Cows that visited the bunk sooner after fresh feed delivery and consumed more feed in this first visit tended to be less feed efficient. These interesting outcomes provide support for continuing investigation that can aid in our understanding of individual cow variation.

Considering our findings in Chapter 2 and 3, several questions and areas for advancement in knowledge still remain. In regards to RFI, this dissertation demonstrated the potential for variation to be partially explained by competition for resources as shown by differences in grouping strategies based on parity and group composition. As previously cited, several physiological mechanisms have been identified as potential contributors to the variation in RFI: 37% protein turnover, tissue metabolism, and stress; 10% digestibility; 10% activity; 9% heat increment of fermentation; 5% body composition, 2% feeding patterns, and 27% other. Competition for resources may contribute to RFI variation in the following categories: feeding patterns, activity, and protein turnover, tissue metabolism, and stress. Both Chapters 2 and 3 showed associations with feed efficiency and feeding patterns, yet the category of feeding patterns is only allocated to approximately 2% of variation. Nonetheless, this contribution in our knowledge toward advancing our understanding of feeding patterns was beneficial, especially replicating associations with eating rate as shown in other studies. Yet, other mechanisms may have larger contributions on a proportion of variation basis (i.e. activity, stress, and other). Therefore, investigation should continue to evaluate activity levels via individual cow involvement in competition behavior at the feed bunk.

Thus far, Chapter 2 and 3 reported tendencies showing less feed efficiency (lower RFI) was associated with cows involved in more competition. Specific interrogation of physiological mechanisms related to energy expenditure in competitive environments may provide more understanding in this area. In addition, individuals involved in highly competitive environments, particularly those that may rank lower in dominance (i.e. lower body weight, younger in age), could be experiencing and perceiving stress differently. Quantifying metabolites related to stress and hunger could provide additional insight on other factors contributing to variation in feed efficiency. In addition, parity has been shown to play a role in social dynamics and feed efficiency. Residual feed intake values range from high to low feed efficiency across both parities; therefore, critical focus on individual strategies and attributes may be necessary to ascertain other factors that play a role in varying feed efficiency.

Potential differentiation in individual cow strategies observed in a competitive feed bunk environment in Chapters 2 and 3 elicited further inquiry to disentangle inter-individual behavioral variation and associated implications. Research presented in Chapter 4 elucidated the impact of feed bunk stocking density on competition behavior and feeding patterns, along with the association between intra-individual behavioral consistency and feed efficiency. Three stocking densities were compared: 1 cow: 1 bin, 2:1 and 4:1. Cows were involved in the greatest competition at the intermediate (2:1) stocking density at the feed bunk. As stocking density increased, cows modified their feeding patterns to partially compensate for reduced access to feed resources. Intra-individual behavioral consistency highlighted the complexities of individual strategy and behavioral responses in that less feed efficient cows were less consistent in competitive behavior, but more consistent in eating rate across stocking densities. Behavioral consistency or plasticity may have benefits for certain individuals, depending on the environment. Future research should continue to investigate the relationship between feed efficiency and intra-individual behavioral consistency under different management environments. This knowledge would contribute to an improved understanding of behavioral strategies and potential implementation of beneficial management practices.

Another future focus that shows promise is expanding the investigation on competition behavior and cows’ roles within these interactions on an hourly basis across an entire day. Recent work from peers in this research group highlighted temporal patterns related to bin visits in 2-h increments. Hours immediately following fresh feed delivery and/or return from the milking parlor are commonly used for competition behavior due to these being peak times where high magnitudes are observed. These high magnitudes are beneficial for understanding what takes place during these high-traffic times at the feed bunk, but literature has also shown that cows are known to adjust their strategies throughout the day to ensure ample intake. Perhaps, focusing on the temporal relationships with competitive interactions, specifically the number of visits that involve competitive interactions (i.e. require competing for resource access) vs. those that do not, could add clarity to this topic. One caveat to this approach is the severe limitation of feasibility for behavioral observation via continuous video. A validated method using electronic Roughage Intake Control (RIC) bin data has been previously used, but is limited to only collecting replacements. This dissertation collected behavior via video observation using multiple behaviors, which highlighted the importance of evaluating the comprehensive behavioral sequence that occurs. Continuous observation is considered the gold standard, so other methods would have to be validated (i.e. computer vision) to ensure robust data collection.

An important consideration for RFI is that the values are inherently related to the sample in which the regression was calculated. Values are not directly comparable between studies, but descriptively observing ranges is still interesting. For example, Chapters 2 – 4 involve RFI values that were similar to other studies conducted in a similar environment at the standard 2:1 stocking density. To aid in comparison, other feed efficiency metrics could be calculated from already existing datasets. Future studies should also consider continuing to quantifying the economic contribution from feed efficiency variation in relation to the long-term management practice and genetic selection impacts.

Regarding practical application of these findings, critical inquiry elicits the question: "how should cows be grouped on-farm?" Chapters 2 and 3 illustrate behavioral and feed efficiency implications of certain grouping strategies that could suggest that grouping cows separately by parity may have benefits. Regardless of grouping strategy, social dynamics will continue to exist within a group-housed dynamic, but practices can be implemented with a goal in mind to reduce excessive competition for resources and enhance benefits of social housing. An important caveat is that this dissertation was unable to evaluate all possible aspects that factor into multi-faceted management practice decisions. Ultimately, an ideal study would have the capability to consider and robustly evaluate all on-farm factors, but this is obviously limited by feasibility. It is worth noting that studies in Chapters 2 – 4 involved very few (if any) health related events, all of which were unrelated to study-assigned treatments. Furthermore, cows enrolled in these studies were also very high producing and remained at high production levels throughout. Each farm makes decisions on a cost-benefit analysis that serves to balance these aforementioned factors; certain priorities may be ranked differently for each farm. Studies with comprehensive datasets, including continuing to use data from the studies herein, provide avenues to evaluate other factors, particularly those related to the economic component, that can assist producers in making these management decisions on-farm.

Chapter 4 strengthened the industry suggestion that cows should be stocked at lower feed bunk densities, particularly targeting 1:1 for ample opportunity for resource access. Intra-individual behavioral consistency showed the complexities of social dynamics. The metric used in this chapter with ISS scores quantified consistency regardless of the directionality on that specific variable. Cows may benefit from consistency in one direction more compared to the other. For example, individuals more consistent in eating at a slower rate may benefit more so (i.e. potentially more feed efficiency) than maintaining consistency across stocking densities with a faster eating rate. The resolution of only 1-h tests provided novel interpretation for behavioral consistency across a short period of time. As mentioned previously, the tendency for cows to change strategies or adjust their behavior throughout a day shows that there is benefit in evaluating behavioral consistency across stocking densities for longer periods of time. In addition, existing literature has shown intriguing impacts of previous experiences in social housing and exposure to different environments on cattle as they age and change environments throughout the industry's modern management systems. Previous exposure to different social dynamics and stocking densities could also impact individual cow strategies and behavioral consistency; an area that continued research could interrogate.

While the first chapters focused on feed bunk resources, cows also compete over different resources within group-housed settings. Research presented in Chapter 5 was the first evaluation of different brush quantities on brush use in any age of cattle. Contrary to predictions, stationary brush quantity (2 vs. 4 brushes for 8 heifers) did not impact the overall duration of brush use or competition. However, when provided more brushes, heifers exhibited longer brush use bouts, which suggested that the provision of four brushes to a group of eight heifers provided greater opportunity for uninterrupted use. Heifers used brushes to express natural behaviors of both grooming and oral manipulation. These findings provided a practical option for producers to implement brushes for dairy heifers to allow for natural behavior and promote positive animal welfare. Additional research should compare brush use and competition using other brush quantities for both stationary and mechanical, rotating brushes to continue to inform resource provision on-farm.

Altogether, this dissertation demonstrated the important considerations of resource provision to minimize excessive competition and enhance the benefits of social group housing for dairy cattle. In lactating cows, Chapters 2, 3, and 4 illustrated that social dynamics and higher stocking densities at the feed bunk have important implications for competition behavior, feeding patterns and feed efficiency. In weaned heifers, Chapter 5 showed that brushes are a practical option that can be provided as a resource to allow natural behavior. Overall, these findings contribute to our knowledge about resource competition that inform grouping strategies on-farm and assist with continuous progress toward improving animal welfare, production, efficiency, and sustainability for the future.

APPENDICES

APPENDIX 1. Chapter 2 Supplemental

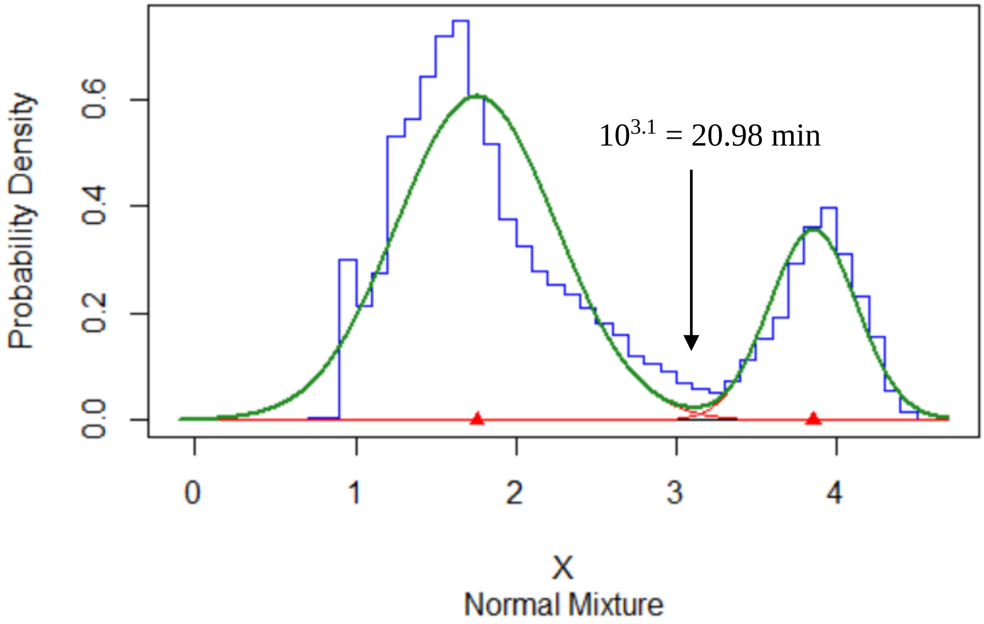
Table 1. Proportion of competition behaviors excluded[1] from analysis by network type[2]

	Excluded out-of-network behavior	
Network Type	Competitive Contacts	Displacements
SM-PR	37.9% (229/605)	18.4% (53/287)
SM-MU	37.1% (300/808)	26.9% (104/387)
MX	29.4% (283/964)	13.9% (74/533)
Total	34.2% (812/2377)	19.1% (231/1207)

[1] Competitive interactions at the feed bunk occurring between 2 cows in different networks (i.e., assigned to different sets of bins) were excluded from the analysis.

[2] Networks of 10 cows sharing sets of bins were created to evaluate parity (PR: primiparous, MU: multiparous) and group composition (SM: same-parity, MX: mixed-parity) combinations; two networks of each type.

Figure 1. Log_{10}-transformed relative frequency distributions of the intervals between the visits at the feed bunk for mid-lactation Holstein cows, fitted with mixture normal distributions. The blue bars represent the frequency of each $\log_{10}$-transformed inter-visit interval. The red lines represent the contribution of individual distributions to the overall probability density (green line).



Training Period Methodology

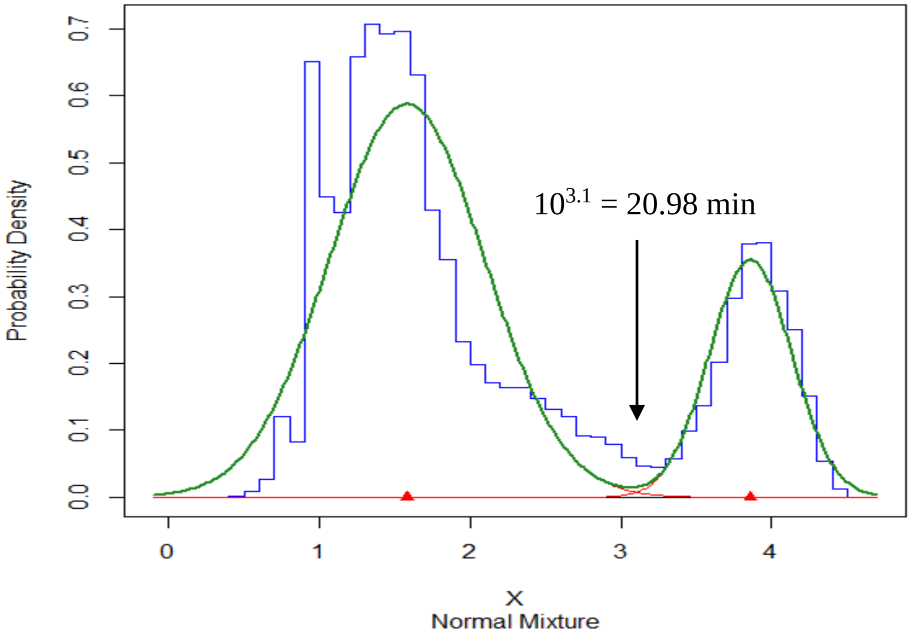
Training cows to eat from specifically assigned sets of 5 RIC feeding bins took place over a 4-wk period. Twenty-two of the MU cows had previous experience with the RIC system. Individual cow intake data from the RIC system was monitored throughout the entire training period to ensure that each cow consumed a minimum amount of feed (10 kg/d as fed during wk 1, 15 kg/d after wk 1) and had at least one 5 min visit at 1 or more of 5 assigned bins.

For the first week, all cows had access to all 30 functional bins for general acclimation to the system. Next, cows were trained to their assigned 5 bins by network. Bin training occurred for 2-3 consecutive d immediately after feed delivery (0900 h or 1500 h) during wk 2, 3, and 4 of the training period. All cows were trained for 10 min daily, with 4 vs. 2 groups trained in the morning vs. afternoon, respectively. The training order was randomized to ensure all cows were trained at both times of day. All cows were locked away from the feed bunk during training, with 10 cows from a single network sorted in a holding pen before the training session began. Each network was trained in 2 separate groups of 5 cows for feasibility and handling ease. These subgroups were the first 5 cows randomly sorted out of the group of 10 in the holding pen. The 5 assigned bins were manually opened and the remaining bins were closed to guide cows to the correct bins. Each network was randomly assigned a unique, colored shape painted onto corrugated plastic signs hung above their assigned bins for the duration of the trial. During training sessions, 2 additional matching signs were attached to both sides of each bin, visible to the cow as she placed her head into the bin to serve as an additional visual cue to assist in learning her assigned bins. Fresh feed was delivered in only the assigned bins immediately before each small group of 5 cows was trained as another cue to encourage cows to seek bins with accessible feed. Two observers recorded each bin visit for each cow in the training group for the duration of the 10-min training session. If cows did not access all 5 assigned bins, they were required to access at least one bin that had not been previously accessed during subsequent training sessions, with observers guiding if necessary using low-stress handling techniques.

During the second week of bin training, outside of the training sessions, cows had access to their 5 assigned bins and 1 additional bin on each side of each assigned bin, resulting in a total of 15 accessible bins. Once all 5 assigned bins were accessed at least once during training, training sessions focused on the least-used bin according to daily intake data. After this, cows only had access to their 5 assigned bins at all times. By wk 4, all cows had accessed all 5 bins during previous training sessions. The second (and final) training day that week only involved those 34 cows (in groups of 2-5 for 5 min) with >20% of daily attempted bin visits to non-assigned bins. Attempted visits to non-assigned bins were discouraged because this behavior reflected a lack of discrimination and could also create undue competition outside of assigned bins. Cows were considered to be trained to their assigned bins once <20% of daily attempted bin visits were directed to non-assigned bins.

APPENDIX 2. Chapter 3 Supplemental

Figure 1. Log_{10}-transformed relative frequency distributions of the intervals between the visits at the feed bunk for mid-lactation Holstein cows, fitted with mixture normal distributions. The blue bars represent the frequency of each log_{10}-transformed inter-visit interval. The red lines represent the contribution of individual distributions to the overall probability density (green line).



Training Period Methodology

Training cows to eat from specifically assigned pairs of RIC feeding bins took place over a 2-wk period. Twenty-two of the multiparous cows had previous experience with the RIC system. For the first week, all cows had access to all 28 functional bins for general acclimation to the system. Individual cow intake data from the RIC system was monitored throughout the entire training period to ensure that each cow consumed a minimum amount of feed (10 kg/d as fed during wk 1, 15 kg/d after wk 1) and had at least one 5-min visit/d at each of her 2 assigned bins.

The following week, cows were trained to their assigned 2 bins by cohort for 10 min/d on 3 consecutive d immediately after AM feed delivery (0900 h). The daily training order was randomized. All cows were locked away from the feed bunk during training, with 4 cows from 2 cohorts (with no shared bins) sorted for training at a time. Yellow caution tape was spanning between the feed bunk and the metal support for the resting stalls was used to separate the 2 cohorts during training. During d 1, the 2 assigned bins for each cohort were manually opened and the remaining bins were closed to guide cows to the correct bins. During d 2 and 3, the bins were set to automatic mode, which closed all bins, and cows had to use their eID ear tags to open their assigned bins.

Each cohort was randomly assigned a unique, colored shape painted onto corrugated plastic signs hung above their assigned bins for the duration of the trial. Due to the overlapping bin assignments, each plastic sign above each bin contained the two unique, colored shapes pertaining to the two cohorts that were able to access that bin. During training sessions, 2 additional matching signs were attached to both sides of each bin, visible to the cow as she placed her head into the bin, to serve as an additional visual cue to assist in learning her assigned bins. Fresh feed was delivered in only the assigned bins immediately before each group was trained as another cue to encourage cows to seek bins with accessible feed. Two observers recorded each bin visit for each cow in the training group for the duration of the 10-min training session. If cows did not access both assigned bins, observers guided them using low-stress handling techniques.

Outside of the training sessions, cows had access to only their 2 assigned bins. Once both assigned bins were accessed at least once during training, training sessions focused on the lesser-used bin according to daily intake data. Cows were considered trained to their assigned bins once <20% of daily attempted bin visits were directed to non-assigned bins; we aimed to minimize such attempts, which reflected a lack of discrimination and could also create undue competition outside of assigned bins.

APPENDIX 3. Chapter 4 Supplemental

Table 1. Calculated ingredient composition and nutrient analysis of the diet fed to primiparous and multiparous mid-lactation Holstein cows

Item, % of DM	Mean
Ingredient composition	
Alfalfa haylage	22.42 ± 1.10
Corn silage	27.42 ± 1.45
Distillers grain	2.26 ± 0.09
Cottonseed	4.86 ± 0.08
Ground corn grain	13.81 ± 0.27
Protein mineral mix[1]	29.22 ± 0.51
Nutrient analysis	
DM, % as fed	51.77
OM	92.32
CP	18.10
NDF	26.17
ADF	21.97
Lignin	4.09
NFC	44.65
Starch	27.27
Fat	4.49
NE_L 3x[2]	1.61

[1] Protein mineral mix was formulated on an as-fed basis to contain fine ground corn (26.33%), soy hull pellet (18.09%), 46% CP soybean meal (17.22%), canola meal (15.60%), SoyPlus (11.60%, Landus Cooperative), calcium carbonate (4.62%), sodium bicarbonate (2.50%), trace mineral salt (1.25%), magnesium oxide (0.82%), urea (0.62%), potassium carbonate (0.30%), Celmanax Dry (0.30%, Arm & Hammer), grease (0.25%), Smartamine M (0.20%, Adisseo), DynaMate (0.15%, The Mosaic Company), and Fortress LG (0.16%, VitaPlus).

[2] Estimated from the NASEM (2022) equations to calculate NE_L at 3× maintenance.

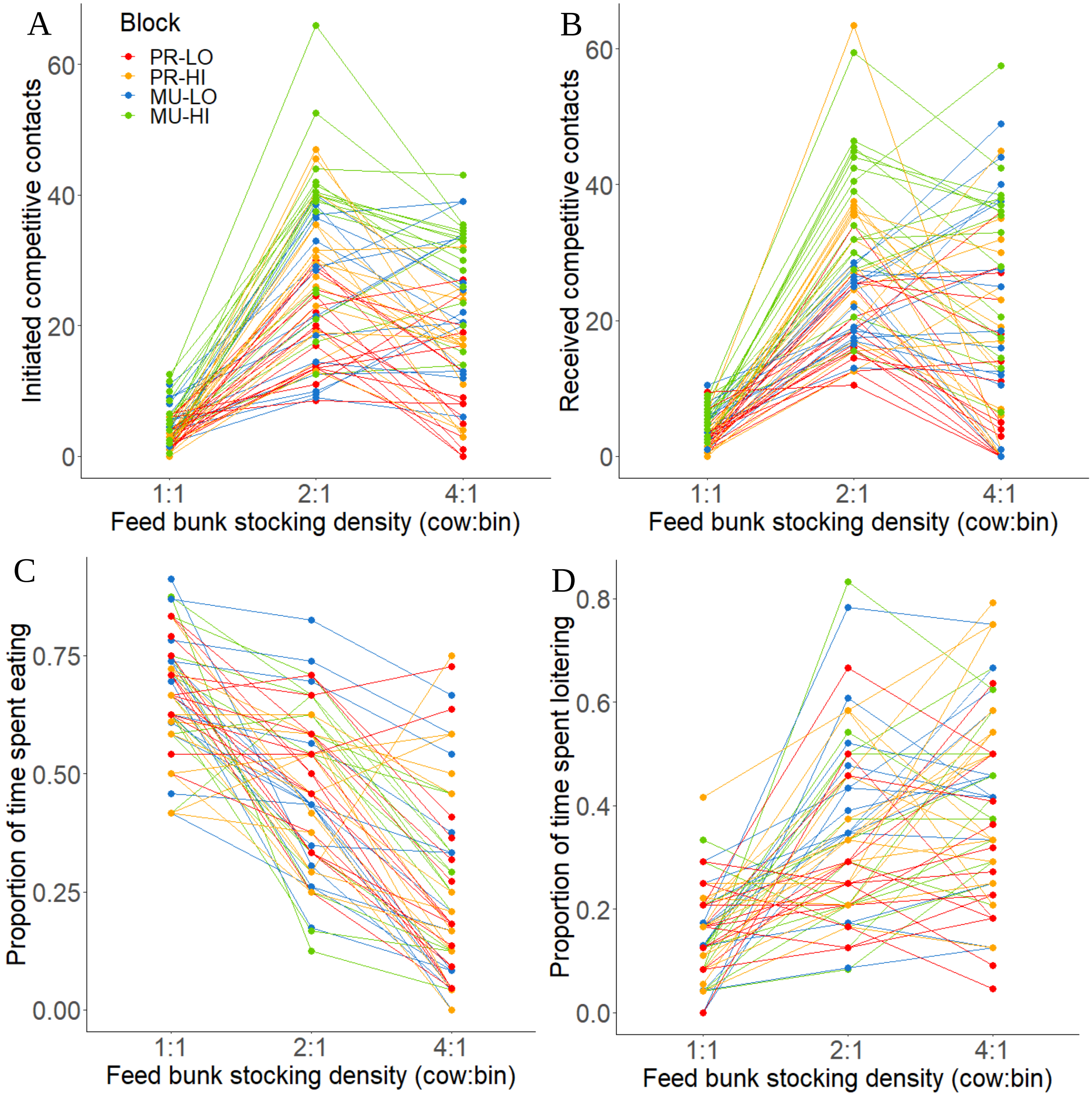
Table 2. Daily intake comparison[1] between test day vs. non-test day intakes (on an as-fed basis) for mid-lactation Holstein cows.

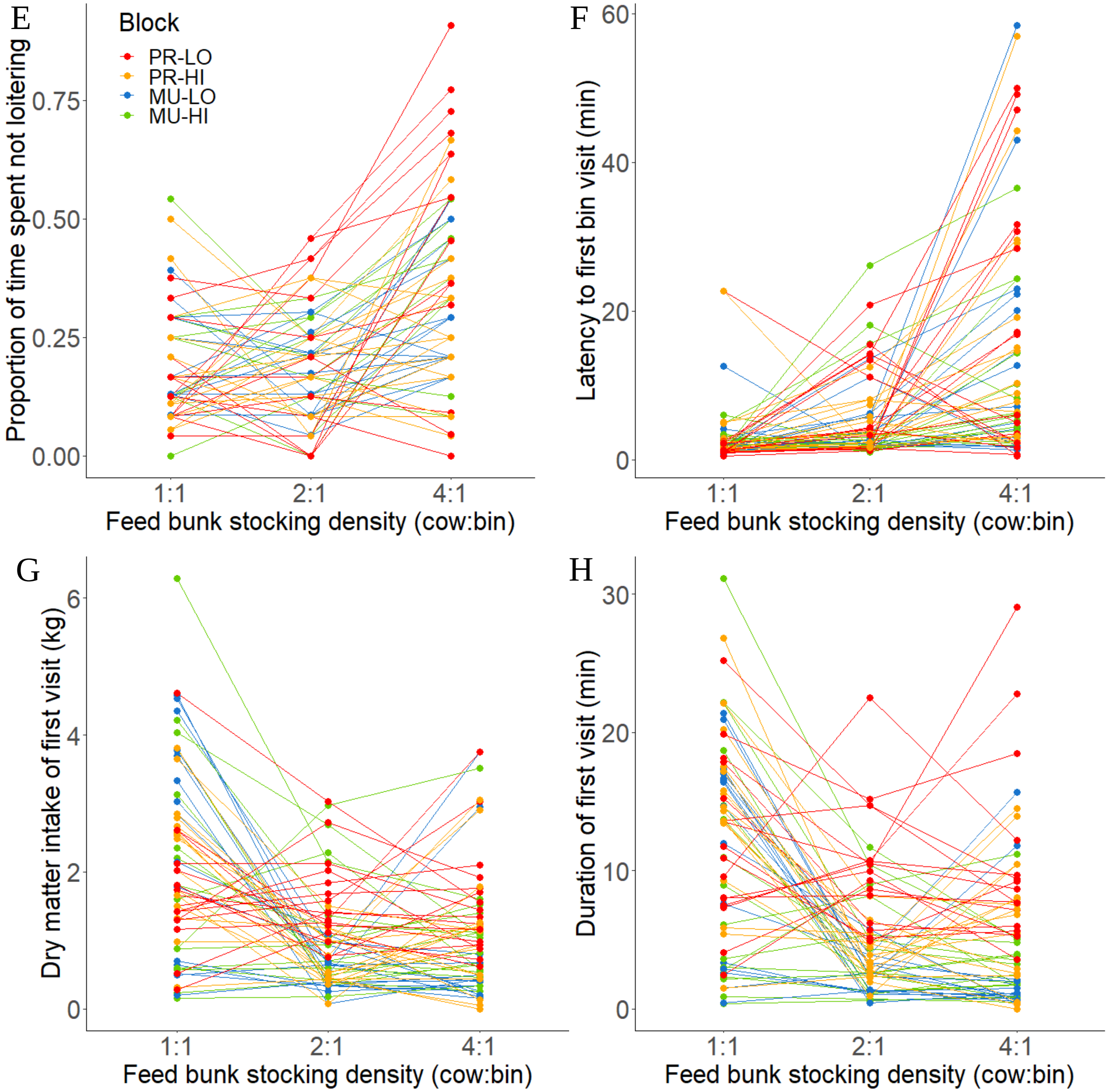
	Intake Type		
Block[2]	Test Day Intake	Non-Test Day Intake	*P*-value
PR-LO			
All cows	47.3 ± 1.1	46.9 ± 0.9	0.36
Subgroup A	49.1 ± 1.6	46.9 ± 1.6	0.0002
Subgroup B	45.6 ± 1.2	46.8 ± 1.0	0.026
PR-HI			
All cows	51.8 ± 1.0	52.6 ± 0.9	0.36
Subgroup A	51.9 ± 1.5	51.6 ± 1.4	0.49
Subgroup B	51.7 ± 1.3	53.8 ± 1.2	0.18
MU-LO			
All cows	61.9 ± 1.2	61.8 ± 1.1	0.89
Subgroup A	63.9 ± 1.4	62.4 ± 1.5	0.33
Subgroup B	59.9 ± 1.9	61.1 ± 1.6	0.25
MU-HI			
All cows	65.3 ± 1.8	64.9 ± 1.4	0.71
Subgroup A	64.1 ± 2.2	65.9 ± 1.9	0.18
Subgroup B	66.5 ± 2.9	64.0 ± 2.1	0.16

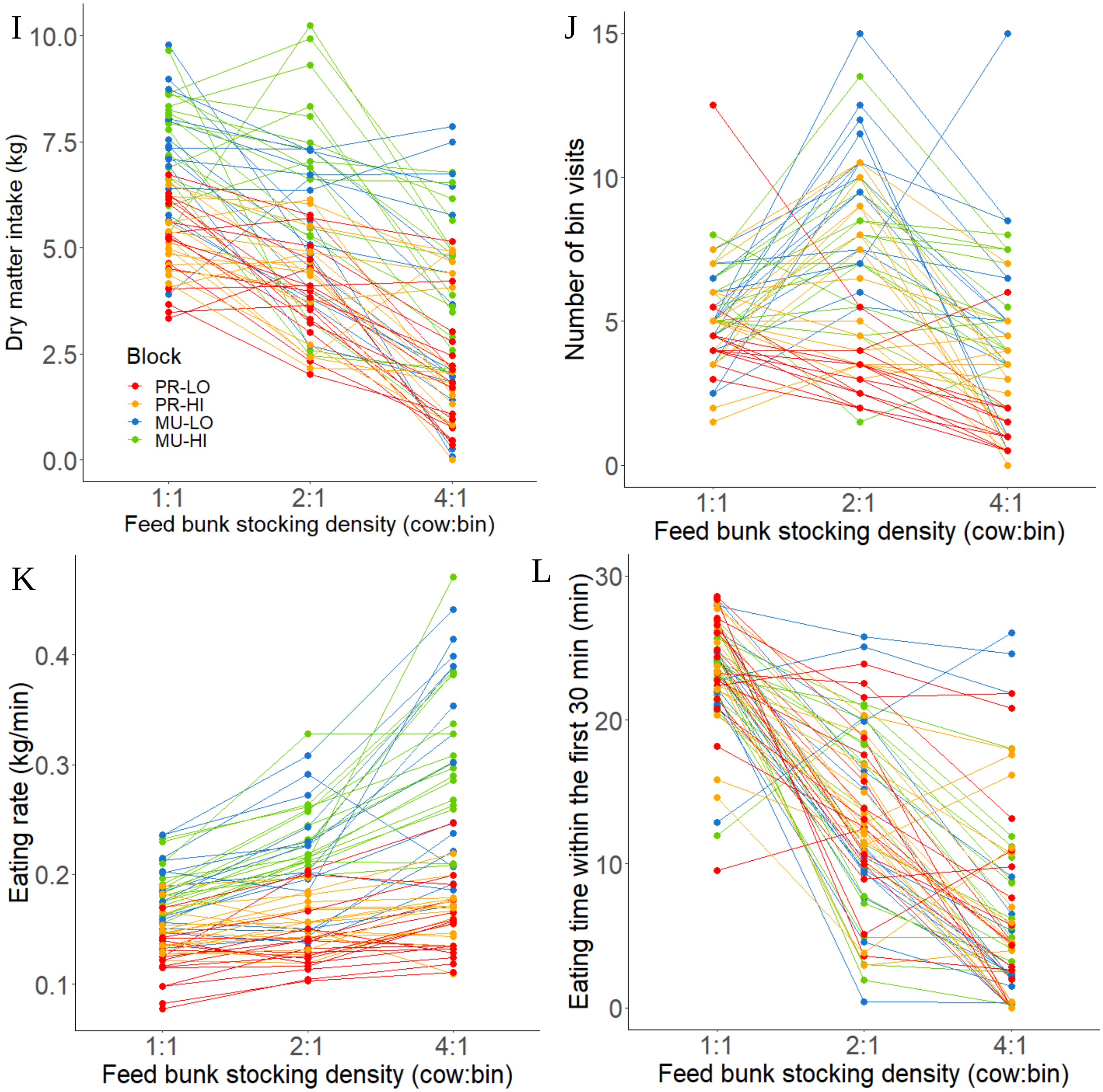
[1] Means and SE reported from a simple pairwise t-test.

[2] Cows were assigned to blocks of 16 cows each by combination of parity (PR: primiparous or MU: multiparous) and BW (LO: low BW or HI: high BW); MU-LO had only 15 cows after removal due to illness unrelated to the study. Within block, cows were randomly assigned to two subgroups of 8 cows each for 1:1 tests.

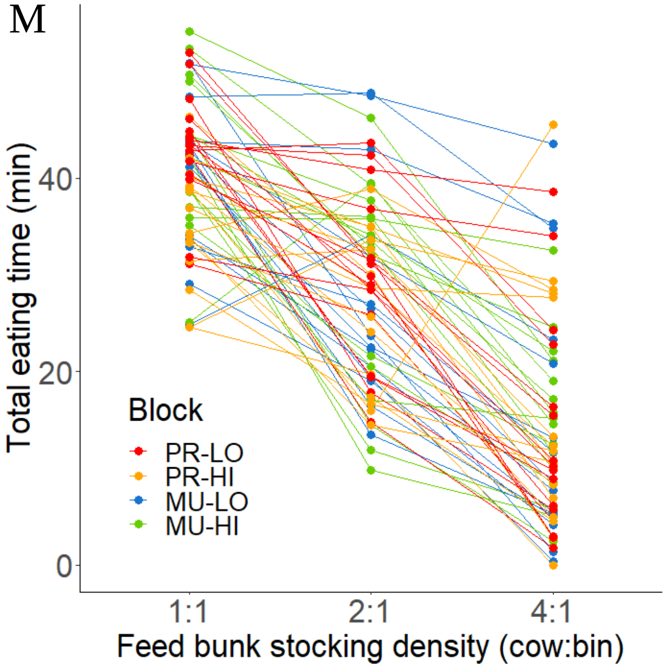
Figure 1. Descriptive behavioral reaction norms showing individual mid-lactation Holstein cows' responses of competition behavior (A-E) and feeding patterns (F-M) to changes in feed bunk stocking density (1 cow: 1 bin, 2:1, or 4:1) in 1-h tests (average of 2 tests per stocking density). Cows were assigned to blocks of 16 cows each by combination of parity (PR: primiparous or MU: multiparous) and body weight (LO: low bodyweight or HI: high bodyweight); MU-LO had only 15 cows after removal due to illness unrelated to the study.




E
Block
PR-LO
PR-HI
MU-LO
MU-HI
Proportion of time spent not loitering
0.00
0.25
0.50
0.75
1:1
2:1
4:1
Feed bunk stocking density (cow:bin)
F
Latency to first bin visit (min)
0
20
40
60
1:1
2:1
4:1
Feed bunk stocking density (cow:bin)
G
Dry matter intake of first visit (kg)
0
2
4
6
1:1
2:1
4:1
Feed bunk stocking density (cow:bin)
H
Duration of first visit (min)
0
10
20
30
1:1
2:1
4:1
Feed bunk stocking density (cow:bin)


I
Dry matter intake (kg)
10.0
7.5
5.0
2.5
0.0
Block
PR-LO
PR-HI
MU-LO
MU-HI
1:1
2:1
4:1
Feed bunk stocking density (cow:bin)
J
Number of bin visits
15
10
5
0
1:1
2:1
4:1
Feed bunk stocking density (cow:bin)
K
Eating rate (kg/min)
0.4
0.3
0.2
0.1
1:1
2:1
4:1
Feed bunk stocking density (cow:bin)
L
Eating time within the first 30 min (min)
30
20
10
0
1:1
2:1
4:1
Feed bunk stocking density (cow:bin)

M


Total eating time (min)
0
20
40
Block
PR-LO
PR-HI
MU-LO
MU-HI
1:1
2:1
4:1
Feed bunk stocking density (cow:bin)

Training Period Methodology

Training cows to eat from specifically assigned 8 RIC feeding bins took place over a 1-wk period. All of the multiparous cows had previous experience with the RIC system, while none of the primiparous cows had previous experience. For the first three days, all cows had access to all 32 functional bins for general acclimation to the system. Individual cow intake data from the RIC system was monitored throughout the entire training period to ensure that each cow consumed a minimum amount of feed (20 kg/d as fed during wk 1) and had at least one 5-min visit/d for at least 1 of 8 assigned bins.

On the fourth day of the training week, cows were trained to their assigned 8 bins by block for three 10 min/training session immediately after AM feed delivery (0900 h). Each block was randomly assigned a unique, colored shape painted onto corrugated plastic signs hung above their assigned bins for the duration of the trial. During training sessions, 2 additional matching signs were attached to both sides of each bin, visible to the cow as she placed her head into the bin to serve as an additional visual cue to assist in learning her assigned bins. Fresh feed was delivered in only the assigned bins immediately before each block of cows was trained as another cue to encourage cows to seek bins with accessible feed. The training order was randomized to expose each block to each of the competitive tests (1 cow:1 bin, 2:1, and 4:1) to be used during the experimental period of the trial. For each 1:1 test, the block was divided into 2 groups of 8 cows, so that only 1 group of 8 cows was tested at once. All cows were locked away from the feed bunk during training, with the exception of 8 or 16 cows from the particular block sorted for training at that time. The feed bunk was divided in half (16 bins/half) by a temporary gate panel. Each group to be trained was provided access to only the half of the feed bunk where their assigned bins were located. Yellow caution tape was spanning across the 8 bins that were not assigned to the group being trained to serve as a visual deterrent to minimize attempts to open an unassigned bin. For the 4:1 tests, caution tape was spanning across 4 bins of the 8 assigned bins (2 bins on each end of the 8 consecutive assigned bins) and were manually closed and deemed inaccessible during the training session. During the first training session for each group, the assigned bins were manually opened and the remaining bins were closed to guide cows to the correct bins. During the second and third session for each group, the bins were set to automatic mode, which closed all bins, and cows had to use their eID ear tags to open their assigned bins. Fresh feed was delivered in only the assigned bins immediately before each block of cows was trained as another cue to encourage cows to seek bins with accessible feed. Two observers recorded each bin visit for each cow in the training group for the duration of the 10-min training session.

Outside of the training sessions, cows had access to only their 8 assigned bins. Cows were considered trained to their assigned bins once < 30% of daily attempted bin visits were directed to non-assigned bins; we aimed to minimize such attempts, which reflected a lack of discrimination and could also create undue competition outside of assigned bins.