

ABSTRACT

KELLY, KATHERINE MARIE. Effects of Environmental Enrichment on Behaviors, Growth, and Stress in Limit-fed Holstein Heifers. (Under the direction of Dr. Stephanie H. Ward).

While limit fed heifers show improved feed efficiency, cost efficiency, and less manure output, the abnormal behaviors demonstrated by these heifers could be considered a welfare concern.

The use of environmental enrichment such as a grooming brush (**BR**) and calf ball (**BA**) could prevent abnormal behaviors and allow for the benefits of a limit fed system. Twenty-four Holsteins heifers (161.9 ± 33 d of age) were housed in 3 pens with either a BR, BA or no enrichment (**NO**). In each pen, heifers received either a high concentrate diet at 85% of DM offer to control group (**LIM**) or 100% (**CON**) of NRC (2001) predicted DMI for six weeks ($n=4$).

Weekly rumen samples, rumination events, blood samples, and body measures were taken.

Rumination events were recorded every 5 mins over 6 hours after feeding once a week.

Individual heifer behaviors such as feed aggression, visits to the feed bunk, use of enrichment, and play behavior were recorded for 24 hours each week. Lying time was recorded every d for six weeks using a leg band activity monitor. The MIXED procedure of SAS was used to analyze the effects of diet, enrichment, date, and all 2-way interactions for all models on body measures, rumen pH and VFA, rumination events, NEFA, and lying behaviors. Enrichment or diet did not affect the average daily gain. Heifers housed with a BR had decreased hip heights ($P = 0.03$) and a tendency for decreased heart girth ($P = 0.06$). Limit-fed heifers consumed less DMI compared with CON (0.70 kg/d DM, $P < 0.01$). However, there were no differences in feed to gain ratio.

There was a tendency for a diet and week interaction for NEFA ($P = 0.08$) where LIM heifers had increased NEFA during week 0 and 5. Control heifers with a BR or NO had 4 and 2.8 more rumination events compared with the LIM in those groups (respectively, $P < 0.01$). Rumen pH was decreased for LIM compared with CON (7.32 ± 0.04 vs. 7.06 ± 0.04 , respectively, $P < 0.01$).

Total VFA for the LIM group increased during week 4 and 5 compared with the CON ($P < 0.01$). Additionally, the molar proportion of acetate ($P = 0.02$), and propionate and butyrate ($P < 0.01$) increased for LIM heifers diet during week 4 and 5. The addition of BA increased play behavior by 8 mins per d ($P = 0.01$). Heifers, no matter the diet, spent 30 mins more using the BR than heifers using the BA ($P = 0.03$). LIM had more aggressive events at the feed bunk ($P < 0.01$) and a tendency for fewer visits to the feed bunk ($P = 0.08$). When the pen was enriched with a BA, LIM had 72 additional mins per d lying down compared with CON ($P = 0.01$). For LIM, the addition of a BR increased lying time by 50 mins per d ($P = 0.05$). The addition of environmental enrichment to heifer pens increased overall social play for both diets. This change in social play and lying time may contribute to increased natural behaviors in dairy heifers with the added benefit of reducing cost using the LIM diet.

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Effects of Environmental Enrichment on Behaviors, Growth, and Stress in Limit-fed Holstein Heifers.

by
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DEDICATION

This thesis is dedication to my family. Without the love and support of my mom, dad, sister and grandmothers, I would not be where I am today. Thank you for supporting my love of dairy cattle.

BIOGRAPHY

Katherine “Katie” Marie Kelly was born on April 30th, 1995 to Mary and Deron Kelly. She grew up in Amelia, Ohio which is about thirty minutes outside of Cincinnati. Katie grew up riding, showing, and owning horses since she was nine years old. After completing high school in 2013, she continued her education in Animal Science at the University of Kentucky.

With dreams of being a vet and little large animal experience, she volunteered with the dairy research group. She quickly fell in love with dairy cattle and the dairy industry, which led to the decision to drop the pre-vet focus and find a career within the dairy industry. During her junior and senior year, Katie developed and successfully completed an undergraduate project focused on trace mineral utilization in late gestation and early lactation dairy cows.

After completing her Bachelor of Animal Science, she found a masters assistantship with Dr. Stephanie Ward at NC State working with heifer development. At NC State, she focuses on helping with dairy extension programs and conducting research in nutritional behaviors in heifers, milk quality in North Carolina, and colostrum management in calves. She expects to obtain her Master of Science in Animal Science in Summer of 2019. Her thesis focuses on how environmental enrichment can affect the behaviors and development of heifers fed a limit-fed diet. After graduation, she plans on obtaining a career where she can communicate one on one with producers and help improve the lives of the animals under their care.

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TABLE OF CONTENTS

LIST OF TABLES	vii
LIST OF FIGURES	viii
Chapter 1: Literature Review	1
1.1 Introduction	1
1.2 Effects of heifer growth on future production	1
1.2.1 Reproductive Success	1
1.2.2 Mammary development and milk production	3
1.3 Current practices for feeding replacement heifers	5
1.4 Limit feeding strategy	8
1.5 Limit feeding and behavior change	11
1.6 Environmental enrichment	13
1.7 Conclusion	17
1.8 References	19
Chapter 2: Effects of environmental enrichment on behaviors, growth, and stress in limit fed Holstein heifers.	26
2.1 Abstract	26
2.2 Introduction	27
2.3 Methods	28
2.4 Results	34
2.5 Discussion	38
2.6 Conclusion	48
2.7 Tables	50
2.8 Figures	55
2.9 References	63

LIST OF TABLES

Table 1.1	NRC (2001) nutritional requirements for Holstein heifers 6 to 12 months of age with a BCS=3 and expected to calve at 24 mos age.....	50
Table 2.1	Ingredients and nutritional analysis of the limit fed and control diet fed to 24 Holstein heifers from mid-November to late December	51
Table 2.2	Ethogram of calf interactions with the brush and ball, play, and feeding behavior.....	52
Table 2.3	Physiological parameters for Holstein heifers consuming a limit-fed or control diet with pens containing a grooming brush, calf ball, or no enrichment from mid-November to late December 2018	53
Table 2.4	Nutrient intakes for Holstein heifers consuming a limit-fed or control diet with pens containing a grooming brush, calf ball, or no enrichment from mid-November to late December 2018	54

LIST OF FIGURES

Figure 2.1	Weekly a) body weight, b) body measures (hip height, wither height, and heart girth), and c) hip width for 24 Holstein heifers on a limit-fed (LIM) or control (CON) diet during mid-November to late December 2018.....	55
Figure 2.2	Weekly serum NEFA concentrations for 24 Holstein heifers on a limit-fed (LIM) or control (CON) diet during mid-November to late December 2018	56
Figure 2.3	Weekly rumination events for 24 Holstein heifers on a limit-fed (LIM) or control (CON) diet during mid-November to late December 2018	57
Figure 2.4	Rumen volatile fatty acids concentrations in 24 Holstein heifers consuming a limit-fed (LIM, solid lines; n=12) or control diet (CON, dashed lines; n=12).....	58
Figure 2.5	Rumen volatile fatty acids molar proportions in Holstein heifers consuming a limit-fed (LIM, solid lines; n=12) or control diet (CON, dashed lines; n=12).....	59
Figure 2.6	Effects of enrichment devices (grooming brush, calf ball or no enrichment) on lying times for 24 Holstein heifers on a limit-fed (LIM) or control (CON) diet during mid-November to late December 2018.....	60
Figure 2.7	Weekly a) social play events, b) mean social play time, and c) total social play time, such as a grooming brush (BR, red line, n=8), a calf ball (BA, black line, n=8), or no enrichment (NONE, grey line, n=8) in 24 Holstein heifers from mid-November to late December 2017	61
Figure 2.8	Weekly treatment enrichment interaction events, and b) visits to the feed bunk, such as a grooming brush (BR, red line, n=8), a calf ball (BA, black line, n=8), or no enrichment (NONE, grey line, n=8) in 24 Holstein heifers from mid-November to late December 2017	62

CHAPTER 1: Literature Review

1.1 Introduction

Replacement heifers are raised to be cost-efficient and environmentally conscience without compromising production. Raising replacement heifers on a dairy farm accounts for 20% of the farm's annual expenses (Heinrichs and Vazquez-Anon, 1993, Gabler et al., 2000). Sixty percent of the cost of raising replacement heifers is related to feed costs (Mourits et al., 1999, Heinrichs et al., 2017). Nutrition programs utilized during the heifer development stage can influence the future production of the heifer (Sejrsen, 1994), and thus has the potential to dictate profitability. Feeding strategies developed for stages from birth to breeding have been shown to affect not only the growth of the dairy heifer but can influence future milk yield (Zanton and Heinrichs, 2005, Heinrichs et al., 2017). Since bodyweight is correlated with puberty benchmarks for growth have been set. These benchmarks ensure optimal rearing cost and milk production in the first lactation (Heinrichs, 1993). Therefore, the goal of this review is to outline 1) the effects of prepubertal heifer growth and development on future production, 2) current practices for feeding prepubertal replacement heifers, 3) alternative feeding strategies (e.g., limit feeding), 4) associated behavioral changes with limit feeding, and 5) incorporation of environmental enrichment.

1.2. Effects of heifer growth on future performance

1.2.1. Reproductive Success

The goal of raising replacement heifers is to reduce the age at first calving (**AFC**). At this point, heifers should be around 85% of their mature BW (NRC, 2001). As of 2014, the average AFC across dairy breeds is approximately 25 months (mo) in the United States. Specifically, in Holsteins, an AFC under 27 mo and body weight (**BW**) around 540 to 650 kg of body weight at

calving, has been shown to increase economic efficiency (Lohakare et al., 2012, Heinrichs et al., 2017). When evaluating 305 d yield records and rearing cost per heifer, expenses are reduced by \$101.50 per head if AFC for Holsteins is reduced from 26 mo to 24 mo (Pirlo et al., 2000).

Another study that focused on milk production, health, and reproduction on three Holstein farms in California found that AFC above 24 mo increased rearing cost per heifer by \$67.55 (Ettema and Santos, 2004). When utilizing a dynamic programming model to evaluate optimal rearing strategies in heifers, it was determined that a prepubertal ADG of 900 g/day was needed to result in a AFC of 21.2 months at a BW of 541 kg for large breeds (Mourits et al., 1999). A study looking at the milk production and phenotypic records of 14 million cows demonstrated that decrease in AFC was correlated with greater fertility and increased milk yield (Hutchison et al., 2017). However, Hutchison et al. (2017) also showed that a decreased AFC was correlated with increased stillbirths in Holsteins. While decreasing AFC has been shown to reduce cost and improve milk yield, it is still important to note that some negative effects may occur with a continual decrease in AFC. A review by Van Amburgh et al. (2018) stated that heifers that have too light of a BW at calving will use more nutrients for growth instead of milk production, while over conditioned heifers will have similar postpartum metabolic responses as high BCS mature calves. Therefore, AFC may be more correlated with weight instead of age.

In order to reduce AFC, heifers must be bred at an earlier age. The onset of puberty has been shown to strongly correlate with BW rather than feeding level or age (Sejrsen, 1994, Macdonald et al., 2005). Large breed heifers, such as Holsteins, average 205 to 280 kg of BW around puberty (Macdonald et al., 2005), and this typically occurs at 9 to 11 mo of age (Lohakare et al., 2012). However, BW may not be adequately monitored by producers. For example, a survey conducted in the Netherlands observed that dairy producers depend on age for

first breeding (13 to 15 months), with only 17% responding that they rely on weight for determining when to breed. Only 74 out of 164 producers could produce the BW measured for post-weaned heifers. Furthermore, the previous survey found that only 14.7% followed a predefined growth strategy for post-weaned heifers (Mourits et al., 2000).

Inconsistent heifer management post-weaning can result in many heifers at the same age varying in BW. Since BW influences age at puberty, thus subsequently breeding age and AFC, it is essential that weight and average daily gain (ADG) be monitored for heifers from weaning to breeding. However, if this ADG is not properly monitored, either due to lack of facilities or employees to weigh heifers, then BW to achieve puberty would not be reached until a later age. Therefore, it is imperative that feeding strategies for prepubescent heifers focus on reaching breeding ADG and thus achieve body weights for breeding earlier without compromising milk production and lifetime productivity.

1.2.2. Mammary development and milk production

While accelerated growth reduces the age of puberty, increases in ADG caused by increased energy in diets may cause an excess of fat that could limit mammary development and future production. Van Amburgh et al. (1998) evaluated first lactation parameters in pre-pubertal Holstein heifers fed rations to achieve three different ADG (0.6, 0.8, and 1.0 kg/d). Heifers fed at a greater ADG showed an increase in BCS suggesting those heifers put on more fat than lean tissue, however, this did not appear to impact milk yield during the first lactation (Van Amburgh et al., 1998). Another study demonstrated accelerated growth rate in prepubertal heifers decreased first lactation fat-corrected milk yield by 7.1% (Lammers et al., 1999). The decrease in growth during these phases and increased pre-pubertal ADG can be related to increased fat deposits in the mammary system causing the limited amount of mammary development,

specifically decreases explicitly in the parenchyma tissues (Lohakare et al., 2012). The parenchyma tissue is the mammary tissue that contains the alveoli (milk secretion cells) and milk ducts. A reduction of parenchyma tissue has been shown to inhibit high milk production (Swanson, 1960). Additionally, accelerated growth demands more energy, which may take away from parenchyma growth (Hoffman and Funk, 1992). Heifers with increased body fat tended to have less parenchymal DNA compared with leaner heifers (Silva et al., 2002).

However, a symposium review by Van Amburgh et al. (2018) stated that heifers that calve within mature BW benchmarks (82 to 85%) will not have the negative effects on milk production no matter the AFC. Van Amburgh et al. (2018) explains that differences in BW in earlier studies could explain the milk yield and composition results. Meyer et al. (2006a) observed no differences in the parenchyma mass for prebuteral heifers fed a elevated or restricted level of nutrients when age at harvest was used as a covariate. Therefore, the parenchyma mass was indirectly affected by nutrient intake and elevated nutrient intake reduced the length of time the gland has to develop. In a companion paper by Meyer et al. (2006b), epithelial cells were not influenced by elevated nutrient intake and total parenchyma DNA.

Feeding rates can also influence milk production when fed at different stages of development. One study showed maximum first lactation milk production was achieved when prepubertal ADG was 799 g/d (Zanton and Heinrichs, 2005). Soberon et al. (2012) reported a 1,168 kg increase in first lactation milk production for every kg increase in ADG during preweaning phase. Heinrichs and Heinrichs (2011) found for every 1 kg increase in dry matter intake (DMI) at weaning; the first lactation 305-d ME milk production increased by 286.7 kg. A positive correlation for milk production also occurred when calves consumed more starter at a younger age (Heinrichs and Heinrichs, 2011). This is believed to be related to body size at

weaning, which could result in greater BW at calving depending on nutritional factors after weaning. Body weight must be accounted for when configuring a ration so that energy is not overfed and result in greater fat deposition (Van Amburgh et al., 2018).

1.3. Current practices for feeding replacement heifers

Nutrition programs for pre-pubertal heifers must address maintenance and growth needs without exceeding or losing too much BW gains. Table 1 depicts the NRC (2001) requirements for Holstein heifers around 6 to 12 months of age. It is important to note these recommendations are based off data from beef breeds. While proper heifer nutrition needs are being met, the feeding strategies used are not the most efficient.

Heifers from weaning to a year of age should receive most of their DMI through grain since their rumen is underdeveloped and cannot hold enough forage to maintain large amounts of growth (Heinrichs, 1996). Generally, heifers this young should be housed indoors as pasture will provide low DM content and not support growth (Heinrichs, 1996). Additionally, the intake of concentrate feeds should result in increased volatile fatty acid (VFA) production which stimulates faster rumen development (Tamate et al., 1962). When heifers are over a year of age, they have a fully-developed rumen and adequate rumen fermentation, meaning they can properly digest forages and grain supplementation is only needed depending on the forage quality (Council, 2001). A good forage hay option for heifers would be legumes or legume/ grass mixes, preferably the second or third cutting as they are palatable and of higher quality (Heinrichs, 1996).

Even for heifers over a year, pasture alone may not be able to meet the energy requirements for growth. Heifers 6 to 12 mo of age need around 0.9 to 1.8 kg of grain supplementation while on pasture (Heinrichs, 1996). Because of this, most heifers are not placed

on pasture, but rather in an open/dry lot or barn (USDA, 2014), however this varies across the United States. Heinrichs et al. (1987) revealed that 46.3% of Pennsylvania producers housed heifers on the pasture for most of the year, with 60% of producers supplementing 2.2 to 2.3 kg of a $15.9 \pm 3.4\%$ crude protein (CP) from 6 mo of age and older. Additionally, it was found that heifers should be 4 to 6 mo before consuming silage or haylage (Heinrichs, 1996). In a survey conducted on 329 dairies in Pennsylvania, it was revealed that corn silage and haylage are the most common forages fed to heifers post-weaning with the first cutting of hay being the second most common forage utilized (Heinrichs et al., 1987). Additionally, a common practice of high production farms in Michigan is to feed the total mixed ration (TMR) refusals from the lactating herd to replacement heifers (Boterman and Bucholtz, 2005). While this could provide some economic benefit, these TMRs are not nutritionally balanced for heifer growth and thus could inhibit the performance of heifers.

As previously mentioned, the lack of nutritional management of heifers could prevent proper developmental growth, thus leading to a decrease in future productivity and profitability. However, feeding more is not a good option either. While greater feeding levels are associated with high milk production in heifers post-breeding, in heifers pre- and peri-pubertal it is negatively correlated with high milk production (Foldager and Sejrsen, 1991). From this research, feeding strategies such as a compensatory feeding program were developed to deliver precise nutrition at specific periods when there is substantial mammary growth (i.e., between 3 to 9 mo of age). The compensatory or stair-step feeding system alternates feeding either above maintenance energy requirements (~130% of metabolizable energy [ME]) or restricted energy requirements (~70% ME) at various stages of development (i. e., pre-puberty, puberty, or late pregnancy). Therefore, the dietary energy is decreased in between developmental states to

promote compensatory mammary development (Choi et al., 1994, Park et al., 1998). The prepubertal phase is feed above maintenance for 3 months and then restricted for 2 months (Park et al., 1998). Compensatory mammary development increases the RNA, casein, and protein found in the mammary tissues and decreases the lipid content in the udder (Park et al., 1998). Additionally, the altering of nutritional requirements during these states can ultimately result in compensatory weight gain. This could increase ADG to help heifers reach breeding weights sooner and decrease AFC and age at breeding.

Specifically, compensatory heifers in these three studies had an increased BW at calving while consuming less DM than the control animals, meaning they had improved feed/growth efficiency (Choi et al., 1994, Park et al., 1998, Ford and Park, 2001). Choi et al. showed that final weights for Holstein heifers were similar, 562 vs. 568 kg, for the control group vs. the stair-step group, respectively. Total gain tended to be increased (373.2 vs. 385 kg) for stair-step heifers (Choi et al., 1994). Ultimately, there was an increase in growth efficiency when Holstein heifers were fed a stair-step feeding program over 18 mo (7.8% vs 8.3%). Ford and Park (2001) demonstrated that there was an increase in ADG for stair-step heifers compared with control (1.01 vs 0.78 kg/d), resulting in an improvement in growth efficiency (7.0 vs 12.2%). Overall, stair-step heifers increased in growth efficiency and gained more or had similar BW compared with control heifers across these studies, thus potentially alleviating extra feed costs associated with replacement heifers.

However, the stair step program requires a high amount of management. Multiple pens would be needed to rotate heifer of different ages through the program along with different diets to make the feeding strategy work. Producers must keep pay close attention to ages of heifers and have systems in place to move heifers into the proper feeding group for her age range.

Smaller producers may not have space or time to house heifers or feed multiple rations. A different strategy may be needed that is simpler and easier to manage while getting similar efficiency results.

1.4. Limit feeding strategy

While many feeding systems have been developed to improve heifer performance and development, limit feeding of heifers at multiple life stages has been shown to reduce nitrogen output, reduce cost, and improve feed efficiency without influencing future milk production (Zanton and Heinrichs, 2015). The concept of limit feeding is to restrict DMI while still delivering the nutritional requirements for a growing or maintenance animal. Limiting the intake has been shown to slow the rate of passage through the rumen, allowing for greater retention times and improved rumen degradation and nutrient utilization (Tamminga et al., 1979, Loerch, 1990).

Limit fed diets have been utilized in growing and gestating beef cattle, sheep and more recently dairy cattle production. Loerch (1996) was successfully able to meet nutritional requirements for gestating, mature beef cattle using a limit fed diet during the winter months. Two trials were completed that had limit fed cows consuming 6 to 7 kg/d DM less than the control group. Limit fed cows maintained an increased body condition score (trial 1) or kept similar body condition as the control (trial 2) throughout the winter and saved \$0.56 to \$0.73 per head per d compared with cows fed a harvested forage-based diet (Loerch, 1996). The calves born from the previous study had increased birth weights, with no differences in calving difficulty between treatments. Limit feeding has been commonly used by beef cattle producers, mostly because of low forage and hay production due to drought conditions in the Midwest (Lalman, 2018). However, this practice has started to gain popularity in the dairy industry.

Limit feeding in multiple stages of dairy heifer development improves nutrient utilization while showing no changes in ADG or milk yield. Heifers at 17 mo of age had similar ADG when fed either 100, 90, and 80% DMI (Hoffman et al., 2007). These same limit fed heifers had similar hip heights, heart girth, and body condition scores compared with the control group fed 100% DMI according to NRC (2001) recommendations. However, the heifers fed 80 and 90% DMI experienced improved feed efficiency by 23.7 and 28.9% compared heifers fed the control. Hoffman et al. (2007) also showed a 12.9 and 34.6% decrease in manure excretion when heifers were limit feed 80 and 90 % DMI, respectively. However, these heifers experienced no change in milk yield, milk fat, or milk protein in the first lactation.

Lascano et al. (2009) demonstrated similar ADG, withers and, hip heights, and heart girth measures between limit feeding heifers 80% DM and the control group (100% DM). These heifers continued to have similar ADG after calving, and both groups produced around 34 kg/d in milk production during the first lactation (Lascano et al., 2009). In a review article that looked at six studies for milk production in limit fed heifers, results from a funnel plot showed no significant differences in lactation performance for limit fed or low forage diets (Zanton and Heinrichs, 2009b).

When heifers (14.5 mos) were fed high forage diets that limited DM to 1.25, 1.50, 1.75, and 2% of BW, changes in the rumen and efficiency of N retention were noted. As intake increased (above 1.50% of BW), the efficiency of nutrient utilization increased (Zanton and Heinrichs, 2008). However, rumen pH decreased, and iso-acids declined as intake increased. Zanton and Heinrichs (2008) showed rumen pH declined as levels of intake increased with rumen pH ranged from 6.86 to 6.00. The study noted that changes were small and may not be biologically different. The normal pH range for dairy heifers is 6.4 to 7.0 pH after removing the

sample from the rumen (Grünberg and Constable, 2009). Additionally, diets in the previous study were not balanced to give similar nutrition, as there was a BW and ME difference between the rations. Additional research observed no differences in rumen pH between a control and limit-fed (85% of control DM) (Kruse et al., 2010).

Zanton and Heinrichs (2008) observed that as DM offered increased, total volatile fatty acids (VFA) increased. Additionally, heifers offered less DM (1.25 or 1.5 % of BW) had increases in the molar proportion of isobutyrate and isovalerate compared with heifers offered DM at 2% of BW but saw no changes in acetate, propionate, butyrate or valerate (Zanton and Heinrichs, 2009a). These results were similar to Lascano et al. (2009), when offering cows high, medium, and low concentrate diets. However, the previous study observed increased propionate and decreased in acetate molar proportions when feeding increased concentrates (Lascano et al., 2009). This change in iso-acids is thought to occur because when fermentable carbohydrates are low, there is an enhanced fermentation of branch-chain amino acids or increased utilization of iso-acids to synthesis branch chain amino acids (Zanton and Heinrichs, 2009a). It is important to note that all studies listed above recorded VFA over a 24-hour period. When dairy cows were switched from a high forage diet to high concentrate diet, the molar proportions of acetic acid decreased, and propionic acid increased eight days after the diet change (Hernandez-Urdaneta et al., 1976). To the author's current knowledge, this is the longest study looking at the effects of changing the diet to high concentrates in dairy cattle. Similar results as the previous study have been noted in goats (Sun et al., 2009).

In a similar study, when nonlactating, multiparous Holstein cows were limited to 71% of NRC requirements and fed an increased percent of shelled corn compared with a high forage diet, there was 15% improvement in DM and organic matter (OM) digestibility, but limit

feeding did not affect starch digestibility (Driedger and Loerch, 1999). Increases in digestibility for limit-fed diets are contributed to longer retention of feed in the gastrointestinal tract and increased available energy in the feedstuff. Driedger and Loerch (1999) also observed a 29% decrease in DM, a 40% decrease in OM excretion, and 13% greater apparent nitrogen digestibility for the limit fed cows. Research in younger beef steers (average BW = 450 ± 18 kg) showed similar reductions in fecal output and increases in digestibility (Clark et al., 2007).

The focus of today's dairy producers is to reach AFC goals will being cost efficient and environmentally friendly. A limit-feeding strategy may provide an opportunity for producers to meet these goals. However, the reduction of feed has been shown to change the behaviors of heifers and may be considered a welfare concern.

1.5. Limit Feeding and behaviors changes

While limit fed heifers tend to maintain similar ADG and milk production as ad libitum heifers, some studies have noted changes in behaviors by limit fed heifers. Hoffman et al. (2007) found a 10 and 15% linear decrease in lying time and a 0.7 and 2 h/d increase in nonrecumbent (time spent standing without eating) time for heifers fed 80% and 90% DM compared with control heifers, respectively. An increased numerical change in vocalization was noted for heifers fed 80% DMI, but no differences were observed between the 100 or 90% DMI heifers (Hoffman et al., 2007). Increases in vocalization are considered a sign of frustration or hunger and could be a sign of decreased welfare (Watts and Stookey, 2000).

Heifers at 9 mo of age, fed a low forage diet, ruminated 150 mins/d less and ate 100 min/d less when compared with control heifers (Greter et al., 2014). Additionally, the control heifers spread their rumination and eating times more evenly across the d compared with limit fed heifers that tended to ruminate and eat within 3 hours after delivery (Greter et al., 2014).

When forages were restricted, heifers increased in oral stereotypies. Heifers exhibited more frequent oral behaviors such as bar chewing and tongue rolling during times of limited feeding (Redbo and Nordblad, 1997). Also, there were increases in licking or biting behaviors and aggressive social behaviors observed in LIM heifers (Redbo and Nordblad, 1997). These changes in behavior suggest stress expressed by the animals, which can negatively impact health and growth.

Lying time has previously been associated with enhanced growth, most likely due to the utilization of energy during times of rest. In calves, lying time has been positively correlated with weight changes ($r=0.32$) over the first 20 weeks of life, with the addition of 6 mins of resting time daily resulting in an additional 0.1 kg in growth (Hänninen et al., 2005). This may occur because sleep is a regulator for growth hormone secretion (Steiger, 2002). Hänninen et al. (2005) believe that better quality sleep due to longer resting times can alter hormone secretion and improve growth. Similar results were observed by Mogensen et al. (1997), where some lying periods were correlated to daily gain. A tendency for a positive correlation was observed between lying time and ADG when ten-month old heifers were housed on slatted floors with no bedding or crowded deep bed pen (Mogensen et al., 1997). However, these correlations were only observed in 2 of the 3 herds in the experiment which may be due to the fact that animals were not for competing for space and had comfortable bedding to all heifers to stand without discomfort (Mogensen et al., 1997). These added comforts could allow all heifers to sleep better compared with heifers housed on slatted floors.

When given access to straw, limit-fed heifers (6 mos) show a 20 min/d decrease in unrewarded visits to the bunk and had a 150 min/d decrease in inactive standing time compared with limit fed heifers without additional straw (Kitts et al., 2011). Additionally, heifers fed a diet

with straw mixed in showed that inactive standing time was decreased by 100 min/d, and a 10 min/d decrease in unrewarded visits to the feeder when compared with limit fed heifers with straw fed separate (Kitts et al., 2011). However, Kitts et al. (2011) showed that heifers that had access to a separate wheat straw diet had a decrease in ADG and feed efficiency when compared with the limit fed group without straw, and there were no differences in lying time or bouts. Limit fed heifers without straw had 13 or fewer displacements at the feed bunk than heifers provided straw (Kitts et al., 2011). Authors of these studies contribute these changes to lack of rumen fill or signs of hunger in limit fed heifers.

An additional study showed a similar trend in unrewarded visits to the feeder and increased feeding time compared with limit fed heifers with no access to straw (Greter et al., 2008, Greter et al., 2011). These changes in unrewarded visits could be an expression of hunger in limit fed heifers. When straw was mixed into a nutrient dense TMR at 10% and 20%, heifers fed more straw increased in the sorting of smaller particles in the ration (Greter et al., 2008). This could increase the risk in of heifers consuming an unbalanced diet and increase chances of getting ruminal acidosis (DeVries and von Keyserlingk, 2009).

1.6. Environmental Enrichment

These stereotypes and abnormal behaviors associated with limit feeding may also become apparent when animals are housed in a bare environment (Pelley et al., 1995). This lack of stimulation could cause boredom, which results in these behaviors. When cattle are housed indoors, the cow or heifer must adjust their time budget to account for less time needed to search for food and to eat (Mandel et al., 2016). Cattle housed indoors now have a substantial period of time that they tend to fill with natural behaviors (i.e., grazing/eating, etc.), and when these behaviors are not fulfilled, these restrictions can cause frustration. Frustration behaviors in cattle

range from leg stomping to nonnutritive oral behaviors (Ninomiya, 2014, Mandel et al., 2016). Left unattended, this frustration can cause the development of abnormal behaviors or the previously described stereotypes (Ninomiya, 2014).

Other behaviors can indicate a positive change in the welfare of the animal. Increases in luxury behaviors, or behaviors that are only observed when physical and mental needs are met, are viewed as an increase in positive wellbeing (Mandel et al., 2016). Luxury behaviors are not seen when animals are sick, hungry, or under environmental stress. Instead, the animal's energy is directed towards the proper immune or other maintenance functions (Weary et al., 2009, Mandel et al., 2013). A typical luxury behavior used to measure welfare in calves is play behavior. Play behaviors are divided into individual play (i.e., galloping and bucking) or social play (i.e., headbutting another animal; (Jensen et al., 1998). Self-care behaviors, such as grooming, can also be a positive indicator of improved welfare (Mandel et al. 2013).

Environmental enrichment is defined as an improvement in the biological function (i.e., health, fitness, and performance; (Newberry, 1995) or the quality of life (Mandel et al., 2016) of a confined animal resulting from the modification of the environment. There are five categories of environmental enrichment: social, occupational, physical, sensory, and nutritional (Bloomsmith et al., 1991). The addition of objects such as grooming brush and a ball or changes to the size and complexity of the enclosure are considered physical enrichment. This type of enrichment has been used extensively in zoo animals (Carlstead and Shepherdson, 2000), laboratory animals (Baumans and Van Loo, 2013), and some livestock such as poultry and swine (Newberry, 1995). The addition of a grooming brush can also be considered tactile enrichment (i.e., a form of sensory enrichment) since it promotes self-grooming (Mandel et al., 2016).

Enrichment in beef cattle is widely studied since feedlot cattle are usually housed in bare environments. The addition of a drum can filled with hay increased active behaviors such as grooming, eating, investigating, and moving, with some carcass characteristics like beef belly thickness and marbling scores being improved in feedlot steers (Ishiwata et al., 2006). When a grooming brush was provided to beef calves, there was an increase in lying with head touching flank or ground and decreased in resting standing time (Ninomiya and Sato, 2009). However, the previous experiment also included items that eliminated competition at the feed bunk and a protective cover to prevent dominate calves from chasing subordinate calves. These additions to the pen could have confounded some of the results. A decrease in lying time was seen for two-week-old calves when provided a grooming brush or Jolly Ball™ (Bulens et al., 2014). Bulens et al. (2014) observed an increase in play and grooming behavior when a calf pen was provided with enrichment. The beef calves used in the previously described Ninomiya and Sato (2009) experiment were ten months old, which may explain the difference in lying behavior between the studies.

Feedlot cattle offered a stationary brush, straw bales, and suspended salt or mineral blocks showed a preference towards the straw but increased the aggressive behavior towards others (Pelley et al., 1995). The straw may have allowed for natural forage behaviors and created competition for use. Pelley et al. (1995) showed the brush led to less aggression, increased in the number of animals lying, and rumination compared with the straw bale. However, the study noted no reasons that changes in the welfare of the animal between the treatments occurred. While the bale was preferred over the brush in the previous experiment, Wilson et al. (2002) observed that beef heifers prefer the brush more frequently and for longer durations compared with scented devices (Wilson et al., 2002).

In the dairy industry, most enrichment is provided for cows and calves. The number of dairy cows being housed indoor year around is increasing each year, and this indoor housing has been seen to add stress to the cow (Mandel et al., 2016). Most dairy calves in the US are housed in individual hutches in order to create sanitary conditions that prevent the spread of disease (USDA, 2012). Individual housing can limit natural behaviors seen when calves are housed in pairs (Jensen and Larsen, 2014). Therefore, calves and cows tend to be enriched in order to provide more natural behaviors, such as grooming, in current housing practices.

When calves were offered a brush or rope, calves had more frequent visits with shorter durations at the brush with the reverse being true for the rope (Zobel et al., 2017). Zobel et al. (2017) saw a tendency towards a positive correlation between play behavior and brush duration ($r = 0.73, p = 0.06$). Calves increased brush and rope use an hour before and 4 hours after feeding. Brush use in calves also promotes self- grooming, which is a sign of positive welfare (Horvath and Miller-Cushon, 2019).

Besides a grooming brush, other enrichment devices like ropes and balls were added to dairy calf environments. Veal calves offered a tire or chain spent less time licking their lips or rolling their tongue compared with calves without enrichment (Veissier et al., 1997). However, there was no change in cortisol concentrations between the two calf groups. Calves housed in outdoor hutches utilized the stationary brush more than items like a chain, artificial teats, or calf “Lollie” (Pempek et al., 2017). The previously mentioned study observed a 50% increase in locomotion play with calves in furnished hutches compared with calves in bare hutches, but no differences were seen in growth, starter intake, or behavioral responses.

1.7. Conclusion

The dairy industry has shifted to breeding heifers at a younger age in order to have an early AFC. Having an AFC below 26 mo has been shown to improve the future milk production resulting in a more profitable animal. However, increasing DMI to obtain a desirable ADG and weight for breeding, may create undesirable fat in the udder and can limit future milk production. Feeding systems, such as the stair-step program, have been developed that change energy intake based on mammary growth. This feeding system allows for better feed efficiency and increased ADG without compiling too much fat. However, this system requires a lot of management and space to operate correctly. Without the proper management of the heifers, the system can fail, thus becoming an economic burden.

Limit feeding is a strategy, popular in beef cattle, that restricts the amount of DM while maintaining similar nutritional values as accelerated fed animals. Limit feeding has been shown in multiple species to improve feed efficiency, maintain high ADG, reduce feeding cost, and reduce manure and nitrogen output while not negatively impacting future milk production. However, studies have observed behavior changes, such as increased time standing around and feed bunk aggression, that could be a sign of reduced welfare. While studies have determined that some of these behaviors can be prevented by feeding straw, the straw would still be an added costs to the farmer, and certain lengths of straws decrease the ADG of the heifer.

Most consider these behavior changes to be signs of hunger, but these changes can also be signs of frustration. These frustration signs are seen in animals such as swine, cattle, and zoo animals that lack a stimulating environment. The use of environmental enrichment devices such as balls, brushes, and tree structures can eliminate these behaviors in multiple species and

provide animals the chance to increase play behavior and expressing natural grooming tendencies that reduce stress.

Current research in limit fed heifers relates the negative behaviors to lack of rumen fill or hunger. To date, no research has been conducted to determine if behaviors observed by limit fed heifers are due to the lack of stimulus in the housing system. Therefore, the objective of this thesis is to elucidate whether the use of environmental enrichment such as a grooming brush or tetherball promotes positive welfare behavior in limit fed heifers. Ultimately, this would allow producers to reap the benefits of a limit fed system and suggest investing in a one time cost (i.e., environmental enrichments) may prevent the occurrence of negative welfare.

1.8. References

- Abeni, F., L. Calamari, L. Stefanini, and G. Pirlo. 2000. Effects of Daily Gain in Pre- and Postpubertal Replacement Dairy Heifers on Body Condition Score, Body Size, Metabolic Profile, and Future Milk Production. *Journal of Dairy Science* 83(7):1468-1478.
- Baumans, V. and P. L. P. Van Loo. 2013. How to improve housing conditions of laboratory animals: The possibilities of environmental refinement. *The Veterinary Journal* 195(1):24-32.
- Bloomsmith, M. A., L. Y. Brent, and S. J. Schapiro. 1991. Guidelines for developing and managing an environmental enrichment program for nonhuman primates. *Laboratory animal science* 41(4):372-377.
- Boterman, E. and H. Bucholtz. 2005. Feeding practices of high-producing herds in Michigan. Pages 119-129 in *Proc. the Proceedings of the Tri-State Dairy Nutrition Conference*. The Ohio State University, Columbus.
- Bulens, A., S. Van Beirendonck, J. Van Thielen, and B. Driessen. 2014. The effect of environmental enrichment on the behaviour of beef calves. Page 235 in *Proc. 6th Int. Conf. Assessment of Animal Welfare Farm and Group Level*, Clermont-Ferrand, France, Wageningen Academic Publishers, Wageningen, the Netherlands.
- Carlstead, K. and D. Shepherdson. 2000. Alleviating stress in zoo animals with environmental enrichment. *The biology of animal stress: Basic principles and implications for animal welfare*:337-354.
- Choi, Y. J., I. K. Han, J. H. Woo, H. J. Lee, K. Jang, K. H. Myung, and Y. S. Kim. 1994. Compensatory Growth in Dairy Heifers: The Effect of a Compensatory Growth Pattern on Growth Rate and Lactation Performance. *Journal of Dairy Science* 80(3):519-524.
- Chuck, G. M., P. D. Mansell, M. A. Stevenson, and M. M. Izzo. 2018. Early-life events associated with first-lactation performance in pasture-based dairy herds. *Journal of Dairy Science* 101(4):3488-3500.
- Clark, J. H., K. C. Olson, T. B. Schmidt, M. L. Linville, D. O. Alkire, D. L. Meyer, G. K. Rentfrow, C. C. Carr, and E. P. Berg. 2007. Effects of dry matter intake restriction on diet digestion, energy partitioning, phosphorus retention, and ruminal fermentation by beef steers. *Journal of Animal Science* 85(12):3383-3390.
- Council, N. R. 2001. *Nutrient Requirements of Dairy Cattle: Seventh Revised Edition, 2001*. The National Academies Press, Washington, DC.
- DeVries, T. J. and M. A. G. von Keyserlingk. 2009. Competition for feed affects the feeding behavior of growing dairy heifers. *Journal of Dairy Science* 92(8):3922-3929.

- Driedger, L. J. and S. C. Loerch. 1999. Limit-feeding corn as an alternative to hay reduces manure and nutrient output by Holstein cows. *Journal of Animal Science* 77(4):967-972.
- Ettema, J. F. and J. E. P. Santos. 2004. Impact of Age at Calving on Lactation, Reproduction, Health, and Income in First-Parity Holsteins on Commercial Farms. *Journal of Dairy Science* 87(8):2730-2742.
- Foldager, J. and K. Sejrsen. 1991. Rearing intensity in dairy heifers and the effect on subsequent milk production. *Beretning fra Statens Husdyrbrugsforsog (Denmark)*.
- Ford, J. A., Jr. and C. S. Park. 2001. Nutritionally Directed Compensatory Growth Enhances Heifer Development and Lactation Potential. *Journal of Dairy Science* 84(7):1669-1678.
- Gabler, M. T., P. R. Tozer, and A. J. Heinrichs. 2000. Development of a Cost Analysis Spreadsheet for Calculating the Costs to Raise a Replacement Dairy Heifer¹. *Journal of Dairy Science* 83(5):1104-1109.
- Greter, A. M., T. J. DeVries, and M. A. G. von Keyserlingk. 2008. Nutrient Intake and Feeding Behavior of Growing Dairy Heifers: Effects of Dietary Dilution. *Journal of Dairy Science* 91(7):2786-2795.
- Greter, A. M., B. L. Kitts, and T. J. DeVries. 2011. *Short communication:* Limit feeding dairy heifers: Effect of feed bunk space and provision of a low-nutritive feedstuff. *Journal of Dairy Science* 94(6):3124-3129.
- Greter, A. M., E. K. Miller-Cushon, B. W. McBride, T. M. Widowski, T. F. Duffield, and T. J. DeVries. 2014. *Short communication:* Limit feeding affects behavior patterns and feeding motivation of dairy heifers. *Journal of Dairy Science* 98(2):1248-1254.
- Grünberg, W. and P. D. Constable. 2009. CHAPTER 6 - Function and Dysfunction of the Ruminant Forestomach. Pages 12-19 in *Food Animal Practice (Fifth Edition)*. D. E. Anderson and D. M. Rings, ed. W.B. Saunders, Saint Louis.
- Hänninen, L., A. M. de Passillé, and J. Rushen. 2005. The effect of flooring type and social grouping on the rest and growth of dairy calves. *Applied Animal Behaviour Science* 91(3):193-204.
- Heinrichs, A., N. Kiernan, R. Graves, and L. Hutchinson. 1987. Survey of calf and heifer management practices in Pennsylvania dairy herds. *Journal of Dairy Science* 70(4):896-904.
- Heinrichs, A. J. 1993. Raising Dairy Replacements to Meet the Needs of the 21st Century. *Journal of Dairy Science* 76(10):3179-3187.
- Heinrichs, A. J. 1996. Nutrition and management of replacement cattle. *Animal Feed Science and Technology* 59(1):155-166.

- Heinrichs, A. J. and B. S. Heinrichs. 2011. A prospective study of calf factors affecting first-lactation and lifetime milk production and age of cows when removed from the herd. *Journal of Dairy Science* 94(1):336-341.
- Heinrichs, A. J. and M. Vazquez-Anon. 1993. Changes in First Lactation Dairy Herd Improvement Records. *Journal of Dairy Science* 76(2):671-675.
- Heinrichs, A. J., G. I. Zanton, G. J. Lascano, and C. M. Jones. 2017. A 100-Year Review: A century of dairy heifer research. *Journal of Dairy Science* 100(12):10173-10188.
- Hernandez-Urdaneta, A., C. E. Coppock, R. E. McDowell, D. Gianola, and N. E. Smith. 1976. Changes in Forage-Concentrate Ratio of Complete Feeds for Dairy Cows. *Journal of Dairy Science* 59(4):695-707.
- Hoffman, P. C. and D. A. Funk. 1992. Applied Dynamics of Dairy Replacement Growth and Management. *Journal of Dairy Science* 75(9):2504-2516.
- Hoffman, P. C., C. R. Simson, and M. Wattiaux. 2007. Limit Feeding of Gravid Holstein Heifers: Effect on Growth, Manure Nutrient Excretion, and Subsequent Early Lactation Performance. *Journal of Dairy Science* 90(2):946-954.
- Horvath, K. C. and E. K. Miller-Cushon. 2019. Characterizing grooming behavior patterns and the influence of brush access on the behavior of group-housed dairy calves. *Journal of Dairy Science* 102(4):3421-3430.
- Hutchison, J. L., P. M. VanRaden, D. J. Null, J. B. Cole, and D. M. Bickhart. 2017. Genomic evaluation of age at first calving. *Journal of Dairy Science* 100(8):6853-6861.
- Ishiwata, T., K. Uetake, N. Abe, Y. Eguchi, and T. Tanaka. 2006. Effects of an environmental enrichment using a drum can on behavioral, physiological and productive characteristics in fattening beef cattle. *Animal Science Journal* 77(3):352-362.
- Jensen, M. B. and L. E. Larsen. 2014. Effects of level of social contact on dairy calf behavior and health. *Journal of Dairy Science* 97(8):5035-5044.
- Jensen, M. B., K. S. Vestergaard, and C. C. Krohn. 1998. Play behaviour in dairy calves kept in pens: the effect of social contact and space allowance. *Applied Animal Behaviour Science* 56(2):97-108.
- Kitts, B. L., I. J. H. Duncan, B. W. McBride, and T. J. DeVries. 2011. Effect of the provision of a low-nutritive feedstuff on the behavior of dairy heifers limit fed a high-concentrate ration. *Journal of Dairy Science* 94(2):940-950.
- Kruse, K. A., D. K. Combs, N. M. Esser, W. K. Coblenz, and P. C. Hoffman. 2010. Evaluation of potential carryover effects associated with limit feeding of gravid Holstein heifers. *Journal of Dairy Science* 93(11):5374-5384.

- Lalman, D. 2018. Limit Feeding Concentrate Diets to Beef Cows as an Alternative to Feeding Hay. Vol. 2019, Oklahoma Cooperative Extension Service
- Lammers, B. P., A. J. Heinrichs, and R. S. Kensinger. 1999. The Effects of Accelerated Growth Rates and Estrogen Implants in Prepubertal Holstein Heifers on Estimates of Mammary Development and Subsequent Reproduction and Milk Production¹. *Journal of Dairy Science* 82(8):1753-1764.
- Lascano, G. J., G. I. Zanton, F. X. Suarez-Mena, and A. J. Heinrichs. 2009. Effect of limit feeding high- and low-concentrate diets with *Saccharomyces cerevisiae* on digestibility and on dairy heifer growth and first-lactation performance¹. *Journal of Dairy Science* 92(10):5100-5110.
- Le Cozler, Y., V. Lollivier, P. Lacasse, and C. Disenhaus. 2008. Rearing strategy and optimizing first-calving targets in dairy heifers: a review. *Animal* 2(9):1393-1404.
- Lengemann, F. and N. Allen. 1955. The development of rumen function in the dairy calf I. Some characteristics of the rumen contents of cattle of various ages. *Journal of Dairy Science* 38(6):651-656.
- Loerch, S. C. 1990. Effects of feeding growing cattle high-concentrate diets at a restricted intake on feedlot performance. *Journal of Animal Science* 68(10):3086-3095.
- Loerch, S. C. 1996. Limit-feeding corn as an alternative to hay for gestating beef cows. *Journal of Animal Science* 74(6):1211-1216.
- Lohakare, J. D., K. H. Südekum, and A. K. Pattanaik. 2012. Nutrition-induced Changes of Growth from Birth to First Calving and Its Impact on Mammary Development and First-lactation Milk Yield in Dairy Heifers: A Review. *Asian-Australasian Journal of Animal Sciences* 25(9):1338-1350.
- Macdonald, K. A., J. W. Penno, A. M. Bryant, and J. R. Roche. 2005. Effect of Feeding Level Pre- and Post-Puberty and Body Weight at First Calving on Growth, Milk Production, and Fertility in Grazing Dairy Cows. *Journal of Dairy Science* 88(9):3363-3375.
- Mandel, R., H. R. Whay, E. Klement, and C. J. Nicol. 2016. *Invited review:* Environmental enrichment of dairy cows and calves in indoor housing. *Journal of Dairy Science* 99(3):1695-1715.
- Mandel, R., H. R. Whay, C. J. Nicol, and E. Klement. 2013. The effect of food location, heat load, and intrusive medical procedures on brushing activity in dairy cows. *Journal of Dairy Science* 96(10):6506-6513.
- Meyer, M. J., A. V. Capuco, D. A. Ross, L. M. Lintault, and M. E. Van Amburgh. 2006a. Developmental and Nutritional Regulation of the Prepubertal Heifer Mammary Gland: I. Parenchyma and Fat Pad Mass and Composition. *Journal of Dairy Science* 89(11):4289-4297.

- Meyer, M. J., A. V. Capuco, D. A. Ross, L. M. Lintault, and M. E. Van Amburgh. 2006b. Developmental and Nutritional Regulation of the Prepubertal Bovine Mammary Gland: II. Epithelial Cell Proliferation, Parenchymal Accretion Rate, and Allometric Growth. *Journal of Dairy Science* 89(11):4298-4304.
- Mogensen, L., C. C. Krohn, J. T. Sørensen, J. Hindhede, and L. H. Nielsen. 1997. Association between resting behaviour and live weight gain in dairy heifers housed in pens with different space allowance and floor type. *Applied Animal Behaviour Science* 55(1):11-19.
- Mourits, M. C. M., R. B. M. Huirne, A. A. Dijkhuizen, A. R. Kristensen, and D. T. Galligan. 1999. Economic optimization of dairy heifer management decisions. *Agricultural Systems* 61(1):17-31.
- Mourits, M. C. M., H. J. Van der Fels-Klerx, R. B. M. Huirne, and M. W. C. Huyben. 2000. Dairy-heifer management in the Netherlands. *Preventive Veterinary Medicine* 46(3):197-208.
- Newberry, R. C. 1995. Environmental enrichment: Increasing the biological relevance of captive environments. *Applied Animal Behaviour Science* 44(2):229-243.
- Ninomiya, S. 2014. Satisfaction of farm animal behavioral needs in behaviorally restricted systems: Reducing stressors and environmental enrichment. *Animal Science Journal* 85(6):634-638.
- Ninomiya, S. and S. Sato. 2009. Effects of 'Five freedoms' environmental enrichment on the welfare of calves reared indoors. *Animal Science Journal* 80(3):347-351.
- Park, C. S., R. B. Danielson, B. S. Kreft, S. H. Kim, Y. S. Moon, and W. L. Keller. 1998. Nutritionally Directed Compensatory Growth and Effects on Lactation Potential of Developing Heifers. *Journal of Dairy Science* 81(1):243-249.
- Pelley, M. C., A. Lirette, and T. Tennessen. 1995. Observations on the responses of feedlot cattle to attempted environmental enrichment. *Canadian Journal of Animal Science* 75(4):631-632.
- Pempek, J. A., M. L. Eastridge, and K. L. Proudfoot. 2017. The effect of a furnished individual hutch pre-weaning on calf behavior, response to novelty, and growth. *Journal of Dairy Science* 100(6):4807-4817.
- Pirlo, G., F. Miglior, and M. Speroni. 2000. Effect of Age at First Calving on Production Traits and on Difference Between Milk Yield Returns and Rearing Costs in Italian Holsteins. *Journal of Dairy Science* 83(3):603-608.
- Redbo, I. and A. Nordblad. 1997. Stereotypies in heifers are affected by feeding regime. *Applied Animal Behaviour Science* 53(3):193-202.
- Sejrsen, K. 1994. Relationships between nutrition, puberty and mammary development in cattle. *Proceedings of the Nutrition Society* 53(1):103-111.

Silva, L. F. P., M. J. VandeHaar, B. K. Whitlock, R. P. Radcliff, and H. A. Tucker. 2002. Short Communication: Relationship Between Body Growth and Mammary Development in Dairy Heifers. *Journal of Dairy Science* 85(10):2600-2602.

Steiger, A. 2002. Sleep and the hypothalamo–pituitary–adrenocortical system. *Sleep Medicine Reviews* 6(2):125-138.

Sun, Y. Z., S. Y. Mao, and W. Y. Zhu. 2009. Rumen chemical and bacterial changes during stepwise adaptation to a high-concentrate diet in goats. *Animal* 4(2):210-217.

Swanson, E. 1960. Effect of rapid growth with fattening of dairy heifers on their lactational ability. *Journal of Dairy Science* 43(3):377-387.

Tamminga, S., C. J. Van Der Koelen, and A. M. Van Vuuren. 1979. Effect of the level of feed intake on nitrogen entering the small intestine of dairy cows. *Livestock Production Science* 6(3):255-262.

Tucker, H. A. 1987. Quantitative Estimates of Mammary Growth During Various Physiological States: A Review¹. *Journal of Dairy Science* 70(9):1958-1966.

USDA. 2012. Dairy Heifer Raiser, 2011: An overview of operations that specialize on raising dairy heifers

Van Amburgh, M. E., D. M. Galton, D. E. Bauman, R. W. Everett, D. G. Fox, L. E. Chase, and H. N. Erb. 1998. Effects of Three Prepubertal Body Growth Rates on Performance of Holstein Heifers During First Lactation. *Journal of Dairy Science* 81(2):527-538.

Van Amburgh, M. E., F. Soberon, M. J. Meyer, and R. A. Molano. 2019. Symposium review: Integration of postweaning nutrient requirements and supply with composition of growth and mammary development in modern dairy heifers. *Journal of Dairy Science* 102(4):3692-3705.

Veissier, I., P. Chazal, P. Pradel, and P. Le Neindre. 1997. Providing social contacts and objects for nibbling moderates reactivity and oral behaviors in veal calves. *Journal of Animal Science* 75(2):356-365.

Watts, J. M. and J. M. Stookey. 2000. Vocal behaviour in cattle: the animal's commentary on its biological processes and welfare. *Applied Animal Behaviour Science* 67(1):15-33.

Weary, D., J. Huzzey, and M. Von Keyserlingk. 2009. Board-invited review: Using behavior to predict and identify ill health in animals. *Journal of animal science* 87(2):770-777.

Wilson, S. C., F. M. Mitlöhner, J. Morrow-Tesch, J. W. Dailey, and J. J. McGlone. 2002. An assessment of several potential enrichment devices for feedlot cattle. *Applied Animal Behaviour Science* 76(4):259-265.

Zanton, G. I. and A. J. Heinrichs. 2005. Meta-Analysis to Assess Effect of Prepubertal Average Daily Gain of Holstein Heifers on First-Lactation Production^{*}. *Journal of Dairy Science* 88(11):3860-3867.

Zanton, G. I. and A. J. Heinrichs. 2008. Rumen Digestion and Nutritional Efficiency of Dairy Heifers Limit-Fed a High Forage Ration to Four Levels of Dry Matter Intake¹. *Journal of Dairy Science* 91(9):3579-3588.

Zanton, G. I. and A. J. Heinrichs. 2009a. Digestion and nitrogen utilization in dairy heifers limit-fed a low or high forage ration at four levels of nitrogen intake¹. *Journal of Dairy Science* 92(5):2078-2094.

Zanton, G. I. and A. J. Heinrichs. 2009b. Review: Limit-Feeding with Altered Forage-to-Concentrate Levels in Dairy Heifer Diets¹. *The Professional Animal Scientist* 25(4):393-403.

Zobel, G., H. W. Neave, H. V. Henderson, and J. Webster. 2017. Calves Use an Automated Brush and a Hanging Rope When Pair-Housed. *Animals* 7(11):84.

CHAPTER 2: Effects of environmental enrichment on behaviors, growth, and stress in limit-fed Holstein Heifers

2.1. Abstract

The use of environmental enrichment such as a grooming brush (**BR**) and calf ball (**BA**) could prevent abnormal behaviors and allow for the benefits of a limit fed system. Twenty-four Holsteins heifers (161.9 ± 33 d of age) were housed in 6 pens with either a BR, BA or no enrichment (**NONE**). In each pen, heifers received either a high concentrate diet at 85% of DM offer to control group (**LIM**), or 100% (**CON**) of NCR (2001) predicted DMI for six weeks ($n=4$ per pen). Weekly rumen samples, rumination events, blood samples, and body measures were taken. Rumination events were recorded every 5 mins over 6 hours after feeding. Individual heifer behaviors such as feed aggression, feeding, use of enrichment, and play behavior were recorded for 24 hours each week. Lying time was recorded every hour for six weeks using a leg band activity monitor. The MIXED procedure of SAS was used to analyze the effects of treatment, date, and all 2-way interactions for all models on body measures, rumen pH, rumination events, and lying behaviors. Enrichment or diet did not affect the average daily gain or body measures. LIM fed heifers consumed less (0.68 kg/d DM, $P < 0.01$). Control heifers with a BR or NONE had 4 and 2.8 more rumination events compared with the LIM in those groups (respectively, $P < 0.01$). Rumen pH was decreased for LIM compared with CON by 0.25 pH for all enrichments ($P < 0.01$). The addition of a BA increased play behavior by 8 mins per d ($P = 0.01$). Heifers, no matter the diet, spent 30 mins more using the BR than heifers using the BA ($P = 0.03$). Treatment did not affect feed aggression or visits to the feed bunk. When the pen was enriched with a BA, LIM had 72 additional mins per d lying down compared with CON ($P =$

0.01). The use of enrichment in limit fed heifers shows some improvement in lying and standing times.

2.2. Introduction

Replacement heifers are raised to be cost-efficient without compromising production. Raising replacement heifers on a dairy farm accounts for 20% of the farm's annual expenses (Gabler et al., 2000) with sixty percent of the cost of raising replacement heifers is related to feed costs (Heinrichs et al., 2017). Since the nutrition programs utilized during the heifer development stage can influence the future production of the heifer (Sejrsen, 1994), the nutrition provided to the heifer thus has the potential to dictate profitability. Feeding strategies developed for stages from birth to breeding have been shown to affect not only the growth of the dairy heifer but can influence future milk yield (Heinrichs et al., 2017). Limit-feeding is the practice of reducing the amount of DM that the animal receives while increasing the nutritant density of the feed. Limit feeding has been shown to improve feed efficiency in dairy heifers (Hoffman et al., 2007, Lascano et al., 2009), reduce feed cost (Loerech, 1996) and improve the digestibility of feed stuffs (Driedger and Loerch, 1999, Clark et al., 2007). While the limit feeding strategy may provide producers a cost efficient way to achieve desired ADG, previous research has noted behavior changes in limit-fed diet. Hoffman et al. (2007) observed increases in vocalization, non-recumbent time and decreases in lying time. Increases in vocalization can be considered a sign of frustration or hunger and demonstrate a decrease in the welfare of the heifer (Watts and Stookey, 2000). Additionally, decreases in lying time and increases in recumbent time can be contributed to increased lameness (Kitts et al., 2011) and decreases in ADG (Mogensen et al., 1997, Hänninen et al., 2005). While some studies contribute these behavior changes to signs that the heifers are hungry (Kitts et al., 2011), these changes in behaviors can also be contributed to lack

of stimulus or frustration since heifers are housed in a bare environment (Pelley et al., 1995). In young dairy calves, environmental enrichment devices such as grooming brushes, ropes and balls, calves are observed to have increased social and locomotion play (Pempek et al., 2017, Zobel et al., 2017). Increases in locomotion play are considered a sign of positive welfare (Jensen et al., 1998). To date, there has been no research conducted to determine if behaviors observed by limit-fed heifers are due to bare environment housing. Therefore, the objective of this study is to elucidate whether the use of environment enrichment such as a grooming brush or tetherball, promotes positive welfare behavior in limit-fed heifers.

2.3. Methods

Housing and Animal Care

Procedures and protocols for heifer experimentation were approved by the North Carolina State University Institutional Animal Care and Use Committee (protocol number 18-117-A). All animals were housed at the North Carolina Department of Agriculture and Consumer Services Piedmont Research Station in Salisbury, NC. Data collection took place between November 2018 and December 2018. Heifers were housed in a three-sided pole barn with Calan bins (American Calan®, Northwood, New Hampshire) located in front of the pole barn on a concrete feeding alley. The pole barn was an open bedded pack containing sawdust with new sawdust added and feed alley scraped weekly. The barn was divided into 6 equal pens using panels with water shared by two pens. The pens were 3.7 X 18.0 meters and provided each animal with 16.7 square meters.

Diets and enrichment

Twenty-four Holstein heifers (161.9 ± 33 d of age, 163.94 kg of initial BW) were randomly assigned to one of the six pens ($n=4$). Two sets of three pens (Replicate 1 and 2)

contained either a hanging ball (**BA**, CalfBall, Future Cow, Longwood, FL), a calf brush (**BR**, Mini Swinging Brush MSB, Delaval, Kansas City, MO), or no behavior modifier (**NA**). The BR and BA were located in the bedded pack portion of the pens. Brushes were set at 71.12 cm off the ground and the ball was hung 78.74 cm off the ground.

All heifers were trained to the Calan bins for one week then enrolled in a split-plot design for five weeks. Two heifers in each pen were fed either a limit fed diet (**LIM**, Table 2.1) or a control diet (**CON**, Table 2.1). The control group will receive a diet that meets NRC (2001) nutritional requirements for heifers planning to calve at 24 mo of age and targeted for an ADG of 0.7 kg/d. Animals fed the LIM diet will be fed 85% of NRC DM requirements for heifers planning to calve at 24 mo of age and targeted for an ADG of 0.7 kg/d but receive all required nutrients to support maintenance and growth. All heifers will be fed fresh feed between 1100 and 1200 with orts weighted and recorded daily. Feed intake was recorded daily by weighing the fresh feed fed the previous d minus the orts recorded the next d. Heifers were given ad libitum access to water.

Individual heifer feed samples were collected weekly. Individual samples were dried in a forced-air oven at 60°C for 36 hours. Individual samples were ground through a 2 mm screen (Wiley Mill, Arthur H. Thomas, Philadelphia, PA). Individual weekly heifer ground samples were compiled into weekly diet samples. Weekly diet sample was analyzed for DM, NDF and ADF, CP, ME and macro minerals Ca, P and K. Samples were sent to Cumberland Valley Analytical Services (Waynesboro, PA) for wet chemistry analysis. All sample were analyzed using procedures form Horwitz (2000) with an ADF modification of Whatman 934-AH glass micro-fiber filters with 1.5µm particle retention used in place of fritted glass crucible.

Sample Collection

Data collection started at the end of the training week (week 0). Data collected continued for 5 weeks (week 1-5). Blood samples, rumen fluid, body weights, and body measures were collected from week 0 to week 5. Behavior data were taken from week 1 to week 5.

All heifers were weighed weekly using a portable scale on a squeeze chute. Weekly hip heights, hip width, wither height, and heart girth was measured using a heifer measuring tape. Hip heights were measured from the hooks to the back-dew claws of the calf. Hip widths were measured from the hook to hook on the calf. Wither heights were measured from the top of the withers to the front dew claws. Heart girths were measured by wrapping the tape around the barrel of the heifer directly behind the elbow and withers where the barrel is at its smallest.

Behaviors

At the beginning of week 0, heifers were equipped with a pedometer (Icecube, IceRobotics, Scotland, UK) that recorded standing, lying time, and lying bouts every hour for 6 weeks. The pedometer was placed on the left hind leg of every heifer. Pedometer data were downloaded from the Icecubes weekly by waving a wand reader across the tag.

Additional behaviors, such as interaction with BA or BR, aggression at the feed bunk reaching for feed, and feeder visits were monitored using a security camera system (8 channel HD-IP H.265 System. GW Security, Inc., South El Monte, CA). Two cameras were positioned between the pack and feed alley with one facing into the pack and one facing into the feed alley. Each camera observed two pens at one time. The last two digits of a heifer's ear tag number were painted across her back with new stray paint applied weekly to help with video identification. Video for all pens was recorded for 24 hours once a week during weeks 1 through 5. A 24 hour time-lapse of week 1 and 5 were then used to determine the most active periods for

each behavior observation and were used to determine what time periods the remaining video was observed. Ball, brush, and social and individual play events were recorded for 24 hour periods on week 2 through 4, but visits to the feed bunk and aggression at the feed bunk were only recorded from 0600 to 1900 on those weeks. Three trained individuals observed behaviors using the ObserveXT (Nolbus, Leesburg, VA). Observed used the ethogram (Table 2.2) and front and back pictures of the animals in each pen to record behaviors. Visits to feed bunk and aggression at feed bunk were recorded as events where social play behaviors and object use was recorded as a timed event. Behavior recording will be viewed on days where other samples were not taken. Week 2 was excluded from analysis due to a camera malfunction. Abnormal events, such as heifers breaking gates and going in a different pen, were excluded in the analysis.

Rumination events were recorded once a week when heifers were not handled through the chute or when behavior observations were being recorded. Heifers were observed every 10 mins for 6 hours, starting one hour after the feed was delivered. A single observer recorded all heifers ruminating during each observation period. Rumination was recorded if the heifer was exhibited chewing motions with her jaw but was not overfeed source.

Rumen Fluid

A rumen fluid sample was taken weekly to assess for temperature, pH, and volatile fatty acids. The fluid was collected by inserting an oral stomach tube down the esophagus into the rumen. A bicycle pump was attached to the other end to extract 20 ml of fluid. The fluid was filtered through two layers of cheesecloth into an insulated cup. Rumen pH and temperature were checked within 30 secs of pulling the sample using a pH probe (Portable Meter Kit, Oakton Instruments, Vernon Hills, IL) and thermometer. Ten ml of fluid was then placed into a sterile 10 ml tube and placed in the cooler containing ice for transport. Samples were stored in a -20°C

freezer until processing. Week 1 was not included in rumen pH or VFA analysis because of a sample collection error, which resulted in only half of the animals receiving rumen samples that day.

Twenty-five g of metaphosphoric acid (**MIS**) was dissolved in 50 ml of deionized water by stirring then, 0.2 g of diethyl acetic acid was added along with 25 ml of water and then mixed through stirring. The final volume was brought to 100 ml using deionized water, and breaker was properly labeled.

One ml of filtered rumen fluid was removed from 10 ml tubes and placed in a microcentrifuge tube. Tubes were centrifuged for 15 mins at 15,000 rpm. One ml of the subsample was removed from the centrifuge and placed in a new microfuge tube where 200 μ l of MIS solution (maintaining a 5:1 ratio) was added as an internal standard. This microfuge tube containing the subsample and MIS solution was then centrifuged for 5 mins at 15,000 rpm. One ml of clear supernatant was removed and placed in a new microfuge container. Volatile Fatty Acids were analyzed by gas-liquid chromatography (model CP-3380; Varian; Walnut Creek, CA) using a fused silica capillary column with 30m x 0.25mm x 0.25 μ m film thickness (Nukol; Supelco Inc., Bellefonte, PA) according to Fellner et al. (1997).

Non-esterified fatty acids.

A serum sample was taken to test for non-esterified fatty acids (**NEFA**) to evaluate metabolic condition. All samples were collected an hour before feeding on sample day each week. Blood samples were collected in a 10 ml red top tube from the jugular vein of each heifer. Non-esterified fatty acids were analyzed using NEFA HR Kit (FUJIFILM Wako Diagnostics, Richmond, VA). Procedures for the in vitro enzymatic colorimetric method assay were followed according to procedures provided by Wako Diagnostics. Samples with a coefficient of variation

over 10 % were reanalyzed. The inter assay and intra assay coefficient of variance were under 10% for all plates.

Statistical Analysis and Calculations

A power test conducted using the Proc Power procedure of SAS (Version 9.4, SAS Institute, Inc., Cary, NC). indicated that an n = 4 would be necessary to detect a difference in behavior at an alpha = 0.05, beta= 0.94. The test was run using a two sample means with acceptable means being determined by Kitt et. al. 2014 and Greter et. al. 2014. From these papers a difference of 1 lb of average daily gain with a standard deviation of 0.5 lb/d and a difference in 30 mins of lying time between the control and treatment groups was determined as the acceptable means.

Data was tested for normality using UNIVARIATE procedure of SAS Potential outliers were tested using the Grubb's test for a single outlier in Microsoft Excel (Office 365). However, no outliers were found. Data was analyzed using a MIXED procedure in SAS 9.4 with the random effect being block (Replicate 1 or 2) and block*enrichment interaction. Heifer was considered the experimental unit. The model for all body measures, ADG, feed:gain ratio, NEFA, VFA, and most behaviors included enrichment (BA vs. BR, vs. NONE), diet (LIM vs. CON), week (date was used for lying time, standing time, and lying bouts), and all 2 way interactions. The model for object interaction included toy (BA vs. BR) diet (LIM vs. CON), week, and all 2-way interactions. The linear statistical model is listed below:

$$Y_{ijk} = \mu + \alpha_i + \eta_{k(i)} + \beta_j + \alpha\beta_{(ij)} + \varepsilon_{k(i)}$$

Where α_i represents the fixed effect of enrichment, β_j represents the fixed effect of diet and $\alpha\beta_{(ij)}$ is the fixed interaction between the enrichment and diet. $\eta_{k(i)}$ represents the whole-plot

error (block error x enrichment error) and $\varepsilon_{k(i)}$ represents the split-plot error (enrichment error x diet error x block error).

The degrees of freedom for the split-plot design in SAS was $df=3$. Results were recorded as least squares means \pm SEM. P values were adjusted using Tukey Kramer for multiple comparisons. A statistical significance was reported at a $P \leq 0.05$. A tendency was reported at a $P \geq 0.05$ and ≤ 0.10 .

2.4. Results

Animal Performance

Body growth measures are summarized in Table 2.1. There were no differences in initial (week 0) BW, hip height (**HH**), hip width (**HW**), wither height (**WH**), or heart girth (**HG**) ($P > 0.05$). Data for initial measures (except for BW) are not shown. Heifers with a BR had the lowest HH overall compared with heifers with a BA or NONE (113.64 ± 0.93 cm vs. 116.86 ± 0.93 and 116.38 ± 0.93 cm, respectively; $P = 0.03$). There was a tendency for heifers with a BR to show a decreased HG by 5.08 cm compared with NONE heifers ($P = 0.06$). As expected, there were weekly increases in BW, HH, HW, WH, and HG (Figure 2.1, $P < 0.01$).

Heifers in the control group consumed 0.70 kg/d more feed than LIM (5.56 ± 0.07 v. 4.87 ± 0.07 kg/d, $P < 0.01$), as intended. However, there was no difference in the overall feed-to-gain ratio ($P > 0.05$). Table 2.4 shows the nutrient intake for diets and enrichment groups.

Non-esterified fatty acid concentrations were decreased ($P < 0.01$) in weeks 1, 3, and 4 compared with weeks 0 and 5 (0.26 ± 0.03 , 0.26 ± 0.03 , and 0.23 ± 0.03 mEq/l vs. 0.39 ± 0.03 and 0.41 ± 0.03 mEq/l, respectively). Week 2 NEFA concentrations were 0.10 mEq/l increased than concentrations in week 4 ($P = 0.03$). There also tended to be a diet by week interaction effect ($P = 0.08$) on NEFA concentrations, as shown in Fig. 2.2.

Rumen function

Rumination events were not recorded during week 0 as a result of inclement weather that prevented travel to the facility. There was an overall week effect ($P < 0.01$) on the weeks with recorded rumination events, with heifers demonstrating significantly more rumination events in week 2 than weeks 1, 3, 4, and 5 (8.36 ± 0.60 events/wk vs. 6.16 ± 0.55 , 6.00 ± 0.56 , 4.53 ± 0.57 , and 6.0 ± 0.56 events/wk, respectively; $P < 0.01$). There was also a diet by week interaction effect on rumination events, as shown in Fig. 2.3 ($P = 0.04$).

Heifers had an average rumen pH of 7.19. Control heifers had increased rumen pH compared with LIM heifers (7.32 ± 0.04 vs. 7.06 ± 0.04 , respectively, $P < 0.01$). Rumen pH declined over the course of the study ($P < 0.01$), resulting in significantly decreased rumen pH during weeks 3, 4, and 5 than weeks 0 and 2 (7.11 ± 0.07 , 7.05 ± 0.07 , and 7.02 ± 0.07 vs 7.40 ± 0.07 and 7.38 ± 0.07 , respectively; $P < 0.01$).

Heifers fed limit-fed diet had greater total VFA concentrations during week 4 and 5 compared with CON (Figure 2.4, $P = 0.03$). Total VFA concentrations were increased during week 5 compared with weeks 0, 2, 3 and 4 (71.76 ± 3.90 mM vs. 46.32 ± 3.90 , 52.09 ± 3.90 , 51.43 ± 3.90 , and 58.64 ± 3.90 mM, respectively, $P < 0.01$). Limit-fed heifers had greater concentrations of total VFA compared with CON heifers overall (63.72 ± 2.47 mM vs. 48.38 ± 2.47 mM, $P < 0.01$). Limit-fed heifers had increased acetic concentrations compared with CON (40.88 ± 1.67 vs. 31.26 ± 1.67 , $P < 0.01$). Butyric, valeric, isobutyric, and isovaleric acid concentrations, as well as propionic acid concentrations ($P = 0.04$), increased during week 4 and 5 for LIM heifers compared with the control diet, as shown in Fig. 2.4.

The molar proportion is the proportion of each VFA compared with the total concentration of VFAs. Limit-fed heifers had greater molar proportions of propionic on week 3

and butyric acid on week 5 (Figure 2.5, $P < 0.01$), as well as acetic acid on week 2 (Figure 2.5, $P = 0.02$). However, CON heifers had greater molar proportions of acetic acid on week 3 (Figure 2.5, $P = 0.02$) and a greater proportion of propionic on week 2 (Figure 2.5, $P < 0.01$). For the molar proportion of acetic acid, weeks 3 and 4 had a decreased percentage compared with weeks 0, 2, and 5 (60.95 ± 0.62 and $61.16 \pm 0.62\%$ vs. 66.51 ± 0.62 , 66.08 ± 0.62 , and $65.68 \pm 0.62\%$, respectively; $P < 0.01$). The molar proportion of propionic acid was increased during week 3 compared with all other weeks ($18.97 \pm 0.72\%$ vs. $16.33 \pm 0.72\%$, respectively; $P < 0.01$). There was a tendency for CON heifers to have a greater molar proportion of propionic acid than LIM ($17.29 \pm 0.60\%$ vs. $16.42 \pm 0.60\%$, respectively; $P = 0.06$). The molar proportion of butyric acid was decreased during week 0 than all other weeks ($9.47 \pm 0.50\%$ vs. $11.54 \pm 0.50\%$, $P < 0.01$). Additionally, the molar proportions of butyric acid in week 4 were increased than weeks 2 and 5 ($12.17 \pm 0.50\%$ vs. 11.02 ± 0.50 and $11.00 \pm 0.50\%$, respectively; $P < 0.02$).

Behavior and lying times

Limit-fed heifers housed with a BA increased in lying time compared with control heifers with a BA and LIM heifers with no enrichment (Figure 2.6, $P < 0.01$). For lying time, CON with BA spent 80.24 mins less lying than LIM with a BA ($P < 0.01$). Control heifers with a BR had increased lying time compared with CON with a BA ($P < 0.01$). Additionally, LIM heifers with a BA had a tendency for increased lying time compared with LIM with no enrichment ($P = 0.06$). Heifers housed with a BR demonstrated increased standing time by 38.87 and 15.70 mins per d compared with the heifers with a BA and NONE, respectively ($P < 0.01$). Heifers housed with a BA stood for 23.17 mins less than NONE heifers ($P < 0.01$). There was also an effect of day for lying time, standing time, and lying bouts ($P < 0.01$).

Week 1 measurements for play behavior, feed bunk behavior, and enrichment interaction was excluded from data analysis because of an error with video downloading that week. However, CON heifers demonstrated fewer aggressive behaviors than LIM (2.30 ± 1.48 vs. 9.18 ± 1.48 , $P < 0.01$).

Heifers interacted with the brush more often than the ball during all 6 weeks of the trial (Figure 2.8, $P < 0.01$). Overall, heifers interacted with the BR more than the BA (15.35 ± 1.08 events/d vs. 4.33 ± 1.08 events/d, respectively; $P = 0.02$). The average time spent with the enrichment was increased for BR vs. BA (2.14 ± 0.17 min vs. 1.13 ± 0.17 min, respectively; $P = 0.04$). The total duration that the heifers spent with the BR was increased than BA (25.76 ± 1.83 min/d vs. 4.14 ± 1.83 min/d, respectively; $P < 0.01$).

Heifers with BA increased social play events, average social play time, and the total duration of social play during weeks 0 and 5 (Figure 2.7, $P < 0.01$). Heifers had increased social play events during week 0 compared with all other weeks (6.25 ± 0.66 events vs. 2.00 ± 0.66 events, respectively; $P < 0.01$). Fewer social play events were recorded during week 2 than week 5 (1.67 ± 0.66 events vs. 2.71 ± 0.66 events, respectively; $P = 0.03$). Heifers with the BA had increased average social play time than heifers with the BR and NONE heifers (2.05 ± 0.29 min vs. 1.12 ± 0.29 and 1.35 ± 0.29 min, respectively; $P < 0.01$).

CON visited the bunk less during week 3 compared with limit-fed heifers (Figure 2.8, $P < 0.01$). Visits to the feed bunk were decreased during weeks 3, 4, and 5 compared with weeks 0 and 2 (19.37 ± 4.54 , 15.29 ± 4.54 , and 18.00 ± 4.54 vs. 39.75 ± 4.54 and 32.13 , respectively; $P < 0.01$). There was a tendency for CON to have greater visits to the feed bunk compared with LIM (26.33 ± 4.32 vs. 23.48 ± 4.32 , respectively; $P = 0.08$).

2.5. Discussion

As seen in previous research, limit feeding heifers did not affect the ADG of the animals (Hoffman et al., 2007, Lascano et al., 2009, Zanton and Heinrichs, 2009). The current study had decreased ADG than expected for prepubertal heifers. The recommended ADG for Holstein heifers is typically 0.80 kg/d for optimal milk production and growth (Zanton and Heinrichs, 2005), with the current study aiming for 0.70 kg/d. The ADG of 0.70 kg/d was decided based on NRC (2001) requirements for large heifers. Dry matter feeding amounts for the CON diet in the current study were based on NRC (2001) DM recommendations. The NRC (2001) DMI recommendations for 150 and 200 kg large breed heifers with an ADG of 0.8 kg/d are 4.2 and 5.2 respectively. Dry matter intakes in the current study both exceed those DMI recommendations. Heifers on the CON group received 5.56 kg/d while the LIM received 4.87 kg/d in the current study. Lascano et al. (2009) provided LIM (8 mo. of age) with 5.34 kg/d of DM and CON with 5.52 kg/d of DM. In another study, six-month-old heifers that were limit-fed a high concentrate diet received 5.70 kg/d of feed (Kitts et al., 2011). Therefore, heifers in the current study may not have received enough feed to meet the recommended ADG. Additionally, the LIM group had a DMI that was only 12% decreased than that of CON, whereas the original goal was for LIM to consume 15% less DM than CON.

Crude protein percentage in the current study is close to recommendations for precision fed heifers (Heinrichs and Zanton, 2016b). While the NDF and ADF in the current experiment are not within range based on NRC (2001) recommendations, Zanton and Heinrichs (2016b) caution that these levels may not be warranted for precision fed animals. An accurate NDF has yet to be determined through research when precision feeding (Zanton and Heinrichs, 2016b).

Based on NRC (2001) recommendations for heifers around 150 kg with an ADG of 0.8 kg/d, net energy for maintenance is 3.57 kg/d and net energy for growth is 1.75 kg/d. Values on the current study did not meet these recommendations. The low ADG and high feed efficiency is likely due to the lack of energy in the diet.

In the current study, heifers housed with a BR had decreased hip height scores than heifers with a BA or NONE. Most research using objects to enrich the environment has only monitored ADG (Wilson et al., 2002, Pempek et al., 2017). The differences in hip height and heart girth could be related to human error. Measures can vary if the heifers are standing on the scale differently than the previous week and depending on the person measuring the heifers. The current study had three different personnel that took measurements. Additionally, the three heifers with the smallest frames were housed in pens with BR, which likely contributed to the differences shown in the current study.

Previous studies have observed a feed efficiency, or feed-to-gain ratio between 6.3 and 6.6 DMI/ADG for LIM around the same age as heifers in the current study (Lascano et al., 2009, Kitts et al., 2011). The present study had exceptional feed efficiency values for not only the LIM group but also the CON group. As previously discussed, heifers did not achieve the ADG typically seen in the industry today, which resulted in the observed increased feed efficiency for the heifers. Additionally, there may not have been enough of a difference in DMI between the feeding groups to observe a difference in feeding efficiency. Limit-fed gravid heifers showed no differences in feed efficiency compared with CON when DM was restricted by 10% (Hoffman et al., 2007). In contrast, Hoffman et al. (2007) observed a 2.9 kg of DM/kg of gain difference when heifers were restricted to 80% of the CON diet DM. Loerch (1990) found no differences in

feed efficiency in growing steers when the diet was limited to 80% of the CON DM. However, Loerch (1990) fed corn silage as the energy source, while the current study used corn grain.

To the author's current knowledge, NEFA concentrations have not been evaluated during limit-fed trials for dairy heifers. When prepartum multiparous dairy cows were offered a limit-fed diet (70% of CON diet DM based off NRC (2001) for 670 kg BW), there were no changes in NEFA concentrations between the two diets (Winkelman et al., 2008), similar to the findings in the current study. This lack of difference is expected since both groups of heifers were consuming diets that were sufficient in energy for maintenance. Non-esterified fatty acid concentrations increase when heifers or cows are not consuming enough energy to meet energy requirements (Vandehaar et al., 1995, Chelikani et al., 2009)

Averaged NEFA concentrations in the current study were 0.30 and 0.33 mEq/L for the CON and LIM, respectively. Bunting et al. (1994) observed an average NEFA concentration of 0.31 mEq/L for heifers fed a similar diet to the CON in the current study. In another study, NEFA concentrations were between 0.40 and 0.30 mEq/L during the first five weeks after a diet change and then declined into the 0.25 to 0.15 mEq/L range by 20 weeks after the diet change (Chelikani et al., 2009). Based on previous studies, heifers experiencing negative energy balance have NEFA concentrations of 0.40 or greater (Villa-Godoy et al., 1990, Vandehaar et al., 1995). The diet by week interaction tendency observed in this study was caused by a difference in NEFA concentrations between the diet groups during week 0. The increased NEFA concentrations exhibited by LIM during week 0 was likely contributed to by natural biological variation between the heifers. Three LIM had notably increased NEFA concentrations during that week than the rest of the LIM. All heifers were handled in the same manner that day, all three heifers consumed all of the feed offered during the previous week, and there was no

difference in activity levels compared with the other heifers that week. Two of the heifers continued to have increased NEFA concentrations compared with the rest of the heifers during the following week. These heifers may naturally have increased NEFA concentrations than the other heifers as a result of biological variation.

The observation that CON had more rumination events than LIM in weeks 1, 2, 4, and 5 was expected because the CON diet contained increased levels of fiber, which promotes rumination (González et al., 2012). Kitts et al. (2011) added straw to the limit-fed ration and only observed a tendency for LIM to have decreased rumination events compared with heifers with straw mixed into the ration. It is essential to note, however, that rumination was only observed for an hour on the four days that were observed, and some animals were still consuming feed at that time (Kitts et al., 2011), which could have prevented the ability to observe a statistical difference between the diet groups. Additionally, Greter et al. (2015) demonstrated similar results to the current study when testing the behavior of LIM. LIM decreased rumination by 134 mins less/d compared with control. However, neither study followed rumination events or times past four days after feeding changes. In the current study, heifers were fed an hour late on week three as a result of equipment issues. Therefore, some CON were still eating at the time of observation. This could be why there were no dietary differences observed in week 3.

The current study showed decreased rumen pH for LIM compared with CON. These results are similar to previous studies (Moody et al., 2007, Zanton and Heinrichs, 2016a) and were expected because the LIM diet had decreased NDF and increased starch content than the CON diet. Normal rumen pH for heifers is between 6.4 and 7.0 pH after removing from the rumen. The increased rumen pH for both diet groups in the current study was likely contributed to by slight saliva contamination since heifers were oral tubed to collect the fluid (Grünberg and

Constable, 2009). No rumen pH samples were in the range for animals with rumen acidosis (< 5.5 pH), rumen stasis (> 8 pH), or urea toxicity (> 8 pH) (Grünberg and Constable, 2009).

The total VFA concentrations observed in the current study are decreased compared with the findings of Zanton and Heinrich (2008), which observed values of 122 mM for the CON and 107 mM for LIM. Moody et al. (2007) showed LIM animals to have a total VFA of 126.4 mM compared with CON at 139.8 mM. Saliva contamination is likely the cause for reduced VFA concentration since Zanton and Heinrich (2008) used cannulated heifers.

Moody et al. (2007) demonstrated increased acetic, propionic, and butyric acid concentrations in LIM cannulated heifers compared with CON. A similar difference was seen in the current study for overall acetic concentrations and, propionic and butyric concentrations during weeks 3, 4, and 5; however differences in the current study were significant. Additionally, the overall values in the current study were decreased than Moody et al. (2007), which is once again likely contributed to by saliva contamination. To the author's knowledge, isovaleric and isobutyric concentrations have not been reported in limit-fed studies. Most limit-fed studies, listed below, only report the molar proportions of VFA.

Molar proportions during weeks 0, 1, and 2 were similar to the molar proportions observed in previous studies (Lascano et al., 2009, Kruse et al., 2010, Zanton and Heinrichs, 2016). Zanton and Heinrichs (2008) observed no changes in the molar proportions of acetate, propionate, butyrate, or valerate, but observed that heifers offered less DM had increased isobutyrate and isovalerate molar proportions compared with CON animals. An additional study demonstrated no differences in any VFA molar proportions between LIM (85% of CON), and CON (Kruse et al., 2010) Lascano et al. (2009) demonstrated no dietary changes in the molar proportions of isobutyric or isovaleric acid, which was also seen in the current study. Lascano et

al. (2009) showed that heifers fed an increased ratio of concentrates (40:60 forage: concentrate) had decreased acetic acid molar proportions compared with the 80:20 ratio. However, there was no difference between the high-concentrate diet (40:60) and the medium-concentrate diet (60:40) (Lascano et al., 2009), which is similar to the CON ration in the current study. It is important to note that a yeast supplement was added to the Lascano et al. (2009) rations, which could have affected the VFA concentrations.

Additionally, for all previous studies mentioned above, rumen sampling only took place over a 24-hr period. Samples were not collected in the weeks after the diet changes. The current study did not observe any VFA concentration differences until week 3. Similar results are seen for VFA molar proportion, where data was not tracked past 24 hrs. When dairy cows were switched from a high-forage diet to a high-concentrate diet, the molar proportions of acetic acid decreased, and the molar proportion of propionic acid increased 8 days after the diet change (Hernandez-Urdaneta et al., 1976). However, these changes did not occur until 14 days after the diet change in the current study. The current study was not as dramatic of a change from forage to concentrate as seen in Hernandez-Urdaneta et al. (1976) and could explain the delay in molar proportion changes. When evaluating the stair-step program in goats, the switch to the increased concentrate ration caused a decline in the molar proportion of acetic acid, an increase in the molar proportion of propionic acid, and no change in the molar proportion of butyric acid about 12 days after concentrates were increased (Sun et al., 2009), which is similar to the changes in molar proportions observed in the current study. More research is needed to determine the changes in molar proportions for valeric, isobutyric, and isovaleric acid over a more extended period.

The current study observed that LIM heifers housed with a BA had an increase in lying time compared with the CON heifers with a BA. There were no dietary differences in lying time for heifers housed with a BR or NONE. However, CON heifer with a BA had greater lying times than CON with a BA. When Japanese black calves (164 ± 17 d of age) were enriched with a brush, log, and wooden wall, Ninomiya and Sato (2009) observed an increase in time lying on the sternum and time lying with the head touching the flank. These sleeping behaviors are related to behavioral satisfaction and decreased levels of stress (Ninomiya, 2014). While the current study did not track sleeping position, the increases in lying time indicated that the BA and BR may help relieve some of the stress observed in LIM heifers. Additionally, the use of an automatic BR has been shown to increase lying time compared with enrichment such as a straw bale during 20-min observation periods (Pelley et al., 1995).

Hoffman et al. (2007) observed an hour decrease in lying time for LIM heifers. However, the current study found that LIM heifers with a BA tended to increase lying time by 50 min compared with LIM heifers with no enrichment. Additionally, the addition of straw to the LIM heifers diet had no impact on lying time for heifers (Kitts et al., 2011). Enrichment may provide a more efficient way of increasing lying time in limit-fed heifers compared with adding straw to the diet.

In the current study, heifers with a BR showed an increase in standing time. Heifers with a BR also had increased mean and total time spent with the object. The increase in standing time is contributed to by the increase in interaction time with the object because the heifer must be standing to interact with the BR. Previous research has shown that LIM heifers have greater inactive standing times (Hoffman et al., 2007, Kitts et al., 2011, Greter et al., 2015). There were no dietary differences in standing time for the current study. However, the current study did not

differentiate between total standing time and inactively standing. Therefore, no dietary difference in standing time could be related to the time spent standing to interact with objects.

Previous research demonstrated no changes in aggressive behaviors (feed bunk displacements) between LIM or CON heifers (Hoffman, 2007). Kitts et al. (2011) observed an increase in displacements at the bunk for LIM heifers with added straw compared with LIM heifers with no straw added. This was attributed to increased motivation to consume extra feed (Kitts et al., 2011). This finding is different from the current research, which observed that LIM heifers had greater aggressive events compared with CON heifers. One explanation could be that since neither group was receiving enough feed to reach optimal ADG, and LIM heifers was fed less DM than CON heifers, heifers had increased aggressive events to access additional feed need to meet growth requirements (Metz, 1983).

Heifers in the current study interacted with the BR more often and for longer periods compared with the ball. Preweaned calves enriched with the same BR used in the current study and a long rope attached to the wall had 56 more visits per d to the BR compared with the rope (Zobel et al., 2017). However, Zobel et al. (2017) observed that calves played with the rope for 20 s per visit longer than the BR. Since the BR had more visits, and the rope had longer visits, there was no difference in the total duration (min/d) for the objects (Zobel et al., 2017). The total duration of BR use in the current study was similar to that seen in Zobel et al. (2017). When preweaned calf hutches were enriched with a stationary brush, Calf Lollie, chain, and artificial teat, calves had a longer duration of a visit for the BR and had a longer frequency of visits to the BR after week 3 (Pempek et al., 2017).

When Ninomiya and Sato (2009) enriched calves with a BR, they observed a decrease in the meantime spent using the BR during d 51 compared with d 3. Pempek et al. (2017) observed

that calves increased the use of the BR by the end of the experiment. Additionally, the interaction with the other enrichments used by Pempek et al. (2017) plateaued at three weeks. The current study observed no change in the interaction events with the BA but observed heifers an increase in the number of visits with the BR on weeks 2 and 4 compared with week 0. Individual calves and heifers have been known to have preferences for certain objects (Newberry, 1995, Zobel et al., 2017). Therefore, variation in visit frequencies and durations between the current study and previous research could be a result of differences in the preferences of individual animals. In the current study, some calves never played with the BR or the BA, while others used it frequently, which is similar to individual calves in previous studies (Zobel et al., 2017, Horvath and Miller-Cushon, 2019). The author could find no current research using the hanging ball to compare visits and duration of use. The BA in the current study had a similar frequency of visits as the chain and similar duration of use as the Lollie in Pempek et al. (2017).

Increases in social play or individual play are signs of improved welfare in calves (Jensen et al., 1998). In the current study, heifers housed with a BA had increased social play events and the total duration of social play during weeks 0 and 5 compared with heifers with a BR or NONE. However, heifers with a BR had increased mean duration of social play compared with BA and NONE. Pempek et al. (2017) observed that calves with furnished hutches spent longer duration in locomotor play and had more bouts of play. There was no social play in the previous study since calves were individually housed in hutches. Play behavior also tended to decrease each week (Pempek et al., 2017). Zobel et al. (2017) demonstrated a tendency for a correlation between the total duration of BR use and play behavior ($r = 0.73$). Most studies recording social play in dairy heifers are related to group housing or pair housing in preweaned heifers (Chua et al., 2002, Jensen, 2015) where heifers housed with other animals are shown to increase social

play. Heifers in the current study had been housed in groups for 4 months prior to the start of the experiment. Social play also decreases as heifers get older (Kerr and Wood-Gush, 1987).

Therefore, all observed changes in social play were likely due to the introduction of enrichment objects to the pens. Overall, the current study indicates that the use of enrichment objects in pens could increase social play in heifers and promote positive welfare.

All heifers continuously decreased visits to the feed bunk over the 6-week period, and heifers with a BA had fewer visits to the bunk during week 3 compared with heifers with a BR or NONE. To the author's current knowledge, there have been no previous studies looking at the effects of environmental enrichment on feeding behaviors. The decrease in visits to the feed bunk is likely due to the heifers adjusting to the diet. It is speculated that the decrease in visits to the bunk demonstrated by heifers with a BA is a result of the animals having numerically increased social play events and total social play durations. Since more energy was used for play, the heifers spent less energy walking to the bunk. Alternatively, the heifers may have preferred to play than forage for feed. Decreases in feed bunk visits in the current study are also similar to previous research. Highly stressed feedlot steers decrease eating duration the longer they remain in the feedlot (Buhman et al., 2000).

The current study observed a tendency for CON heifers to visit the bunk more often compared with LIM. Previous research has observed that LIM spend less time eating, which is expected since LIM have less DM to consume and decreased amounts of fibrous feed (Hoffman, 2007, Kitts et al., 2011, Greter et al., 2014). Others studies using crossbred heifers demonstrated that heifers fed ad libitum had more frequent visits to the bunk (Schwartzkopf-Genswein et al., 2002). Additionally, Kitts et al. (2011) observed that LIM without straw had increased unawarded visits to the feeder. The current study did not have the ability to separate actual

feeding times from unawarded visits. The increased aggression at the feed bunk by LIM likely pushed away the smaller CON housed in the same pen. Small CON were often seen visiting the feed bunk when LIM were lying down a few hours after the feed was delivered. Greter et al. (2014) also observed that LIM consumed most of their feed within 3 hours of delivery while CON distributed their time across the d. This could also account for the more frequent visits.

However, restriction of milk intake in preweaned calves has demonstrated increases in aggressive behaviors at the automatic feeding system (De Paula Vieira et al., 2008). De Paula Vieira et al. (2008) also observed restricted calves visiting the feeder more often and ingested milk allowance more rapidly compared to ad libitum heifers. The authors of the previous study relate behavior changes to hunger in the restricted milk fed calves. Therefore, limit-fed heifers may be experiencing sign of hunger in the current experiment.

2.6 Conclusion

While heifers were not consuming enough feed to support a high rate of ADG, the current study had enough dietary difference to provide diet by week interactions that demonstrate the longer-term effects of limit-feeding heifers. The current study showed that limit-fed heifers had increases in total VFA concentrations, and some individual VFAs the longer the heifer was consuming the diet. However, more research is needed on the long-term effects of LIM on rumination events and VFA development with heifers that are consuming enough DM to reach optimal ADG. In future research, an additional week should be added before the start of the study to determine the maximum intake for the heifers. Then the LIM diet should be calculated based on the intake from that week. Additionally, the follow up study should expand to a longer time period to allow heifers more adjustment time.

Providing a BA for limit fed heifers helped to increase lying time compare to CON with a BA. Additionally, the heifers provided with a BR had a numerical increase in lying time compared with heifers with NONE. The addition of enrichment objects may help prevent the reduction in lying time demonstrated by LIM. However, more research is need with heifers fed for optimal ADG. Heifers in the current study had more frequent visits to the BR and spent longer durations there, showing a preference for the BR compared with the BA. This translated into heifers housed with either a BA or BR demonstrating significant or numerical increases in social play compared with heifers with no enrichment. This is a sign that providing enrichment to heifers' pens can improve the welfare of the heifers. Overall, this study demonstrated that the use of environmental enrichment could improve welfare and potentially mitigate some of the negative behaviors typically observed in LIM.

2.7. Tables

Table 1.1. NRC (2001) nutritional requirements for Holstein heifers 6 to 12 month of age with a BCS=3 and expected to calve at 24 month age.

	6 months	12 months
Dry Matter, kg	5.2	7.1
ME, Mcal/day	10.6	16.2
MP, g/d	415	550
RDP, g/d	481	667
RUP, g/d	176	209
CP ^a , %	12.7	12.3
NDF, min %	30-33	30-34
ADF, min %	20-21	20-22
NFC, max %	34-38	34-39
Ca, g	11.3	15
P, g	9.1	10.6
Mg, %	0.11	0.11
Cl, %	0.11	0.12
K, %	0.47	0.48
Na, %	0.08	0.08
S, %	0.2	0.2
Co, mg/kg	0.11	0.11
Cu, mg/kg	10	10
I, mg/kg	0.27	0.3
Fe, mg/kg	43	31
Mn, mg/kg	22	20
Se, mg/kg	0.3	0.3
Zn, mg/kg	32	27
Vit A, IU/kg	16000	24000
Vit D, IU/kg	6000	9000
Vit E, IU/kg	160	240

^a Equivalent to crude protein (CP) if rumen degradable protein (RDP) and rumen undegradable protein (RUP) are balanced

Table 2.1. Ingredients and nutritional analysis of the limit-fed and control diet fed to 24 Holstein heifers from mid-November to late December.

	Control Diet (100% NRC)	Limit-fed diet (85% NRC)
<i>Ingredients, kg DM per day</i>		
Corn Grain, ground	2.27	2.50
Soybean meal	0.95	1.04
Grass hay	1.81	-
Cottonseed, hulls	1.51	1.67
Vitamin Premix	0.01	0.01
Salt	0.03	0.03
Di-Calcium Phosphate	0.07	0.07
<i>Nutrient Analysis</i>		
DM, %	87.12	86.95
OM, %	95.14	95.78
TDN	59.24	65.50
NEm,	0.58	0.68
NEg, kcal	0.32	0.40
CP, %DM	14.48	17.86
CF, %DM	2.60	3.02
ADF, %DM	33.68	25.84
aNDF, %DM	51.40	34.26
Ash, %DM	4.86	4.22
Ca, %DM	0.37	0.35
P, %DM	0.44	0.52
Mg, %DM	0.23	0.21
K, %DM	1.64	1.20
Na, %DM	0.11	0.21
Mn, PPM	81.20	85.80
Zn, PPM	84.20	102.20
Cu, PPM	15.80	25.00

Table 2.2. Ethogram of calf interactions with the brush and ball, play, and feeding behavior.

Behavior	Description
Play with others	Frontal pushing and butt head to head with another heifer (Jenson et al., 1998)
Individual Play	Galloping, bucking or ground play behavior as describe by Jenson et al. (1998)
Ball use	Any part of the body in contact with the ball or chain. Behavior ends if heifer is standing by object for 10secs with no contact or if heifer walks away from object
Brush use	Any part of body in contact with mechanical brush. Behavior ends when brush stops spinning (10secs with no contact) or if heifer walks away from object
Feed bunk visit	Heifer opened Calan gate and placed head in her assigned bin
Aggression at bunk	Making physical contact with feeding heifer and takes her place at the bin.

Table 2.3. Physiological parameters for Holstein heifers consuming a limit-fed or control diet with pens containing a grooming brush, calf ball, or no enrichment from mid-November to late December 2018.

	Diet ^{1,2}		SEM	Enrichment ²			SEM	P-values		
	Control	Limit-fed		Ball	Brush	No enrichment		Diet	Enrichment	Diet×Toy
Initial BW, kg	162.73	165.14	10.31	169.72	147.10	175.00	12.62	0.87	0.28	0.62
ADG, kg/d	0.48	0.51	0.06	0.51	0.51	0.44	0.07	0.75	0.70	0.87
Feed:gain ⁴	12.59	10.52	1.78	9.72	10.96	14.74	2.22	0.45	0.27	0.89
Hip Height, cm	115.19	116.08	0.76	116.87 ^a	113.64 ^b	116.38 ^a	0.94	0.41	0.03	0.21
Hip Width, cm	30.45	31.19	0.53	31.32	29.64	31.50	0.84	0.11	0.35	0.63
Wither Height, cm	108.51	109.88	0.94	110.39	107.47	109.70	1.37	0.19	0.41	0.10
Heart Girth, cm	131.39	131.95	1.27	132.31	128.80	133.91	1.55	0.76	0.06	0.14

^{a,b,c} Within row, means without a common superscript significantly differ ($P \leq 0.05$)

¹Values are reported as least square means and SEM for the experiment

²Control: 100% DM recommendation by NRC (2001) standards. Limit-fed: 85% DM recommendation by NRC (2001) standards. All nutritional requirements for growth and maintenance were met.

³Expressed as average DMI/hd/d

⁴Calculated by dividing DMI/hd/d and ADG

Table 2.4. Nutritional intake for Holstein heifers consuming a limit-fed or control diet with pens containing a grooming brush, calf ball, or no enrichment from mid-November to late December 2018.

Nutrient intake ³	Diet ^{1,2}			Enrichment ²				P-value		
	Control	Limit-fed	SEM	Ball	Brush	No enrichment	SEM	Diet	Toy	Diet*Toy
DMI	5.55 ^a	4.87 ^b	0.07	5.28	5.09	5.26	0.08	<0.01*	0.20	0.16
CP	0.86	0.81	0.02	0.80	0.81	0.82	0.20	0.66	0.55	0.46
Lignin	0.15	0.15	0.01	0.15	0.15	0.16	0.01	0.60	0.47	0.56
ADF	1.63	1.59	0.05	1.56	1.60	1.66	0.06	0.56	0.56	0.49
aNDF	2.34	2.27	0.09	2.24	2.30	2.38	0.11	0.58	0.63	0.53
Ash	0.25	0.24	0.01	0.24	0.24	0.25	0.01	0.54	0.48	0.41
Energy Intake										
NE _M , Mcal/d	1.45	1.43	0.03	1.41	1.43	1.47	0.04	0.64	0.52	0.45
NE _G , Mcal/d	0.83	0.82	0.03	0.81	0.82	0.84	0.03	0.78	0.76	0.71

^{a,b,c} Within row, means without a common superscript significantly differ ($P \leq 0.05$)

¹Values are reported as least square means and SEM for the experiment

²Control: 100% DM recommendation by NRC (2001) standards. Limit-fed: 85% DM recommendation by NRC (2001) standards.

All nutritional requirements for growth and maintenance were met.

³Expressed as kg/d unless stated otherwise

**P-values* <0.05, determined significant

2.8. Figures

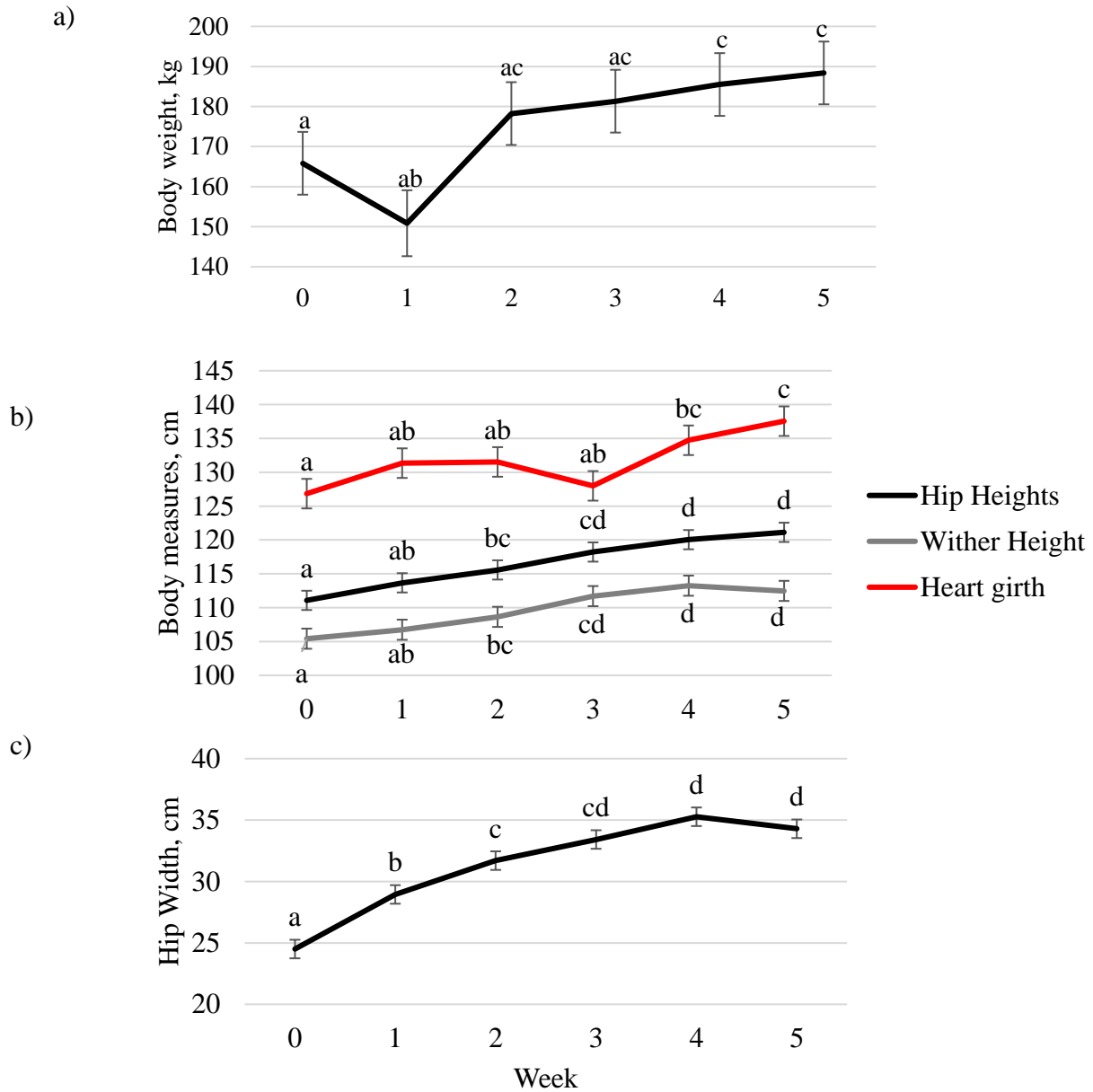


Figure 2.1. Weekly a) body weight, b) body measures (hip height, wither height, and heart girth), and c) hip width for 24 Holstein heifers on a limit-fed (LIM) or control (CON) diet during mid-November to late December 2018. ^{a,b,c} Means without a common superscript differ ($P \leq 0.05$).

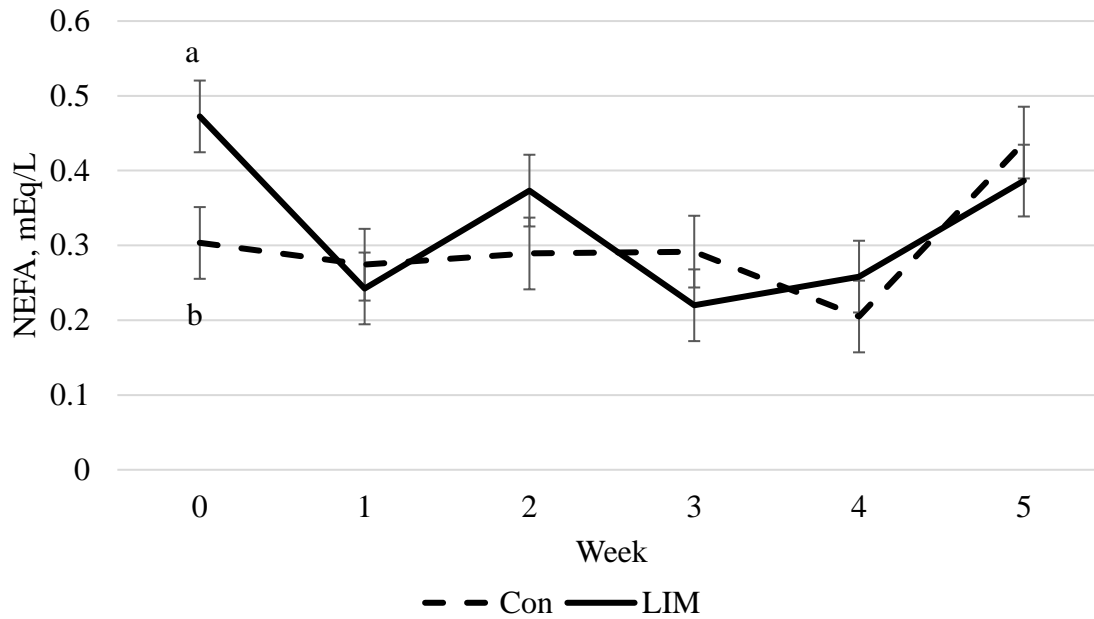


Figure 2.2. Weekly serum NEFA concentrations for 24 Holstein heifers on a limit-fed (LIM) or control (CON) diet during mid-November to late December 2018. ^{a,b,c} Means within a given week without a common superscript differ ($P \leq 0.05$).

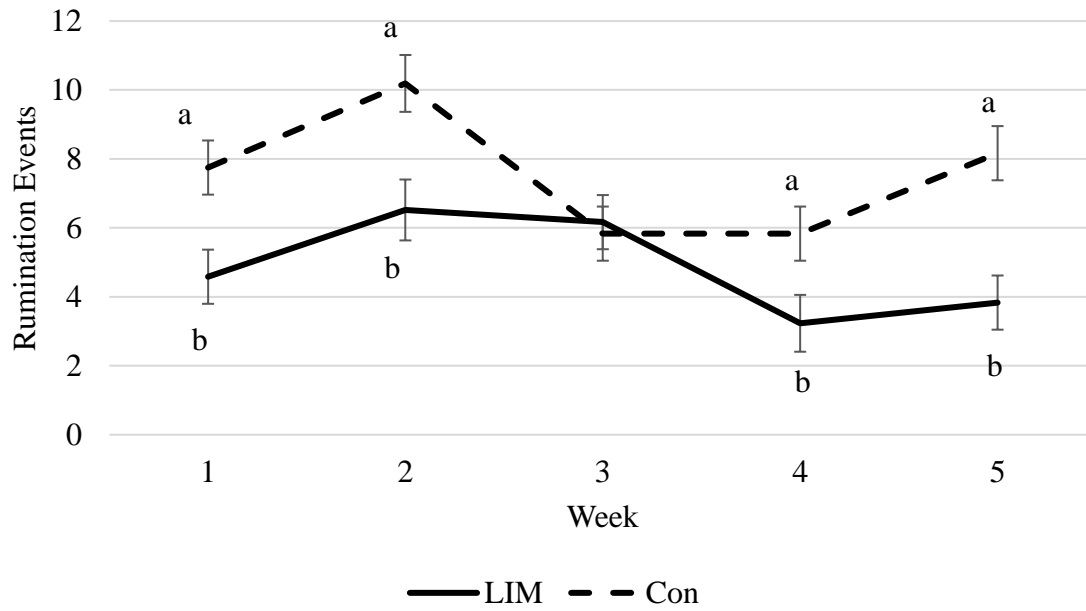


Figure 2.3. Weekly rumination events for 24 Holstein heifers on a limit-fed (LIM) or control (CON) diet during mid-November to late December 2018. ^{a,b} Means within a given week without a common superscript differ ($P \leq 0.05$). Weekly difference not shown.

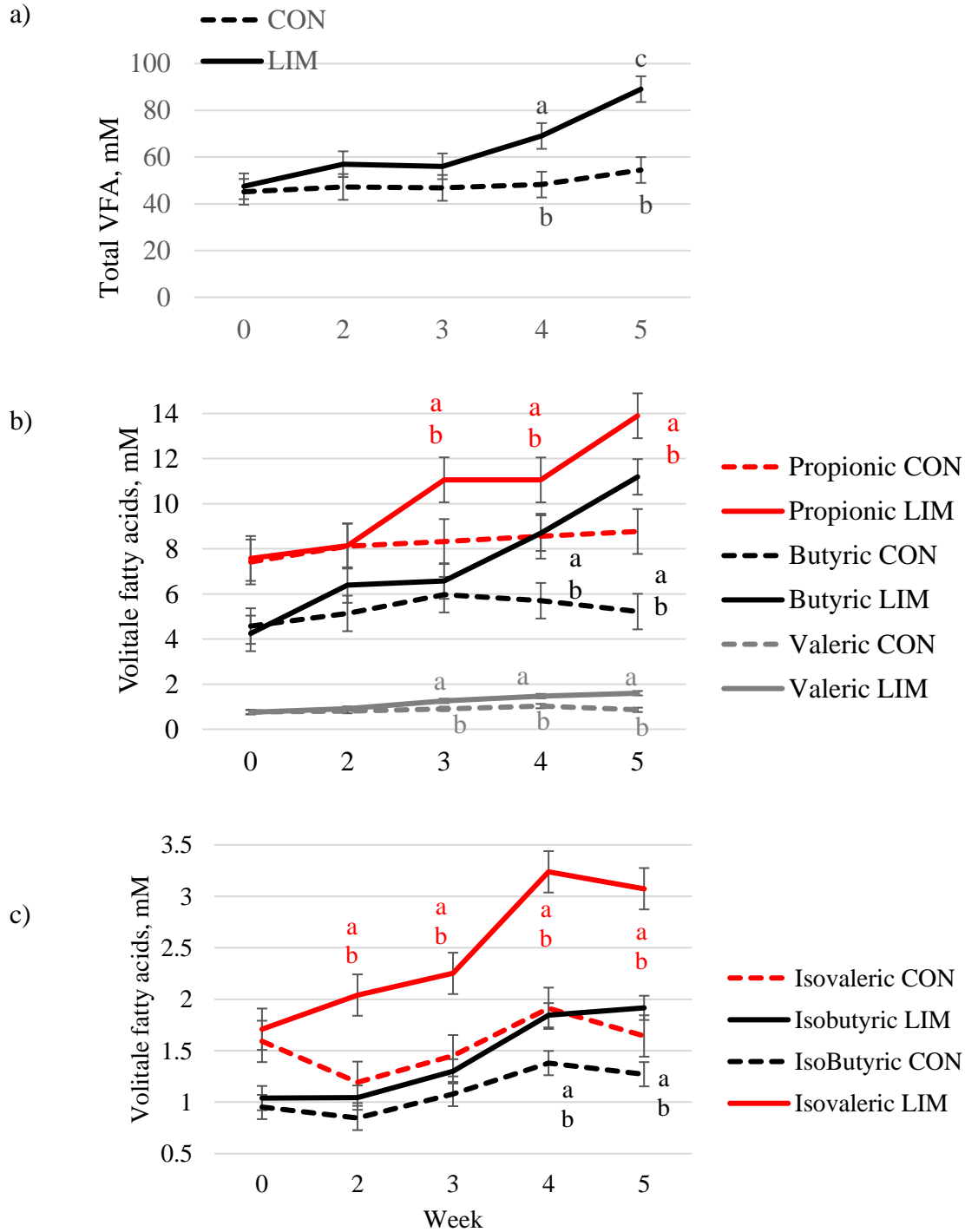


Figure 2.4. Rumen volatile fatty acids concentrations in 24 Holstein heifers consuming a limited (LIM, solid lines; n=12) or control diet (CON, dashed lines; n=12). a) total VFAs. b) volatile fatty acids: propionic (red lines), butyric (black lines), and valeric acid (grey lines). c) volatile fatty acids: iso-valeric (red lines) and iso-butyrac acid (black lines) ^{a,b}. Means within a given week without a common superscript differ ($P \leq 0.05$). Weekly difference not shown.

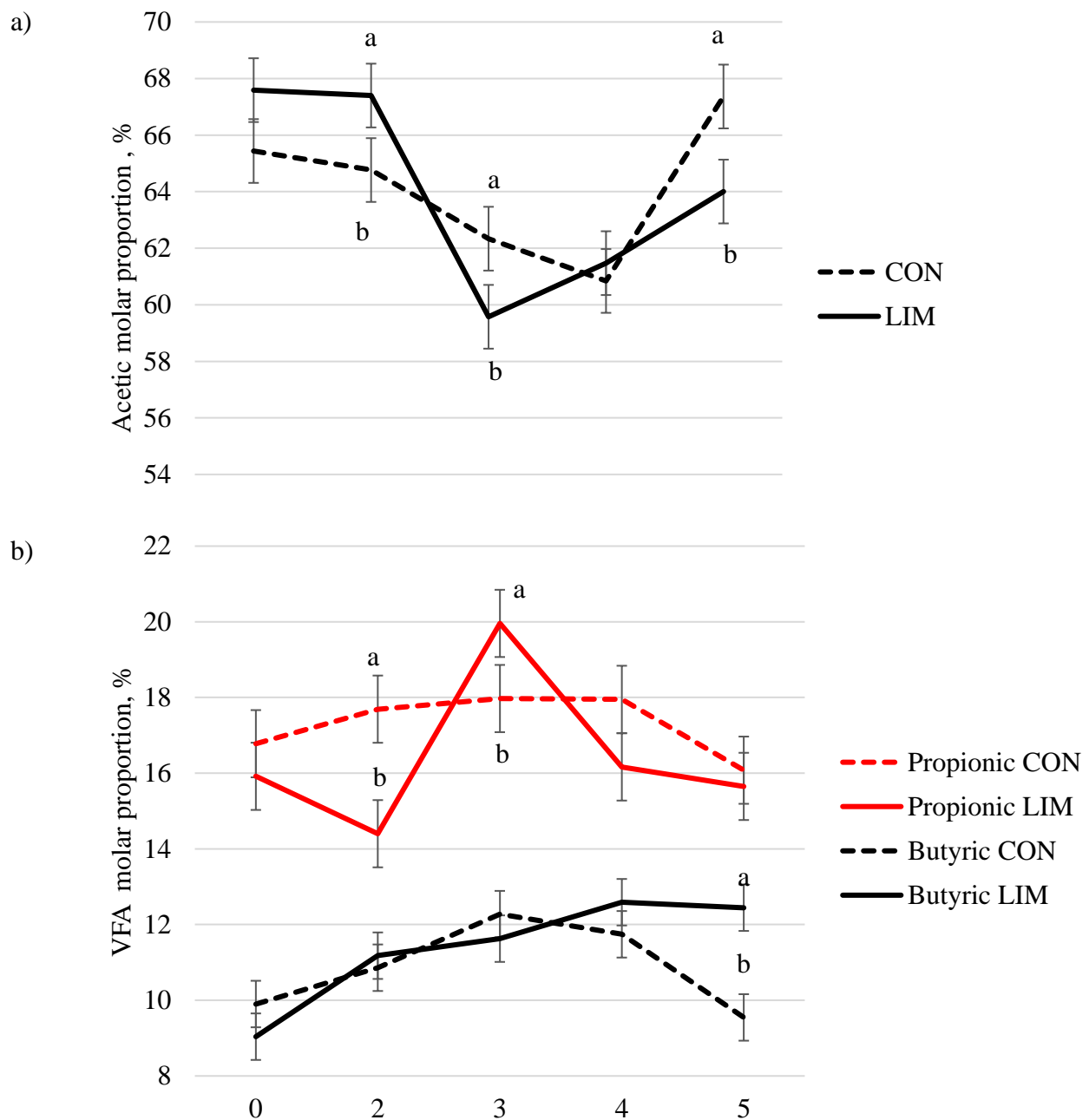


Figure 2.5. Rumen volatile fatty acids molar proportions in Holstein heifers consuming a limited (LIM, solid lines; n=12) or control diet (CON, dashed lines; n=12). a) acetic molar proportion. b) volatile fatty acids: propionic (red lines) and butyric (black lines). ^{a,b} Means within a given week without a common superscript differ (P ≤ 0.05). Weekly difference not shown.

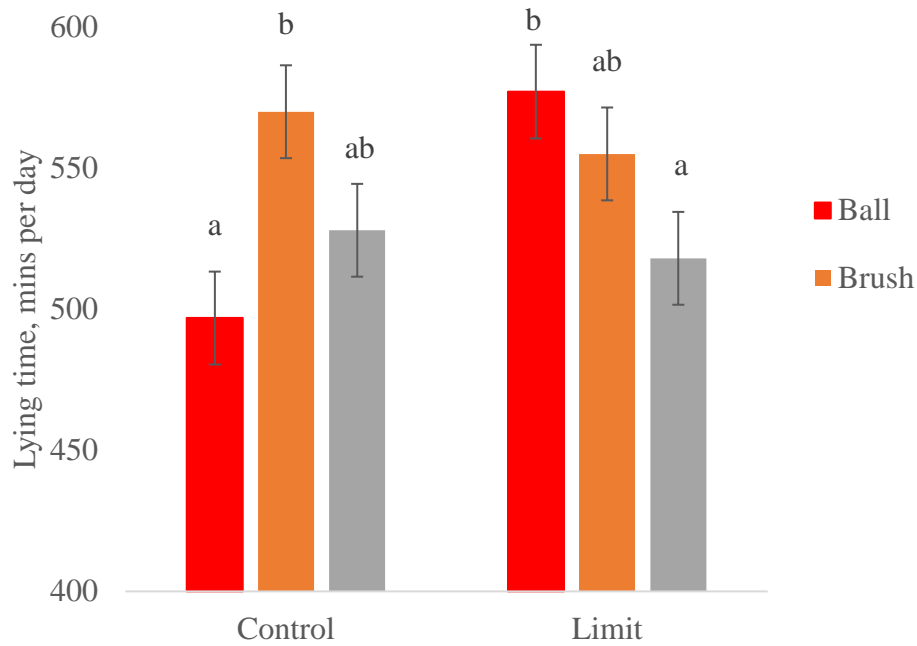


Figure 2.6. Effects of enrichment devices (grooming brush, calf ball or no enrichment) on lying times for 24 Holstein heifers on a limit-fed (LIM) or control (CON) diet during mid-November to late December 2018. ^{a,b} means without a common superscript differ ($P \leq 0.05$).

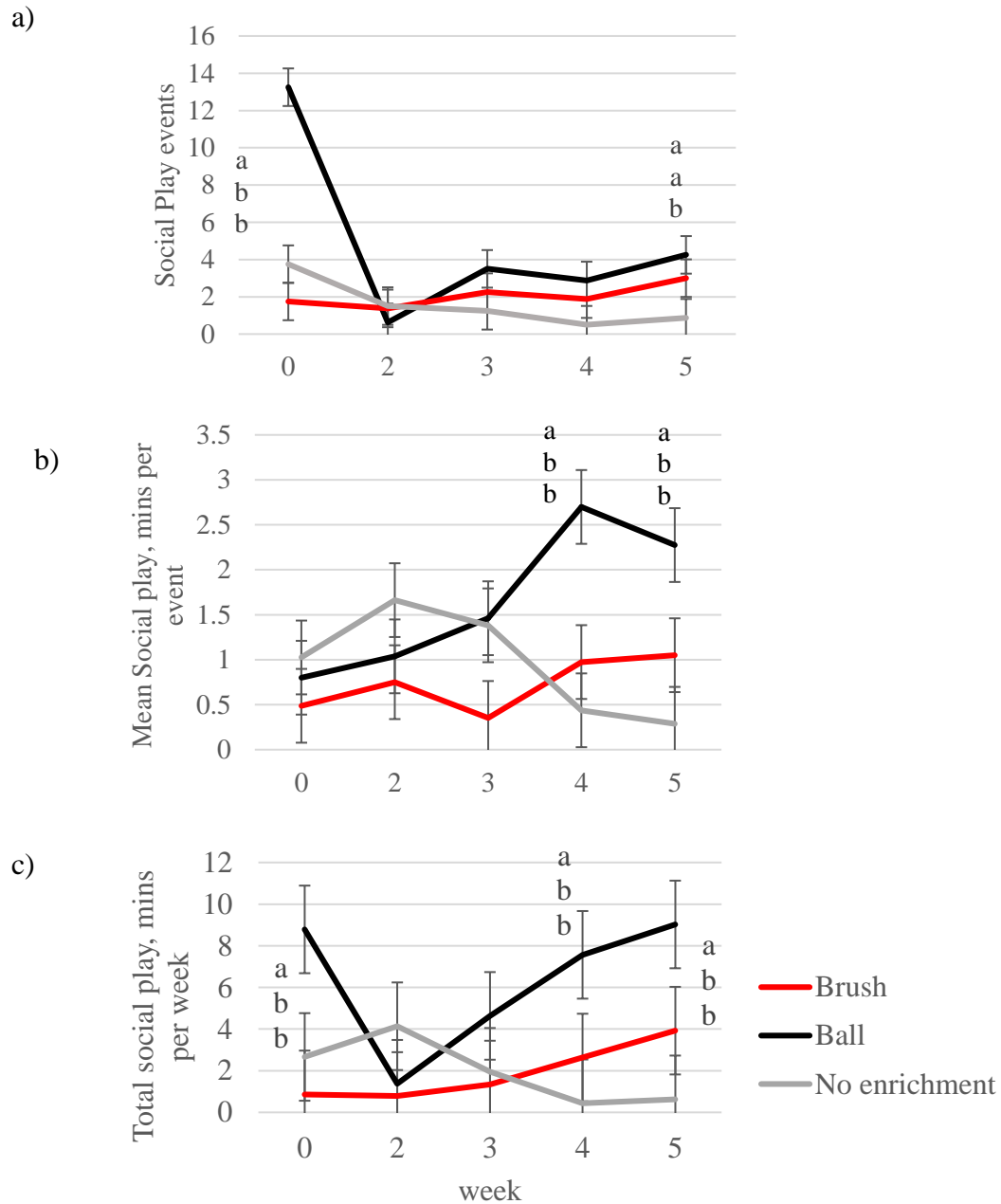


Figure 2.7. Weekly a) social play events, b) mean social play time, and c) total social play time, such as a grooming brush (BR, red line, n=8), a calf ball (BA, black line, n=8), or no enrichment (NONE, grey line, n=8) in 24 Holstein heifers from mid-November to late December 2017.

a)BA: Social play events increased during week 0 compared with all other weeks ($P < 0.01$) and week 2 play decreased compared with week 3 ($P < 0.01$). NONE: heifers had increased play in week 0 compared with 2, 4 and 5 ($P < 0.01$). b) BA: Week 0 and 2 had decreased mean social play compared with week 4 and 5 ($P < 0.01$). Week 3 was less than week 4 ($P < 0.01$). NONE: Week 2 and 3 had increased social play mean times than week 4 and 5. c) BA: week 0, 4 and 5 increased compared with week 2 and 3. ^{a-c} Within week, means without a common superscript differ ($P \leq 0.05$).

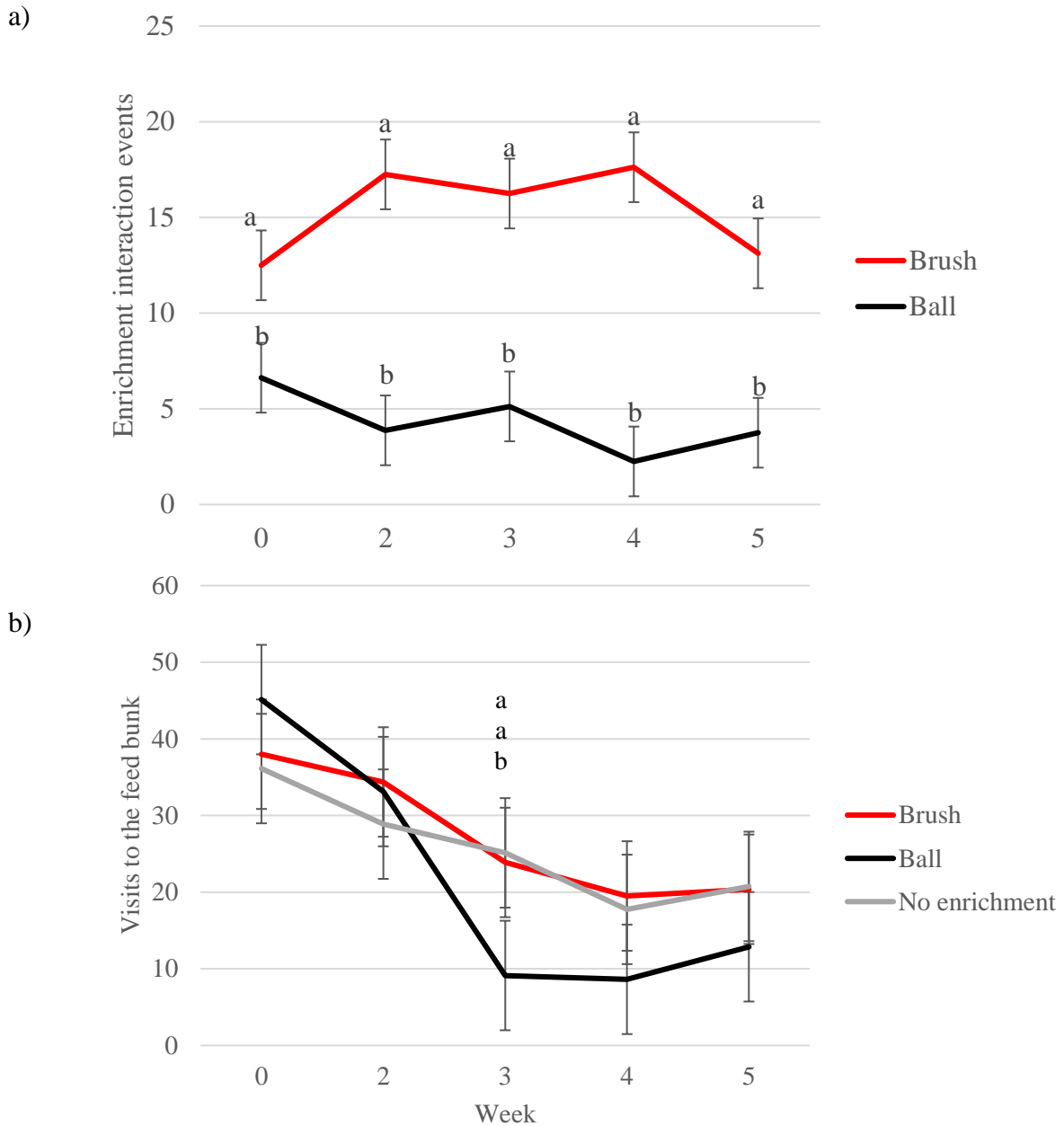


Figure 2.8. Weekly treatment a) enrichment interaction events, and b) visits to the feed bunk, such as a grooming brush (BR, red line, n=8), a calf ball (BA, black line, n=8), or no enrichment (NONE, grey line, n=8) in 24 Holstein heifers from mid-November to late December 2017. a) BR events increased in week 2 and 4 compared with week 0 ($P < 0.01$). b) BA: Week 0 and 2 were increased compared with 3, 4, and 5 ($P < 0.01$). NONE: Week 0 and 2 were increased compared with week 4 and 5 ($P < 0.01$). BR: Week 0 and 2 were increased compared with 3, 4, and 5 ($P < 0.01$). Enrichment interaction events does not include NONE in analysis since no object was provided for heifers to interact with. 6. ^{a-c} Within week, means without a common superscript differ ($P \leq 0.05$).

2.9. References

- Buhman, M. J., L. J. Perino, M. L. Galyean, and R. S. Swingle. 2000. Eating and Drinking Behaviors of Newly Received Feedlot Calves. *The Professional Animal Scientist* 16(4):241-246.
- Chelikani, P. K., D. J. Ambrose, D. H. Keisler, and J. J. Kennelly. 2009. Effects of dietary energy and protein density on plasma concentrations of leptin and metabolic hormones in dairy heifers. *Journal of Dairy Science* 92(4):1430-1441.
- Chua, B., E. Coenen, J. van Delen, and D. M. Weary. 2002. Effects of Pair Versus Individual Housing on the Behavior and Performance of Dairy Calves. *Journal of Dairy Science* 85(2):360-364.
- De Paula Vieira, A., V. Guesdon, A. M. de Passillé, M. A. G. von Keyserlingk, and D. M. Weary. 2008. Behavioural indicators of hunger in dairy calves. *Applied Animal Behaviour Science* 109(2):180-189.
- González, L. A., X. Manteca, S. Calsamiglia, K. S. Schwartzkopf-Genswein, and A. Ferret. 2012. Ruminal acidosis in feedlot cattle: Interplay between feed ingredients, rumen function and feeding behavior (a review). *Animal Feed Science and Technology* 172(1):66-79.
- Greter, A. M., E. K. Miller-Cushon, B. W. McBride, T. M. Widowski, T. F. Duffield, and T. J. DeVries. 2014. *Short communication*: Limit feeding affects behavior patterns and feeding motivation of dairy heifers. *Journal of Dairy Science* 98(2):1248-1254.
- Greter, A. M., E. K. Miller-Cushon, B. W. McBride, T. M. Widowski, T. F. Duffield, and T. J. DeVries. 2015. *Short communication*: Limit feeding affects behavior patterns and feeding motivation of dairy heifers. *Journal of Dairy Science* 98(2):1248-1254.
- Grünberg, W. and P. D. Constable. 2009. CHAPTER 6 - Function and Dysfunction of the Ruminant Forestomach. Pages 12-19 in *Food Animal Practice (Fifth Edition)*. D. E. Anderson and D. M. Rings, ed. W.B. Saunders, Saint Louis.
- Hernandez-Urdaneta, A., C. E. Coppock, R. E. McDowell, D. Gianola, and N. E. Smith. 1976. Changes in Forage-Concentrate Ratio of Complete Feeds for Dairy Cows. *Journal of Dairy Science* 59(4):695-707.
- Hoffman, P. 2007. The potential to limit feed dairy replacement heifers. Pages 186-192 in *Proc. MINNESOTA NUTRITION CONFERENCE*.
- Hoffman, P. C., C. R. Simson, and M. Wattiaux. 2007. Limit Feeding of Gravid Holstein Heifers: Effect on Growth, Manure Nutrient Excretion, and Subsequent Early Lactation Performance. *Journal of Dairy Science* 90(2):946-954.
- Horvath, K. C. and E. K. Miller-Cushon. 2019. Characterizing grooming behavior patterns and the influence of brush access on the behavior of group-housed dairy calves. *Journal of Dairy Science* 102(4):3421-3430.

Horwitz, W. 2000. Official methods of analysis of AOAC international 17th edition. Association of Analytical Chemists International, Gaithersburg, MD.

Jensen, M. B., K. S. Vestergaard, and C. C. Krohn. 1998. Play behaviour in dairy calves kept in pens: the effect of social contact and space allowance. *Applied Animal Behaviour Science* 56(2):97-108.

Jensen, M. B. e. a. 2015. Pair housing and enhanced milk allowance increase play behavior and improve performance in dairy calves. *Journal of dairy science. American Dairy Science Association* 98:2568-2575.

Kerr, S. G. C. and D. G. M. Wood-Gush. 1987. The development of behaviour patterns and temperament in dairy heifers. *Behavioural Processes* 15(1):1-16.

Kitts, B. L., I. J. H. Duncan, B. W. McBride, and T. J. DeVries. 2011. Effect of the provision of a low-nutritive feedstuff on the behavior of dairy heifers limit fed a high-concentrate ration. *Journal of Dairy Science* 94(2):940-950.

Kruse, K. A., D. K. Combs, N. M. Esser, W. K. Coblenz, and P. C. Hoffman. 2010. Evaluation of potential carryover effects associated with limit feeding of gravid Holstein heifers¹. *Journal of Dairy Science* 93(11):5374-5384.

Lammers, B. P., A. J. Heinrichs, and R. S. Kensinger. 1999. The Effects of Accelerated Growth Rates and Estrogen Implants in Prepubertal Holstein Heifers on Estimates of Mammary Development and Subsequent Reproduction and Milk Production¹. *Journal of Dairy Science* 82(8):1753-1764.

Lascano, G. J., G. I. Zanton, F. X. Suarez-Mena, and A. J. Heinrichs. 2009. Effect of limit feeding high- and low-concentrate diets with *Saccharomyces cerevisiae* on digestibility and on dairy heifer growth and first-lactation performance¹. *Journal of Dairy Science* 92(10):5100-5110.

Metz, J. 1983. Food competition in cattle. *Farm Animal Housing and Welfare*:164-170.

Moody, M. L., G. I. Zanton, J. M. Daubert, and A. J. Heinrichs. 2007. Nutrient Utilization of Differing Forage-to-Concentrate Ratios by Growing Holstein Heifers¹. *Journal of Dairy Science* 90(12):5580-5586.

Newberry, R. C. 1995. Environmental enrichment: Increasing the biological relevance of captive environments. *Applied Animal Behaviour Science* 44(2):229-243.

Ninomiya, S. 2014. Satisfaction of farm animal behavioral needs in behaviorally restricted systems: Reducing stressors and environmental enrichment. *Animal Science Journal* 85(6):634-638.

Pelley, M. C., A. Lirette, and T. Tennessen. 1995. Observations on the responses of feedlot cattle to attempted environmental enrichment. *Canadian Journal of Animal Science* 75(4):631-632.

Pempek, J. A., M. L. Eastridge, and K. L. Proudfoot. 2017. The effect of a furnished individual hutch pre-weaning on calf behavior, response to novelty, and growth. *Journal of Dairy Science* 100(6):4807-4817.

Schwartzkopf-Genswein, K. S., S. Atwood, and T. A. McAllister. 2002. Relationships between bunk attendance, intake and performance of steers and heifers on varying feeding regimes. *Applied Animal Behaviour Science* 76(3):179-188.

Sun, Y. Z., S. Y. Mao, and W. Y. Zhu. 2009. Rumen chemical and bacterial changes during stepwise adaptation to a high-concentrate diet in goats. *Animal* 4(2):210-217.

Vandehaar, M. J., B. K. Sharma, and R. L. Fogwell. 1995. Effect of Dietary Energy Restriction on the Expression of Insulin-like Growth Factor-I in Liver and Corpus Luteum of Heifers¹. *Journal of Dairy Science* 78(4):832-841.

Villa-Godoy, A., T. L. Hughes, R. S. Emery, W. J. Enright, A. D. Ealy, S. A. Zinn, and R. L. Fogwell. 1990. Energy balance and body condition influence luteal function in holstein heifers. *Domestic Animal Endocrinology* 7(2):135-148.

Wilson, S. C., F. M. Mitlöhner, J. Morrow-Tesch, J. W. Dailey, and J. J. McGlone. 2002. An assessment of several potential enrichment devices for feedlot cattle. *Applied Animal Behaviour Science* 76(4):259-265.

Winkelman, L. A., T. H. Elsasser, and C. K. Reynolds. 2008. Limit-Feeding a High-Energy Diet to Meet Energy Requirements in the Dry Period Alters Plasma Metabolite Concentrations but Does Not Affect Intake or Milk Production in Early Lactation. *Journal of Dairy Science* 91(3):1067-1079.

Zanton, G. I. and A. J. Heinrichs. 2005. Meta-Analysis to Assess Effect of Prepubertal Average Daily Gain of Holstein Heifers on First-Lactation Production^{*}. *Journal of Dairy Science* 88(11):3860-3867.

Zanton, G. I. and A. J. Heinrichs. 2009. Review: Limit-Feeding with Altered Forage-to-Concentrate Levels in Dairy Heifer Diets¹. *The Professional Animal Scientist* 25(4):393-403.

Zanton, G. I. and A. J. Heinrichs. 2016a. Efficiency and rumen responses in younger and older Holstein heifers limit-fed diets of differing energy density^{>1}. *Journal of Dairy Science* 99(4):2825-2836.</sup>

Zobel, G., H. W. Neave, H. V. Henderson, and J. Webster. 2017. Calves Use an Automated Brush and a Hanging Rope When Pair-Housed. *Animals* 7(11):84.