

Effect of Grouping Strategy and Stocking Density on the Behavior  
of Prepartum Dairy Cows and the Association between Behavior  
and Periparturient Cow Health

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Karen Marie Luchterhand

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Dr. Marcia I. Endres

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## Table of Contents

List of Tables .....	viii
List of Figures .....	x
<b>PERIPARTURIENT DAIRY COW PHYSIOLOGY, BEHAVIOR, AND MANAGEMENT .....</b>	<b>1</b>
Introduction.....	1
Biology and Physiology .....	2
Transition Cow Management.....	6
Prepartum Pen Management .....	8
Dairy Cattle Behavior .....	12
Agonistic Interactions .....	13
Displacements .....	14
Lying Behavior .....	16
Feeding Behavior .....	19
Housing and Management .....	21
Overcrowding .....	21
Regrouping.....	23

**EXPERIMENT 1: EFFECT OF PREPARTUM GROUPING STRATEGY ON DISPLACEMENTS**

**FROM THE FEED BUNK AND FEEDING BEHAVIOR OF DAIRY COWS .....24**

Summary .....24

Introduction.....25

Materials and Methods.....27

*Animals and Housing*.....27

*Experimental Treatments and Design*.....28

*Behavior Measurements*.....29

*Statistical Analysis*.....31

Results.....32

*Social Behavior*.....32

*Feeding Behavior*.....33

Discussion.....33

Conclusions.....37

**EXPERIMENT 2: EFFECT OF STOCKING DENSITY ON SOCIAL, FEEDING, AND**

**LYING BEHAVIOR OF PREPARTUM DAIRY ANIMALS .....42**

Summary .....42

Introduction.....43

Materials and Methods.....46

*Animals and Housing*.....46

*Experimental Treatment and Design*.....47

*Behavior*.....48

*Feeding Behavior*.....49

<i>Lying Behavior</i> .....	49
<i>Statistical Analysis</i> .....	50
Results.....	51
<i>Social Behavior</i> .....	51
<i>Feeding Behavior</i> .....	52
<i>Relationship between Social Rank and Daily Feeding Time</i> .....	52
<i>Lying Behavior</i> .....	52
<i>Relationship between Social Rank and Lying Behavior variables</i> .....	53
Discussion.....	54
Conclusions.....	60

**EXPERIMENT 3: EVALUATION OF PREPARTUM FEEDING TIME AS A**

**PREDICTOR OF PERIPARTURIENT HEALTH DISORDERS IN DAIRY COWS .....67**

Summary.....	67
Introduction.....	68
Materials and Methods.....	70
<i>Animals, Management, and Housing</i> .....	70
<i>Feeding Behavior Measurements</i> .....	71
<i>Body Condition and Locomotion Scoring</i> .....	72
<i>Blood Sampling and Analysis of Metabolites in Plasma</i> .....	72
<i>Clinical Examination and Definition of Diseases</i> .....	72
<i>Production Parameters</i> .....	73
<i>Statistical Analysis</i> .....	73
Results.....	74

<i>Displaced Abomasum</i> .....	75
<i>Metritis</i> .....	76
<i>Retained Fetal Membrane</i> .....	76
<i>Mastitis up to 14 DIM</i> .....	77
<i>Lame at DIM 1 and DIM 35</i> .....	77
<i>Milk Yield and Composition</i> .....	78
Discussion.....	79
Conclusions.....	84
<b>EXPERIMENT 4: ASSOCIATIONS BETWEEN SOCIAL RANKING AND HEALTH OF</b>	
<b>PERIPARTUM DAIRY COWS</b> .....	<b>90</b>
Summary.....	90
Introduction.....	91
Materials and Methods.....	94
<i>Study Design, Animals, and Housing</i> .....	94
<i>Behavior Measurements</i> .....	95
<i>Social Behavior</i> .....	95
<i>Evaluating Social Rankings</i> .....	96
<i>Body Condition and Locomotion Score</i> .....	97
<i>Blood Sampling and Analysis of Metabolites in Plasma</i> .....	97
<i>Clinical Examination and Definition of Diseases</i> .....	98
<i>Production Parameters</i> .....	98
<i>Reproductive Parameters</i> .....	98
<i>Statistical Analysis</i> .....	99

Results.....	100
<i>Displacement Index</i> .....	100
<i>Health</i> .....	100
<i>Reproduction</i> .....	101
<i>Metabolites</i> .....	101
<i>Milk Yield and Composition</i> .....	101
Dominance Category – Galindo and Broom.....	101
<i>Health</i> .....	101
<i>Reproduction</i> .....	102
<i>Metabolites</i> .....	102
<i>Milk Yield and Composition</i> .....	103
Dominance Category – Top and Bottom 10 <sup>th</sup> Percentile .....	104
<i>Health</i> .....	104
<i>Reproduction</i> .....	104
<i>Metabolites</i> .....	105
<i>Milk Yield and Composition</i> .....	105
Discussion.....	105
Conclusions.....	109

## **EXPERIMENT 5: PREPARTUM LYING BEHAVIOR AND POSTPARTUM HEALTH**

<b>DISORDERS IN JERSEY COWS.....</b>	<b>122</b>
Summary.....	122
Introduction.....	123
Materials and Methods.....	125
<i>Animals and Housing</i> .....	125
<i>Experimental Treatment and Design</i> .....	126
<i>Lying Behavior</i> .....	126
<i>Health and Measurements</i> .....	127
<i>Body Condition and Locomotion Score</i> .....	127
<i>Blood Sampling and Analysis of Metabolites in Plasma</i> .....	127
<i>Clinical Examination and Definition of Diseases</i> .....	128
<i>Statistical Analysis</i> .....	128
Results.....	129
<i>Lameness</i> .....	130
<i>Subclinical Ketosis</i> .....	130
<i>Metritis and Acute Metritis</i> .....	131
Discussion.....	132
Conclusions.....	135
<b>BIBLIOGRAPHY .....</b>	<b>141</b>

## List of Tables

### EXPERIMENT 2: EFFECT OF STOCKING DENSITY ON SOCIAL, FEEDING, AND LYING BEHAVIOR OF PREPARTUM DAIRY ANIMALS

<b>Table 1.</b> Lying time (h/d) during the prepartum period according to parity, treatment (80D vs. 100D) and social rank category .....	61
<b>Table 2.</b> Feeding times (min/d) during the prepartum period according to parity (parous vs. nulliparous), treatment (80D vs 100D) and social rank category.....	62

### EXPERIMENT 3: EVALUATION OF PREPARTUM FEEDING TIME AS A PREDICTOR OF PERIPARTURIENT HEALTH DISORDERS IN DAIRY COWS

<b>Table 1.</b> Incidence of displaced abomasum (DA), metritis, acute metritis, retained fetal membrane (RP), subclinical ketosis, mastitis up to 14 DIM, lame at DIM 1 and DIM 35, for nulliparous, primiparous, and multiparous Jersey cows. ....	85
<b>Table 2.</b> Mean ( $\pm$ SEM) daily feeding time (min/d) of healthy (n = 661) and metritic (n = 159) Jersey cows by parity from 21 to 1 d before calving.....	86
<b>Table 3.</b> Mean ( $\pm$ SEM) daily feeding time (min/d) of healthy (n = 661) and retained fetal membrane (n = 69) Jersey dairy cows by parity from 21 to 1 d before calving.....	87

**EXPERIMENT 4: ASSOCIATIONS BETWEEN SOCIAL RANKING AND HEALTH OF PERIPARTUM DAIRY COWS**

**Table 1.** Frequencies of social rank categories based on 2 methods calculating social rank based on Galindo and Broom (2000).....110

**Table 2.** Adjusted odds ratios (AOR) and 95% confidence intervals (95% CI) of displacement index calculated during the close-up prepartum period and transition health disorders of Jersey .....111

**Table 3.** Incidence and adjusted odds ratios (AOR) with 95% confidence intervals (95%CI) of transition health events up to 60 days in milk in Jersey cows by social rank (high, middle, low) .....112

**Table 4.** Least square means (LSM) and standard error (SE) of energy corrected milk of Jersey cows up to 150 DIM by social rank .....113

**Table 5.** Adjusted odds ratios (AOR) and 95% confidence intervals (95% CI) of top and bottom 10<sup>th</sup> percentile social rank in Jersey cows .....114

**Table 6.** Least square mean (LSM) and standard error (SE) milk yield of top and bottom 10<sup>th</sup> percentile social rank of Jersey cows Housing and Management.....115

**EXPERIMENT 5: PREPARTUM LYING BEHAVIOR AND POSTPARTUM HEALTH DISORDERS IN JERSEY COWS**

**Table 1.** Incidence of transition health events up to 60 days in milk in Jersey cows .....136

## List of Figures

### EXPERIMENT 1: EFFECT OF PREPARTUM GROUPING STRATEGY ON DISPLACEMENTS FROM THE FEED BUNK AND FEEDING BEHAVIOR OF DAIRY COWS

- Figure 1.** Mean number of displacements (n/d) from the feed bunk for cows housed in a stable (AIAO, all-in-all-out) or weekly entrance of new animals (TRD, traditional) pen management during the close-up prepartum period.....38
- Figure 2.** Mean rate of displacements from the feed bunk for cows housed in a stable (AIAO, all-in-all-out) or weekly entrance of new animals (TRD, traditional) pen management during the close-up prepartum period .....39
- Figure 3.** Feeding times of cows housed in a stable (AIAO, all-in-all-out) or weekly entrance of new animals (TRD, traditional) pen management during the close-up prepartum period .....40
- Figure 4.** Percentage of cows eating at the feed bunk over a 24-h period for a stable (AIAO, all-in-all-out) or weekly entrance of new animals (TRD, traditional) pen management during the close-up prepartum period (data were averaged over 30 d and only included days when the pen housed greater or equal to 40 cow).....41

**EXPERIMENT 2: EFFECT OF STOCKING DENSITY ON SOCIAL, FEEDING, AND LYING  
BEHAVIOR OF PREPARTUM DAIRY ANIMALS**

**Figure 1.** Average stocking density based on the number of headlocks and number of stalls according to treatment. There were 8 experimental units (4 replicates x 2 pens/treatment per replicate). Treatments: 80D = cows housed in prepartum pens with 80% target stocking density (38 cows/pen; each pen with 48 headlocks and 44 stalls) and 100D = cows housed in prepartum pens with 100% target stocking density (48 cows/pen). .....63

**Figure 2.** Mean daily displacements from the feed bunk for treatments: 80D = cows housed in prepartum pens with 80% target feed bunk stocking density (38 cows/48 headlocks) and 100D = cows housed in prepartum pens with 100% target feed bunk stocking density (48 cows/48 headlocks). There were 8 experimental units (4 replicates x 2 pens/treatment per replicate). Treatment affected the mean ( $\pm$  SEM) of the number of displacements from the feed bunk (80D =  $15.2 \pm 1.0$ , 100D =  $21.3 \pm 1.0$ ;  $P < 0.001$ ).....64

**Figure 3.** Mean daily lying times for 80 (**80D**) and 100% (**100D**) prepartum feed bunk stocking density. There were 8 experimental units (4 replicates x 2 pens/treatment per replicate). Treatments: 80D = cows housed in prepartum pens with 80% target feed bunk stocking density (38 cows/48 headlocks) and 100D = cows housed in prepartum pens with 100% target feed bunk stocking density (48 cows/48 headlocks). .....65

**Figure 4.** Mean daily feeding times for 80 (**80D**) and 100% (**100D**) prepartum feed bunk stocking density relative to calving. There were 8

experimental units (4 replicates x 2 pens/treatment per replicate).

Treatments: 80D = cows housed in prepartum pens with 80% target feed

bunk stocking density (38 cows/48 headlocks) and 100D = cows housed in

prepartum pens with 100% target feed bunk stocking density (48 cows/48

headlocks). .....66

**EXPERIMENT 3: EVALUATION OF PREPARTUM FEEDING TIME AS A PREDICTOR OF PERIPARTURIENT HEALTH DISORDERS IN DAIRY COWS**

**Figure 1.** Mean ( $\pm$ SEM) daily feeding time (min/d) of multiparous Jersey cows; healthy (n = 210) and lame at DIM 1 (n = 9) from 21 to 1 d before calving.....88

**Figure 2.** Mean ( $\pm$ SEM) daily feeding time (min/d) of Jersey cows (prepartum lactation  $\geq$  1); healthy (n = 451) and lame at DIM 1 (n = 13) from 21 to 1 d before calving.....89

**EXPERIMENT 4: ASSOCIATIONS BETWEEN SOCIAL RANKING AND HEALTH OF PERIPARTUM DAIRY COWS**

**Figure 1.** Frequency of displacement index rounded to the nearest tenth of 953 prepartum Jersey cows.....116

**Figure 2.** Adjusted odds of curvilinear association of displacement index and metritis for nulliparous, primiparous, and multiparous Jersey cows .....117

**Figure 3.** Non-esterified fatty acid (NEFA) concentrations of Jersey cows by parity and social rank.....118

**Figure 4.** Milk yield (kg/d) of multiparous Jersey cows by social rank.....119

<b>Figure 5.</b> Log count somatic cell (LogSCC) of Jersey cows by social rank and parity .....	120
<b>Figure 6.</b> Non-esterified fatty acid concentration (NEFA) of Jersey cows by top and bottom 10 <sup>th</sup> percentile of social rank.....	121
 <b>EXPERIMENT 5: PREPARTUM LYING BEHAVIOR AND POSTPARTUM HEALTH DISORDERS IN JERSEY COWS</b>	
<b>Figure 1.</b> Daily lying time (h/d), lying bouts (n/d) and lying bout duration (min/bout) of nulliparous, primiparous, and multiparous prepartum Jersey cows .....	137
<b>Figure 2.</b> Daily lying time of prepartum Jersey cows (h/d) diagnosed lame at DIM 1 or healthy.....	138
<b>Figure 3.</b> Daily lying bouts of prepartum Jersey cows (no. bouts/d) diagnosed lame at DIM 1 .....	139
<b>Figure 4.</b> Daily lying time of prepartum Jersey cows (h/d) diagnosed with metritis or healthy .....	140

# LITERATURE REVIEW

## PERIPARTURIENT DAIRY COW PHYSIOLOGY, BEHAVIOR, AND MANAGEMENT

### **Introduction**

The transition dairy cow is one of the highest risk animals for falling ill or dying on the dairy farm. Typically this period is described as 3 weeks before and after calving (Grummer, 1995). During this period cows experience physiological, immune, and nutritional changes making the cow at risk for metabolic and infectious diseases (Goff and Horst, 1997). Up to 25% of cows are culled or die during the first 60 DIM (Godden et al., 2004), which may be attributed to an unsuccessful transition period. Concern over animal well-being and loss of farm profitability due to morbidity and mortality losses have increased research in the area of transition cow management and better understanding of behavior to facilitate improved management of these cows.

There has been increasing interest in improving the welfare of the transition dairy cow. Broom (1991) describes welfare as the state of an individual in relation to its environment and that an animal can have both good and poor welfare. By evaluating cow comfort, nutrition, housing, and management steps can be taken to improve the transition cow welfare. To assess welfare Winckler et al. (2003) recommends evaluating lameness incidence and prevalence, injuries, body condition score, cleanliness, lying behavior, agonistic social behavior, abnormal behavior, animal-human relationship and stockmanship. Other factors often included when evaluating welfare include housing factors, disease incidence and mortality. Nutrition should be evaluated to prevent over-

conditioned dry cows and that all nutrient requirements are met. This literature review will focus on transition cow management including environment, nutrition, and behavior and its relationship with the biology and physiology of the dairy cow to ultimately improve the well-being of the dairy cow.

### **Biology and Physiology**

Dramatic changes occur in the dairy cow during the transition period including hormonal, feed intake, nutrient requirements, and immune function. These changes are primarily driven by the synthesis of milk components and the development of the mammary glands and to a lesser extent to the increasing demands of the fetus towards the end of gestation. It has been cited that many of the metabolic and health problems that occur in the dairy cow are due to the increased productivity (Jones et al., 1994). Approximately 14 d prior to calving there is a dramatic decrease in feed intake (Grummer et al., 2004) with prepartum dry matter intake (**DMI**) decreasing from 2 to 1.4% of body weight. After parturition it will take approximately 1 week for DMI to exceed consumption that occurred during late gestation (Grant and Albright, 1995) and steadily increase for a few weeks after parturition (Annen et al., 2004). However, during this time the cow will be in negative energy balance often up to 6 or more weeks postpartum due to the nutrient demands of milk production. The mobilization of adipose tissue is normal at the beginning of lactation; however, if the magnitude and speed of mobilization exceeds the metabolic ability of the liver then the incidence of metabolic problems will increase. If not properly managed, cows can have greater problems during and after parturition. The goals of the transition dairy cow management program are to minimize

negative energy balance, maintain blood calcium levels and maintain a strong immune system.

The drive to produce milk is given priority over nearly all other physiological processes during this time. In addition to the needs of lactation, the growth of the fetus also increases nutrient needs. The growth of the fetus utilizes 3-5 Mcal of net energy, 46% of maternal glucose and 360 g of metabolizable protein during the final weeks of gestation (Bell, 1995). A cow producing 30 kg of milk/d uses at least 2 kg of blood glucose to synthesize lactose for milk (Bell, 1996). An up-regulation of gluconeogenesis by the liver is critical while other organs and tissues have to adapt to the reduced availability of glucose.

Negative energy balance and homeorhetic adaptations during the transition from non-lactating to lactating are characterized by a continual decline in plasma insulin concentration until calving (Doepel et al., 2002) and a decrease in the sensitivity of adipose tissue to insulin (Bell and Bauman, 1997). Somatotropin increases rapidly towards the end of gestation and postpartum (Bauman and Vernon, 1993) while plasma progesterone which is high during gestation rapidly falls at calving. There is a transitory elevation in estrogen and glucocorticoids during the transition period. These hormonal changes contribute to a decline in DMI and the mobilization of adipose tissue and stimulate the liver to increase glucose production (Grummer, 1995). When adipose tissue is mobilized there is an increase in plasma non-esterified fatty acid (**NEFA**) concentrations and rapidly increases during the last 3 days of gestation (Grummer, 1995). Non-esterified fatty acids are used by many tissues as an energy source during periods of

negative energy balance (Grummer et al., 2004). The higher circulating NEFA concentrations lead to great uptakes of fatty acids by the liver. When the increase in fatty acid supply to the liver exceeds the capacity for oxidation, the liver produces ketone bodies and stores triglycerides in the liver (Drackley et al., 2001). The reduction of oxidation capacity and production of ketone bodies can lead to the development of fatty liver disease and ketosis. Nonesterified fatty acid concentrations during the prepartum period have been associated with increased risk of developing displaced abomasum (LeBlanc et al., 2005), retained placenta (LeBlanc et al., 2004) and culling (Duffield et al., 2009).

Serum calcium concentrations are under strict homeostatic control and are regulated by the endocrine system. Calcium concentrations are maintained by intestinal absorption, bone resorption or deposition, renal reabsorption and urinary excretion, fecal excretion, salivary recycling, fetal deposition and milk secretion (Overton and Waldron, 2004). Calcium is also necessary for muscle contractions, transmission of nerve signals and signal transducer in many types of cells, including immune cells (Bradford, 2011). There is an increase in the calcium requirements for the transition dairy cow with the onset of lactation. On the first day of lactation calcium requirements can increase by 3-fold and will continue as milk yield increases (Horst et al., 2005). If blood calcium is not replaced as rapidly as absorption from the intestine and resorption from bone release, cows can develop hypocalcemia or clinical milk fever, which puts the cow at risk for other diseases. Cows with clinical hypocalcemia have been observed to have an increased risk of postpartum diseases such as mastitis, retained placenta, metritis and

displaced abomasum (Goff and Horst, 1997; Mulligan et al., 2006, Goff, 2008). Martinez et al. (2012) noted cows with subclinical hypocalcemia were at greater risk for developing metritis, whereas Chapinal et al. (2011) reported subclinical hypocalcemia was not associated with metritis or retained placenta, but was associated to an increased risk of displaced abomasum. Chamberlin et al. (2013) observed hypocalcemic cows had significantly higher NEFA concentrations on d 0 and had more lipid in hepatocytes on d 7 and d 35 postpartum. The use of anionic prepartum diets has been successful in the reduction of clinical and sub-clinical hypocalcemia in cows predisposed to milk fever (Horst et al., 1997).

During the transition period there is a decrease in both adaptive and innate immune systems. While the etiology is not well understood it seems to be due to physiologic changes associated with parturition and metabolic factors related to the onset of lactation (Overton and Waldron, 2004). Decreased function of lymphocytes and neutrophils has been measured during the transition period (Mallard et al., 1998). Monocytes, the largest leukocyte, respond to stimuli with an increased release of inflammatory cytokines during this period (Sordillo et al., 1995). Cytokines activate the production of acute phase proteins such as haptoglobin and serum amyloid A, which are produced by the liver and are elevated around calving (Bionaz et al., 2007). These acute phase proteins are generally low in the bloodstream, but concentrations are elevated during systemic activation. Glucocorticoids are known for being involved in immunosuppression, while not completely understood it has been suggested that the rise in cortisol around calving coupled with hormonal changes might contribute to

immunosuppression (Weber et al., 2001). Elevated NEFA and beta-hydroxybutyrate (**BHBA**) concentrations may impair neutrophil viability and function (Scalia et al., 2006). Cows without fatty liver cleared bacterial endotoxin from circulation within 30 minutes of administration whereas cows with fatty liver were unable to clear the toxins even after 6 hours (Andersen et al., 1996). When lactating cows were subjected to endotoxin administration there were dramatic changes in circulating concentrations of cortisol, glucagon and insulin in order to maintain glucose homeostasis (Waldron et al., 2003a) and decreased levels of blood calcium and phosphorus (Waldron et al., 2003b). Overton and Waldron (2004) hypothesized immunosuppression during the transition period may be normal and protective to prevent the development of a secondary metabolic disorder.

### **Transition Cow Management**

As previously described, the transition period is a critical time for the dairy cow. In Pennsylvania herds 5 percent died and 7.6 percent were culled within 60 days in milk (Dechow and Goodling, 2008). Providing good nutrition and management are essential for a successful transition from dry to milking. When cows were changed to a close-up diet 21 days prior to parturition, those cows tended to have increased yields of fat, 3.5 percent fat-corrected milk, and protein during the first 5 months of lactation compared to a 60-d dry cow diet; however, there were no differences in milk yield (Contreras et al., 2004). Having cows at the proper body condition score can help prevent metabolic disorders (Heuer et al., 1999) and increase milk yield. Cows with a body condition score of 3.0 or less tended to produce more milk in early lactation than cows with a body condition score of 3.25 or greater (Contreras et al., 2004). Previous research observing

the transition dairy cow determined close-up dry cows within 10 days of calving averaged 86.8 min/d feeding and decreased to 61.7 min/d post calving (Huzzey et al., 2005). Time spent drinking averaged 5.5 min/d and close-up cows averaged 12.3 h/d standing which increased slightly to 13.4 h/d after parturition with standing bouts reasonably consistent pre-and post-calving 11.7 and 13.1 bouts/d, respectively (Huzzey et al., 2005). Similarly, lying time of the periparturient cow decreased from 13.5 h/d to nadir (10.6 h/d) on d 1 in freestall housed cows (Calderon and Cook, 2011). As cows approached parturition the number of lying bouts increased while the lying bout duration decreased (Calderon and Cook, 2011).

Nordlund et al. (2006) used time lapse video and recorded the proportion of cows feeding throughout the day. The highest occupancy rate was 80%, which occurred shortly after feed delivery. When calculated it equated to approximately 30 inches (0.76 m) of linear bunk space per Holstein sized cow. Though this was calculated in a lactating pen, it was recommended that close-up and fresh pens do not exceed this linear bunk space to prevent exacerbated reductions in feeding behavior due to competition. Nordlund et al. (2006) speculated the current industry standard of 24 inch (0.61 m) headlocks is smaller than the width of the average Holstein cow.

When designing transition cow pens it is important to know herd weekly calvings. Kammell and Graves (2007) describe if a pen is only built to the average weekly calvings, 45 out of 100 weeks the pen would be overstocked. It is recommended pen capacity meets the herd needs 90 percent of the time, reducing overstocking to only 10 out of 100 weeks (Kammell and Graves, 2007). Both Nordlund et al. (2006) and

Kammell and Graves (2007) recommend only using 2-row instead of 3-row freestalls to provide enough bunk space for transition cows. If a bedded pack is being used for transition cows, a resting area of 9.5 -19 m<sup>2</sup> per cow (Holstein) is recommended.

### **Dry Cow Pen Management**

The transition from housing cattle in tie-stall barns and managing them individually to group housing in freestall barns has brought change to the lifestyle of the dairy cow. Most management and activities occurred in the tie-stall including eating, drinking, milking, lying, and health treatments (Cook and Nordlund, 2004). In freestall systems, depending on the management of the farm, transition cows could be moved up to 5 different pens which results in new pen mates with each change. The changes in grouping increase social interactions, often agonistic, before the group stabilizes (Cook and Nordlund, 2004). After changing the social hierarchy of a pen, it typically takes a minimum of 3 days to stabilize and usually no more than 7 days (Grant and Albright, 2001).

It has been speculated that grouping heifers with cows would result in negative effects (Nordlund et al., 2006). The lower-ranking animals, typically the smaller and younger animals, may be the most affected by regrouping; these animals spent more time standing without eating and ate at a faster rate, putting the animal at risk for ruminal acidosis and lameness events (Proudfoot et al., 2009). One day after regrouping and moving into a new pen cows had reduced feed intake, rumination time and feeding rate (Schirrmann et al., 2011). It is during the transition period that any changes in behavior may put the cow at risk for a health disorder.

Researchers in Wisconsin have proposed producers create stable pens during the dry period in addition to creating a monitoring program of fresh cow performance called the Transition Cow Index™ (TCI; Nordlund, 2006). The TCI uses DHIA data from the previous lactation to predict performance at the first test day of the new lactation and calculates the difference in actual performance versus predicted. Additionally, on-farm disease events are used in the calculation of TCI.

To create these stable pens, cows with similar expected calving dates are grouped together. These cows will then stay with their penmates until parturition, and then they will be moved into a calving pen followed by a fresh pen. Farms that participated in the study and changed dry cow grouping management saw a response of greater than 1,400 lbs TCI® which led to the expected benefit of \$170 of income over feed cost per cow per year (Nordlund, 2010). However, the farms that participated built a new transition barn with larger freestalls (52 inch wide by nine feet), 30 inch headlocks with one headlock per cow, and overbuilt the barn for times of high occupancy of cows. Since this was an observational study and many different factors were changed on these herds, it is difficult to determine which variable producers should change in their facilities or management of pre-fresh cows if they are unable to build an entire new facility.

Few studies have investigated grouping strategies for prepartum cows. Coonen et al. (2011) have the most similar experimental design to our Experiment 1 with 2 treatments (stable housed and dynamic) and 3 replicates. Cows entered bedded pack pens approximately 28 d before expected calving date. New cows were added to the dynamic pen up to 2 times per week to maintain a pen stocking density of 10 animals. Collected

data included pen average dry matter intake, NEFA concentrations, milk production and displacements from the feed bunk. Limitations included only watching for displacements from the feed bunk for 1 h after feeding delivery and only 2 times per week. In both the dynamic and stable pens, feed bunk space was adjusted up to 4 times per week to reach 0.76 m of linear space, which would not occur in most commercial conditions.

Overstocking greater than 1 cow/stall, is a common practice on dairy farms, especially during times of calving surges, anticipation for filling a new facility, or recommendations from financial advisers. Current industry recommendations for close-up and fresh cows are providing approximately 0.76 m (30 inches or approximately 80 percent stocking rate) of linear bunk space for Holstein and similar sized cattle and 1 stall per cow (Nordlund, 2006). It is likely the recommendation came from an observational study that showed a decrease of 0.7 kg/d of milk yield of heifers that were mixed in a pen with multiparous cows for every 10 percent unit increase in stocking density (Oetzel et al., 2007). It is known that increasing stocking density in both stall and linear feed bunk space can affect dry matter intake (Proudfoot et al., 2009), lying behavior (Fregonesi et al., 2007), and increase agonistic behaviors (DeVries et al., 2004).

Fregonesi et al. (2007) evaluated different stall stocking densities in lactating Holstein dairy cattle with a switchback design. All cows spent 1 week in each treatment and returned back to 100 percent after exposure to other treatments. When cows were overstocked by 150 percent there was a reduction in lying time by 1.7 h/d and more displacements from the stall compared to 100 percent stocking rate (Fregonesi et al., 2007). Wierenga and Hopster (1990) evaluated rank and stocking density. Lower ranked

cows had their lying schedule affected when the stocking density reached 125 percent. The lower ranked cows tended to shift their lying times to the earlier evening as stalls available during the night were reduced (Wierenga and Hopster, 1990). However, lying time of low ranking animals was reduced 154 min/d when the pen was stocked at 155 percent.

When the feed bunk was restricted to 2 cows per 1 feed bin, cows had similar daily dry matter intake and number of visits to the feeder, but tended to spend less time feeding compared to cows with 1 feed bin per cow (Collings et al., 2011). Cows overstocked for feed bin access consumed 11 percent less feed during the 2 hours after fresh feed delivery compared to non-overstocked (Collings et al., 2011). The authors noted the cows compensated by fulfilling their dry matter intake outside of the 2 hour window of fresh feed delivery.

During times of higher stocking density at the feed bunk there has been an increase in the number of displacements at the feed bunk (DeVries et al., 2004; Proudfoot et al., 2009) which can reduce dry matter intake (von Keyserlingk et al., 2008). Collings et al. (2011) reported overstocking the feed bins by 2 cows to 1 feed bin resulting in a greater number of displacements compared to stocking densities of 1 cow to 1 feed bin when feed access was offered for 24 or 14 hours. Transition cows 1 week prior to calving were placed in a competitive (2 cows to 1 feed bin) or non-competitive environment (1 cow to 1 feed bin). Both primiparous and multiparous cows were displaced more in the competitive environment; however, competitively fed multiparous cows spent 28 percent less time feeding, resulting in a tendency for reduced

dry matter intake, while there was no effect of competition for primiparous cows (Proudfoot et al., 2009).

### **Dairy Cattle Behavior**

Cattle evolved as grazing herbivores that gathered into herds for protection from predators. Group size was typically determined by the availability of food and access to mates (Huxley, 2006). They are social creatures by nature and the social competition between animals is often thought to be regulated primarily through dominance relationships (Drews, 1993). Domesticated cattle are imposed to comingling by age, sex, stage of gestation and exposed to multiple regroupings during the course of a year or a lactation. There has been an increased interest concerning the social relationships and the effects of social rank on the behavior and performance of dairy cattle (Arave and Albright, 1981). Observation of 27 herds in Wisconsin established social dominance was most correlated with age and weight, while stage of lactation, milk yield and temperament were not related to dominance (Dickson et al., 1970). Phillips and Rind (2002) observed in a grazing herd that social dominance was positively correlated with body weight and lactation number, but was negatively correlated with grazing time.

Social interactions and rank have the most impact when there are limited resources. There is a trend in the U.S. dairy industry from housing cows individually in tie-stalls to housing cows in pens with access to stalls or a bedding pack. This intensification has resulted in large group sizes with animals often competing for resources i.e. feed, water, and lying spaces (Val-Laillet et al., 2008b). Certain animals may be unable to access these resources due to more dominant animals blocking access

by threats, displacements and bunts (Grant and Albright, 2001). Social behavior can modify dry matter intake and productivity (Grant and Albright, 1995). Kondo and Hurnik (1990) defined social stabilization when non-physical agonistic interactions predominate and the ratio of physical to non-physical interactions remains fairly stable. A lower ranked animal may have reduced access to food, water, shade, and resting areas (Barroso et al., 2000). Although cattle will engage in aggressive interactions when kept on pasture (Phillips and Rind, 2001), a noticeable increase in the number of interactions occurs when space constraints are imposed (Fregonesi et al., 2007). When dominant and subordinate cows were grazed in separate pastures, both groups gained more weight and spent more time lying than when they were co-mingled in a paddock together (Phillips and Rind, 2002).

### ***Agonistic Behavior***

Agonistic behavior is often defined with physical and non-physical interactions. Types of agonistic behaviors include displacements, bunting, pushing, and fighting (physical) and threats and avoidance (non-physical; Castro et al., 2011; Kondo and Hurnik, 1990; Zobel et al., 2011). Kondo and Hurnik (1990) reported almost 80 percent of interactions were physical on the first day of regrouping and stabilized to approximately 40 percent after six days. Cows that were moved into a new pen where only 2 out of 15 animals were able to eat at the same time, average agonistic interactions (including displacements and maintaining ground) were 6.3 per day for beef feedlot heifers and also most occurred shortly after the first feed delivery (Zobel et al., 2011). When dairy cattle had their feeding space doubled from 0.5 m to 1.0 m per cow,

aggressive interactions were reduced by 57 percent (DeVries et al., 2004). As stocking density increased, there was a curvilinear increase in the number of times cows were displaced from the feed bunk and cows fed at a post and rail were more likely to be displaced than cows fed in headlocks (Huzzey et al., 2006).

The Héren breed in Switzerland is known for their dominant behavior and use in cow fights. During the winter cows are often housed for many months in tie-stalls due to weather; however, the cattle must eventually be allowed to go outside for exercise due to animal welfare requirements mandated in Sweden. Researchers noted when interval between days increased there was also an increase in the frequency of agonistic behavior (Castro et al., 2011). In addition, the duration of fights increased when the interval between exercises was longer than three days. Providing routine exercise may reduce agonistic behaviors in some breeds of cattle.

### *Displacements*

Displacements are a way to measure social hierarchy and aggressive behaviors. An interaction between two cows is considered a displacement from the feed bunk when physical contact initiated by one cow causes the receiving cow to stop feeding, back out and entirely remove her head from the headlock (Endres et al., 2005). Galindo and Broom (2000) calculated an index of displacements as the number of times a cow displaces other individuals divided by the number of times she displaced another cow plus the number of times she was displaced. Cows with an index value of 0.4-0.6 were considered middle ranking, while those with an index above 0.6 were high ranking, and those lower than 0.4 were low ranking animals (Galindo and Broom, 2000).

Val-Laillet et al. (2008b) examined three areas to assess consistency in competition for the following resources; feed bunk, stalls, and a mechanical brush for total confinement freestall housed dairy cattle. They reported that individual measures of competitive success were not highly correlated between the three resources and found 88 percent of the displacements happened at the feed bunk indicating access to feed was a high priority (Val-Laillet et al., 2008b). The finding of feed being a high priority for dairy cows appears to be confirmed with other researchers. When feed bunk space per animal was decreased from 1.0 m to 0.5 m the frequency of displacements was greater (1.6 vs. 0.7, respectively; DeVries et al., 2004). Approximately 5 displacements per day were recorded when the linear bunk space was 0.81 m per cow and increased to 12 displacements per day at 0.21 m linear bunk space in headlocks (Huzzey et al., 2006). With post and rail, displacements were 8 per day at 0.81 m linear bunk space and increased to 17 displacements at 0.21 m linear bunk space (Huzzey et al., 2006).

Galindo et al. (2000) found that lame cows were displaced less than cows that did not become lame. In a different study, Galindo and Broom (2002) reported that lame cows were less likely to initiate an aggressive behavior, however received similar aggressive behavior as non-lame cows. They also noted there were no differences in time spent licking other cows, however the frequency of times being licked was higher in lame cows. The authors hypothesized that the lame cows licking their herd mates may be a way to maintain stable relationships or as a way to cope with the discomfort of the injury by looking for comfort from other individuals.

### ***Lying Behavior***

Cows lie down for approximately 10-14 hours per day (Barberg et al., 2007; Haley et al, 2001; Jensen et al., 2005, Munksgaard and Simonsen, 1996). Lying surfaces should be soft, dry, and comfortable and offer good traction to allow the cow to rise and lie down normally without slipping (Chaplin et al., 2000). Lying has a higher priority for cows than eating and social contact when these behaviors are restricted (Munksgaard et al., 2005). When cows were deprived of 3 h/d of lying, within 12 h they had made up 50 percent of the lost lying time (Metz, 1985). Cow comfort can be evaluated by observing cow's behavior, time lying and standing in the stall, and stall surface preference (Tucker and Weary, 2004). Overton et al. (2002) observed the greatest proportion of cows lying (65 percent) in a freestall herd was during the hours of 3:00 am to 9:00 am, with the exception of when cows left for the morning milking and returned 1 hour later.

Lying surface can have an effect on lying times. Herlin (1997) had 12.2, 12.5, and 14.8 h/d lying time when cows had lying surfaces of concrete, standard rubber mat, and a soft rubber mat, respectively. Cows in a large pen with rubber flooring, spent 14.7 h/d lying down compared to 10.6 h/d for cows housed in concrete tie-stalls (Haley et al., 2000). Another study reported that cows spent 12.3 h/d lying down on mattress tie-stalls compared to 10.4 h/d for concrete tie-stalls (Haley et al., 2001). Lying bout duration was shorter on mattresses (62 min) than concrete stalls (77.7 min), but the frequency of lying was higher on mattresses (13 vs. 9; Haley et al., 2001). This can indicate cows will get up and change positions more frequently when given a softer surface. Cows in strawyards spent more time lying and ruminating than cows in cubicles (Fregonesi and

Leaver, 2000). Cows housed in compost bedded pack barns had daily lying time of 9.3 h/d with 11.0 bouts/d and lying bout duration of 50.8 min/bout (Endres and Barberg, 2007). Primiparous cows housed on pasture spent 1 h/d fewer lying than multiparous cows (Sepúlveda-Varas et al., 2014). It appears when cows are free of restrictions and are housed on softer surfaces, pasture or bedding packs, likely provides a better surface for performing these changes in posture compared with harder surfaces such as mattresses. In addition to the stall surface, stall maintenance and stall is important. When sand was 6.2 cm below the freestall curb, daily lying time was decreased by 1.15 hours (Drissler et al., 2005). Poorly designed stalls that are too short or narrow can affect lying times and possibly create injuries when the individual is rising. Total lying time improved when stall width increased from 106 cm (12.3 h/d) to 126 cm (13.0 h/d; Tucker et al., 2004). Tucker et al. (2004) also looked at stall length to determine preference. Cows spent less time with the front two hooves in the stall when the length was increased from 229 cm (173 min) to 274 cm (131 min). Cows that spend more time standing with 2 feet in the stall are at a greater risk of lameness (Cook, 2004).

Additional factors that can alter lying time are overcrowding and regrouping social dynamics. Producers often have more cows than stalls in each pen and the lower ranked animals will be displaced by more dominant individuals. When there was 25 percent overcrowding, the mean reduction was 44 min and subordinate animals had an average of 82 less minutes of lying time (Wierenga, 1983). Lactating dairy cows averaged 12.9 h/d of lying at 100 percent stocking of the freestalls. When overstocking increased to 150 percent lying times were reduced to 11.2 h/d (Fregonesi et al., 2007).

Cows that were regrouped socially during mid lactation had reduced time lying compared to prior to regrouping (von Keyserlingk et al., 2008). Charlton et al. (2014) reported that as the number of cows in the pen increased, lying daily bout frequency increased and lying bout duration decreased in a field study of 111 commercial freestall dairies.

Forced reduction of lying times can affect health of cattle. Jersey cows that were restricted on the amount of time to lie down, had greater basal plasma cortisol concentrations, reduced adrenocorticotrophic hormone (ACTH) and cortisol response following corticotrophin-releasing hormone (CRH) challenge (Fisher et al., 2002). While short term increased cortisol secretion allows an animal to cope with stress or stimuli, long term increase in cortisol concentrations reduce growth and impair immune system function (Fisher et al., 2002). Restriction of lying affected cortisol levels more than the restriction of feed (Fischer et al., 2002).

Lying behavior has been documented by data loggers or visual observation in person or with the use of time-lapse video. The use of continuous recording to track individual animals can be used (Ito et al., 2009; Overton et al., 2002). Data loggers can be placed on the neck with a collar or on the leg of the animal (Endres and Barberg, 2007) to allow for accurate collection of lying behavior. Ito et al. (2009) reported that 3 days of 1 minute continuous scan samples on 30 focal cows could accurately describe lying behavior for commercial dairy cows. Ten minute scan sampling from continuous recorded video was accurately able to describe lying behavior (Fregonesi et al., 2007). A validation study conducted by Ledgerwood et al. (2010) found 6 s and 30 s sample interval accurately measured all aspects of lying behavior when short readings of lying

and standing behavior were filtered from the data set. Recording capacity was approximately 11 d for the Onset Pendant G data logger recording 30 s intervals (Ledgerwood et al., 2010). Schirmann et al. (2011) used 1 minute scan intervals to record lying behavior with data loggers attached to the hind leg.

The use of loggers could potential indicate cows that are risk for periparturient period disorders or lameness. Cows that were lame spent more time lying down than nonlame cows whether it was on pasture (Sepúlveda-Varas et al., 2014) or housed indoors (Galindo and Broom, 2000; Ito et al., 2010; Calderon and Cook, 2011).

### ***Feeding Behavior***

Cows spend approximately 5 to 6 hours a day eating (Val-Laillet et al., 2008b) and daily time spent is second to lying. Feed is a highly sought after resource for dairy cows and overcrowding can lead to aggressive behaviors at the feed bunk (DeVries et al., 2004). Heifers that were competitively feed (2 heifers to 1 bunk) tended to have shorter feeding times and consumed 9% fewer meals per day; however, the duration of meals was 10% longer and tended to be 13% larger (DeVries and von Keyserlingk, 2009). In competitive situations, animals will still try to maintain intake, but will shift their eating patterns to compensate.

Second to lying in daily time budgets, feeding is a high priority for cattle. Monitoring feeding behavior has the potential to detect changes in dairy cattle health. González et al. (2008) reported ketosis cows were detected by a rapid daily decrease in feed intake, feeding time, and feeding rate approximately 4 days before it was diagnosed by farm staff. Approximately 1 wk prior to diagnosis, lame cows decreased feed intake

and feeding time but increased their feeding rate (González, et al., 2008). Cows that were metritic spent 22 less minutes per day at the feed alley during the transition period than non-metritic cows (Urton et al., 2005). For every 10 minute decrease in average daily feeding time cows were twice as likely to be diagnosed with metritis (Urton et al., 2005).

When feeding space per cow was doubled from 0.5 m to 1.0 m, feeding time increased by 24% and was most evident for the subordinate group of cows (DeVries et al., 2004). Both DeVries et al. (2004) and Huzzey et al. (2006) hypothesized feeding times would increase if cows were provided more than 0.6 m of bunk space. Cows that were higher ranking spent a greater percentage of their time at the feed bunk compared to the lower ranking cows (43.8 vs. 36.7 percent, respectively; Val-Laillet et al., 2008a). As competition increased at the feed bunk, cows spent 19% less time eating and increased consumption rate by 27% (Olofsson, 1999).

Feeding behavior can be captured with continuous video recording. Endres et al. (2005) used a 10 minute scan sampling that captured 96 percent of the variation in feeding behavior in comparison to a 1 minute scan sample. Additionally, computerized systems are able to monitor feed intake, number of visits, and duration (Bach et al., 2004; González, et al., 2008; Collings et al., 2011).

Feeding behavior may be used to detect illness in transition cattle. Severely metritic cows consumed less feed and reduced daily feeding time than healthy cows beginning 2 weeks prior to parturition and remained lower for the following 3 weeks and 2 weeks, respectively (Huzzey et al., 2007). Mildly metritic cows had reduced dry matter intake 1 week prior to parturition and for the following 3 weeks compared to healthy

cows and feeding time was reduced 24.7 min/d in mildly metritic cows 1 week after calving (Huzzey et al., 2007).

## **Housing and Management**

As dairy farms increase in size, producers are now able to group and manage their cattle more effectively. Cattle can be grouped based on stage of lactation, parity, and breeding status. However, management issues arise that are not ideal for the welfare of the animal. Often dairy producers have to overcrowd pens due to economic reasons or overabundance of animals in a particular stage of lactation. During these overcrowding situations, less dominant animals may be undernourished from restriction to the feed bunk. Social dominance has been highly correlated with body size and age (Dickson et al., 1970) often leaving the younger and smaller animals at a disadvantage for resources or altering peak times to utilize the resource. Additionally with a large number of animals to care for, there is less time to deal with each individual animal. Industry recommends grouping primiparous and multiparous animals separately since primiparous animals are more submissive and may have different nutritional needs.

## **Overcrowding**

Overcrowding is a common occurrence on dairy farms. Due to a large group of calvings, stocking density can increase outside of ideal. Current industry recommendation for lactating dairy cattle is 0.6 m linear feed bunk space to ensure all cows can feed simultaneously (Grant and Albright, 2001). In a situation with 135 percent stocking density at the feed bunk supplemental concentrate was consumed in 15 minutes and there was a high correlation between time spent eating and dominance (Friend and

Polan, 1978). However, when variables such as milk production, percent milk fat, and mature equivalent milk production were included in the model, dominance had negligible effect. In a second study by Friend and Polan (1978), time spent at the feed bunk was not significantly reduced until feed bunk space was limited from 0.5 to 0.1 m/cow. Daily feeding times decreased as linear feed bunk space decreased from 0.81 to 0.21 (Huzzey et al., 2006). Headlocks further reduced daily feeding times compared to post and rail (Huzzey et al., 2006).

As cited previously, lying is an important behavior for cattle, especially for cattle in high stress situations such as the transition period and during high milk production. When 1 stall was available for 2 cows, daily resting period was decreased from 14.2 to 10.7 h/d and cows were forced to lie in the alleyways due to no available stalls (Friend and Polan, 1978). Wierenga and Hopster (1990) evaluated stocking densities of 125, 133, and 155 % compared to 100% (one stall and one headlock per cow). When stocking density reached 155% mean lying times decreased. However, after evaluating rank, lower ranked cows were affected when stocking density reached 125%. The lower ranked cows tended to shift their lying times to the earlier evening as stalls available during the night were reduced (Wierenga and Hopster, 1990).

The cow comfort index (CCI) can be used as a snapshot to assess cow comfort in the stalls. It is calculated by dividing the number of cows lying in a stall by the number of cows lying in a stall plus the number of cows standing in a stall multiplied by 100. Cow comfort index is recommended to be greater than 85 percent (Nelson, 1996).

Krawczel et al. (2008) compared stocking densities of 100, 113, 131, and 142 % and the CCI was 80.7, 81.9, 82.9, 82.6, respectively with no difference among stocking densities.

Stall usage index is similar to cow comfort index, but the denominator includes all animals in the pen not eating. Stall usage index is more affected by stocking density than CCI because it includes animals standing idle in the alleys. When stocking density increased from 113 to 142 % the SUI decreased from 70.2 to 66.3 (Krawczel et al., 2008).

### **Regrouping**

Regrouping cattle is a common management practice on commercial dairy farms. Cows are often grouped by pregnancy status, stage of lactation, milk production, and parity (Grant and Alright, 2001). When new cows are entered in a pen, there is a reestablishing of social relationships often through threats, bunting, and other physical interactions (Lamb, 1975). High production cows that were regrouped and moved into a new pen spent 15 minutes less eating during the first hour following regrouping, reduced the number of lying bouts, and endured more than double the number of displacements from the feed bunk than from the previous 3 days (von Keyserlingk et al., 2008). Primiparous heifers that were regrouped reduced milk production by 3.5% compared to prior to regrouping (Hasegawa et al., 1997). Kondo and Hurnik (1990) reported an increase of adverse physical interactions for 2 to 3 days after the introduction of new animals to a group.

# **EXPERIMENT 1: EFFECT OF PREPARTUM GROUPING STRATEGY ON DISPLACEMENTS FROM THE FEED BUNK AND FEEDING BEHAVIOR OF DAIRY COWS**

## **SUMMARY (J. Dairy Sci. 97:2800-2807)**

The objective of the current study was to determine whether providing a stable pen management affected displacements from the feed bunk and feeding behavior of prepartum dairy cows. Two hundred and twenty-four nonlactating Jersey primiparous and multiparous cows were enrolled in the study. The 2 treatments were all-in-all-out (**AIAO**; 44 cows were moved into the close-up prepartum pen as one group with no additions during the 5-wk rep) or traditional (**TRD**; with weekly entrance of new cows to maintain pen density of 44 cows). Cows ( $253 \pm 3$  d of gestation) were balanced for parity and projected 305-d mature equivalent and assigned randomly to either AIAO or TRD treatments. At enrollment cows with a body condition score  $< 2$  or  $> 4$  (1-5 scale; 1=emaciated, 5=obese) or with a locomotion score  $> 3$  (1-5 scale; 1=normal gait, 5=severely lame) were not included. Displacements from the feed bunk were measured weekly for both treatments when TRD cows were moved into the close-up pen (d 0) and additionally on d 1, 2, 3, and 7 for 3 h after fresh feed delivery. A displacement rate was created to take into account differences in stocking density throughout the experiment. Displacement rate was calculated as the number of displacements divided by the number of cows in the pen at that time. Feeding behavior was measured using video 10-min scan sampling for 24 h periods at d 0, 1, 2, and 7. Displacements and feeding behavior were

recorded for all 5 wk of each repetition. There were treatment by week interactions for number of displacements and displacement rate. The TRD treatment had more displacements from the feed bunk than AIAO on wk 1, 3, and 5 with no differences on wk 2 and 4. Similarly, the TRD treatment had a greater displacement rate than the AIAO treatment on wk 1 and 5 with a tendency on wk 3. There were no differences between the treatments on wk 2 and 4. There was a treatment by week interaction for feeding time. Cows housed in the AIAO treatment had longer average feeding times for wk 2 with a tendency for wk 3, but spent 39 fewer minutes eating than the TRD treatment during the wk 1 of the study. Housing prepartum close-up cows in a stable pen management reduced displacements from the feed bunk and altered average daily feeding times.

## **INTRODUCTION**

The transition period (considered 3 wk before to 3 wk after parturition; Grummer, 1995) is a critical time for the dairy cow. During this period cows experience physiological, immune, and nutritional changes that make the cow at risk for metabolic and infectious diseases (Goff and Horst, 1997). Dry matter intake depression occurs during the final 2 to 3 weeks before parturition, yet is a time when demand for nutrients is increasing (Grummer et al., 2004). Up to 25% of cows are culled or die during the first 60 days in milk (Godden et al., 2003), which could be attributed to an unsuccessful transition period. Minimizing stressors during the transition period may allow for a successful transition from the nonlactating to the lactating stage.

On large commercial dairy farms, cows are commonly subjected to many pen changes during their lactation and dry period (Grant and Albright, 2001). One strategy recommended to nutritionally manage prepartum dairy cows is to have a far-off and a close-up dry period (Watters et al., 2008). These pens are typically subjected to once a week or twice a week inclusions of new animals after cows leave the pen for calving to maintain a desired stocking density. These pen movements may disrupt the social dynamics of the group and negatively impact DMI (Cook and Nordlund, 2004). When dairy cattle are subjected to regrouping there are increased physical agonistic behaviors such as displacements, threats, and butting and a decrease in milk production (von Keyserlingk

et al., 2008). Prepartum cows moved into a new pen had reductions in DMI and rumination time, and these moved cows displaced other cows already previously in the pen twice as frequently after regrouping (Schirmann et al., 2011). These negative physical interactions usually moderated 3 d after regrouping (Kondo and Hurnik, 1990). The lower-ranking animals seemed to be the most affected from regrouping. These animals spent more time standing without eating and ate at a faster rate, putting the animal at risk for ruminal acidosis and lameness events (Proudfoot et al., 2009).

Nordlund et al. (2006) recommended that producers adopt a stable social grouping during the prepartum period to minimize social disruptions. Cows with a similar calving date can be grouped together during the prepartum close-up period and stay in that pen until calving or calve in bedding pack pens (Cook, 2009). During this time no new cows are added to the pen until all to most of the current animals have calved. A disadvantage

of the stable pen management system is the need for additional pens which increases building cost. Cook (2009) recommends sizing the pens to accommodate 140 percent of the average weekly calving rate with the stable pen management. At times, pens will be underutilized while a few remaining cows are awaiting parturition. A study that examined a stable pen management versus a dynamic pen did not find a difference on the number of displacements from the feed bunk, DMI, plasma NEFA concentrations and milk production up to 30 DIM (Coonen et al., 2011). In that study, approximately 1 cow was added at a time to a small pen with only 10 cows. Additionally, cows in their study calved in the dry cow pen. It is unknown whether these results would be similar to movements of small groups of cows into a larger pen with cows being moved to a calving pen as they near parturition, similar to conditions experienced on large commercial dairies.

The objective of our study was to examine whether having a stable social group or all-in-all-out (**AIAO**) during the close-up prepartum period would alter the number of displacements from the feed bunk or total daily feeding time compared to cows housed in a traditional pen management (**TRD**) with weekly entrance of new animals in a large dairy setting. We hypothesized the AIAO treatment would result in fewer displacements from the feed bunk and a longer daily feeding time than the TRD treatment.

## **MATERIALS AND METHODS**

### ***Animals and housing***

A total of 224 primiparous and multiparous nonlactating Jersey cows were allocated to 4 groups (2 treatments x 2 replications) from June to September 2011. The

study was conducted at a large commercial dairy farm (6,400 lactating animals) in south-central Minnesota. Prepartum cows were provided a TMR once daily at approximately 0500 h and fed from a feed alley by headlocks. Feed was pushed up frequently throughout the day by farm personnel.

When cows demonstrated signs of calving, farm personal moved the cows to an individual box stall. At d 1 post-calving cows were moved into a freestall pen with 240 stalls and 260 headlocks stocked at 100% based on the number of stalls for 21 d. Video observation ceased when the cows left the dry period treatment pens.

### ***Experimental treatments and design***

At enrollment all cows were  $\geq 1$  lactation. Cows were required to have a body condition score between 2 and 4 (1-5 scale; 1=emaciated, 5=obese) and a locomotion score  $< 3$  (1-5 scale; 1=normal gait, 5=severely lame) or were not included in the study. Cows  $253 \pm 3$  d in gestation were balanced for parity (1 or  $\geq 2$  lactation) and projected 305-d mature equivalent milk yield at enrollment and were assigned to 1 of the 2 study pens. The 2 treatments were AIAO – 44 cows assigned to a pen as a group and no new cows added during a 5-wk period ( $n = 2$  with a total of 88 cows) or TRD treatment – weekly entrance of new cows to maintain a pen density of 44 cows after cows in the pen calved ( $n = 2$  with a total of 136 cows). During the initial week of each replicate, 44 cows entered the AIAO treatment with no new cows entering the pen during the 5-wk observation period. During the initial week, cows enrolled in the TRD treatment were added to a group of close-up cows to reach a pen stocking density of 44 cows. New cows were enrolled weekly in the TRD treatment pen to maintain a stocking density of 44 cows

(92% feed bunk; 100% stall stocking density) across the study period. At the end of the 5-wk replicate, a new TRD and AIAO group started, but treatments switched pens to avoid location bias.

Two experimental pens housing 44 cows each were used. The pens were on either side of a feed lane in a 12-row low profile cross-ventilated freestall barn and located on one end of the middle rows of the barn adjacent to the dairy's additional, non-experimental close-up prepartum pens. Both experimental pens had the same measurements of 31.7 m x 11.0 m, and had 44 sand bedded freestalls (229L x 107W x 114H cm) with a head-to-head configuration and 48 0.61 m headlocks (92% feed bunk stocking density). Two water troughs were located in the pen and measured 366 cm x 56 cm. One water trough was located at the end of the bank of freestalls and a shared water trough was located between the treatment pen and an adjacent non-experimental pen. Hourly pen temperatures were collected for the duration of the study using a data logger (Hobo H8 Pro Series, temperature accuracy  $\pm 0.5^{\circ}\text{C}$ , Onset Corp, Bourne, MA) placed 3 m above the bedding surface. Temperatures were averaged over the 3 h of feed bunk displacement observation period and averaged over 24 h for the feeding behavior analysis.

### ***Behavior measurements***

All enrolled cows were identified with a unique alphanumeric symbol on both sides of their rib cage using permanent hair dye either in black or blonde. Hair dye was applied at either d -1 or 0 prior to cows moving to the treatment pens.

To observe social interactions while feeding, each pen was equipped with 3 video cameras (Weldex, Cypress, CA) connected to a digital video recording system (Channel Visions, Costa Mesa, CA). Displacements from the feed bunk were measured continuously for 3 h on the day of move-in immediately after cows were moved at 1300 h (d 0) and following fresh feed delivery (0500 h) on d 1, 2, 3, and 7 of each 5-wk observation period. From continuous video observation, an interaction between 2 cows was considered a displacement from the feed bunk when physical contact initiated by 1 cow caused the receiving cow to stop feeding, back out and entirely remove her head from the headlock (Endres et al., 2005). To account for changes in stocking density, a displacement rate was calculated as daily displacements divided by the number of cows in the pen for each day of observation. This correction was needed for both treatments, because cows were removed after calving in both pens and cows were added to the TRD treatment only once a week (similarly to what would happen in a dairy farm). Both displacements from the feed bunk and the displacement rate were averaged daily for each week of the entire 5-wk observation period.

Feeding time was measured using 10-min video scan sampling for 24-h periods at d 0, 1, 2, and 7 (Endres et al., 2005). A cow was considered eating when the cow's ears were on the feed alley side of the headlocks. Daily feeding times in minutes were calculated by summing the daily 10-minute scan samples and multiplying that value by 10. To determine the percentage of cows eating at the feed bunk, the number of cows eating was summed at each 10-minute scan sample. Only observation days with 40 or more cows in the pen were included in the percentage of cows at the feed bunk analysis.

### *Statistical Analysis*

The UNIVARIATE procedure of SAS was used to examine normality and for the presence of outliers for the number of displacements, displacement rate and feeding times. A linear mixed model (MIXED procedure, SAS Institute Inc., Cary, NC) was built to evaluate the effect of close-up prepartum grouping management and the outcome variables of number of displacements, displacement rate and feeding time. Pen was used as the experimental unit. Cow within pen was used as a random effect for feeding behavior and observation day within week was a repeated measure for both feeding and displacement behaviors. The REPEATED statement was used for analysis of repeated measurements, the compound symmetry structure of covariance was chosen according to the Bayesian Akaike information criteria. Treatment (TRD vs. AIAO) was the fixed effect for all models. Covariates examined for number of displacements and displacement rate included average daily pen parity, average days prepartum, barn temperature, week, and the treatment by week interaction. Covariates examined for feeding time included parity, average daily barn temperature, stall stocking density, days to parturition, week, and treatment by week interaction. Least squares means and standard errors were determined using the LSMEANS statement in the MIXED procedure. Least squares means comparisons of the treatment by week interaction were separated with PDIFF. The TTEST procedure was used to determine if the percentage of cows eating during a 24 h period differed between the treatments at each observed time point.

## RESULTS

Mean temperature in the barn during the study was 21.6 °C and ranged from 11.4 to 30.7 °C. Mean relative humidity was 80.0 and ranged from 42.8 to 100 %. There was no difference between treatments. Average parity did not differ between AIAO and TRD treatments ( $1.6 \pm 0.1$  vs.  $1.7 \pm 0.1$ ; LSMean  $\pm$  SE, respectively). Once weekly, a mean of  $10.4 \pm 3.1$  cows, range 6-16 cows, were added to the TRD group.

### *Social Behavior*

A treatment  $\times$  week interaction was observed for mean daily number of displacements from the feed bunk (Figure 1). The TRD treatment had more displacements from the feed bunk during wk 1, 3, and 5 ( $P < 0.05$ ) than AIAO treatment, whereas there were no differences between the treatments during wk 2 and 4.

Within the AIAO treatment there were fewer displacements during wk 5 than during wk 1-4 ( $P < 0.05$ ), but number of displacements during wk 1-4 did not differ. Week 1 of the TRD treatment had a greater frequency of displacements from the feed bunk than during wk 2-5 ( $P < 0.05$ ). Additionally, there were a greater number of displacements during wk 3 of the TRD treatment compared to wk 4 ( $P < 0.05$ ).

There was a treatment  $\times$  week interaction for displacement rate between the AIAO and TRD treatments (Figure 2). The TRD treatment had greater ( $P < 0.05$ ) displacement rate than the AIAO treatment during wk 1 and 5 ( $0.78 \pm 0.07$  and  $0.46 \pm 0.07$  vs.  $0.33 \pm 0.06$  and  $0.19 \pm 0.06$ , respectively), a tendency ( $P = 0.066$ ) for greater displacements during wk 3 ( $0.56 \pm 0.06$  vs.  $0.40 \pm 0.06$ , respectively) and was similar to AIAO in week 2 and 4.

### ***Feeding Behavior***

There was a treatment by week interaction for daily feeding times (Figure 3,  $P < 0.001$ ). Cows housed in the AIAO treatment spent 39 fewer minutes per day eating during wk 1 than TRD treatment. During wk 2 of the study the AIAO treatment had a longer average daily feeding time by 25 min than TRD treatment ( $P < 0.05$ ), with a tendency of longer feeding time during wk 3 of the study ( $P = 0.054$ ). There were no differences between the treatments in feeding times during week 4 and 5.

Within the AIAO treatment, wk 1 feeding times were shorter than wk 2 and 3, with a tendency during wk 4 ( $P = 0.092$ ), and did not differ from wk 5. No other weeks within the AIAO treatment differed. Within the TRD treatment, wk 1 had a longer feeding time than wk 2 and 3 and did not differ from wk 4 and 5. During wk 3, cows in the TRD treatment had shorter daily feeding times than week 4 and 5 and did not differ from wk 2.

There were no differences in maximum feed bunk occupancy occurring at fresh feed delivery (0500 h) with 64.9 and 68.6% of cows eating at that time for the TRD and AIAO, respectively (Figure 4). In general, the AIAO treatment had a greater percentage of feed bunk occupancy during periods of low feeding activity.

## **DISCUSSION**

Kondo and Hurnik (1990) defined social stabilization as when the ratio of non-physical to physical interactions remains fairly stable. Based on data from von Keyserlingk et al. (2008) physical interactions appear to decrease 3 d after regrouping for lactating dairy cattle with approximately 60% of interactions non-physical and 40%

physical (Kondo and Hurnik, 1990). The AIAO grouping strategy was proposed to minimize physical social interactions during the transition period. Previous research investigating a stable versus a dynamic pen management did not find a difference in feed bunk displacements for prepartum cows (Coonen et al., 2011). Those researchers speculated there was minimal disruption with only a small number of animals moving into the dynamic pen. In their study, approximately 1 cow was moved into the pen and this occurred up to 2 times a week. In the current study approximately 10 cows were moved into the pen once weekly, which may have made the social disruptions more noticeable. Schirmann et al. (2011) observed that prepartum cows that were moved into a new pen displaced cows at the feed bin more frequently than cows that stayed in the home pen. Kondo and Hurnik (1990) reported the total number of interactions was greatest immediately after regrouping. In the TRD pen each week there was a group of cows moved into the pen, likely increasing the social disruption. There was no noticeable pattern in the TRD treatment in regards to the average weekly number of displacements; we suspect the number of displacements was more determined by dynamics of the group. In the AIAO treatment, the average weekly displacements were fairly stable from week 1 to 3 of the study when only a few cows calved. During wk 4 and 5 there was a marked decrease in displacements, likely due to the increased feeding space available.

The AIAO grouping strategy resulted in a lower stocking density and increased feed bunk space compared to the TRD treatment; while this was unavoidable, we calculated a displacement rate to compensate for this potential confounder. The TRD treatment had 1.6 times greater displacement rate than the AIAO grouping strategy, even

after accounting for stocking density. Cows in this study were provided 0.61 m of feed bunk space when the pen was stocked at the maximum of 44 animals per pen. Feed bunk space per cow varied from 0.61 to 1.04 m in the TRD treatment and from 0.61 to 14.6 m in the AIAO treatment throughout the study. Even during the weeks when the AIAO had similar feed bunk space to TRD, the TRD grouping strategy still had more displacements from the feed bunk than AIAO. When feed bunk space is reduced, an increase of physical displacements is observed (DeVries et al., 2004, Proudfoot et al., 2009). DeVries et al. (2004) reported 57% fewer aggressive interactions when feed bunk space increased from 0.5 to 1.0 m per cow. Proudfoot et al. (2009) examined competition at the feed bin (1:1 cow:feed bin or 2:1 cows:feed bin) for 1 wk prepartum and 2 wk postpartum and found that although competition increased displacements, the competition affected primiparous and multiparous animals differently. Multiparous cows housed in the competitive feeding environment spent more time standing without eating than multiparous cows not housed in the competitive environment and no differences were observed in the primiparous groups. Competitively fed multiparous animals spent less time feeding 1 wk prepartum and had a tendency for lower DMI, whereas postpartum these cows did not differ on DMI. However, they compensated with a greater feed intake rate (Proudfoot et al., 2009). In another study, when competition was increased from 1 to 4 cows per feeding station, the lower-ranking animals altered their feed consumption to less preferred times of the day (Olofsson, 1999). In the current study, we did not examine social rank in relation to feeding time of the day due to study design; however,

we speculate that the lower ranking animals may be more affected by the treatment than the group averages might indicate.

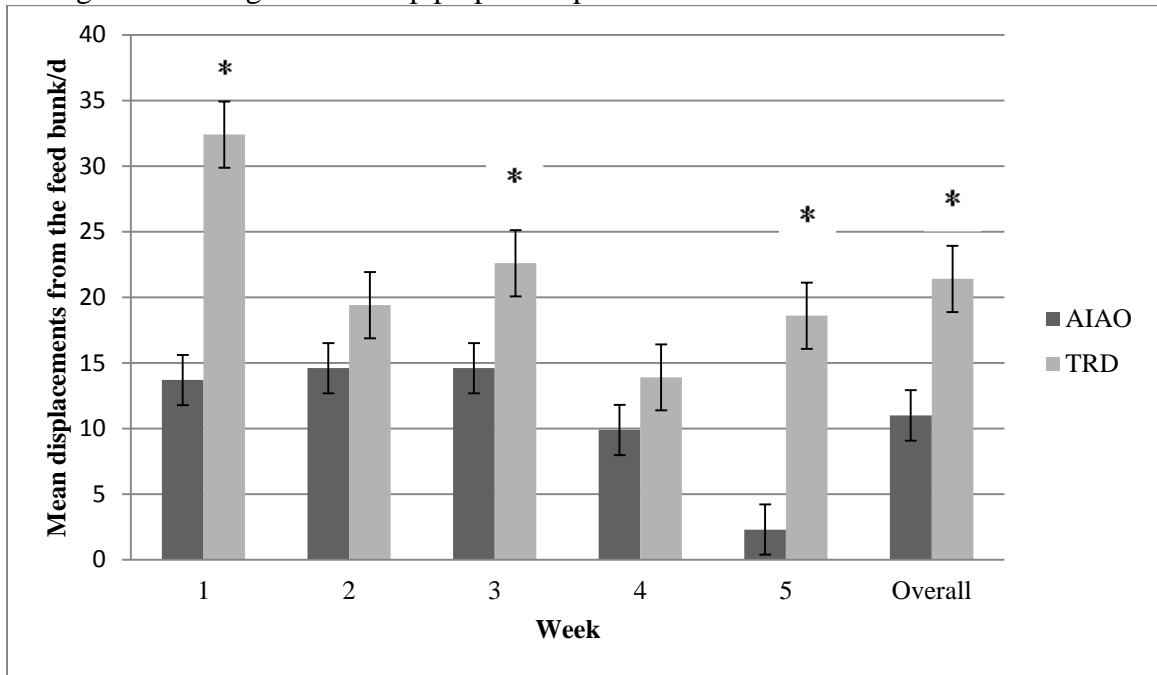
It has been hypothesized that creating a stable prepartum group would minimize physical social interactions that would ultimately affect DMI and health during this critical time (Nordlund et al., 2006). When lactating dairy cows were regrouped, they spent 15 min less time eating during the first hour after regrouping; however, total feeding time did not differ (von Keyserlingk et al., 2008). Prepartum cows that were regrouped and moved into a new pen saw a decrease in DMI from baseline values, while regrouped cows that stayed in their home pen did not differ from their baseline values (Schirrmann et al., 2011). In the current study, cows housed in the AIAO treatment had reduced daily feeding time during the first week being housed in the pen than the TRD treatment. We hypothesize that because there were resident cows in the TRD treatment with approximately 10 study cows added to the pen in the beginning of that replication, the pen average may have masked the potential reduction in feeding time experienced by those newly introduced cows or cows already in the pen. Further research on the effect of pen residence time for each individual cow is warranted. Similar to the decrease in DMI that occurs during the last 7 to 10 days of pregnancy (Bertics et al., 1992), cows in the AIAO treatment had a gradual decrease in feeding time from wk 2 to wk 5 prior to parturition; however, this reduction was not significant. Feeding times did not have a specific pattern within the weeks for the TRD treatment, likely due to the variation of gestation length within that group. Coonen et al. (2011) examined DMI for 28 d prepartum comparing a stable and a dynamic prepartum grouping strategy with no

differences between the treatments. Dry matter intake was not examined in the current study because the study was performed on a large commercial dairy. However, in a companion study (Silva et al., 2013) we showed that the AIAO grouping strategy did not differ from the TRD treatment on number of disease events, NEFA and BHBA concentrations, or milk production postcalving. Additionally, Coonen et al. (2011) found that a stable pen treatment did not affect plasma NEFA concentrations or milk production up to 30 DIM with Holsteins.

### **CONCLUSIONS**

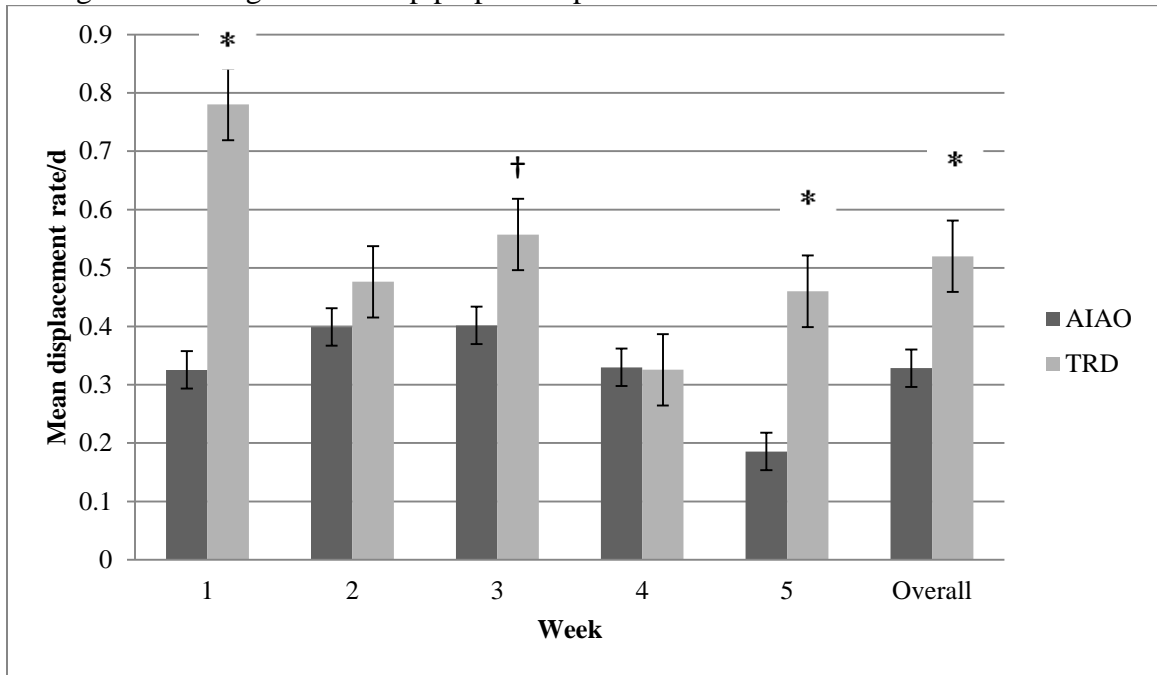
In the current study, the AIAO grouping strategy had fewer displacements from the feed bunk than the TRD and this was evident during all weeks of the repetition except during wk 2 and 4 when the treatments did not differ. Even when accounting for changes in stocking density the AIAO treatment still had a lower displacement rate than the TRD treatment. The AIAO cows spent fewer minutes eating daily than the TRD cows during wk 1, whereas having a longer feeding time during wk 2. Our results (with Jersey cows and approximately 92% feed bunk stocking density) indicate the AIAO treatment reduced negative social behaviors and altered daily feeding times. More research is needed with various pen stocking densities and other breeds, such as Holstein.

**Figure 1.** Mean number of displacements (n/d) from the feed bunk for cows housed in a stable (AIAO, all-in-all-out) or weekly entrance of new animals (TRD, traditional) pen management during the close-up prepartum period



\* denotes significant differences between treatments ( $P < 0.05$ ) within week

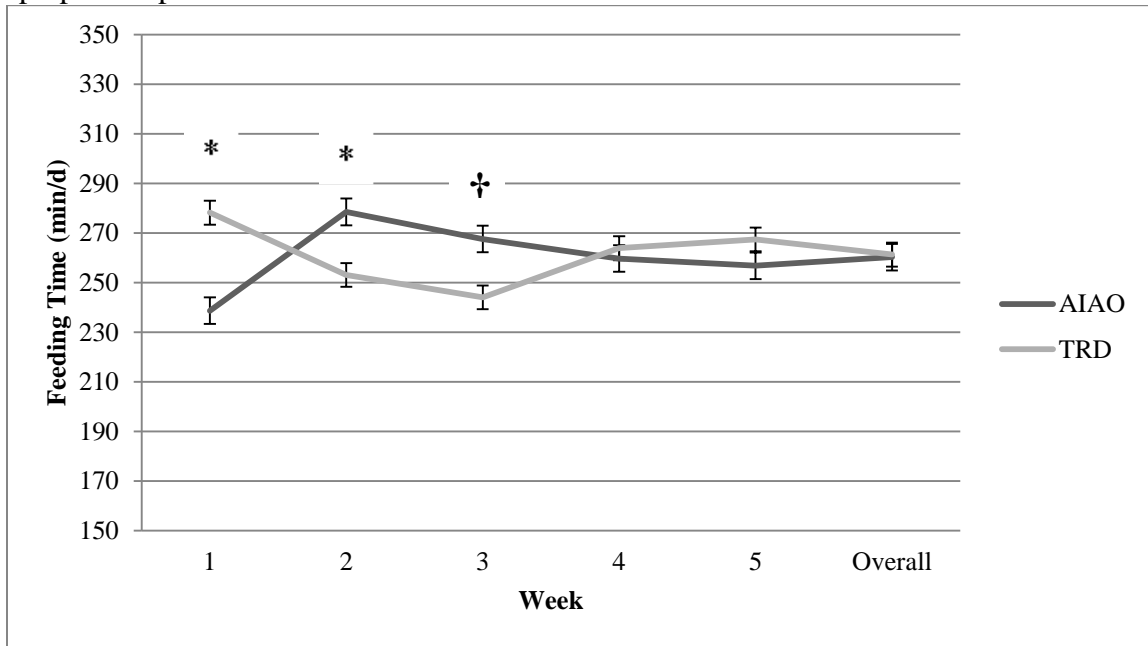
**Figure 2.** Mean rate of displacements from the feed bunk for cows housed in a stable (AIAO, all-in-all-out) or weekly entrance of new animals (TRD, traditional) pen management during the close-up prepartum period



\* denotes significant differences between treatments ( $P < 0.05$ ) within week

† denotes a tendency between treatments ( $P = 0.056$ ) within week

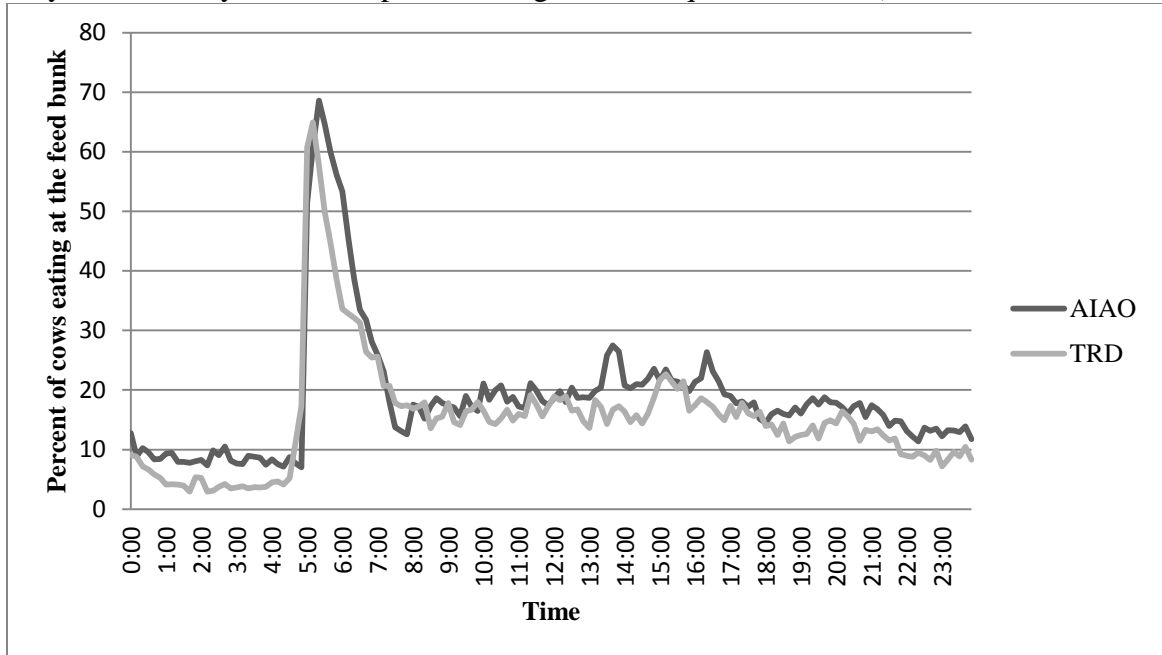
**Figure 3.** Feeding times of cows housed in a stable (AIAO, all-in-all-out) or weekly entrance of new animals (TRD, traditional) pen management during the close-up prepartum period



\* denotes significant differences between treatments ( $P < 0.05$ ) within week

† denotes a tendency between treatments ( $P = 0.054$ ) within week

**Figure 4.** Percentage of cows eating at the feed bunk over a 24-h period for a stable (AIAO, all-in-all-out) or a weekly entrance of new animals (TRD, traditional) pen management during the close-up prepartum period (data were averaged over 30 d and only included days when the pen housed greater or equal to 40 cows)



Significant differences in percentage eating found for feeding periods 0030-0450, 1320-1330, 1650, 1720, 2200-2210, and 2320-2340 h.

## **EXPERIMENT 2: EFFECT OF STOCKING DENSITY ON SOCIAL, FEEDING AND LYING BEHAVIOR OF PREPARTUM DAIRY ANIMALS**

### **SUMMARY**

The objectives of this study were to determine the effects of prepartum stocking density on social, lying, and feeding behavior of dairy animals and to investigate the relationship between social rank and stocking density. In total, 756 Jersey animals were enrolled in the study approximately 4 wk prior to expected calving date. There were 8 experimental units (4 replicates x 2 pens/treatment per replicate) and at each replicate one pen each of nulliparous and ‘parous’ (primiparous and multiparous) animals per treatment were enrolled. The 2 treatments were 80% stocking density (**80D**, 38 animals/pen; each pen with 48 headlocks and 44 stalls) and 100% stocking density (**100D**, 48 animals/pen). Parous animals were housed separately from nulliparous animals. Animals at  $254 \pm 3$  d of gestation were balanced for parity (parous vs nulliparous) and projected 305-d ME milk yield (only parous animals) and randomly assigned to either 80D or 100D. Displacements from the feed bunk were measured for 3 h after fresh feed delivery on d 2, 5, and 7 of each week. Feeding behavior was measured for 24-h periods (using 10-min video scan sampling) on d 2, 5 and 7 on wk 1 of every replicate and d 2 and 5 for the following 4 wk. A displacement index (proportion of successful displacements from the feed bunk relative to all displacements the animal was involved in) was calculated for each animal and used to categorize animals into ranking categories of high, middle and low. Seventy nulliparous and 64 parous focal animals in

the 80D treatment and 89 nulliparous and 74 parous focal animals in the 100D were used to describe lying behavior (measured with data loggers). Animals housed at 80D had fewer daily displacements from the feed bunk than those housed at 100D ( $15.2 \pm 1.0$  vs.  $21.3 \pm 1.0/d$ ). Daily feeding times differed between nulliparous and parous animals at the 2 stocking densities. Nulliparous 80D animals spent  $12.4 \pm 5.0$  fewer min/d feeding than nulliparous 100D animals whereas 100D parous animals tended to spend  $7.6 \pm 4.5$  fewer min/d feeding than 80D parous animals. There were no differences in number of lying bouts or lying bout duration between the 2 treatments; lying time was longer for 100D on d -33, -29, and -26 and shorter on d -7, -5 and 0 than 80D. The interaction between treatment, parity and social rank was associated with lying and feeding times. In summary, animals in the 80D treatment had a lower number of displacements from the feed bunk and spent more time lying down near parturition than 100D animals, and 80D nulliparous animals had reduced daily feeding time than 100D nulliparous animals. Although these results showed some potential behavior benefits of a prepartum stocking density of 80% compared to 100%, observed changes were small. However, greater stocking density cannot be recommended; more research is needed to evaluate the effects of stocking densities greater than 100% and with other breeds of cattle besides Jersey.

**Key words.** Prepartum cow, stocking density, feeding behavior, lying behavior, social behavior

## INTRODUCTION

The transition dairy cow is one of the highest risk animals for falling ill or dying on the dairy farm. Typically the transition period is described as 3 weeks before and after

calving (Grummer, 1995). During this period cows experience physiological, immune, and nutritional changes making the cow at risk for metabolic and infectious diseases (Goff and Horst, 1997). Up to 25% of cows are culled or die during the first 60 DIM (Godden et al., 2003), which may be attributed to an unsuccessful transition period. Concern over animal well-being and reduction on farm profitability due to morbidity and mortality losses have stimulated more research in the area of transition cow management and behavior in order to improve transition cow success.

Dry matter intake decreases 3 wk prior to calving (Hayirli et al., 2002) and more severe reductions in DMI may put the cow at risk for metabolic disorders such as ketosis and fatty liver. Cows will typically occupy 80% of the feed bunk linear space during the peak feeding time after fresh feed delivery (Huzzey et al., 2006, Nordlund et al., 2006). The current industry recommendations for prepartum freestall housed dry cows is to provide a minimum of 0.76 m of linear bunk space/cow (or stock at 80% of 0.61 m headlocks) with at least 1 stall/cow for resting space (Nordlund et al., 2006). In a field study with prepartum nulliparous and parous animals housed together it was reported that for every 10% increase in stocking density above 80% of headlocks there was a 0.7 kg/d decrease in milk yield for first lactation cows in that lactation (Oetzel et al., 2007). Increasing linear feeding space has been observed to reduce competition at the feed bunk (Huzzey et al., 2006) and may benefit lower ranking animals. There were fewer aggressive interactions and increased feeding activity during the 90 min after fresh feed delivery when lactating dairy cattle had access to 1.0 m feeding space per cow compared to 0.5 m per cow (DeVries et al., 2004). Krawczel et al. (2012) reported changes in

social behavior of lactating cows with a linear increase in displacements from the feed bunk as stall stocking density increased from 100 to 142%, but they observed no differences in feeding or rumination time.

Two resources highly valued by cows are lying and feeding space. Lactating cows spend approximately 12-13 h/d lying down (Fregonesi et al., 2007) and 5-6 h/d feeding (Val-Laillet et al., 2008). Lying has a higher priority for cows than eating and social contact when these behaviors are restricted (Munksgaard et al., 2005). Lying time was linearly reduced when stocking density increased from 100 to 150% (Fregonesi et al., 2007). Krawczel et al. (2012) reported lying time was reduced for stocking densities of 131 and 142% compared to 100 or 113%. Late lactation cows stocked at 100% of stalls spent less time lying down compared to cows stocked at 25% of stalls (Telezhenko et al., 2012).

The feed bunk can be an area of competition causing changes in feeding behavior. Multiparous cows in a competitive feed environment (2 cows/1 feed bin) had a shorter feeding time and ate at a faster rate for up to 2 wk post-calving, whereas the feeding behavior of primiparous cows did not differ (Proudfoot et al., 2009). In other studies when the number of cows to feed bin increased, the competitively fed cows did not differ in dry matter intake or daily feeding time, but cows had fewer meals per day with the tendency of larger and longer meals (Olofsson, 1999; Hosseinkhani et al., 2008). Additionally, as competition at the feed bunk increased, idle standing time also increased (Olofsson, 1999) which has been associated with an increase in lameness prevalence (Cook et al., 2004).

Only a few studies have evaluated stocking density during the prepartum period and in conditions similar to commercial dairies. Data collected by Oetzel et al. (2007) demonstrated a decrease in milk yield for primiparous cows in a mixed pen with multiparous cows when pre-fresh pen stocking densities exceeded 80%. Huzzey et al. (2012) reported greater DMI, plasma non-esterified fatty acids and glucose concentrations with a tendency for greater fecal cortisol metabolite for cows housed at a higher pen stocking density (1 stall/cow and 0.67 m linear feed bunk space compared to 0.5 stall/cow and 0.34 m linear feed bunk space). To our knowledge no research on stocking density during the prepartum period has been conducted with Jersey cows, and results could differ from Holstein cows.

The objectives of this study were to determine whether increasing stocking density in a commercial Jersey dairy farm from 80 to 100% would affect social, feeding and lying behavior of prepartum nulliparous and parous dairy animals and to investigate whether behaviors varied among animals of different social rank at the 80 or 100% stocking density.

## **MATERIAL AND METHODS**

### ***Animals and Housing***

A total of 756 nulliparous and parous (primiparous and multiparous) Jersey animals were allocated to 2 treatments from October 2012 to March 2013. The study was conducted at a large commercial dairy farm (6,400 lactating dairy cows) in south-central Minnesota. Prepartum animals were provided a TMR (balanced to meet nutrient requirements) once daily at approximately 0700 h and fed from a feed alley by headlocks.

Four experimental pens housing either 38 or 48 animals each were used in each replicate period with 2 pens per treatment enrolled at each replicate. Two pens (1 per each treatment) housed primiparous and multiparous cows together referred to as ‘parous’ and two pens (1 per each treatment) housed nulliparous animals. All experimental pens had the same measurements of 31.7 m x 11.0 m and had 44 deep sand bedded freestalls (229L x 107W x 114H cm) with a head-to-head configuration and 48 0.61-m headlocks. Sand bedding was added once weekly and pens were scraped once daily. Two water troughs were located in the pen and measured 366 cm x 56 cm. One water trough was located at the end of the bank of freestalls and a shared water trough was located between the treatment pen and an adjacent non-experimental pen. After each replicate, treatment within parity (nulliparous or parous) was switched to the opposite pen to prevent location bias.

When animals demonstrated signs of calving, farm personal moved them to an individual box stall. At d 1 post-calving cows were moved into a freestall pen with 240 stalls and 260 headlocks, stocked at 100% based on the number of stalls for 21 d. Parous and nulliparous animals were housed separately during the first 21 DIM.

### ***Experimental Treatments and Design***

Animals at  $254 \pm 3$  d from expected calving date were balanced for parity (parous vs. nulliparous) and projected 305-d mature equivalent (parous animals only) and were allocated to 80% (**80D**; 38 animals/pen; each pen with 48 headlocks and 44 stalls) or 100% (**100D**; 48 animals/pen) stocking density. Animals were required to have a body condition score between 2 and 4 (1-5 scale; 1=emaciated, 5=obese) and a locomotion

score  $\leq 3$  (1-5 scale; 1=normal gait, 5=severely lame) or were not included in the study. Four hundred and eighteen animals were enrolled in 100D and 338 animals in 80D over the study period. Pens were stocked twice weekly (on Mondays and Thursdays) and groups of 2 to 15 animals (median = 9 animals) were moved to the 80D and 100D pens to re-establish the targeted stocking density.

### ***Behavior Measurements***

All enrolled animals were identified with a unique alphanumeric symbol on their back using permanent hair dye either in black or blonde. Hair dye was applied at either d 0 prior to animals moving to the treatment pens or d 1.

***Social Behavior.*** To observe social interactions while feeding, we used 3 video cameras (Weldex, Cypress, CA) per pen located 5 m above the feed bunk and connected to a digital video recording system (Channel Visions, Costa Mesa, CA). Displacements from the feed bunk were measured continuously during 3 h following fresh feed delivery (07:00 $\pm$ 1:00) on d 2, 5, and 7 of each 5-wk observation period. Previous research has shown that displacements from the feed bunk for 2 h following feeding explained the majority of the variation associated with displacements recorded over a full 24 h ( $R^2 = 0.96$ ;  $P < 0.001$ ; Collings et al., 2011). Observation days were chosen based on days that animals were not scheduled to be locked up by farm staff or research personnel. From continuous video observation, an interaction between 2 animals was considered a displacement from the feed bunk when physical contact initiated by 1 animal caused the receiving animal to stop feeding, back out and entirely remove her head from the headlock (Endres et al., 2005). Displacements from the feed bunk were summed daily

for the entire 5-wk observation period. A displacement index (**DI**) was calculated (Galindo and Broom, 2000) as the proportion of successful displacements from the feed bunk relative to all displacements in which the animal was involved. An overall social rank was calculated for each animal during her time in the pen. Animals with a  $DI > 0.6$  were classified as high ranking,  $0.4 - 0.6$  were middle ranking, and animals with a  $DI < 0.4$  were classified as low ranking. Although we acknowledge the group composition in the pens was dynamic, it is suggested that this DI represented the typical rank of the cow.

**Feeding Behavior.** Feeding time for all marked animals in the pen was measured using 10-min video scan sampling for 24-h periods (Endres et al., 2005) on d 2, 5, and 7 for wk 1 of each replicate and at d 2 and 5 for wk 2-5. An animal was considered eating when her ears were on the feed alley side of the headlocks. Video observations ceased when the animal left the prepartum period treatment pen.

**Lying Behavior.** A total of 297 focal animals were used to describe pen lying behavior. The 100D and 80D pens averaged  $20 \pm 3$  and  $17 \pm 3$  (Mean  $\pm$  SD) focal animals per pen, respectively. Lying time, lying bouts, and lying bout duration were measured using HOBO Pendant G data loggers (Onset, Bourne, MA). Data loggers were set to collect lying behavior at 30-s intervals (Ledgerwood et al., 2010) and placed on the cow's right hind leg 1 d after entrance into the pen. Loggers were kept on the cow for 12 d and removed for 7 d and reattached for another 12 d or until the cow calved. Animals that calved early had loggers removed within 2 d of calving and data post calving was removed from the data set. Daily lying times, frequency of lying bouts, and lying bout

duration were computed for each cow using a macro in SAS (SAS Institute Inc., Cary, NC) developed by N. Chapinal (University of British Columbia, Pers. Comm.).

### *Statistical Analysis*

The UNIVARIATE procedure of SAS (SAS Institute Inc., Cary, NC) was used to examine normality and detect the presence of outliers for number of displacements, feeding times and lying behavior by pen. A linear mixed model (MIXED procedure, SAS Institute Inc., Cary, NC) was built to evaluate the effect of close-up prepartum feed bunk stocking density and the outcome variables of daily displacements from the feed bunk, daily feeding time, and lying behavior (daily lying time, number of lying bouts/d and lying bout duration). Pen was used as the experimental unit. The structure of covariance (auto-regressive, unstructured, or compound symmetry) was chosen according to the Bayesian Akaike information criteria for repeated measurements. A repeated statement included day and cow nested within replicate as the subject. Pen was used as a random effect for social, feeding and lying behaviors. Covariates examined for social behavior included treatment (80D vs. 100D), parity (nulliparous vs. parous), average day relative to calving, replicate (1-4), and day of replicate (d 1-35). Covariates examined for lying and feeding behavior included treatment, parity, day relative to calving, day of replicate, and the interaction of treatment  $\times$  day relative to calving. Additionally, the interaction of parity (nulliparous and parous) and treatment was examined for all models and if not significant, results were combined. Least squares means and standard errors were computed using the LSMEANS statement in the MIXED procedure. Least squares means comparisons were separated with PDIFF.

An additional analysis was conducted to determine whether animals of different social ranking behaved differently at the 2 different stocking densities. Daily feeding times and lying behavior were analyzed with a mixed effects linear regression model using PROC MIXED of SAS (v. 9.2 SAS Institute Inc., Cary, NC). Cow was treated as a random effect (df = 722). The repeated measures of day were modeled for each cow based on Bayesian information criterion. Covariates included treatment, parity, social category (high, middle, or low-ranking), and day relative to calving. The interaction of social rank, treatment, and parity were examined. Least squares means and standard errors were separated with PDIFF.

## **RESULTS**

Daily average stocking densities based on number of headlocks or stalls were different between treatments (Figure 1;  $P < 0.01$ ). Headlocks were stocked on average at  $74.1 \pm 0.4\%$  and  $94.5 \pm 0.3\%$  for the 80 D and 100D treatments, respectively; stalls were stocked on average at  $80.8 \pm 0.4\%$  and  $103.1 \pm 0.4\%$  for the 80D and 100D treatments, respectively. Overall, the targeted stocking density difference of 20 percentage units between the 2 treatments was achieved during the study period.

### ***Social Behavior***

There was no effect of parity or parity by treatment interaction on displacements from the feed bunk; therefore, results were combined. Animals housed in the 80D feed bunk stocking density averaged  $15.2 \pm 1.0$  displacements/d (LSMean  $\pm$  SE) whereas the 100D pen averaged  $21.3 \pm 1.0$  displacements/d ( $P < 0.01$ ; Figure 2).

### ***Feeding Behavior***

There was a treatment  $\times$  parity interaction (Figure 4;  $P = 0.003$ ). Overall, parous animals spent  $46.9 \pm 6.6$  min/d more time feeding than nulliparous animals ( $P < 0.01$ ). Mean daily feeding times for parous 100D, parous 80D, nulliparous 100D and nulliparous 80D were  $293.6 \pm 5.1$ ,  $301.2 \pm 5.4$ ,  $256.7 \pm 5.2$ , and  $244.3 \pm 5.5$  min/d, respectively. Nulliparous animals at 100D stocking density spent  $12.4 \pm 5.0$  more min/d feeding than 80D nulliparous animals ( $P = 0.013$ ), whereas 100D parous animals tended to spend  $7.6 \pm 4.5$  fewer min/d feeding than 80D parous animals ( $P = 0.095$ ).

### ***Relationship between Social Rank and Daily Feeding Time***

There was an interaction among treatment, parity, and social rank for daily feeding time. Social rank was not associated with feeding time among 80D nulliparous, 100D nulliparous, and 80D parous animals. However, middle ranking parous animals housed in 100D spent  $27.2 \pm 8.7$  ( $P = 0.002$ ) and  $15.0 \pm 7.4$  ( $P = 0.042$ ) more min/d eating than high and low ranking animals, respectively (Table 2). High and low ranking 100D parous animals did not differ in their daily feeding time.

### ***Lying Behavior***

Both 80D and 100D spent  $13.0 \pm 0.1$  h/d lying down (LSMean  $\pm$  SE;  $P > 0.05$ ). Nulliparous animals spent  $0.4 \pm 0.2$  h/d less time lying down than multiparous animals ( $12.9 \pm 0.1$  and  $13.2 \pm 0.1$ , respectively;  $P = 0.028$ ). A treatment  $\times$  day relative to calving effect was observed (Figure 3;  $P = 0.004$ ). Animals (nulliparous and parous) stocked at 100D had longer lying times than 80D on d -33, -29, and -26 with a tendency on d -24 prepartum. On d -7, -5 and 0, 80D had longer lying times than 100D animals ( $P < 0.05$ ).

The 80D and 100D treatments were not different for number of lying bouts per day ( $15.3 \pm 0.5$  and  $14.9 \pm 0.5$ , respectively). Nulliparous animals had more ( $P < 0.01$ ) lying bouts per day ( $16.5 \pm 0.5$ ) than parous animals ( $13.7 \pm 0.5$ ). Lying bout duration did not differ between the 80D or 100D stocking density treatments ( $1.1 \pm 0.03$  h/bout). There was a difference in lying bout duration between nulliparous and parous animals ( $P < 0.01$ ). Lying bout duration for nulliparous animals was  $0.35 \pm 0.04$  h/bout less than parous animals ( $0.9 \pm 0.03$  and  $1.3 \pm 0.03$  h/bout, respectively).

### ***Relationship between Social Rank and Lying Behavior Variables***

The interaction among treatment, parity and social rank was associated with lying time ( $P = 0.024$ ; Table 1). High ranking parous animals in the 80D stocking density spent  $1.0 \pm 0.4$  h/d less time lying down than middle ranking animals ( $P = 0.017$ ) and did not differ from low ranking animals. No differences were observed in daily lying time for middle and low ranking 80D parous animals. Middle ranking 100D nulliparous animals spent  $0.8 \pm 0.3$  ( $P = 0.016$ ) and tended to spend  $0.7 \pm 0.4$  ( $P = 0.089$ ) fewer h/d lying down than low and high ranking nulliparous animals, respectively. High and low ranking 100D nulliparous animals did not differ in daily lying time.

The interaction between treatment and social rank did not affect number of lying bouts per day or lying bout duration; however, differences existed between parities. High ranking parous animals had fewer lying bouts/d ( $P = 0.034$ ) than middle ranking animals ( $12.3 \pm 0.9$  vs.  $14.8 \pm 0.7$  lying bouts/d) and did not differ ( $P = 0.11$ ) from low ranking animals ( $14.2 \pm 0.8$ ). Middle and low ranking parous animals did not differ in the number of lying bouts/d. Nulliparous animals averaged  $18.4 \pm 0.9$ ,  $16.0 \pm 0.7$ , and  $16.5 \pm$

0.8 lying bouts per day for high, middle and low ranking, respectively. High ranking nulliparous animals averaged  $2.4 \pm 1.1$  more bouts than middle ranking animals ( $P = 0.038$ ) and did not differ from low ranking animals. Middle and low ranking nulliparous animals did not differ in the number of lying bouts/d.

The lying bout duration (h/bout) of high ranking parous animals was longer than middle ranking ( $1.4 \pm 0.06$  vs  $1.2 \pm 0.05$ ;  $P = 0.049$ ) and tended to be longer than low ranking parous animals ( $1.2 \pm 0.05$ ;  $P = 0.079$ ). No differences were observed between middle and low ranking parous animals for lying bout duration. Social rank was not associated with lying bout duration for nulliparous animals and averaged  $0.9 \pm 0.5$  h/bout.

## **DISCUSSION**

The purpose of this study was to replicate conditions commonly observed on commercial dairy farms with the addition of new animals to the prepartum pen which creates socially unstable groups and reduces stall, feed bunk and overall pen space as pen density increases. Our results confirm previous research indicating that when more space was provided at the feed bunk the number of displacements during the 3 hours after fresh feed delivery was reduced, regardless of parity. Several studies have demonstrated that increasing stocking density affects feeding and lying behavior (Olofsson, 1999, DeVries et al., 2004, Proudfoot et al., 2009). DeVries et al. (2004) reported there were reduced aggressive interactions and increased feeding activity after fresh feed delivery when feed bunk space was increased from 0.5 to 1.0 m per cow. Hill et al. (2009) did not notice an increase in displacements from the feed bunk with 4 stocking densities ranging from 100 to 142%. They maintained pen size, but blocked access to stalls and headlocks to achieve

the desired stocking density. Additionally, groups were kept socially stable, which may contribute to a reduction on the number of displacements from the feed bunk. Lobeck-Luchterhand et al. (2014) reported fewer displacements from the feed bunk for close-up prepartum animals housed in a socially stable group versus a pen with weekly entrances of new animals and this difference was still apparent even after accounting for differences in pen stocking density.

Due to the different nutrient demands of nulliparous animals than the rest of the herd, some large commercial dairies house nulliparous animals separately from parous animals. In our study nulliparous and parous animals were housed separately per protocol of the dairy. Boyle et al. (2013) reported that nulliparous animals mixed with multiparous animals prepartum received fewer butts and had greater locomotion than nulliparous animals housed with other nulliparous animals. Most of our differences in lying behavior can be attributed to the nulliparous animals and their tendency to be more mobile than their older counterparts (locomotion score at enrollment  $1.0 \pm 0.01$  vs.  $1.1 \pm 0.01$ ;  $P < 0.001$ ). Nulliparous animals spent less time lying down per day, had shorter lying bout duration with more lying bouts per day than parous animals. Boyle et al. (2013) reported that there were no differences in lying behavior (lying time, number of lying bouts, and lying bout duration) of nulliparous animals that were mixed with multiparous animals compared to nulliparous animals housed with other nulliparous animals during the 3 wk prepartum and stocked at 100% of stalls.

Prepartum animals in this study were housed in pens with stall stocking density averaging 103.1% (maximum of 109%) and overall lying behavior was not adversely

affected compared with animals housed at 80.8%. However, animals in the 80D treatment spent more time lying down near parturition than 100D animals whereas earlier in the prepartum period the opposite was observed, with 100D animals lying down longer than 80D. These results could indicate that cows normally compete and gain access to the stalls when stocked at higher densities, but near parturition when lying times normally are reduced, under stocking is protective and allows cows to maintain lying time for a longer period. Hill et al. (2009) reported as pen stocking density (headlocks and stalls) of a mixed pen of multiparous and primiparous cows increased from 100 up to 142%, the percentage of cows and time spent lying decreased. Similarly, Fregonesi et al. (2007) reported lying time was reduced by 2 h/d when stocking density of stalls was increased from 100 to 150%. Krawczel et al. (2012) reported a decrease in lying time when stocking density exceeded 131% compared to 100 and 113%. The time budgets of prepartum cows tend to be interrupted less than lactating dairy cows. Data from lactating dairy cows on commercial herds showed that as the number of cows in the pen increased, lying daily bout frequency increased and lying bout duration decreased (Charlton et al., 2014). Based on our results and previous studies, it appears that lying time is not significantly affected for prepartum cows up to 103% of stall stocking density.

Stocking animals at 100% had a tendency to affect mean daily feeding times. Nulliparous animals stocked at 100% spent a longer time feeding than nulliparous animals stocked at 80%. The opposite tended to be observed with parous animals stocked at 100% spending less time eating than parous animals stocked at 80%. These results tend to contrast with Hill et al. (2009) and Krawczel et al. (2012) who found no

differences in mean daily feeding time for lactating cows at 4 stocking densities of 110, 113, 131 or 142%. Significant differences in mean feeding times were observed between nulliparous and parous animals, which in the current experiment were housed separately. In the previous studies mentioned, nulliparous and parous animals were housed together and this could be one reason for contrasting results. Azizi et al. (2009) also reported that prepartum nulliparous animals spent less time eating than parous animals, which was also observed for early lactation cows. Future work should document priority of access to the two resources (stalls and feed) separately and with both, mixed and separate parity groups.

An additional analysis was conducted to determine if certain social groups of animals were affected by the increased competition for feed and lying space. Differences were noticed between treatment and parity. Nulliparous animals housed at either 80 or 100% stocking density did not differ in daily feeding times; however, middle ranking parous animals spent more time feeding than both high and low ranking animals. Huzzey et al. (2006) reported there was an increase in feeding time when stocking density decreased and this was most apparent in lower ranking cows. Proudfoot et al. (2009) observed competition had a tendency to decrease feed intake of prepartum multiparous cows the week before calving, although postpartum intakes did not differ. Olofsson (1999) observed that there was an increase in DMI as competition increased and this was driven by an increased feeding rate (Olofsson, 1999). In the current study, dry matter intake data were not collected because the experiment was conducted in a commercial dairy.

Studies have shown that when competition increased there was an increase in standing time (Huzzey et al., 2006) and a decrease in daily lying time (Fregonesi et al., 2007). High ranking parous animals spent less time lying down in the 80D treatment and had fewer and longer lying bouts than middle ranking animals. Middle ranking nulliparous animals at 100D spent less time lying down than both high and low ranking animals and this was attributed to the decrease in the number of lying bouts. Although animals in this experiment were categorized on rank according to their ability to access the feed bunk, Val-Laillet et al. (2008) reported individual measurements of success to displace another cow were not highly correlated with the three resources tested: feed alley, stalls, and rotating brush. In a study evaluating social hierarchy on cow's use of shade and water, social hierarchy did not influence drinking behavior when the water trough was inside the paddock; however, when the water trough was located in the alley, the number of drinking events and the time spent drinking were greater for dominant cows in comparison to subordinate cows (Coimbra et al., 2012). Social hierarchy did not influence the number of visits or the time spent in the shade, irrespective of the location of the water trough (Coimbra et al., 2012). The average feeding time of lower-ranked cows was significantly longer than dominant cows (Hasegawa et al., 1997). The use of feed barriers increased cow feeding time and decreased the number of feeding bouts in relation to the total time feed was available and was notably observed in subordinate cows (Hetti Arachchige et al., 2014). It appears social rank may alter prepartum animals' behaviors for both feeding and lying resources; social rank relationships are complicated and still not completely understood.

We found in a previous study that reducing stocking density for close-up prepartum animals from 100 to 80% did not result in fewer health events, increased milk yield or greater percent of animals diagnosed pregnant after first postpartum AI (Silva et al., 2014). In addition, we observed no differences between the 80 and 100% stocking densities for number of animals removed from the herd or metabolic parameters (Silva et al., 2014). Our evaluation of a sub-group of animals for the effect of prepartum stocking density on immune parameters showed no differences between treatments for polymorphonuclear leukocyte count or phagocytic activity (Dresch et al., 2013). Other research groups showed similar results. Stocking rate did not affect pregnancy rates, immunological parameters or health status of grazing dairy cattle (McCarthy et al., 2012). Boyle et al. (2013) reported no differences in milk production for nulliparous cows mixed with multiparous cows when compared to an all nulliparous pen stocked at 100%.

Most studies during the transition period involved Holstein cows (Huzzey et al., 2005; Proudfoot et al., 2009; Krawczel et al., 2012). Olson et al. (2011) reported that Holstein-Jersey cows had significantly greater odds of mastitis than pure Holstein cows, whereas there was a trend for Holstein-Jersey cows to have lower odds of metritis than pure Holstein cows. The incidence of transition health events were: DA – 14 vs. 0%, ketosis – 9 vs. 9%, mastitis – 2 vs. 27% and metritis – 19 vs. 0% in 43 Holstein and 22 purebred Jersey cows, respectively (Olson et al., 2011). Holstein cows had greater days open than Jerseys (169 vs. 132 d), whereas Jerseys had a higher frequency of mastitis than Holsteins (10.3 vs. 1.1%, respectively; Brown et al., 2011). Holstein cows mobilized more body energy in early lactation than Danish Red or Jerseys (Friggens et

al., 2007). To our knowledge this is the first stocking density study of prepartum Jersey cows in a commercial setting evaluating behavior.

### **CONCLUSIONS**

Housing Jersey prepartum animals at 100% stocking density increased negative social behaviors, affected lying behavior differently depending on time prior to parturition, increased daily feeding time of nulliparous animals, and had a tendency to reduce daily feeding time of parous animals compared to 80% stocking density. Social rank was associated with feeding and lying behaviors and this association varied depending on parity and stocking density. Therefore, although these results showed some potential behavior benefits of a low prepartum stocking density of 80%, more research is needed to evaluate the effect of stocking densities greater than 100% and with other breeds of dairy cattle, such as Holstein.

**Table 1.** Lying time (h/d) during the prepartum period according to parity, treatment (80D vs 100D)<sup>1</sup> and social rank category

		Social Rank Category <sup>2</sup>		
		High	Middle	Low
Parous <sup>3</sup>	80D	12.6 ± 0.3 <sup>a</sup>	13.5 ± 0.3 <sup>b</sup>	13.2 ± 0.3 <sup>a,b</sup>
	100D	13.0 ± 0.3	13.2 ± 0.3	13.6 ± 0.2
Nulliparous	80D	13.3 ± 0.3	13.1 ± 0.3	12.7 ± 0.3
	100D	13.1 ± 0.3 <sup>a,b,†</sup>	12.4 ± 0.3 <sup>b,†</sup>	13.2 ± 0.3 <sup>a</sup>

<sup>a,b</sup>Means within row with different superscripts differ ( $P < 0.05$ ).

<sup>†</sup>High and middle ranking nulliparous animals tend to differ ( $P < 0.10$ ).

<sup>1</sup>80D = animals housed in prepartum pens with 80% target feed bunk stocking density (38 cows/48 headlocks); and 100D = animals housed in prepartum pens with 100% target feed bunk stocking density (48 cows/48 headlocks).

<sup>2</sup>The displacement index (DI) for each cow was calculated as the number of displacements as actor divided by total displacements. Social categories were low:  $DI < 0.40$ ; middle:  $0.40 \leq DI \leq 0.60$ ; and high:  $DI > 0.60$ .

<sup>3</sup>Parous animals were a mix of prepartum lactations  $\geq 1$  and were housed separately from nulliparous animals.

**Table 2.** Feeding times (min/d) during the prepartum period according parity (parous vs. nulliparous), treatment (80D vs. 100D)<sup>1</sup> and social rank category

Parous <sup>3</sup>		Social Rank Category <sup>2</sup>		
		High	Middle	Low
Parous <sup>3</sup>	80D	300.7 ± 7.4	301.3 ± 6.2	294.9 ± 5.9
	100D	272.7 ± 6.8 <sup>a</sup>	300.0 ± 5.6 <sup>b</sup>	284.9 ± 4.9 <sup>a</sup>
Nulliparous				
Nulliparous	80D	255.3 ± 8.5	250.1 ± 6.2	238.1 ± 7.0
	100D	265.9 ± 8.5	256.4 ± 5.4	258.9 ± 6.0

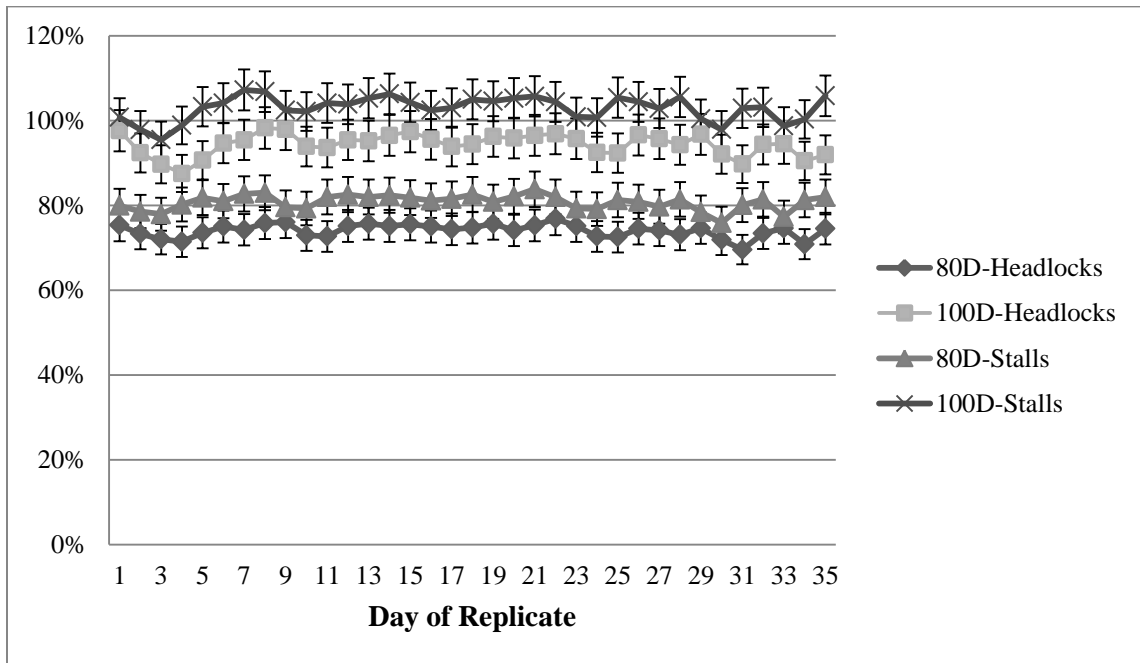
<sup>a,b</sup>Means within row with different superscripts differ ( $P < 0.05$ ).

<sup>1</sup>80D = animals housed in prepartum pens with 80% target feed bunk stocking density (38 cows/48 headlocks); and 100D = animals housed in prepartum pens with 100% target feed bunk stocking density (48 cows/48 headlocks).

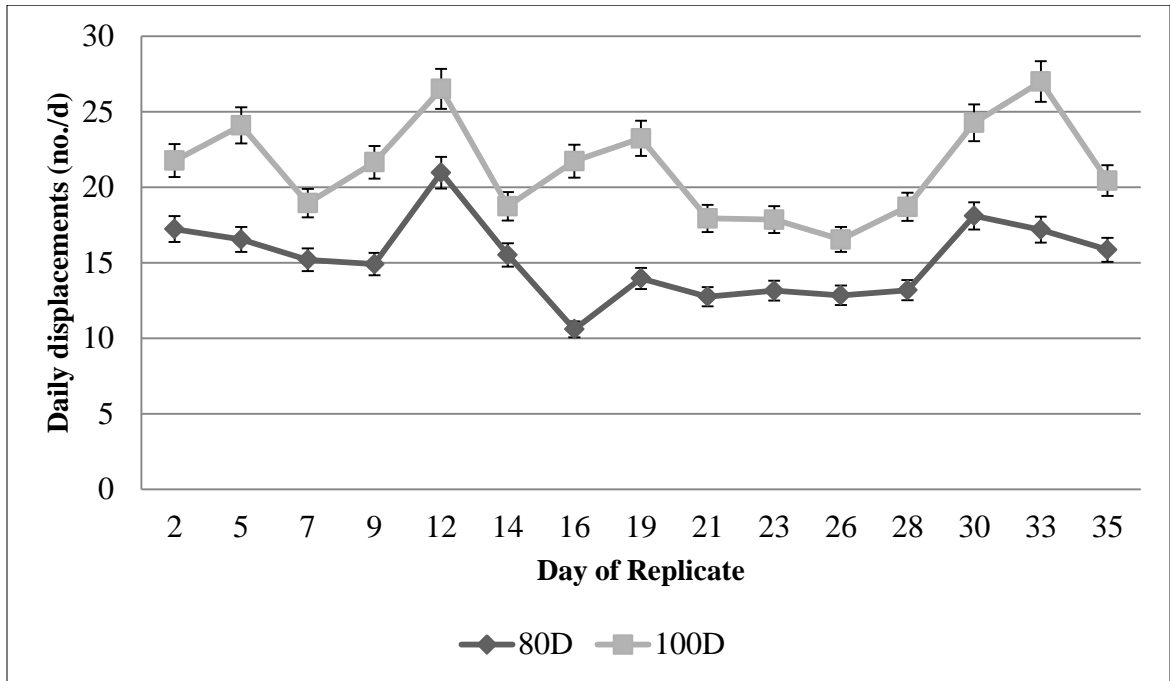
<sup>2</sup>The displacement index (DI) for each cow was calculated as the number of displacements as actor divided by total displacements. Social categories were low:  $DI < 0.40$ ; middle:  $0.40 \leq DI \leq 0.60$ ; and high:  $DI > 0.60$ .

<sup>3</sup>Parous animals were a mix of prepartum lactations  $\geq 1$  and were housed separately from nulliparous animals.

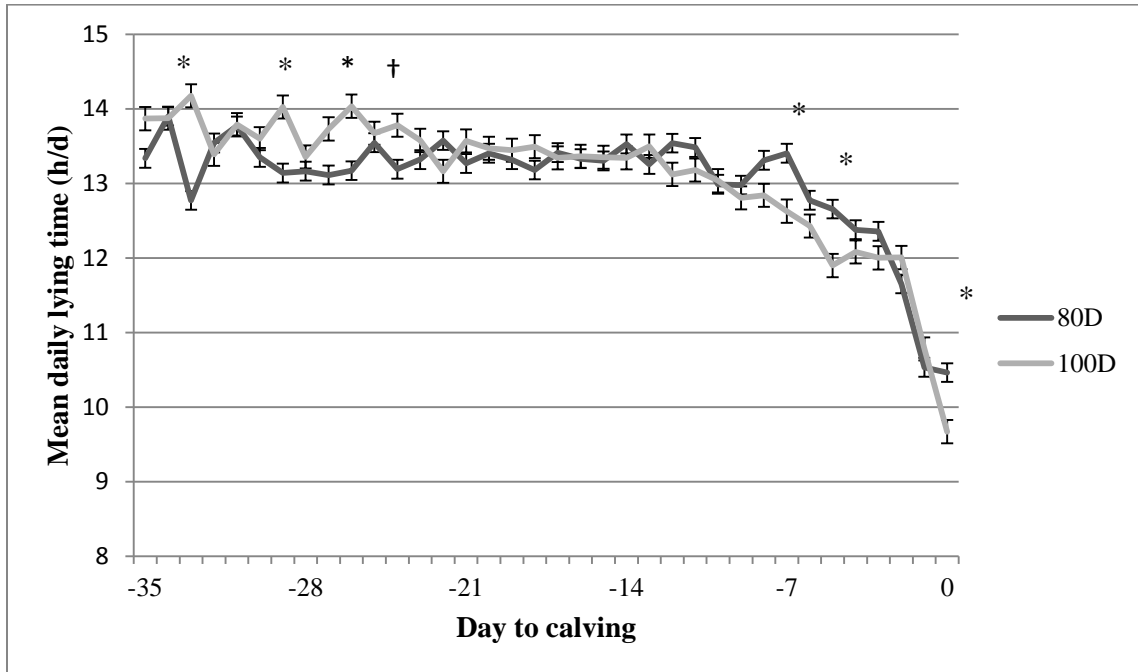
**Figure 1.** Average stocking density based on the number of headlocks and number of stalls according to treatment. There were 8 experimental units (4 replicates x 2 pens/treatment per replicate). Treatments: 80D = cows housed in prepartum pens with 80% target stocking density (38 cows/pen; each pen with 48 headlocks and 44 stalls) and 100D = cows housed in prepartum pens with 100% target stocking density (48 cows/pen).



**Figure 2.** Mean daily displacements from the feed bunk for treatments. Treatments: 80D = cows housed in prepartum pens with 80% target stocking density (38 cows/pen; each pen with 48 headlocks and 44 stalls) and 100D = cows housed in prepartum pens with 100% target stocking density (48 cows/pen). There were 8 experimental units (4 replicates x 2 pens/treatment per replicate). Treatment affected the mean ( $\pm$  SEM) of the number of displacements from the feed bunk (80D =  $15.2 \pm 1.0$ , 100D =  $21.3 \pm 1.0$ ;  $P < 0.001$ ).



**Figure 3.** Mean daily lying times for 80 (80D) and 100% (100D) prepartum feed bunk stocking density. There were 8 experimental units (4 replicates x 2 pens/treatment per replicate). Treatments: 80D = cows housed in prepartum pens with 80% target stocking density (38 cows/pen; each pen with 48 headlocks and 44 stalls) and 100D = cows housed in prepartum pens with 100% target stocking density (48 cows/pen).

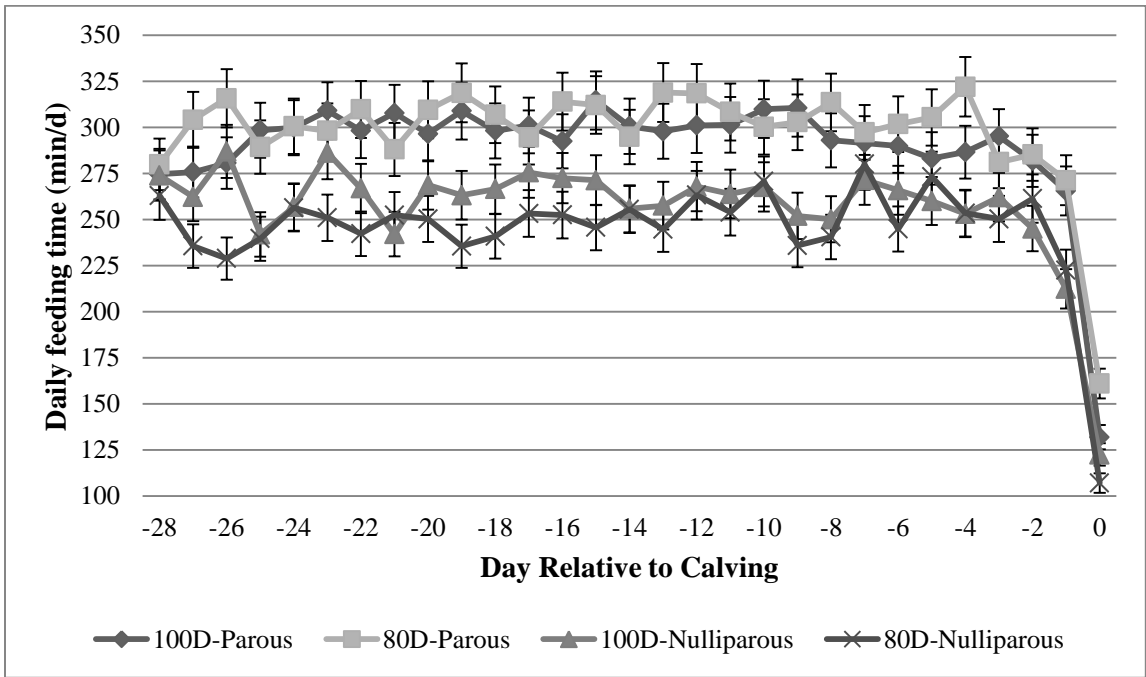


\*Significant differences between treatments ( $P < 0.05$ ).

†Denotes a tendency between treatments ( $P < 0.10$ ).

Treatments differed in lying time on d -33, -29 -26, -7, -5, and 0.

**Figure 4.** Mean daily feeding times for 80 (**80D**) and 100% (**100D**) prepartum feed bunk stocking density relative to calving. There were 8 experimental units (4 replicates x 2 pens/treatment per replicate). Treatments: 80D = cows housed in prepartum pens with 80% target stocking density (38 cows/pen; each pen with 48 headlocks and 44 stalls) and 100D = cows housed in prepartum pens with 100% target stocking density (48 cows/pen). Overall 80D nulliparous animals spent  $12.4 \pm 5.0$  less min/d feeding than 100D nulliparous animals ( $P < 0.05$ ). Parous 80D parous (prepartum lactation  $\geq 1$ ) animals tended to spend  $7.6 \pm 4.5$  more min/d feeding than 100D parous animals ( $P = 0.095$ ).



# **EXPERIMENT 3: EVALUATION OF PREPARTUM FEEDING TIME AS A PREDICTOR OF TRANSITION HEALTH DISORDERS IN DAIRY COWS**

## **SUMMARY**

The objective of this study was to investigate the relationship between prepartum feeding times and periparturient health disorders, first test milk yield and milk composition in Jersey cows. Jersey cows  $253 \pm 3$  d of gestation were marked with unique alphanumeric symbols and were moved into a close-up dry pen. At enrollment, cows with a body condition score  $< 2$  or  $> 4$  (1-5 scale; 1=emaciated, 5=obese) or with a locomotion score  $> 3$  (1-5 scale; 1=normal gait, 5=severely lame) were not included. Feeding time was measured using 10-min video scan sampling for 24-h periods 2-4 d per wk of the study. From preliminary analysis it was determined d -21 to -1 prepartum were most accurate for describing changes in feeding behavior. A total of 925 cows were eligible for analysis. Parity was based on lactation number at time of enrollment and classified as nulliparous, primiparous and multiparous (lactation  $\geq 2$ ). Receiver operator curve (**ROC**) analysis was used to determine sensitivity and specificity at the critical threshold level. Prepartum feeding time from d -14 to d -1 were averaged to 1 value per cow for ROC analysis. Multiparous cows subsequently diagnosed with metritis, retained fetal membrane, displaced abomasum (**DA**), lame at 1 DIM or lame 35 DIM had decreased prepartum feeding times. Receiver operating curve analysis determined a critical threshold for retained fetal membrane of 257.5 min/d (Se = 64.7%, Sp = 71.1%). Multiparous cows that were diagnosed lame at 1 DIM spent  $52.6 \pm 20.4$ ,  $76.7 \pm 22.2$  and

54.8 ± 29.0 min/d less feeding during wk -3, wk -2, and wk -1, respectively, and the critical threshold was 224.2 min/d (Se = 71.4%, Sp = 89.2%). Primiparous cows diagnosed with a DA had decreased prepartum feeding time up to 2 wk prior to calving compared with healthy primiparous cows. Primiparous cows diagnosed lame at 1 DIM decreased their feeding time on wk -2 and had a critical threshold of 278.6 min/d (Se = 100%, Sp = 68.5%). Nulliparous cows with a case of mastitis up to 14 DIM were observed to alter their prepartum feeding behavior on wk -1 with a reduction 63.1 ± 25.2 min/d. Monitoring of prepartum feeding behavior of multiparous cows, even at a limited number of days, appeared to be beneficial in predicting certain transition disorders. Real-time daily feeding behavior monitoring technologies are now available in the US which might prove to be even more helpful in identifying cows at risk for transition disorders.

## **INTRODUCTION**

Early detection of health disorders would potentially prevent or reduce the duration of disease events and reduce on farm mortality. The periparturient period usually defined as 3 wk before and 3 wk after calving (Grummer et al., 1995) is considered one of the most critical periods of a dairy cow's life, with up to 25% of cows being culled or dying during the first 60 days in milk (Godden et al., 2003). During the periparturient period cows are at risk for metabolic and infectious disorders, which can ultimately affect reproductive performance (Gröhn et al., 2003). It has been estimated that approximately 50% of cows have one or more adverse health events during this period (Ferguson, 2001). Reducing morbidity and mortality can improve animal welfare and farm profitability by reducing treatment costs, preventing reductions of milk yield,

improving reproductive performance, and minimizing premature culling or death (González et al., 2008).

Changes in feeding times may become particularly useful for early detection of health disorders with the increased use of automated technologies occurring on dairy farms. Some monitoring systems have been validated and the data generated by them are highly correlated to direct observation (Chapinal et al., 2007). Previous research with dairy cattle has indicated cows that had a case of metritis had reductions in feeding time (Urton et al., 2005) and dry matter intake (Huzzey et al., 2007) during the transition period compared to healthy cows. Cows diagnosed with ketosis had rapid decreases in feeding time, feed intake, and feeding rate approximately 4 d before diagnosis by farm staff (González et al., 2008). Additionally, cows diagnosed lame spent approximately 20 less min/d feeding 7 d prior to diagnosis (González et al., 2008). Healthy feedlot steers spent more time at the feed bunk than morbid steers (Sowell et al., 1998). The use of radio frequency technology to obtain individual time spent at the feed bunk was able to detect morbid steers approximately 4 d earlier than trained feedlot personnel (Quimby et al., 2001).

The objective of this study was to investigate the relationship between prepartum feeding times and transition health disorders, first test milk yield and milk composition in Jersey cows.

## MATERIALS AND METHODS

### *Animals, Management, and Housing*

The present study was based on data collected during 2 previous experiments conducted at a large commercial dairy farm (6,400 lactating dairy cows) in south-central Minnesota, USA. Close-up prepartum cows were housed in a 12-row low profile cross-ventilated barn and provided TMR once daily with frequent push-ups. Prepartum cows were fed from a feed alley by headlocks. All experimental pens (2 pens utilized in study 1 and 4 pens in study 2) had the same measurements of 31.7 m x 11.0 m and had 44 deep sand bedded freestalls (229L x 107W x 114H cm) with a head-to-head configuration and 48 - 0.61 m headlocks. Two water troughs were located in the pen and measured 366 cm x 56 cm. One water trough was located at the end of the bank of freestalls and a shared water trough was located between the treatment pen and an adjacent non-experimental pen. Temperature and relative humidity were recorded hourly in each study pen throughout the experiment.

Jersey cows were enrolled in the experiment 4 wk prior to expected calving date and all cows had a body condition score between 2 and 4 (1-5; scale 1=emaciated, 5=obese) and locomotion score < 3 (1-5 scale; 1=normal, 5=severely lame). A total of 925 cows were used in the current study. In study 1, 209 prepartum primiparous and multiparous cows were enrolled. Cows were either exposed to an all-in-all-out grouping strategy or a traditional pen management with once weekly entrance of new cows. In study 2, 316 nulliparous and 400 primiparous and multiparous Jersey cows were used in the dataset. In study 2, nulliparous animals were housed separately from the mixed pen

of primiparous and multiparous cows. Prepartum parity (nulliparous, primiparous and multiparous) was tested independently after univariate analysis detected differences in feeding behavior.

When cows demonstrated signs of calving, farm personal moved the cows to an individual box stall. At d 1 post-calving cows were moved into a freestall pen with 240 stalls and 260 headlocks, stocked at 100% based on the number of stalls for 21 d. Parous and nulliparous cows were housed separately during the first 21 d. Video observation ceased when cows left the prepartum period treatment pens.

### ***Feeding Behavior Measurements***

All enrolled cows were identified with a unique alphanumeric symbol on their backs using permanent hair dye either in black or blonde. Hair dye was applied from 0 ± 1 relative to entering the treatment pens. To observe feeding behavior, each pen was equipped with 3 video cameras (Weldex, Cypress, CA) connected to a digital video recording system (Channel Visions, Costa Mesa, CA). Feeding time was measured using 10-min video scan sampling for 24-h periods (Endres et al., 2005). Study 1 feeding times were collected on d 1, 2, 3, and 7 of each week of the 5-wk replicate, whereas for study 2 data were collected on d 2, 5, and 7 for wk 1 of each replicate and d 2 and 5 for wk 2-5. A cow was considered eating when the cow's ears were on the feed alley side of the headlocks.

### ***Body Condition and Locomotion Score***

At enrollment and on d  $1 \pm 1$ ,  $28 \pm 3$ , and  $56 \pm 3$  postpartum all cows were scored for body condition (1 = emaciated and 5 = obese; Ferguson et al., 1994) and locomotion (1 = normal and 5 = severely lame; Sprecher et al., 1997).

### ***Blood Sampling and Analysis of Metabolites in Plasma***

Blood samples were collected from all cows on  $-18 \pm 3$ ,  $-11 \pm 3$ ,  $-4 \pm 3$ ,  $3 \pm 3$ ,  $10 \pm 3$ ,  $17 \pm 3$  and  $24 \pm 3$  d relative to calving from the coccygeal vein or artery immediately after feeding while cows were restrained in self-locking headlocks. Samples were collected into evacuated tubes containing K2 EDTA (Becton Dickinson Vacutainer Systems, Franklin Lakes, NJ). Tubes were placed in ice until centrifugation for plasma separation ( $1,200 \times g$  for 15 min at  $4^{\circ}\text{C}$ ). Plasma was aliquoted into microcentrifuge tubes and stored at  $-32^{\circ}\text{C}$  until analysis.

Samples collected weekly from DIM -18 to -4 were analyzed for concentrations of NEFA using a colorimetric assay (Wako Chemicals USA, Richmond, VA; Ballou et al., 2009). Concentrations of BHBA were determined enzymatically (Ranbut, Randox Laboratories, Antrim, UK; Ballou et al., 2009) from samples collected weekly from DIM 3 to 24.

### ***Clinical Examination and Definitions of Diseases***

All cows were examined on DIM  $1$ ,  $4 \pm 1$ ,  $7 \pm 1$ ,  $10 \pm 1$ , and  $13 \pm 1$  for the diagnosis of retained fetal membrane and metritis. Retained fetal membrane (**RFM**) was defined as retention of a fetal membrane past 24 h postpartum. Metritis was defined as cows with watery, pink or brown, and fetid uterine discharge. Acute metritis included the

symptoms of metritis and cows that presented fever ( $> 39.5^{\circ}\text{C}$ ), anorexia or depression. Cows were classified with subclinical ketosis (**SCK**) when BHBA concentrations were  $\geq 1200 \mu\text{mol/L}$ . All cows were observed once daily for displacement of abomasum (**DA**) and thrice daily for mastitis. Cows were followed up to 14 DIM for mastitis and 60 DIM for DA. Cows were considered healthy ( $n = 676$ ) were not diagnosed with metritis, RP, SCK, DA, or mastitis up to 14 DIM and were no lame at DIM 1.

### ***Production Parameters***

Cows were milked thrice daily. Monthly milk yield, milk fat and protein contents, and SCC were recorded for individual cows during Dairy Herd Improvement Association test. Data regarding milk composition were collected up to first test (Mean  $\pm$  SD;  $18 \pm 8$  DIM) as a proxy for health status. Energy-corrected milk yield was calculated for each cow using the formula  $\text{ECM (kg)} = [(\text{kg of milk} \times 0.327) + [(\text{kg of fat}) \times 12.95] + [(\text{kg of protein}) \times 7.2]$  (Orth, 1992).

### ***Statistical Analysis***

Data were analyzed with SAS (v 9.2 SAS Institute Inc., Cary, NC). Cow was used as the experimental unit ( $n = 925$ ). Preliminary statistical analysis determined no difference related to study and data from the 2 studies were combined. Differences in feeding behavior of cows diagnosed with a health disorder vs healthy counterparts were detected up to 21 d prepartum. Average daily feeding times were average among three prepartum periods that were categorized by week prepartum: wk -3 (d -21 to d -15), wk -2 (d -14 to d -8) and wk -1 (d -7 to d -1). Day of calving was excluded from analysis due to the cow leaving the treatment pen. A repeated statement included day and cow as the

subject. The structure of covariance (compound, unstructured, or autoregressive) for the repeated statement was chosen according to the Bayesian Akaike information criteria. Fixed effects to the model included health status (disease event of interest vs. healthy), wk relative to calving, parity (nulliparous, primiparous, multiparous), and the interaction of health status by wk relative to calving. Other covariates offered to the model included the interaction of health status by parity and wk relative to calving, average daily pen temperature and pen stocking density and change in body condition score.

The LOGISTIC procedure (SAS Institute) was used to perform multivariate logistic regression, receiver operating characteristic (**ROC**) and area under the curve (AUC) analyses. Preliminary analysis determined average daily feeding time from d -14 to -1 provided the highest AUC values. Variables included average daily feeding time, average day relative to calving, and parity. Critical threshold, sensitivity and specificity analyses were completed using MedCalc (MedCalc Software, Mariakerke, Belgium).

## **RESULTS**

A total of 210, 241, and 210 nulliparous, primiparous and multiparous animals, respectively, were not diagnosed with metritis, acute metritis, RP, mastitis, SCK or lame at DIM 1 and were considered healthy. Table 1 shows the frequency and incidence of health events by parity. There were no associations of prepartum daily feeding time (min/d) and cows that had SCK versus healthy cows for nulliparous ( $245 \pm 12$  vs.  $255 \pm 4$ ;  $P = 0.44$ ), primiparous ( $334 \pm 30$  vs.  $304 \pm 3$ ;  $P = 0.31$ ) or multiparous ( $296 \pm 16$  vs.  $293 \pm 4$ ;  $P = 0.85$ ), respectively. Daily feeding time did not differ between nulliparous ( $253 \pm 8$  vs  $251 \pm 4$ ;  $P = 0.80$ ), primiparous ( $294 \pm 11$  vs  $305 \pm 3$ ;  $P = 0.37$ ) and

multiparous ( $286 \pm 12$  vs  $294 \pm 3$ ;  $P = 0.51$ ) cows that had acute metritis versus healthy cows, respectively.

### ***Displaced Abomasum***

Only 2 primiparous cows and 5 multiparous cows were diagnosed with a DA. One multiparous cow was removed from the analysis with outlying feeding times than other multiparous cows diagnosed with a DA. Nulliparous cows were removed from the analysis due to not having a case. With the remaining primiparous and multiparous cows, the interaction among DA, parity, and week ( $P = 0.034$ ) was associated with primiparous cows that had a DA tended to spend  $82.7 \pm 42.9$  ( $P = 0.054$ ) less min/d feeding during wk -3 and spent  $85.2 \pm 42.9$  ( $P = 0.047$ ) and  $92.3 \pm 42.9$  ( $P = 0.032$ ) less min/d during wk -2, and wk -1, respectively. Multiparous cows that had a DA reduced feeding time only on wk -2 ( $82.0 \pm 31.7$ ) fewer min/d feeding;  $P = 0.001$  compared with their healthy counterparts.

Area under the curve analysis for primiparous cows with a DA was 0.97 (95% CI; 0.95-0.99). The critical threshold was  $\leq 202$  min/d total feeding time with sensitivity of 100% and specificity of 97.1%. The AUC for multiparous cows was 0.76 (95% CI; 0.70-0.82). The critical threshold was  $\leq 277$  min/d for multiparous cows with a DA (Se = 100%, Sp = 56%). When primiparous and multiparous cows were combined the AUC was 0.86 (95% CI; 0.82-0.89) with a critical threshold of  $\leq 202$  min/d (Se = 67%, Sp = 96%).

### ***Metritis***

Incidence of metritis for nulliparous, primiparous, and multiparous animals were 22.8, 15.7 and 12.7%, respectively. Primiparous and nulliparous cows did not have significant changes in prepartum daily feeding time versus healthy counterparts (Table 2;  $P = 0.003$ ). Multiparous cows diagnosed with metritis spent less time feeding during wk -3 and wk -2 prepartum ( $P = 0.037$  and  $P = 0.004$ , respectively) than healthy counterparts, however, did not differ between healthy cows on wk -1 ( $P = 0.15$ ).

Area under the curve analysis determined no association of prepartum feeding time and metritis diagnosis for nulliparous and primiparous cows. The AUC for multiparous cows was 0.64 (0.58-0.70; 95% CI) with a critical threshold of  $\leq 316.7$  min/d. At the critical threshold, sensitivity was 94.7% and specificity was 32.4%.

### ***Retained Fetal Membrane***

The incidence of RP was 4.4% for nulliparous, 8.8% for primiparous and 9.3% for multiparous cows. No significant differences of average daily feeding time were observed for nulliparous cows during wk -3 and wk -2 (Table 3;  $P = 0.037$ ). However, nulliparous cows diagnosed with RP tended ( $P = 0.081$ ) to eat 39 min longer than their healthy counterparts on wk -1 prepartum. Primiparous cows with RP tended to spend  $25 \pm 15.1$  ( $P = 0.098$ ) fewer min/d feeding during wk -3 and spent  $28.5 \pm 14.2$  ( $P = 0.045$ ) fewer min/d feeding on wk -2 than healthy primiparous cows. There was a numeric decrease of  $21.1 \pm 14.3$  min/d ( $P = 0.14$ ) during wk -1 for primiparous cows with a RP. Multiparous cows with RP spent  $33.4 \pm 15.5$  ( $P = 0.031$ ) and tended to spend  $26.0 \pm 15.0$  ( $P = 0.083$ ) fewer min/d eating during wk -2 and wk -1 than healthy multiparous cows.

Prepartum feeding times of nulliparous and primiparous cows diagnosed with RP were not significant in the ROC analysis. The AUC for multiparous cows diagnosed with RP was 0.71 (0.65-0.77; 95% CI). The critical threshold was 257.5 min/d with a sensitivity of 65% and specificity of 71%.

### ***Mastitis up to 14 DIM***

Seven nulliparous, 4 primiparous and 6 multiparous cows had a case of mastitis by 14 DIM. There was a tendency for an interaction of parity, week relative to calving, and cows diagnosed with mastitis ( $P = 0.062$ ). No differences were observed in prepartum feeding times and health status for multiparous and primiparous cows. Nulliparous cows that had a clinical case of mastitis up to 14 DIM spent  $63.1 \pm 25.2$  fewer min/d feeding during wk -1 than healthy counterparts ( $P = 0.013$ ). Feeding times during wk -3 and wk -2 did not differ between health status for nulliparous cows.

### ***Lame at DIM 1 and DIM 35***

A total of 0, 4, and 9 cows were diagnosed lame 1 d post calving for nulliparous, primiparous and multiparous cows, respectively. Nulliparous cows were removed from the analysis. There was an interaction of lameness, parity and week prepartum ( $P = 0.021$ ). Multiparous cows diagnosed lame at DIM 1 (Figure 1) spent  $52.6 \pm 20.4$  ( $P = 0.010$ ),  $76.7 \pm 22.2$  ( $P = 0.001$ ), and  $63.3 \pm 21.5$  ( $P = 0.003$ ) fewer min/d feeding during wk -3, wk -2, and wk -1, respectively, than healthy multiparous cows. On wk -2, lame primiparous cows tended to spend  $54.8 \pm 29.0$  ( $P = 0.059$ ) less min/d feeding than healthy primiparous cows. There were no significant differences between healthy and lame primiparous cows on wk -3 or wk -1 relative to calving.

Area under the curve for multiparous cows diagnosed lame at DIM 1 were 0.85 (95% CI; 0.79-0.89). The critical threshold was 224.2 min/d with a sensitivity of 71% and specificity of 89%. The AUC for primiparous cows were 0.83 (0.78-0.88) with a critical threshold of 278.6 min/d with 100% sensitivity and 69% specificity. Since both primiparous and multiparous cows were housed together, an analysis with parity was combined. When feeding times of multiparous and primiparous were combined AUC was 0.84 (95% CI; 0.81-0.88) with a critical threshold of 260 min/d, 82% sensitivity and 74% specificity.

By 35 DIM 3 nulliparous, 10 primiparous, and 23 multiparous cows were diagnosed lame. Among nulliparous and primiparous cows no differences in prepartum feeding time were observed between healthy cows and those diagnosed lame at DIM 35. Multiparous cows diagnosed lame spent  $28.8 \pm 13.0$  ( $P = 0.027$ ) less min/d feeding during wk -2 and  $29.4 \pm 13.3$  ( $P = 0.028$ ) less min/d feeding during wk -1 than healthy multiparous cows. Area under the curve was 0.69 (95% CI; 0.63 – 0.76) for multiparous cows. A critical threshold of  $\leq 268$  min/d with sensitivity of 74% and specificity of 65% was determined for daily feeding times up to 2 wk prior to calving.

### ***Milk Yield and Composition***

There were no associations of prepartum daily feeding time and milk yield ( $P = 0.43$ ), ECM ( $P = 0.68$ ), FCM ( $P = 0.42$ ), milk protein yield ( $P = 0.13$ ), milk protein % ( $P = 0.19$ ), milk fat % ( $P = 0.20$ ), fat yield ( $P = 0.80$ ), somatic cell score ( $P = 0.11$ ), or 305ME ( $P = 0.98$ ).

## DISCUSSION

Our results provide some evidence for using prepartum feeding time to identify primiparous and multiparous cows at risk for health disorders early after calving with limited sampling (2-4 d/wk). Decreased prepartum feeding times were associated with metritis, RP, DA, lameness at 1 DIM and lameness at 35 DIM. To our knowledge this study was the first to show an association between feeding behavior and RP and DA. Previous research has detected associations between feeding time of dairy cows and metritis (Urton et al., 2005; Huzzey et al., 2007, Patbandha et al., 2012), clinical ketosis (Goldhawk et al., 2009) and lameness (González et al., 2008; Palmer et al., 2012).

In the current study nulliparous cows were housed separately from primiparous and multiparous cows. Parity was examined as 3 categories due to the differences in feeding times that existed. Nulliparous cows spent fewer min/d feeding than both primiparous and multiparous cows, regardless of health status. Although primiparous and multiparous cows were housed together, differences in feeding times existed and therefore they were not combined in the mixed models. Huzzey et al. (2007) reported primiparous cows had a slower feeding rate than multiparous cows and in the current study primiparous Jersey animals spent more time feeding than multiparous cows. Prepartum nulliparous cows typically have lower DMI/d than multiparous cows (Janovick and Drackley, 2010) and shorter feeding times could be considered a proxy for DMI.

Overall disease incidence was lower than previously reported values. Combined parity incidence was 17.2% for metritis, 9.1% for acute metritis, 0.8% for DA, 7.5% for

RP, 1.8% for mastitis, 2.5% for subclinical ketosis, 1.4% for lameness at 1 DIM, and 3.9% for lameness at 35 DIM. Olson et al. (2011) reported 0 DA, 2 cases (9%) of ketosis, 6 (27%) cases of mastitis and 0 cases of metritis in a study with 22 first lactation Jersey cows. Patbandha et al. (2012) reported a 40% incidence of metritis in crossbred cows and Urton et al. (2005) reported 69% incidence in Holstein cows and heifers.

Although the incidence of DA was very low (0.6% for primiparous and 1.7% for multiparous cows) there was a dramatic decrease in prepartum feeding times among cows diagnosed with DA compared with healthy cows. Accuracy of a diagnostic test is evaluated through sensitivity and specificity (Greiner et al., 2000). The ROC technique optimizes cut-off values with sensitivity and specificity with regards to a given prevalence in the population (Greiner et al., 2000). A test with a high sensitivity, when negative, is useful to rule out disease. Conversely, a test with a high specificity, when positive, rules in disease. Combining parities to minimize multiple thresholds decreased the sensitivity while maintaining a high specificity. However, due to the limited number of animals and cows having other diseases present the results must be interpreted with caution. Typically periparturient health disorders are interrelated rather than one single health even. LeBlanc et al. (2005) indicated that RP, metritis and increased concentrations of BHBA were associated with an increased risk for DA. Cows with a DA had an average BHBA of 514  $\mu\text{mol/L}$  whereas healthy cows had 474  $\mu\text{mol/L}$ . Of the cows with a DA in our study, 2 had both RP and metritis, 1 only RP and 1 cow only metritis. No cows with a DA had subclinical ketosis or were lame at 1 or 35 DIM.

Nulliparous cows had the greatest incidence of metritis (28%), although our incidence was much lower than 49% reported in Holsteins by Giuliadori et al. (2013). This is most likely due to the reduced calving difficulty in the Jersey breed (Cole et al., 2005) and reducing the trauma to the uterine wall from assisted calvings. Metritic nulliparous cows were not observed altering feeding times from healthy counterparts. However, it is unknown whether they altered other feeding characteristics, such as dry matter intake or feeding rate.

When examining ROC for metritis, only multiparous cows had an AUC value that was better than just chance. Patbandha et al. (2012) reported a critical value of 302 min/d with a sensitivity and specificity of 75% and 66.7%, respectively. Our results with a critical threshold of 316.7 min/d had a high sensitivity of 94.7%, but a low specificity of 32.4%. Therefore, only few cows not having metritis would actually test negative. One limitation of our study was feeding time was not measuring feeding time throughout the entire prepartum feeding behavior and using the cow as her own baseline. Feeding times can be influence by diet with a high forage diet taking longer to consume than a lower forage diet (Greter et al., 2012). Huzzey et al. (2007) reported DM intake of severely metritic cows was depressed 2 wk prior to calving and those cows continued to consume less feed 3 wk after calving. A decrease in feeding time was observed 2 wk prior to the diagnosis of clinical metritis (Huzzey et al., 2007). Patbandha et al. (2013) recorded prepartum feeding time of 20 multiparous Holstein-Friesian crossbred cows and reported that cows with daily feeding time of 284.5 min/d during the period d -6 to d -2 were more likely to develop metritis (Se = 75% and Sp = 91.7%) compared to cows above that

threshold. Those metritic cows had lower number of feeding bouts and higher inactive standing time compared to normal cows.

Previous studies have cited RP and dystocia as risk factors for metritis (Giuliodori et al., 2013; Bruun et al., 2002; Huzzey et al., 2007). Additional risk factors for metritis included breed, parity, calving season, ketosis, milk fever, mastitis during the dry period (Bruun et al., 2002), stillborn birth and elevated NEFA levels prepartum (Giuliodori et al., 2013). Retained placenta has been linked to immune suppression and elevated NEFA concentrations with ketosis being a risk factor for RP (Qu et al., 2014). Cows with RP had significantly lower neutrophil function before calving and up to 2 wk postpartum (Kimura et al., 2002). Elevated lipid mobilization increases the risk for fatty liver. Insulin resistance and the production of ketones reduce DMI. While some previous studies removed cows with more than one disorder, we decided not to do so because of the interrelationship of transition disorders and compared them to healthy counterparts that were free of any infectious or metabolic disorders up to 60 DIM. For cows diagnosed with metritis, 25.9% also had RP, 1.8% DA, 5.9% subclinical ketosis, 1.8% lameness and 2.3% a mastitis case by 14 DIM.

To our knowledge this is the first study to find an association between prepartum feeding time and RP. Primiparous and multiparous cows diagnosed with RP decreased their feeding time by 29 min/d and 26 min/d at wk -2, respectively. However, at wk -1 there was only a numeric decrease in feeding time for primiparous cows and a tendency for multiparous cows. Receiver operator curve for multiparous cows was fairly good for detecting a multiparous cow with RP. A sensitivity of approximately 65% would not be

able to detect all cows that would have RP, but are similar to previous reported values of using prepartum feeding time as an indicator.

No association between subclinical ketosis ( $\text{BHBA} \geq 1200 \mu\text{mol/L}$ ) and prepartum feeding time was observed in the current experiment. Gonzalez et al. (2008) reported a 45.5 min/d decrease in feeding time for 1 d prior to diagnosis of ketosis and a 19 min/d reduction up to 8 d prior to diagnosis. Those authors noted that once clinical ketosis was diagnosed it affected feeding behavior for a short duration of time. In our study, subclinical ketosis diagnosis averaged  $14 \pm 6$  DIM, which was beyond our feeding behavior measurement period. This may explain our lack of association of prepartum feeding time and subclinical ketosis. However, Goldhawk et al. (2009) reported that every 10-min decrease in daily time spent at the feeder during the week before calving, resulted in a 1.9 times increase in the risk of subclinical ketosis.

Multiparous cows diagnosed lame at 1 DIM had a significant decrease in prepartum feeding time up to 3 wk prior to calving. At enrollment cows were excluded from the study if the locomotion score was  $\geq 3$ . It is unknown when animals became lame because they were not scored again until 1 DIM. It has been reported that hoof lesions may take up to 2 mo for locomotion to become affected (Bergsten, 2003). The types of lesions were not recorded in our study. Previous studies have evaluated milk yield and feed intake as indicators of lameness. Bareille et al. (2003) observed that milk yield and feed intake were slightly depressed in cows diagnosed lame. González et al. (2008) reported daily feeding time was the characteristic most consistently changed in cows that were diagnosed lame.

Infectious diseases, such as mastitis, typically occur during the first 2 wk of lactation (Goff and Horst, 1997). Having a mastitis case up to 14 DIM was the only model that detected changes in feeding behavior of nulliparous cows. Up to 1 wk prior to calving nulliparous animals diagnosed with mastitis had decreased feeding time by 63 min/d. Among nulliparous animals diagnosed with mastitis, 1 had both RP and metritis, 3 had metritis and 1 cow had subclinical ketosis. Gonz ales et al. (2008) did not analyze acute udder disorders because of the great variation in feeding times with some cows eating for longer or shorter than healthy cows. Goff and Horst (1997) reported a high proportion of mammary infections occurs during the 1<sup>st</sup> wk of the dry period when milk flow ceases to flush bacteria from the canal. Bareille et al. (2003) observed a decrease in DMI as early as 5 d prior to diagnosis for systemic mastitis and 4 d prior to diagnosis for one affected quarter.

## CONCLUSIONS

Our analysis provides evidence that monitoring prepartum feeding time aid in the identification of cows at higher risk of a periparturient disorder. Prepartum multiparous cows (lactation  $\geq 2$ ) decreased their prepartum feeding time compared to healthy counterparts more than did nulliparous and primiparous animals. More research is needed to investigate the use of real time monitoring systems that could automate the measurement of individual cow feeding behavior. Additionally, more research is needed to determine whether deviations from each animal's own daily feeding times would be a more accurate measurement, especially for nulliparous animals which tended to not alter their feeding times compared to healthy counterparts.

**Table 1.** Incidence of displaced abomasum (DA), metritis, acute metritis, retained fetal membrane (RP), subclinical ketosis (SCK), mastitis up to 14 DIM, lame at DIM 1 and DIM 35 for nulliparous, primiparous, and multiparous Jersey cows.

	Nulliparous <sup>1</sup>		Primiparous <sup>1</sup>		Multiparous <sup>1</sup>		All Cows	
	(n = 316)		(n = 318)		(n = 291)		(n = 925)	
	n	%	n	%	n	%	n	%
DA	0	0	2	0.6	5	1.7	7	0.8
Metritis	72	22.8	50	15.7	37	12.7	159	17.2
Acute Metritis	43	13.5	21	6.6	20	6.9	84	9.1
RP	14	4.4	28	8.8	27	9.3	69	7.5
SBK	14	4.4	2	0.6	7	2.4	23	2.5
Mastitis	7	2.2	4	1.3	6	2.1	17	1.8
Lame 1 DIM	0	0	4	1.3	9	3.1	13	1.4
Lame 35 DIM	3	0.9	10	3.1	23	7.9	36	3.9

<sup>1</sup> Parity classified at time of enrollment when entering prepartum close-up pens.

**Table 2.** Associations between metritis and daily feeding time of Jersey cows from wk -3 to wk -1 according to parity.

Parity	Week Prepartum	Healthy (min/d)	Metritis (min/d)	Difference (min/d)	Health Status ( <i>P</i> -value)
Multiparous <sup>1</sup>	-3	292.7 ± 4.2	269.8 ± 10.8	23.0 ± 11.6	0.047
	-2	304.0 ± 4.2	272.2 ± 10.0	31.7 ± 10.8	0.003
	-1	282.4 ± 4.3	267.2 ± 9.7	15.1 ± 10.6	0.152
Primiparous <sup>1</sup>	-3	304.3 ± 3.9	291.5 ± 8.7	12.8 ± 9.5	NS
	-2	308.3 ± 3.9	297.9 ± 8.5	10.4 ± 9.3	NS
	-1	300.9 ± 3.9	295.9 ± 8.5	5.0 ± 9.4	NS
Nulliparous <sup>1</sup>	-3	255.2 ± 4.4	260.3 ± 7.0	-5.0 ± 8.1	NS
	-2	253.5 ± 4.5	249.0 ± 7.0	4.5 ± 8.1	NS
	-1	246.0 ± 4.5	260.9 ± 7.0	-14.9 ± 8.1	0.067

<sup>1</sup>Parity classified at time of enrollment in prepartum close-up pens.

NS – not significant values ( $P \geq 0.2$ )

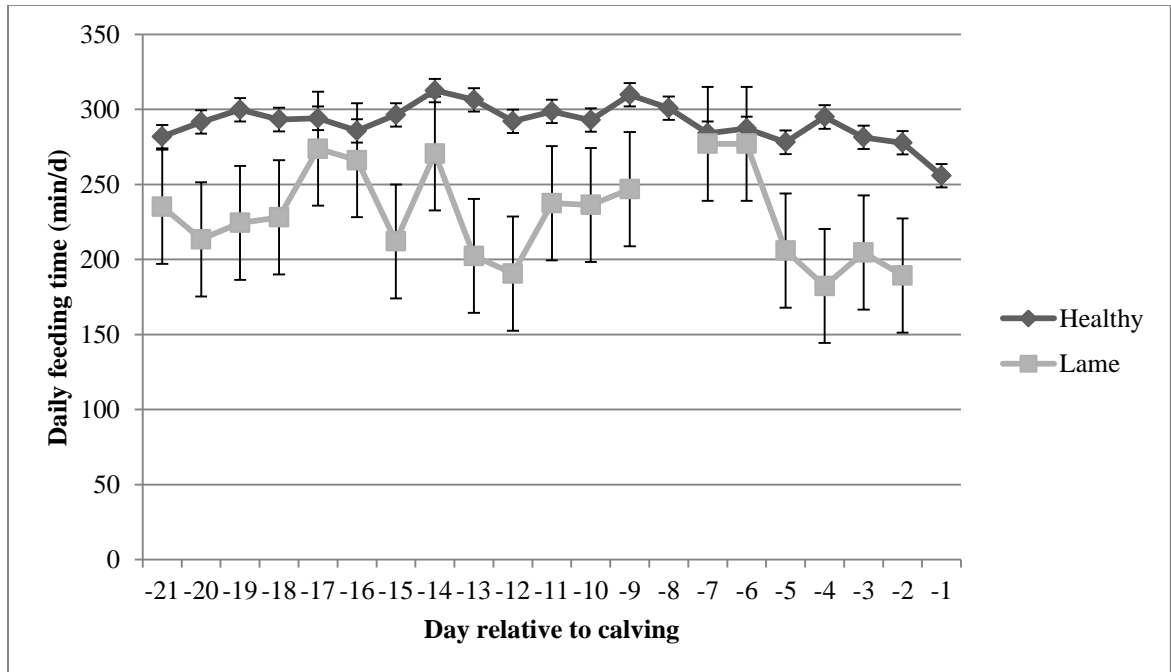
**Table 3.** Associations between retained fetal membrane and daily feeding time of Jersey dairy cows by parity from wk -3 to wk -1 before calving according to parity

Parity	Week Prepartum	Average feeding time (min/d)			Health Status
		Healthy	Metritis	Difference	P-value
Multiparous <sup>1</sup>	-3	292.7 ± 4.2	284.5 ± 17.6	8.2 ± 18.1	NS
	-2	302.9 ± 4.2	269.5 ± 14.9	33.4 ± 15.5	0.031
	-1	281.1 ± 4.3	255.4 ± 14.4	26.0 ± 15.0	0.083
Primiparous <sup>1</sup>	-3	304.3 ± 3.9	279.3 ± 14.6	25.0 ± 15.1	0.098
	-2	308.2 ± 3.9	279.7 ± 13.7	28.5 ± 14.2	0.045
	-1	300.6 ± 4.0	279.6 ± 13.8	21.1 ± 14.3	0.142
Nulliparous <sup>1</sup>	-3	255.0 ± 4.5	263.2 ± 23.0	-8.1 ± 23.4	NS
	-2	253.3 ± 4.6	241.7 ± 22.4	11.6 ± 22.8	NS
	-1	245.7 ± 4.7	285.0 ± 22.1	-39.2 ± 22.5	0.081

<sup>1</sup> Parity classified at time of enrollment during prepartum period.

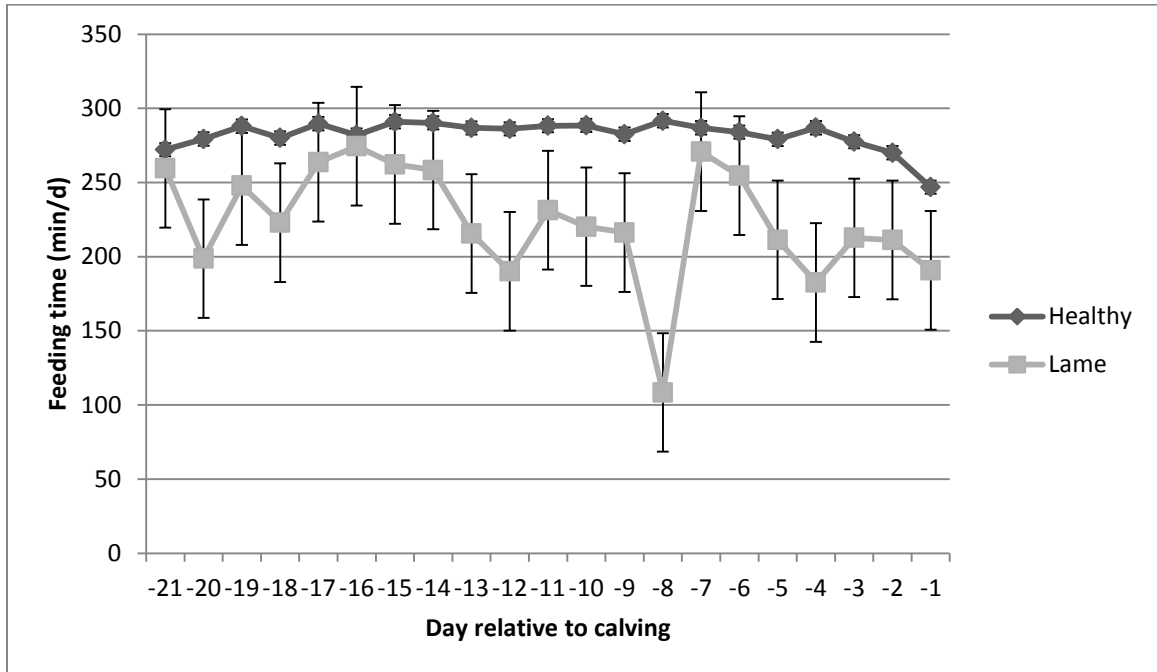
NS – not significant values ( $P \geq 0.2$ )

**Figure 1.** Mean ( $\pm$  SEM) daily feeding time (min/d) of multiparous Jersey cows; healthy (n = 210) and lame at DIM 1 (n = 9) from 21 to 1 d before calving.



Parity classification was based on lactation number at time of enrollment during prepartum period. Lame multiparous cows spent  $64.2 \pm 18.1$  fewer minutes/day feeding during the 3 weeks relative to calving than healthy counterparts ( $P < 0.001$ ).

**Figure 2.** Mean ( $\pm$  SEM) daily feeding time (min/d) of Jersey cows (prepartum lactation  $\geq 1$ ); healthy (n = 451) and lame at DIM 1 (n = 13) from 21 to 1 d before calving.



Lame multiparous cows spent  $42.7 \pm 15.6$  fewer minutes/day feeding during the 3 weeks prior to calving than healthy counterparts ( $P = 0.007$ ).

## **EXPERIMENT 4: ASSOCIATION BETWEEN SOCIAL RANKING AND HEALTH OF PERIPARTUM DAIRY COWS**

### **SUMMARY**

The aim of this study was to determine whether social dominance, determined by displacements from the feedbunk prepartum and 3 different methods, was associated with health, reproduction, and milk yield of transition cows on a large commercial dairy. A total of 953 cows were eligible for the analysis. Cows were enrolled 4 wk before expected calving date and were marked with unique alphanumeric symbols. At enrollment all cows had a body condition score between 2 and 4 (1-5; scale 1=emaciated, 5=obese) and locomotion score  $\leq 3$  (1-5 scale; 1=normal, 5=severely lame). Displacements were observed for 2-4 d/wk. A displacement index (**DI**) was calculated for each cow as the number of displacements as actor divided by total displacements as actor or reactor. Displacement index was used on a continuous scale and categorized into social rankings; cows with a DI of less than 0.4 were considered low-ranking, 0.4-0.6 were considered middle-ranking, and cows greater than 0.6 were considered high-ranking cows. Additionally, the top and bottom 10th percentile of DI were evaluated. A 1-unit increase in DI increased the odds retained fetal membrane by 1% (1.00-1.03) and decreased the odds of metritis by 1% (0.97-1.00). There was a curvilinear relationship of DI and metritis with cows with DI values closer to 0.5 having a lower risk of metritis than cows on either end of the DI scale. High ranking cows were 2.0 times more likely to have an RP event than low ranking cows (1.1-3.7) with no differences among middle and

high or low ranking cows. Social rank was not associated with metritis, subclinical ketosis, displaced abomasum, mastitis or lameness. The bottom 10th percentile of DI cows were 4.7 times (1.6-13.4) more likely to have a case of metritis than the top 10<sup>th</sup> percentile ranking cows. No other health events were associated with top or bottom 10th percentile DI values. A cow's ability to displace another cow as a determinant of her social rank was not very helpful or consistent in predicting the odds of having a transition health disorder.

## **INTRODUCTION**

The intensification of dairy production in the United States has resulted in larger dairy farms housing cows in loose housing rather than tie-stalls and has resulted in larger group sizes, often with animals competing for resources i.e. feed, water, and lying spaces (Val-Laillet et al., 2008a). Certain lower ranking animals may be unable to access these resources due to dominant animals blocking access by displacements, threats, and bunts (Grant and Albright, 2001). During the transition period cows are predisposed to reductions in DMI, negative energy balance, and suppressed immune system (Goff and Horst, 1997). Most metabolic diseases occur within 2 wk of parturition making this one of the most stressful periods for a cow. Most displacements occur at or near the feedbunk (Val-Laillet et al., 2008b); during this time of reduced DMI we hypothesized that a cow's displacement index during the prepartum period may be associated with her health and performance up to 60 DIM.

The concept of social dominance has varied from a linear hierarchy (Kendall, 1962) to categorizing cattle into social dominance groups (Galindo and Broom, 2000). In

larger groups of cows where a linear hierarchy is difficult to calculate, a dominance index can help describe the effectiveness for a cow to displace another from the feed bunk (Val-Laillet et al., 2008a). Different methods have been proposed to categorize cattle into dominance groups and have similar calculations but deal with either the number of displacements or the number of individuals that a cow displaces. The displacement index (**DI**) categories proposed by Galindo and Broom (2000) are based on the number of agonistic encounters that an animal won or lost and is calculated as the number of times a cow displaces other individuals (actor) divided by the times the cow displaces another cow plus the number of times the cow is displaced (reactor). This index ranges from 0 to 1 with cows less than 0.4 considered low-ranking, 0.4 - 0.6 middle-ranking, and greater than 0.6 high-ranking individuals. This method has been the one of the most adopted method of separating cows into social groups (Hohenbrink and Meinecke-Tillmann, 2012; Huzzey et al., 2012 and Val-Laillet et al., 2008b). The Galindo and Broom (2000) method places emphasis on frequency of wins, rather than the number of individuals that a cow displaces. Mendl et al. (1992) created an index for pigs that was calculated by the number of individuals that a pig was able to dominate and was adopted by DeVries et al. (2004) and Huzzey et al. (2006) for dairy cattle. The index is calculated as number of cows an individual is able to displace divided by the number of cows the individual is able to displace plus the number of cows that are able to displace the individual and multiplied by 100. The Mendl et al. (1992) method emphasizes the number of individuals displaced, rather than the number of displacements. A third method developed by Kondo and Hurnik (1990) describes the dominance of an individual in

pairwise interactions. Strong correlations have been determined between the three indices (Val-Laillet et al., 2008a) and for the purpose of this study we decided to use the index created by Galindo and Broom (2000).

Ranking animals into social dominance categories and how it correlates to certain behaviors and health are not well understood. Lower ranking animals (those who were displaced more than they displaced) ate meals faster (Proudfoot et al., 2009) and spent less time in the feeding area than higher-ranking individuals (Val-Laillet et al., 2008b); however, no indication was made whether these animals had more health disorders. Lower ranking animals spent less time lying and a longer time standing idle than both middle- and high-ranking animals (Galindo and Broom, 2000). In addition, low-ranking animals were more likely to become lame than high-ranking cows (Galindo and Broom, 2000). Low-ranking late gestation cows exposed to high stocking rates (5 stalls/10 cows and .34 m of bunk space) for 14 d had higher daily nonesterified fatty acid concentrations and a greater peak insulin response than high-ranking cows (Huzzey et al., 2012), potentially making these cows more at risk for postpartum disorders. In pigs that were grouped into social categories, the high success pigs gained the most weight whereas the high success and no success groups had lower levels of salivary cortisol than pigs from the low success group (Mendle et al., 1992). Additionally, the high success group gave birth to heavier total weights of piglets than the no success group. To our knowledge no studies have determined prepartum social rank and then followed those cows up to 60 DIM to determine whether social dominance was associated with postpartum health, reproduction, and milk yield and composition.

The objective of this study was to examine displacement index in prepartum Jersey cows as calculated by Galindo and Broom (2000) used as a continuous value, grouped into social index categories according to Galindo and Broom (2000), or grouping cows into 3 categories as top, intermediate and bottom 10th percentile DI and their associations with postpartum health, reproduction, and milk composition.

## **MATERIAL AND METHODS**

### ***Study Design, Animals and Housing***

A total of 987 nulliparous, primiparous and parous Jersey cows were enrolled in the current study. Cows were removed for not calving within 6 wk of expected date or missing health data leaving 953 cows for analysis. The study was conducted at a large commercial dairy farm (6,400 lactating dairy cows) in south-central Minnesota, USA. Close-up prepartum cows were housed in a 12-row low profile cross-ventilated barn and fed a TMR (balanced to meet nutrient requirements; NRC, 2001) once daily from a feed alley by headlocks with frequent feed push-ups. All experimental pens had the same measurements of 31.7 m x 11.0 m and included 44 deep sand bedded freestalls (229L x 107W x 114H cm) with a head-to-head configuration and 48 0.61-m headlocks. Two water troughs were located in the pen and measured 366 cm x 56 cm. One water trough was located at the end of the bank of freestalls and a shared water trough was located between the treatment pen and an adjacent non-experimental pen.

Cows were enrolled in the study 4 wk prior to expected calving date. All cows had a body condition score between 2 and 4 (1-5; scale 1=emaciated, 5=obese) and locomotion score < 3 (1-5 scale; 1=normal, 5=severely lame). Cows evaluated were

from 2 experiments. In experiment 1, 112 prepartum primiparous and 113 multiparous Jersey cows for a total of 225 were enrolled. Cows were either exposed to an all-in-all-out grouping strategy (n = 88 cows) or a traditional pen management with once weekly entrance of new cows (n = 137). In experiment 2, 324 nulliparous, 192 primiparous and 212 multiparous Jersey cows were enrolled for a total of 728 cows. Nulliparous cows were housed separately from the mixed pen of primiparous and multiparous cows. There were 183 nulliparous and 231 primiparous and multiparous cows assigned to 100% stocking density and 141 nulliparous and 173 primiparous and multiparous cows assigned to 80% stocking density.

### ***Behavior Measurements***

All enrolled cows were identified with a unique alphanumeric symbol on their back using permanent hair dye either in black or blonde. Hair dye was applied from d -1 to 1 d after cows entered the pen.

***Social behavior.*** To observe social interactions while feeding, each pen was equipped with 3 video cameras (Weldex, Cypress, CA) mounted above the pen and connected to a digital video recording system (Channel Visions, Costa Mesa, CA). In Experiment 1 displacements from the feed bunk were measured continuously during 3 h following entrance into the pen at d 0 (13:00±1:00) and after fresh feed delivery (05:00±1:00) on d 1, 2, 3, and 7 each week of the 5-wk observation period. In Experiment 2 displacements were measured for 3 h following fresh feed delivery (07:00±1:00) on d 2, 5, and 7 of each week of the 5-wk observation period. Previous research indicated a majority of displacements occurred 2 h following the first feeding

(Collings et al., 2011). Observation days were chosen based on days that cows were not scheduled to be locked up by farm staff or research personnel. From continuous video observation, an interaction between 2 cows was considered a displacement from the feed bunk when physical contact initiated by 1 cow caused the receiving cow to stop feeding, back out and entirely remove her head from the headlock (Endres et al., 2005).

### ***Evaluating Social Dominance***

For each cow a displacement index (**DI**) was calculated by the methods proposed by Galindo and Broom (2000):

$$= \frac{\text{total times cow initiated displacement}}{\text{total times cow initiated displacement} + \text{total times cow received displacement}}$$

Displacement index values were between 0 and 1. These values were used in 3 different approaches to determine whether prepartum social classification is related to transition cow health and performance.

1. **Displacement Index (Method 1)** – DI values were used and examined in the model as continuous and quadratic. Cows were not categorized into social dominance groups.
2. **Social Dominance Categories (Method 2)** - Cows were grouped in dominance categories (Galindo and Broom, 2000); cows with a DI of less than 0.4 were considered low-ranking, 0.4-0.6 were considered middle-ranking, and cows greater than 0.6 were considered high-ranking cows.
3. **Top and Bottom 10<sup>th</sup> percentile (Method 3)** - The distribution of the DI were evaluated with PROC UNIVARIATE. The bottom and top 10<sup>th</sup> percentile were selected to examine cows on the extreme ends of DI. Cows were categorized into

3 groups low- ranking  $DI < 0.19$ , middle-ranking  $0.19-0.71$ , and high-ranking  $DI > 0.71$ .

### ***Body Condition and Locomotion Score***

At enrollment and on d  $1 \pm 1$ ,  $28 \pm 3$ , and  $56 \pm 3$  all cows were scored for body condition (BCS, 1 = emaciated and 5 = obese; 0.25 unit increments; Ferguson et al., 1994) and locomotion (1 = normal and 5 = severely lame; Sprecher et al., 1997). Change in BCS was calculated from BCS at enrollment subtracted from BCS at DIM 1. Cows were classified as lame when locomotion score was  $\geq 3$ .

### ***Blood Sampling and Analysis of Metabolites in Plasma***

Blood samples were collected from all cows on DIM  $-18 \pm 3$ ,  $-11 \pm 3$ ,  $-4 \pm 3$ ,  $3 \pm 3$ ,  $10 \pm 3$ ,  $17 \pm 3$  and  $24 \pm 3$  from the coccygeal vein or artery immediately after feeding while cows were restrained in self-locking headlocks. Samples were collected into evacuated tubes containing K2 EDTA (Becton Dickinson Vacutainer Systems, Franklin Lakes, NJ). Tubes were placed in ice until centrifugation for plasma separation ( $1,200 \times g$  for 15 min at  $4^\circ\text{C}$ ). Plasma was aliquoted into microcentrifuge tubes and stored at  $-32^\circ\text{C}$  until analysis.

Samples collected weekly from DIM -18 to 24 were analyzed for concentrations of NEFA using a colorimetric assay (Wako Chemicals USA, Richmond, VA; Ballou et al., 2009). Concentrations of BHBA were determined enzymatically (Ranbut, Randox Laboratories, Antrim, UK; Ballou et al., 2009) from samples collected weekly from DIM 3 to 24.

### ***Clinical Examination and Definitions of Diseases***

All cows were examined on DIM 1,  $4 \pm 1$ ,  $7 \pm 1$ ,  $10 \pm 1$ , and  $13 \pm 1$  for the diagnosis of retained fetal membrane and metritis. Retained fetal membrane (**RP**) was defined as retention of a fetal membrane past 24 h postpartum. Metritis was defined as cows with watery, pink or brown, and fetid uterine discharge. A cow was diagnosed with subclinical ketosis (**SCK**) when BHBA concentrations were  $\geq 1200 \mu\text{mol/L}$ , regardless of sample. All cows were observed once daily for displacement of abomasum (**DA**) and thrice daily for mastitis. Mastitis, DA, and cows removed (sold or dead) from the herd were followed up to 60 DIM.

### ***Production Parameters***

Cows were milked thrice daily. Monthly milk yield, milk fat and protein contents, and SCC were recorded for individual cows during DHIA test. Data regarding milk composition were collected up to 150 DIM. Energy-corrected milk was calculated for each cow using the formula (Orth, 1992).

$$ECM (kg) = [(kg \text{ of milk}) \times 0.327] + [(kg \text{ of fat}) \times 12.95] + [(kg \text{ of protein}) \times 7.2]$$

All cows received recombinant bST (500 mg of Posilac; Elanco Animal Health, Greenfield, IN) every 10 d starting at DIM  $57 \pm 3$ .

### ***Reproduction Parameters***

All cows had ovaries examined by ultrasound (5 MHz, Ibex Lite, E.I. Medical Imaging, Loveland, CO) on DIM  $39 \pm 3$  and  $53 \pm 3$ . Cows without a CL on DIM 39 and 53 were considered anovular.

Cows were all subjected to same reproductive program. Cows were presynchronized with 2 injections of PGF<sub>2a</sub> on DIM 39 ± 3 and 53 ± 3. Cows were inseminated if observed in estrus after DIM 50. Cows not observed were enrolled in a synchronization protocol (Ovsynch56) and artificially inseminated. Cows were examined for pregnancy 31 ± 3 after AI and pregnant cows were reexamined 66 ± 3 and 178 ± 3 after AI.

### ***Statistical Analysis***

There was no effect of experiment therefore data were combined for use in the current study. A displacement rate was calculated for each animal that was enrolled and observed in the study. The FREQUENCY procedure of SAS (SAS Institute Inc., Cary, NC) was used to determine the percentage of cows in each social category for both methods 2 and 3. Associations of social dominance category or DI and events of health, reproductive, and milk composition were analyzed with the LOGISTIC procedure of SAS. Fixed effects were social dominance category (high, middle, or low, Method 2; top 10th, middle, or bottom 10th percentile, Method 3) or DI. Additional covariates offered to the multivariable logistic regression model were parity (nulliparous, primiparous, and multiparous (lactation ≥ 2)), twin pregnancy, calf sex, stillborn, lame at DIM 0, change of BCS, health events including metritis, subclinical ketosis, RP, DA, and mastitis. The dominance index models were offered the covariate DI × DI to examine for a curvilinear association. A backwards procedure was used to remove the least significant variables until variables  $P < 0.1$  remained. Adjusted odds ratios and 95% confidence intervals were reported.

Metabolites (BHBA and NEFA) were analyzed with MIXED procedure of SAS with repeated measures. The repeated statement included time and subject with cow nested within treatment. The structure of covariance (autoregressive, compound symmetry or unstructured) were chosen according to the Bayesian Akaike information criteria. Cow was included in the random statement. Fixed effects were social dominance category (high, middle, or low; top 10th, middle, or bottom 10th percentile; or DI. Additional covariates offered to the multivariable logistic regression model were parity (nulliparous, primiparous, and multiparous (prepartum lactation  $\geq 2$ )), twin pregnancy, calf sex, stillborn, lame at DIM 0, change of BCS, health events including metritis, subclinical ketosis, RP, DA, and mastitis. The interaction of social dominance category  $\times$  parity  $\times$  sampling period was examined. The dominance index models were offered the covariate DI  $\times$  DI to examine for a curvilinear association. Least squares means were separated with the PDIFF statement.

## RESULTS

Percentages of cows in each social ranking category calculated by both method 2 and 3 are listed in Table 1. The distribution of the frequency of DI is shown in Figure 1.

### *Displacement index*

**Health.** There were no associations of DI and SCK, DA, endometritis, acute metritis, or cows that died or sold up to 60 DIM (Table 2). There were no associations with lame at DIM 1, DIM 35 or DIM 56 and DI.

There was a curvilinear association between DI and metritis (Figure 2; quadratic effect:  $P = 0.018$ ; linear effect  $P = 0.004$ ). Displacement index was associated with RP

(AOR = 1.02, 95% CI 1.00-1.03;  $P = 0.021$ ) and incidence of mastitis up to 60 DIM (0.99, 0.97-1.00;  $P = 0.045$ ). For every 1-unit increase in DI resulted in a 1% decrease in odds of a case of mastitis.

**Reproduction.** Displacement index was not associated with first AI pregnancy conception, first AI pregnancy loss, or days to first breeding.

**Metabolites.** Displacement index was not associated with BHBA concentrations. Additionally, there were no associations of DI and NEFA concentrations.

**Milk Yield and Composition.** For every 1-unit increase in DI resulted in  $0.02 \pm 0.01$  kg/d of milk yield ( $P = 0.035$ ). Similarly, for every 1-unit increase in DI resulted in  $0.02 \pm 0.01$  kg/d more of ECM ( $P = 0.032$ ). As DI increased by 1-unit there was an increase in linear SCC of  $0.005 \pm 0.002$  ( $P = 0.031$ ). There were no associations of DI and milk protein percent, milk protein yield, milk fat percent, or milk fat yield.

#### ***Dominance Category – Galindo and Broom***

Body condition score at the time of calving was greater for high ranking cows ( $2.97 \pm 0.02$ ) than low-ranking cows ( $2.90 \pm 0.01$ ;  $P = 0.001$ ) with no difference from middle-ranking cows ( $2.94 \pm 0.01$ ). Middle ranking cows had an average BCS  $0.07 \pm 0.02$  greater than low-ranking cows ( $P = 0.034$ ).

**Health.** There was no association of dominance category with incidence of metritis, subclinical ketosis, DA, endometritis, acute metritis, or mastitis (Table 3). Social dominance category was not associated with death or sold up to 60 DIM. Risk of lameness in the first 60 DIM did not differ between social categories. Retained fetal membrane was the only peripartum health event associated with dominance category ( $P =$

0.041). High-ranking cows were 2.0 times (1.08-3.70, 95% CI) more likely to have RP than low-ranking cows with no differences between low- and middle-ranking cows.

**Reproduction.** There was an interaction of dominance category and parity for first artificial insemination pregnancy rate ( $P = 0.045$ ). High-ranking nulliparous cows were 2.2 times more likely to become pregnant after first insemination than middle-ranking nulliparous cows (95% CI; 1.13 - 4.36). No other interactions were significant. There were no associations of social rank on pregnancy loss from first artificial insemination.

**Metabolites.** Social dominance categories were not associated with BHBA concentrations on DIM 0, 7, and 14. There was an interaction of social dominance category  $\times$  parity  $\times$  day relative to calving (Figure 3;  $P < 0.001$ ) and NEFA concentrations. Within multiparous cows at DIM 0, low-ranking cows had greater NEFA concentrations than high-ranking cows (LSMean  $\pm$  SE,  $411.7 \pm 18.8$  vs.  $365.9 \pm 19.0$ ;  $P = 0.042$ ). No other associations were found among rank and parity within multiparous cows. There were no associations of social dominance category for nulliparous cows. On DIM -14 and 0 high-ranking primiparous cows had a tendency for greater NEFA concentrations than middle-ranking primiparous cows (DIM -14,  $184.0 \pm 25.2$  vs.  $131.5 \pm 15.7$ ;  $P = 0.059$ ; DIM 0,  $375.8 \pm 22.4$  vs.  $325.1 \pm 18.5$ ;  $P = 0.059$ , respectively). Additionally, on DIM 0 high-ranking primiparous cows had greater NEFA concentrations than low-ranking primiparous cows ( $319.6 \pm 16.7$ ;  $P = 0.029$ ). No other associations were observed for primiparous cows.

**Milk Yield and Composition.** There was an interaction of social dominance category  $\times$  parity  $\times$  test interaction (Table 4; Figure 4;  $P < 0.001$ ). High-ranking

multiparous cows produced (LSM  $\pm$  SE)  $4.2 \pm 1.4$  ( $P = 0.003$ ) and  $4.7 \pm 1.3$  ( $P < 0.001$ ) more kg of ECM than middle- and low-ranking cows, respectively, at first DHI test. The high-ranking multiparous cows produced more ECM than low-ranking cows for tests 2 ( $P = 0.042$ ) and 3 ( $P = 0.004$ ) with a tendency on test 4 ( $P = 0.056$ ). There were no differences between high- and middle-ranking multiparous cows for tests 2-5. There were no differences in ECM among the social categories for primiparous cows. A difference of ECM was only observed during test 2 for nulliparous cows. High-ranking nulliparous cows produced  $3.2 \pm 1.6$  ( $P = 0.041$ ) kg more milk than low-ranking nulliparous cows with a tendency to produce more than middle-ranking cows ( $P = 0.057$ ).

There was an association between milk protein percent and social dominance category ( $P = 0.043$ ). Milk protein percentages were  $3.57 \pm 0.04$ ,  $3.61 \pm 0.03$  and  $3.56 \pm 0.03$  for high-, middle- and low-ranking cows, respectively. Middle-ranking cows had  $0.05 \pm 0.02$  greater milk protein percent than low-ranking cows ( $P = 0.014$ ). No other differences were observed for milk protein percent. There were no differences in milk fat percentages (high,  $4.54 \pm 0.28$ ; middle,  $4.59 \pm 0.28$ ; low  $4.56 \pm 0.28$ ) among the social dominance categories.

There was an interaction of social dominance category  $\times$  parity  $\times$  test for linear SCC (Figure 5;  $P = 0.018$ ). There were no associations of linear SCC and social dominance category for multiparous cows. High-ranking primiparous cows had greater linear SCC than low-ranking cows and a tendency compared to middle-ranking cows for tests 3, 4, and 5. High-ranking nulliparous cows had greater linear SCC than low-ranking cows at test 5 ( $P = 0.037$ ).

### ***Social Rank – Top and Bottom 10th Percentile***

Body condition score of bottom-ranking cows was  $0.13 \pm 0.04$  and  $0.09 \pm 0.02$  lower than top and middle-ranking cows ( $P = 0.001$  and  $P = 0.002$ , respectively). Body condition score did not differ between top and middle-ranking cows.

***Health.*** The bottom-ranking cows were 4.7 (95% CI, 1.6-13.4) times more likely to have a metritis event than the top 10th percentile ranking cows (Table 5). No differences were observed between top- and middle-ranking cows for metritis.

There were no associations of rank for RP, DA, mastitis, subclinical ketosis, endometritis, or acute metritis. There was no association of social rank and cows that were sold or died. Odds of lameness at DIM 1, DIM 35, and DIM 56 did not differ by social rank.

***Reproduction.*** There were no associations of rank on first AI conception (Table 5). There was an association of rank and pregnancy loss after first AI. Middle ranking cows were 1.6 times (1.02-2.64) more likely to have pregnancy loss after first AI than top-ranking cows. There were no differences between top and bottom-ranking cows on first AI pregnancy loss. There was a tendency for middle-ranking cows to have fewer days to first breeding than top-ranking cows (LSMeans  $\pm$  SE,  $66.5 \pm 1.8$  vs.  $66.6 \pm 2.1$ ;  $P = 0.055$  respectively). Bottom-ranking cows did not differ from top- or middle-ranking cows for days to first breeding ( $66.6 \pm 2.1$ ).

***Metabolites.*** There were no associations of rank and BHBA concentrations. Non-esterified fatty acid concentrations differed between rankings on DIM 0. Top-ranking cows had higher NEFA concentrations than bottom-ranking cows ( $P = 0.008$ ) and a

tendency of higher concentrations than the middle-ranking cows ( $P = 0.059$ ).

Additionally, there was a tendency for middle-ranking cows to have higher concentrations than the bottom-ranking cows ( $P = 0.096$ ). No other associations were found during the other sampling periods.

***Milk Yield and Composition.*** There was an interaction of social ranking  $\times$  parity  $\times$  test on daily milk yield (Table 6,  $P < 0.001$ ), ECM yield ( $P < 0.001$ ), protein yield ( $P < 0.001$ ), protein percent ( $P < 0.001$ ) and linear SCC ( $P = 0.048$ ). There were no associations between daily fat yield or fat percent and social ranking. Differences were observed among social rank up to  $80 \pm 8$  DIM for multiparous and primiparous cows with high ranking cows typically producing more milk than middle and low ranking cows.

## DISCUSSION

All 3 of the proposed methods were limited in their ability to determine association with a health category and consistency among the methods. Displacement index (Method 1) was only able to find a significant association with metritis, RP and mastitis event up to 60 DIM. Galindo and Broom categories (Method 2) were only associated with RP. Examining the top and bottom 10th percentile DI (Method 3) resulted in a significant association with metritis, but not with any other health event.

Social dominance has been established in dairy cattle by using physical or non-physical threats. Dominance rank tends to be stable for high and low ranking animals, but tends to vary for middle-ranking cows (Arave and Albright, 1976). Dominance does not appear to be highly correlated across all 3 resources (feeder, stalls, and brush) available to indoor housed cattle (Val-Laillet et al., 2008b). Cows that were high-ranking

for the feeder were not necessarily high ranking for access to the stalls. High-ranking cows spent more time at the feeder for the 2 h following fresh feed delivery than low-ranking cows, potentially altering the feed composition available during non-peak times (Val-Laillet et al., 2008b). There was a significant correlation of BCS and social rank with socially dominant cows having a greater BCS than lower ranking cows (Hohenbrink and Meinecke-Tillmann, 2012). In the current study, results were similar with high-ranking animals having a greater BCS than lower ranking cows.

Previous research has indicated that regrouping dairy cows leads to increased agonistic behaviors. Schirmann et al. (2011) observed prepartum cows that were moved into a new pen displaced cows at the feed bin more frequently than cows that stayed in the home pen. Physical interactions appeared to decrease 3 d after regrouping (von Keyserlingk et al., 2008). Due to the nature of close-up pens cows were moved out of the pen to the maternity pen for calving and pens were restocked 1-2 times/wk. Cows that were housed in a stable grouping management had fewer displacements from the feed bunk than cows housed in a dynamic pen with weekly restocking (Lobeck-Luchterhand et al., 2014).

Of the health events associated with rank these events were primarily infectious with the exception of RP which is often considered metabolic (Goff and Horst, 1997). Some previous work examining social behavior and health observed metritic cows up to 1 wk before calving displaced other cows less often and were displaced more often than healthy cows (Patbandha et al., 2012). Similarly, Huzzey et al. (2007) reported cows that were diagnosed with metritis were less aggressive during the prepartum period than

healthy cows. Metritic cows in the current study were involved in fewer displacements as the reactor (14.6 vs. 13.1;  $P = 0.03$ ), but did not differ from non-metritic cows in the number of displacements they initiated. During the week before calving cows with subclinical ketosis initiated fewer displacements at the feed bunk compared with animals that remained healthy after calving (Goldhawk et al., 2009). It is unknown whether the cow was actually low-ranking or if sickness behavior dictates whether the cow wants to be involved or avoid aggressive behaviors at the feed bunk. Animals sick from acute bacterial, protozoan or viral infections display a set of non-specific symptoms including changes in body temperature, uneasiness, loss of interest for daily activities and reduction of feed intake (Aubert, 1999). Research has indicated a cross-talk between neuroendocrine and immune systems suggesting the evolution of behavioral strategies in addition to immune strategies in order to fight infection (Aubert, 1999). Cows that were sick around the time of calving and were housed indoor with partially covered pens ate less, tended to spend more time lying down, and spent more time in the corner of the pen compared with healthy cows (Proudfoot et al., 2014). Healthy feedlot steers spent 30% more time at the feed bunk than morbid steers (Sowell et al., 2009). Sickness behavior may make these animals socially subordinate rather than subordinate animals becoming sick.

In the current study, lameness was not associated with DI or social rank. Galindo and Broom (2000) reported low ranking cows were more likely to become lame during the housing period than middle and high ranking cows. Low ranking cows spent less time lying, more idle time standing and spent more time standing with 2 feet in the stall

than middle and high ranking cows (Galindo and Broom, 2000). Similarly, a significant correlation existed between DI and lameness (Hohenbrink and Meineck-Tillmann, 2012) with cows with a higher DI showing a lower degree of lameness. In the current study, numerically more lame cows were low ranking (9) than either high (2) or middle (2) ranking cows. Lame cows were less likely to initiate an aggressive interaction, but there were no differences in receiving an aggressive interaction (Galindo and Broom, 2002). The authors speculated that lame cows may lose their social position, rather than social rank being a risk factor for lameness.

Limited research has examined social and health traits, typically due to the laborious observation of animals and large number of animals required to examine health traits. In a small study with 20 cows, Patbandha et al. (2012) observed metritic cows were less likely to displace other cows approximately 3.5 fewer times/d and were displaced more frequently (3.5 times/d) than healthy cows. The metritic cows had lower DI values compared to healthy cows. When cows were stocked at high densities the low ranking cows had greater daily NEFA and 11,17-dioxoandrostane concentrations relative to cows in the high-ranking group (Huzzey et al., 2012).

Both dominant and submissive cows moved to a new pen had reductions of milk yield during the first week after calving (Arave and Albright, 1976). Daily milk yield of mid lactation middle-ranking primiparous cows was greater than dominant and subordinate cows (Hasegawa et al., 1997). In the current study, milk yield and ECM were associated with social rank for multiparous cows. High ranking cows tended to produce more milk than low-ranking cows up to approximately 80 DIM. In competitive

situations the socially lower ranked cows are most likely being chased and displaced by higher rank cows.

## **CONCLUSIONS**

In the current study social rank was limited in its ability to determine a cow's odds of having a transition health disorder and was not consistent among the 3 methods utilizing displacement index values. As DI values increased there were increased odds of RP and decreased odds of mastitis incidence. Displacement index had a curvilinear relationship with mastitis with high and low DI. Based on the Galindo and Broom (2000) social ranking categories high ranking cows were more likely to have an RP than low ranking cows. When examining the top and bottom 10th percentile of DI, the bottom ranking cows were more likely to have a metritis event than low ranking cows. Although ranking cows on social dominance at the feed bunk appears to have some merit for predicting odds of a health disorder after parturition, the lack of consistency among the 3 methods indicated that social rank did not seem beneficial for predicting cows at risk for transition health disorders.

**Table 1.** Frequencies of social rank categories based on 2 methods calculating social rank based on Galindo and Broom (2000)

Social Categories	Galindo and Broom Method		Top and Bottom 10 <sup>th</sup> Percentile	
	%	n	%	n
High-rank	22.3	212	10.1	96
Middle-rank	33.5	319	80.0	762
Low-rank	44.3	422	10.0	95

Observations as actor and reactor were totaled then displacement index was calculated. Displacement index = total displacements initiated / total displacements initiated and received. Galindo and Broom method – High-rank  $\geq 0.6$ , middle-rank 0.4 – 0.6, low-rank  $\leq 0.4$ . Top and bottom 10<sup>th</sup> percentile - cows were categorized into 3 groups high-rank DI  $> 0.71$ , middle-rank 0.19-0.71, low- rank DI  $< 0.19$ .

**Table 2.** Adjusted odds ratios (AOR) and 95% confidence intervals (95% CI) of displacement index calculated during the close-up prepartum period and transition health disorders of Jersey cows.

Disorder	AOR	95% CI	P-value
Subclinical ketosis	0.99	0.97-1.02	0.70
Metritis <sup>1</sup>	---	---	---
Endometritis <sup>2</sup>	0.99	0.98-1.01	0.27
Acute Metritis	1.01	0.99-1.03	0.42
Retained fetal membrane	1.01	1.00-1.03	0.021
Displaced abomasum	1.01	0.98-1.04	0.68
Mastitis	0.99	0.97-1.00	0.045
Died	0.99	0.97-1.02	0.65
Sold	1.01	0.99-1.03	0.31
Removed	1.00	0.99-1.02	0.69
Lame DIM 1	0.99	0.97-1.02	0.54
Lame DIM 35	1.00	0.99-1.02	0.94
Lame DIM 56	1.00	0.99-1.02	0.93
1 <sup>st</sup> AI Pregnancy	1.00	0.99-1.00	0.27
Pregnancy Loss <sup>3</sup>	1.00	0.99-1.00	0.25

<sup>1</sup> – There was a curvilinear association with metritis ( $P = 0.018$ ), linear ( $P = 0.004$ ).

<sup>2</sup> – Endometritis was collected on 682 cows.

<sup>3</sup> – Pregnancy loss occurring after first breeding.

**Table 3.** Incidence and adjusted odds ratios (AOR) with 95% confidence intervals (95% CI) of transition health events up to 60 days in milk in Jersey cows by social rank (high, middle, low)<sup>1</sup>.

Health Event	Frequency		High		Middle		Low	
	n	(%)	AOR	95% CI	AOR	95% CI	Ref.	P-value
DA	8	0.9	0.78	0.12-4.96	0.62	0.12-3.29	--	0.85
RP	69	7.3	1.99	1.08-3.70	0.95	0.50-1.79	--	0.04
Ketosis	26	2.7	0.49	0.16-1.56	0.71	0.29-1.73	--	0.44
Mastitis	53	5.7	0.42	0.14-1.05	1.21	0.63-2.29	--	0.13
Metritis	174	18.5	0.92	0.56-1.50	0.77	0.50-1.20	--	0.51
Acute metritis	86	9.1	1.18	0.47-2.93	1.33	0.60-2.95	--	0.77
Endometritis <sup>2</sup>	48	7.0	0.64	0.27-1.43	0.65	0.31-1.32	--	0.39
Removed	56	5.9	1.27	0.49-3.10	1.4	0.64-3.10	--	0.69
Died	15	1.6	0.54	0.08-2.28	0.36	0.05-1.49	--	0.39
Sold	41	4.3	2.13	0.68-6.65	2.40	0.87-6.64	--	0.22
Lame DIM 1	13	1.4	0.38	0.08-1.82	0.38	0.08-1.84	--	0.29
Lame DIM 35	37	4.2	0.92	0.39-2.14	0.89	0.40-1.99	--	0.95
Lame DIM 56	36	4.1	1.16	0.51-2.66	1.01	0.44-2.31	--	0.93
First AI conception	402	48.4	0.83	0.58-1.19	0.80	0.58-1.10	--	0.34
Pregnancy Loss <sup>3</sup>	27	2.9	0.76	0.53-1.08	0.81	0.59-1.10	--	0.21

<sup>1</sup> – Categories by Galindo and Broom, 2000. Displacement index = total displacements initiated / total displacements initiated and received. High-rank  $\geq 0.6$ , middle-rank 0.4 – 0.6, low-rank  $\leq 0.4$ .

<sup>2</sup> – Endometritis was collected on 682 cows

<sup>3</sup> – Pregnancy loss occurring after first breeding.

**Table 4.** Least square means (LSM) and standard error (SE) of energy corrected milk (kg/d) of Jersey cows up to 150 DIM by social rank<sup>1</sup>

	DIM (Mean ± SD)	Test No.	Social Rank		
			High (LSM ± SE)	Middle (LSM ± SE)	Low (LSM ± SE)
Multiparous	18 ± 8	1	37.9 ± 1.7 <sup>a</sup>	33.7 ± 1.8 <sup>b</sup>	33.2 ± 1.6 <sup>b</sup>
	48 ± 8	2	35.3 ± 1.5 <sup>a</sup>	33.8 ± 1.6 <sup>a,b</sup>	32.6 ± 1.4 <sup>b</sup>
	79 ± 7	3	34.9 ± 1.5 <sup>a</sup>	32.6 ± 1.6 <sup>a,b</sup>	30.9 ± 1.4 <sup>b</sup>
	110 ± 8	4	30.3 ± 1.8	28.6 ± 1.9	27.5 ± 1.6
	139 ± 8	5	24.5 ± 2.1	23.5 ± 2.2	24.0 ± 2.1
Primiparous	19 ± 8	1	32.2 ± 1.9	32.4 ± 1.7	33.4 ± 1.6
	48 ± 8	2	30.0 ± 1.7	32.4 ± 1.5	31.3 ± 1.4
	80 ± 7	3	30.3 ± 1.9	31.9 ± 1.5	31.5 ± 1.4
	112 ± 8	4	28.0 ± 2.1	28.9 ± 1.8	30.3 ± 1.6
	140 ± 8	5	23.4 ± 2.5	22.9 ± 2.2	24.5 ± 2.0
Nulliparous	18 ± 8	1	22.5 ± 1.9	22.2 ± 1.7	21.1 ± 1.7
	48 ± 9	2	25.9 ± 1.7 <sup>a</sup>	23.0 ± 1.4 <sup>a,b</sup>	22.6 ± 1.5 <sup>b</sup>
	79 ± 9	3	21.8 ± 1.9	21.9 ± 1.5	22.1 ± 1.5
	109 ± 9	4	21.0 ± 2.2	21.4 ± 1.8	19.2 ± 1.8
	136 ± 9	5	17.2 ± 2.6	19.9 ± 2.3	19.9 ± 2.3

<sup>a,b</sup> – Different superscripts LSMeans differ ( $P < 0.05$ ).

<sup>1</sup> – Categories by Galindo and Broom, 2000. Displacement index = total displacements initiated / total displacements initiated and received. High-rank  $\geq 0.6$ , middle-rank  $0.4 - 0.6$ , low-rank  $\leq 0.4$ .

**Table 5.** Adjusted odds ratios (AOR) and 95% confidence intervals (95% CI) of top and bottom 10<sup>th</sup> percentile social rank in Jersey cows.

Health Event	Frequency		Dominance Category					
	n	(%)	Top 10%	Middle		Bottom 10%		P-value
			Ref	AOR	95% CI	AOR	95% CI	
Subclinical ketosis	26	2.7	--	0.8	0.2-2.7	1.5	0.3-7.3	0.45
Metritis	164	17.4	--	1.7	0.7-4.3	4.7	1.6-13.4	0.003
RP	69	7.3	--	0.4	0.1-1.2	0.5	0.2-1.1	0.15
DA	8	0.9	--	1.2	0.1-7.9	0.4	0.0-4.7	0.47
Mastitis	53	5.7	--	1.4	0.5-4.1	0.9)	0.2-3.7	0.50
Endometritis <sup>2</sup>	48	7.0	--	1.1	0.3-3.9	1.8)	0.4-8.0	0.55
Acute metritis	86	9.1	--	0.7	0.2-2.7	0.7)	0.1-3.3	0.85
Died	15	1.6	--	1.3	0.2-10.7	0	0	0.96
Sold	41	4.3	--	0.7	0.2-2.6	1.6	0.4-7.2	0.36
Removed	56	5.9	--	1.0	0.2-5.4	1.4	0.2-9.4	0.67
Lame DIM 1	13	1.4	--	2.3	0.3-18.6	1.0	0.1-16.6	0.56
Lame DIM 35	37	4.2	--	1.0	0.4-2.7	0.5	0.1-2.2	0.54
Lame DIM 56	36	4.1	--	0.8	0.2-2.6	0.8	0.2-2.6	0.33
1 <sup>st</sup> AI Pregnancy	402	48.4	--	1.6	0.9-2.5	1.8	0.9-3.4	0.13
Pregnancy Loss <sup>3</sup>	27	2.9	--	1.6	1.0-2.6	1.7	0.9-3.1	0.125

<sup>1</sup> – Displacement index = total displacements initiated / total displacements initiated and received. Top and bottom 10<sup>th</sup> percentile - cows were categorized into 3 groups high-rank DI > 0.71, middle-rank 0.19-0.71, low- rank DI < 0.19.

<sup>2</sup> –Endometritis was collected on 682 cows

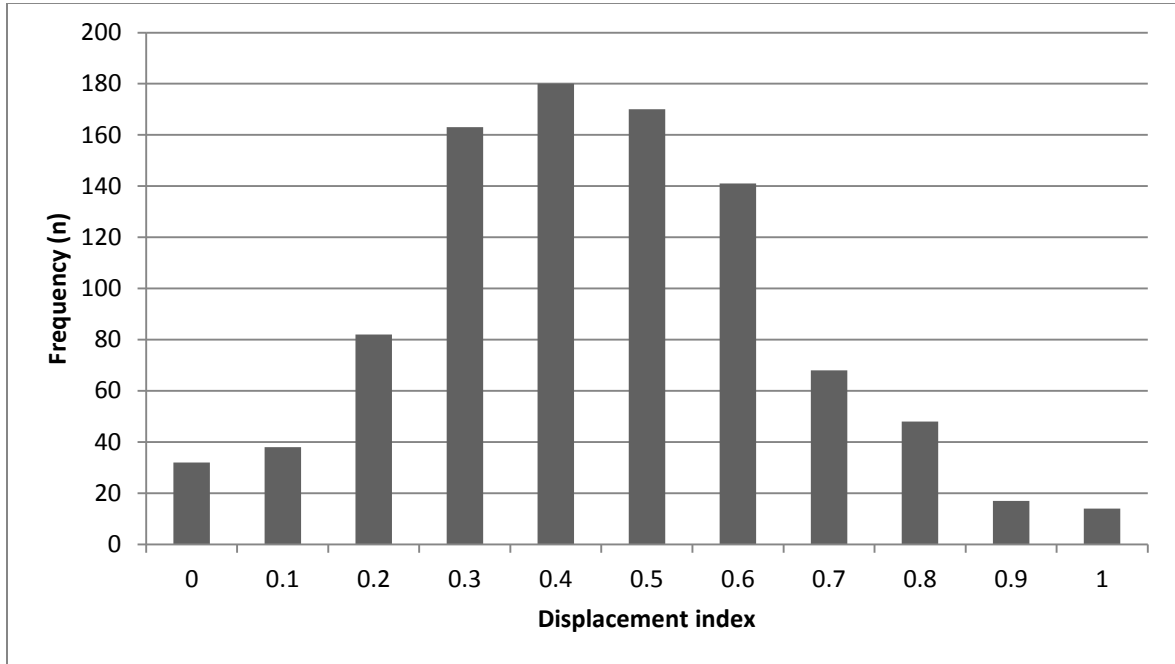
<sup>3</sup> – Pregnancy loss occurring after first AI.

**Table 6.** Least square mean (LSM) and standard error (SE) milk yield of top and bottom 10<sup>th</sup> percentile social rank of Jersey cows.

	DIM (Mean ± SD)	Test	Ranking Category <sup>1</sup>		
			Top 10% (kg) (LSM ± SE)	Middle (kg) (LSM ± SE)	Bottom 10% (kg) (LSM ± SE)
<b>Multiparous</b>					
	18 ± 8	1	27.4 ± 2.3 <sup>a</sup>	23.6 ± 2.0 <sup>b</sup>	25.2 ± 2.3 <sup>a,b</sup>
	48 ± 8	2	26.4 ± 2.2 <sup>a</sup>	23.0 ± 1.8 <sup>b</sup>	23.1 ± 2.2 <sup>b</sup>
	79 ± 7	3	24.8 ± 2.1 <sup>a</sup>	21.2 ± 1.8 <sup>b</sup>	23.1 ± 2.2 <sup>a,b</sup>
	110 ± 8	4	19.0 ± 2.3	18.2 ± 2.0	18.0 ± 2.4
	139 ± 8	5	12.2 ± 2.6	13.9 ± 2.2	16.2 ± 2.6
<b>Primiparous</b>					
	19 ± 8	1	24.6 ± 2.5 <sup>a</sup>	22.8 ± 2.0 <sup>a,b</sup>	20.8 ± 2.4 <sup>b</sup>
	48 ± 8	2	22.8 ± 2.4	22.1 ± 1.9	19.5 ± 2.2
	80 ± 7	3	23.7 ± 2.5 <sup>a</sup>	20.6 ± 1.8 <sup>b</sup>	19.0 ± 2.2 <sup>b</sup>
	112 ± 8	4	18.0 ± 2.7	18.0 ± 2.0	18.3 ± 2.5
	140 ± 8	5	16.0 ± 2.8	13.4 ± 2.2	13.1 ± 2.7
<b>Nulliparous</b>					
	18 ± 8	1	14.5 ± 2.9	13.9 ± 2.0	12.7 ± 2.7
	48 ± 9	2	18.1 ± 2.6	15.7 ± 1.9	14.0 ± 2.6
	79 ± 9	3	15.6 ± 2.8	14.1 ± 1.9	13.5 ± 2.9
	109 ± 9	4	13.1 ± 3.4	12.3 ± 2.0	10.3 ± 3.1
	136 ± 9	5	9.5 ± 3.1	10.2 ± 2.3	9.5 ± 3.1

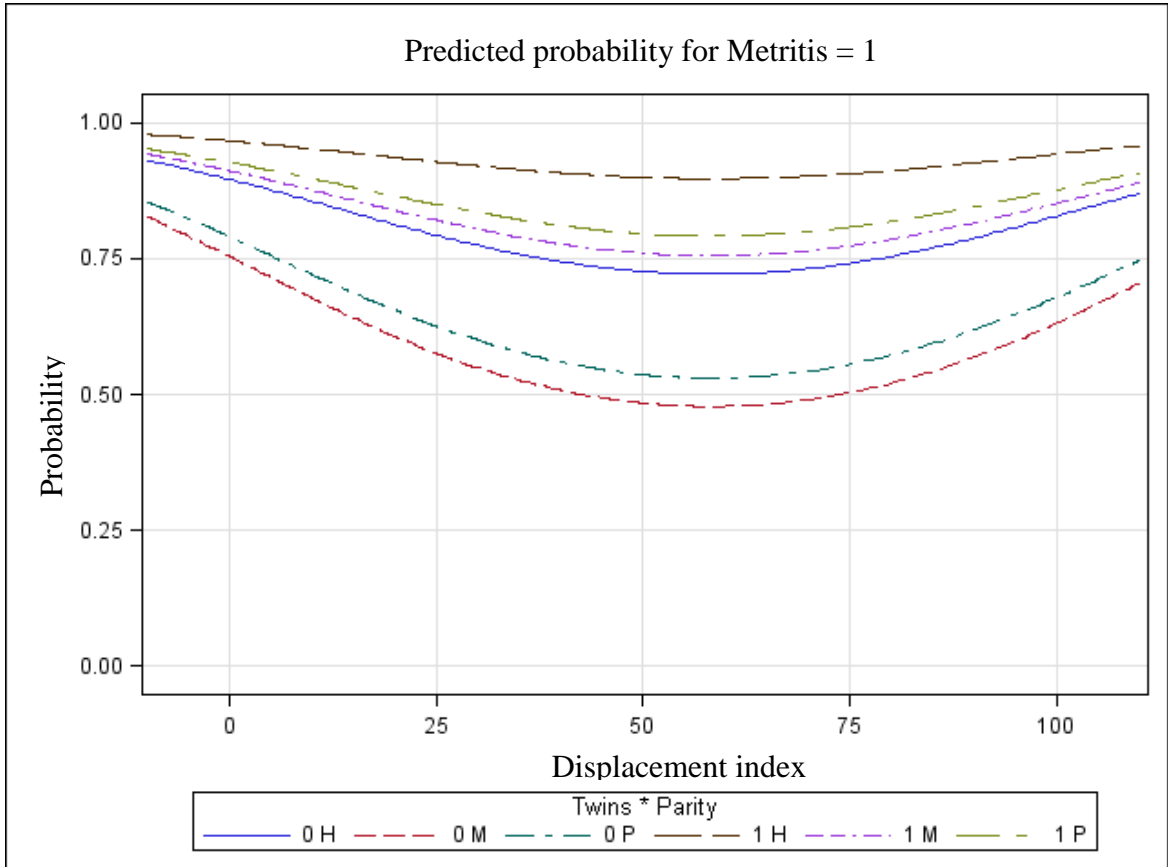
<sup>1</sup> – Displacement index = total displacements initiated / total displacements initiated and received. Top and bottom 10<sup>th</sup> percentile - cows were categorized into 3 groups high-rank DI > 0.71, middle-rank 0.19-0.71, low- rank DI < 0.19.

**Figure 1.** Frequency of displacement index rounded to the nearest tenth of 953 prepartum Jersey cows.



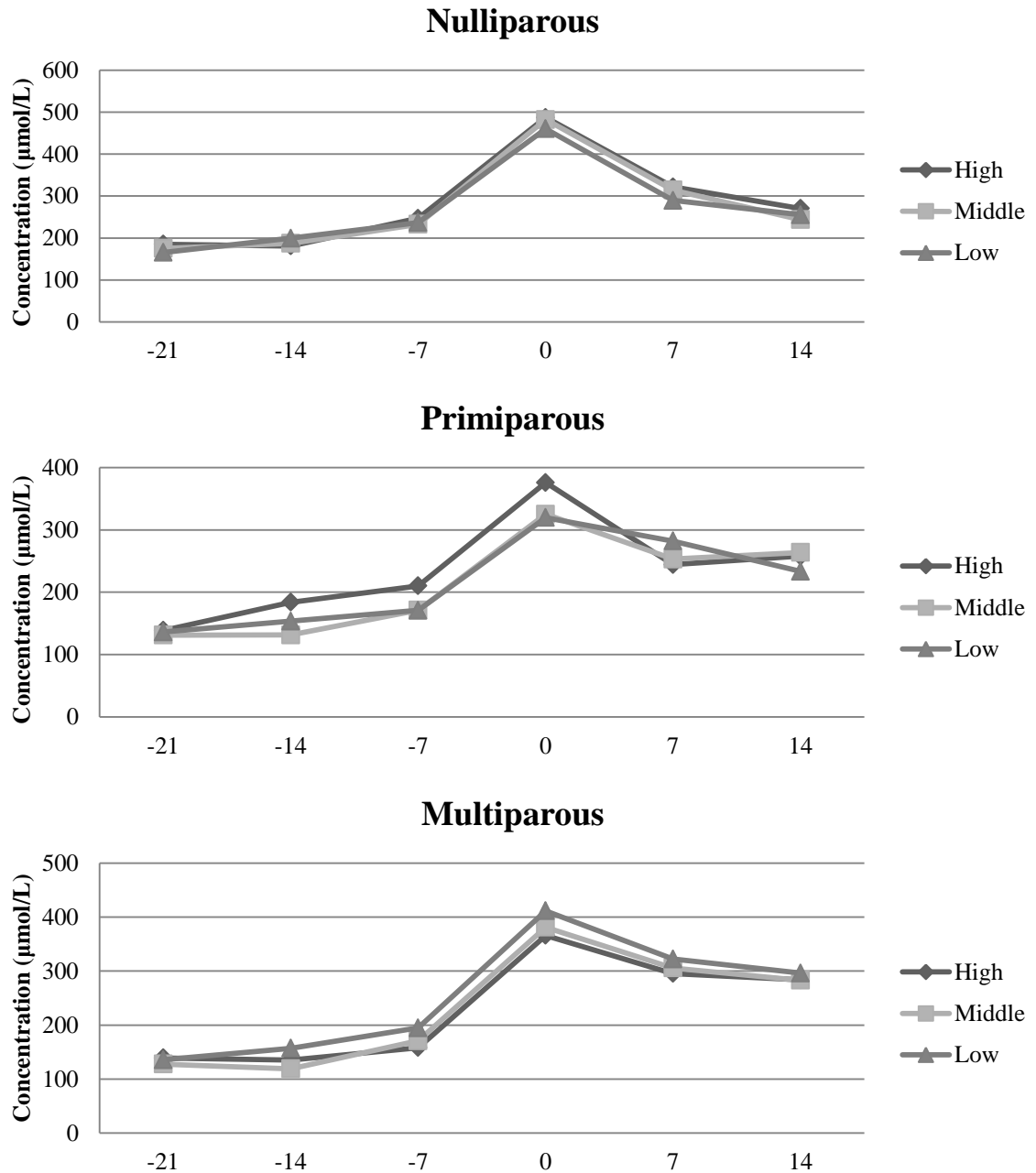
Displacement index = total displacements initiated / total displacements initiated and received

**Figure 2.** Adjusted odds of curvilinear association of displacement index and metritis for nulliparous, primiparous, and multiparous Jersey cows.



Displacement index = total displacements initiated / total displacements initiated and received. Curvilinear association between DI and metritis (quadratic effect:  $P = 0.018$ ; linear effect  $P = 0.004$ ).

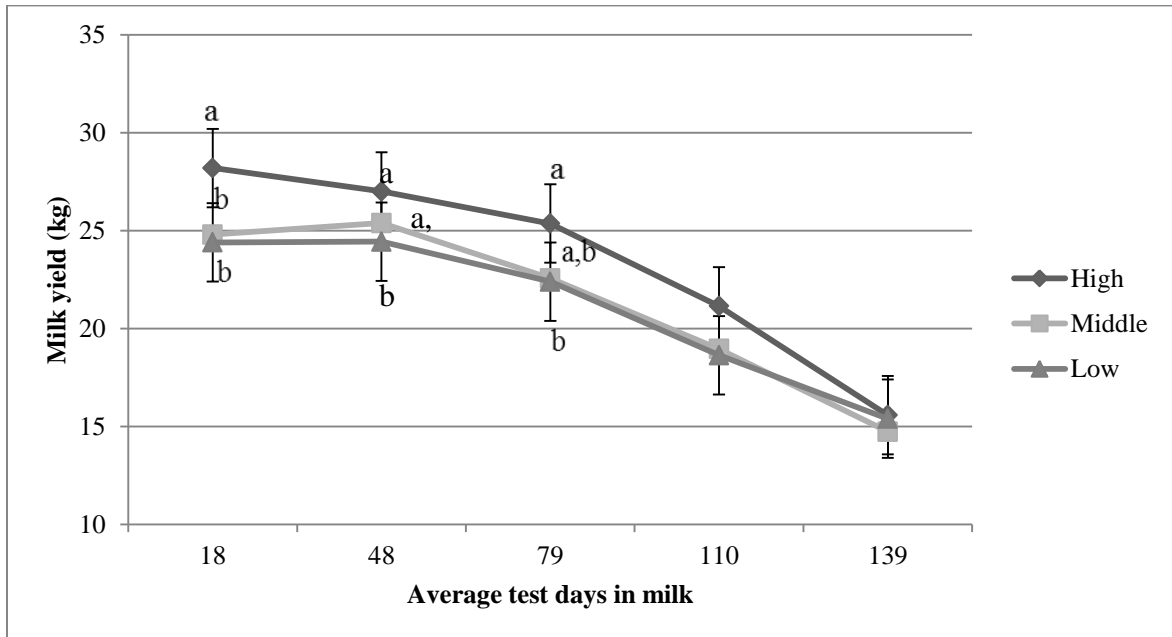
**Figure 3.** Non-esterified fatty acid (NEFA) concentrations of Jersey cows by parity and social rank



Primiparous cows – High ranking cows had greater NEFA concentrations than middle ranking cows on DIM -14 ( $P < 0.05$ ). On DIM 0, high ranking cows had greater concentrations than middle and low ranking cows ( $P < 0.05$ ).

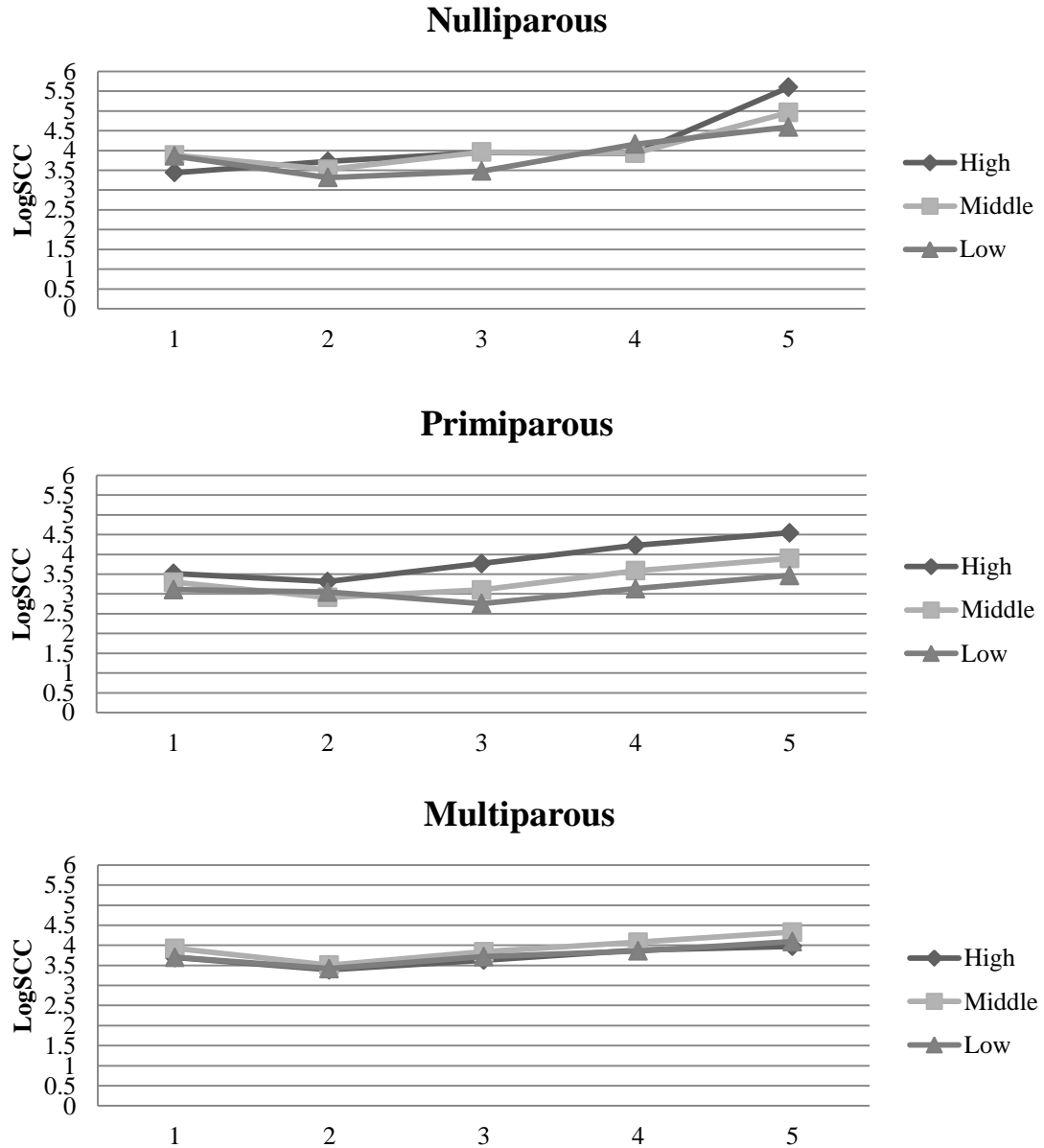
Multiparous cows – On DIM 0 high ranking cows had greater NEFA concentrations than low ranking cows ( $P < 0.05$ ).

**Figure 4.** Milk yield (kg/d) of multiparous Jersey cows by social rank



<sup>a,b</sup> – Different superscripts least square means differ ( $P < 0.05$ ). Categories by Galindo and Broom, 2000. Displacement index = total displacements initiated / total displacements initiated and received. High-rank  $\geq 0.6$ , middle-rank 0.4 – 0.6, low-rank  $< 0.4$

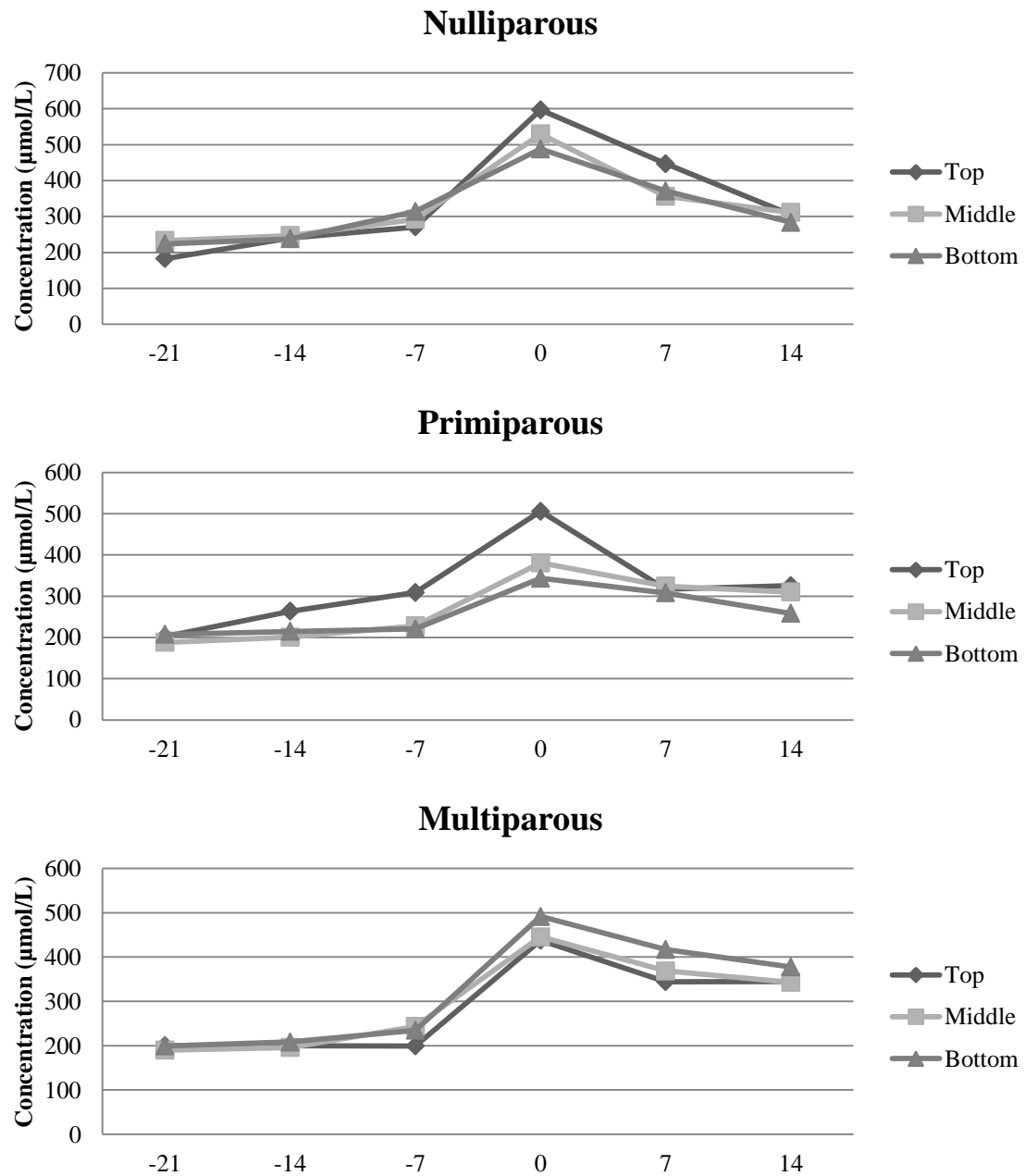
**Figure 5.** Log count somatic cell (LogSCC) of Jersey cows by social rank and parity



Primiparous cows – Test 3, High ranking cows had greater LogSCC than middle and low ranking cows ( $P < 0.05$ ). Test 4 and 5, High ranking cows had greater LogSCC than low ranking cows ( $P < 0.05$ ) and did not differ from middle ranking cows.

Nulliparous cows – Test 5, high ranking cows had greater LogSCC than low ranking cows ( $P < 0.05$ ) and did not differ from middle ranking. Displacement index = total displacements initiated / total displacements initiated and received. High-rank  $\geq 0.6$ , middle-rank  $0.4 - 0.6$ , low-rank  $\leq 0.4$ .

**Figure 6.** Non-esterified fatty acid concentration (NEFA) of Jersey cows by top and bottom 10<sup>th</sup> percentile of social rank



Nulliparous cows DIM 7, top 10<sup>th</sup> percentile cows had greater NEFA concentrations than bottom 10<sup>th</sup> percentile ( $P < 0.05$ ). Primiparous cows DIM -7, top 10<sup>th</sup> percentile had greater NEFA concentrations than middle and bottom 10<sup>th</sup> percentile ( $P < 0.05$ ). Multiparous cows DIM 0, bottom 10<sup>th</sup> percentile cows had greater NEFA concentrations than top 10<sup>th</sup> percentile ( $P < 0.05$ ) and tended to have greater concentrations than middle ranked cows ( $P < 0.10$ ).

## **EXPERIMENT 5: PREPARTUM LYING BEHAVIOR AND POSTPARTUM HEALTH DISORDERS IN JERSEY COWS**

### **SUMMARY**

The objective of the current study was to determine whether lying behavior (duration, number of bouts, and lying bout duration) was associated with postpartum health events up to 60 DIM. A total of 278 cows were used in the analysis. Parity (nulliparous, primiparous, and multiparous) was classified at the time of enrollment. Cows were enrolled 4 wk before expected calving date, had a body condition score between 2 and 4 (1-5 scale) and were not lame (<3 on a 1-5 scale). Nulliparous cows were housed separately from primiparous and multiparous cows. Daily lying time, number of lying bouts and bout duration were measured with data loggers. Data loggers were attached to the cow 1 d after enrollment, and stayed on the cow for 12 d, then loggers were removed for 7 d and reattached for another 12 d or until the cow calved. Primiparous cows spent more time lying down ( $13.6 \pm 0.2$  h/d) than nulliparous ( $12.7 \pm 0.1$ ;  $P < 0.001$ ) and multiparous cows ( $13.0 \pm 0.2$ ). As parity increased the number of lying bouts/d decreased. Lying bouts were  $11.9 \pm 0.2$  for nulliparous,  $10.3 \pm 0.2$  primiparous and  $9.4 \pm 0.2$  bouts/d for multiparous cows. Nulliparous lying bout duration was  $106.0 \pm 2.6$  min/bout, primiparous ( $140.0 \pm 3.6$  min/bout) and multiparous ( $158.5 \pm 3.9$  min/bout) and it was different among parities. Lame cows spent  $2.7 \pm 1.0$  more h/d lying than non-lame cows and had a bout duration  $86 \pm 31$  min/bout longer than non-lame cows. During wk -1 subclinical ketosis nulliparous cows had more lying bouts/d

( $21.3 \pm 2.4$ ) than healthy nulliparous cows ( $16.1 \pm 0.5$ ). During wk -2 and wk -1 subclinical ketosis nulliparous cows had lying bout duration of  $41 \pm 20$  and  $35 \pm 15$  min/bout shorter than healthy nulliparous cows, respectively. Metritic primiparous cows spent  $1.0 \pm 0.5$  and  $1.2 \pm 0.5$  fewer h/d lying than healthy primiparous cows during wk -2 and wk -1, respectively. Similarly, during wk -2 and wk -1 acute metritic primiparous cows spent  $1.4 \pm 0.6$  and  $1.7 \pm 0.7$  fewer h/d lying than healthy primiparous cows, respectively. In conclusion, lying behavior during the prepartum period was associated with postpartum health status of Jersey cows and therefore could potentially be used as a predictor of cows at risk for transition disorders.

## **INTRODUCTION**

Due to high incidence of health disorders that occur during the transition period there is increasing interest to find indicators to predict cows that will be at risk for health events. The increasing use of data loggers to automatically measure lying behavior (Ledgerwood et al., 2010) allows for quick identification of animals that deviate from herd mates or their baseline measurements. Resting behavior can be an important measure of cow comfort with cows typically spending 10-14 h/d lying, 10.5 lying bouts/d and bout duration of 1.2 h/bout (Charlton et al., 2014; von Keyserlingk et al., 2009). During the transition period, cows reduced their lying time from 11.7 h/d in prepartum period to 10.6 h/d in the postpartum period (Huzzey et al., 2005). Similarly, cows on mattress during the close-up prepartum period averaged 10.6 to 13.5 h/d lying down (Calderon and Cook, 2011) cows reduced lying times in the days prior to parturition.

Reductions in lying time have been associated with an increased risk for lameness (Cook et al., 2004). Increases in pen stocking density can alter lying behavior. When competition in the pen increased there was an increase in standing time (Huzzey et al., 2006) and a decrease in daily lying time (Fregonesi et al., 2007). Cows that were severely lame spent more h/d lying than non-lame cows (12.8 vs. 11.2 h/d, respectively; Ito et al., 2010). When cows had lying bouts greater than 90 min/bout those cows were 3 times more likely to be severely lame (Ito et al., 2010). Cows diagnosed with hoof lesions up to 15 wk post fresh spent more time standing during the 2 wk prior to calving than cows not diagnosed with hoof lesions (Proudfoot et al., 2010). In addition, lame cows were at risk for ketosis with elevated  $\beta$ -hydroxybutyrate concentrations (Calderon and Cook, 2011).

Other health disorders that have been associated with changes in lying behavior include mastitis (Cyples et al., 2012), subclinical hypocalcemia (Jawor et al., 2012) and primiparous cows with 2 or more clinical disorders (Sepúlveda-Varas et al., 2014). Recent research has indicated dairy cows with mastitis will spend more time standing than cows without mastitis (Cyples et al., 2012; Zimov et al., 2011). When cows were challenged with *E.coli* they reduced lying time from 11.8 to 10.6 h/d (Cyples et al., 2012). It is speculated cows spend more time standing due to the discomfort of inflammation in the udder (Zimov et al., 2011). Cows with subclinical hypocalcemia stood for longer during the 24-h period before parturition and spent less time standing 1 d post fresh (Jawor et al., 2012). Primiparous cows that developed more than one clinical

disease spent more time lying and tended to have longer lying bouts during d 3-7 after calving (Sepúlveda-Varas et al., 2014).

The objective of this study was to determine if lying behavior (time, number of bouts, and bout duration) during the prepartum period was associated with transition health disorders in Jersey dairy cows.

## **Material and Methods**

### ***Animals and Housing***

A total of 287 prepartum nulliparous and parous Jersey cows were enrolled in the study from October 2012 to March 2013. The study was conducted at a large commercial dairy farm (6,400 lactating dairy cows) in south-central Minnesota, USA. Close-up prepartum cows were housed in a 12-row low profile cross-ventilated barn and provided TMR (balanced to meet nutrient requirements; NRC, 2001) once daily with frequent push-ups. Prepartum cows were fed from a feed alley by headlocks. All 4 experimental pens had the same measurements of 31.7 m x 11.0 m. Cows were housed in pens with 44 deep sand bedded freestalls (229L x 107W x 114H cm) with a head-to-head configuration and 48 0.61-m headlocks. Two water troughs were located in the pen and measured 366 cm x 56 cm. One water trough was located at the end of the bank of freestalls and a shared water trough was located between the treatment pen and an adjacent non-experimental pen.

When cows demonstrated signs of calving, farm personal moved the cows to an individual box stall. At d 1 post-calving cows were moved into a freestall pen with 240 stalls and 260 headlocks, stocked at 100% based on the number of stalls for 21 d. Parous

and nulliparous cows were housed separately for the first 21 d. For cows that calved early, loggers were removed within 2 d of calving.

### ***Experimental Treatment and Design***

The data set came from a prepartum stocking density study (Lobeck-Luchterhand et al., unpublished data). A total of 278 cows at  $254 \pm 3$  d from expected calving date were allocated to 80% (**80D**; 38 animals/pen; each pen with 48 headlocks and 44 stalls) or 100% (**100D**; 48 animals/pen) stocking density. Animals were required to have a body condition score between 2 and 4 (1-5 scale; 1=emaciated, 5=obese) and a locomotion score  $<3$  (1-5 scale; 1=normal gait, 5=severely lame) or were not included in the study. Pens were stocked twice weekly and groups of 2 to 15 animals (median = 9 animals) were moved to the 80D and 100D pens to re-establish the targeted stocking density. Two pens housed primiparous and multiparous cows and two pens housed nulliparous animals with stocking density treatments applied to both nulliparous and the mixed parity pens. After each replicate, treatment within parity (nulliparous or mixed parity) was switched to the opposite pen to prevent location bias. Preliminary analysis did not find an association between prepartum stocking density and health, therefore data from both treatments were combined for the analysis in the current study.

***Lying Behavior.*** Lying time, lying bouts, and lying bout duration were measured using HOBO Pendant G data loggers (Onset, Bourne, MA). Data loggers were set to collect lying behavior at 30-s intervals (Ledgerwood et al., 2010) and placed on the cow's right hind leg 1 d after entrance into the pen. Loggers were kept on the cow for 12 d and removed for 7 d and reattached for another 12 d or until the cow calved. Animals that

calved early had loggers removed within 2 d of calving and data post calving were removed from the data set. Daily lying times, frequency of lying bouts, and lying bout duration were computed for each cow using a macro in SAS (SAS Institute Inc., Cary, NC) developed by N. Chapinal (University of British Columbia, Pers. Comm.).

### ***Health and Measurements***

***Body Condition and Locomotion Score.*** At enrollment and on d  $1 \pm 1$ ,  $28 \pm 3$ , and  $56 \pm 3$  all cows were scored for body condition (BCS, 1 = emaciated and 5 = obese; 0.25 unit increments; Ferguson et al., 1994) and locomotion (1 = normal and 5 = severely lame; Sprecher et al., 1997). Change in BCS was calculated from BCS at enrollment subtracted from BCS at DIM 1. Cows were classified as lame when locomotion score was  $\geq 3$ .

***Blood Sampling and Analysis of Metabolites in Plasma.*** Blood samples were collected from all cows on DIM  $-18 \pm 3$ ,  $-11 \pm 3$ ,  $-4 \pm 3$ ,  $3 \pm 3$ ,  $10 \pm 3$ ,  $17 \pm 3$  and  $24 \pm 3$  from the coccygeal vein or artery immediately after feeding while cows were restrained in self-locking headlocks. Samples were collected into evacuated tubes containing K2 EDTA (Becton Dickinson Vacutainer Systems, Franklin Lakes, NJ). Tubes were placed in ice until centrifugation for plasma separation ( $1,200 \times g$  for 15 min at  $4^{\circ}\text{C}$ ). Plasma was aliquoted into microcentrifuge tubes and stored at  $-32^{\circ}\text{C}$  until analysis.

Samples collected weekly from DIM -18 to 24 were analyzed for concentrations of NEFA using a colorimetric assay (Wako Chemicals USA, Richmond, VA; Ballou et al., 2009). Concentrations of BHBA were determined enzymatically (Ranbut, Randox

Laboratories, Antrim, UK; Ballou et al., 2009) from samples collected weekly from 3 to 24 DIM.

*Clinical Examination and Definitions of Diseases.* All cows were examined on DIM 1,  $4 \pm 1$ ,  $7 \pm 1$ ,  $10 \pm 1$ , and  $13 \pm 1$  for the diagnosis of retained fetal membrane and metritis. Retained fetal membrane (**RP**) was defined as retention of a fetal membrane past 24 h postpartum. Metritis was defined as cows with watery, pink or brown, and fetid uterine discharge. Acute metritis was defined when cow had the previous symptoms with a fever  $> 39.5$  °C. A cow was diagnosed with subclinical ketosis (**SCK**) when BHBA concentrations were  $\geq 1200$   $\mu\text{mol/L}$ , regardless of sample. All cows were observed once daily for displacement of abomasum (**DA**) and thrice daily for mastitis. Cases of mastitis were examined up to 14 DIM. The health records of DA and cows removed (sold or dead) from the herd were followed up to 60 DIM.

### *Statistical Analysis*

The UNIVARIATE procedure of SAS (SAS Institute Inc., Cary, NC) was used to examine normality and detect the presence of outliers for daily lying time, lying bouts and lying bout duration. Two hundred cows did not have a DA, RP, metritis, subclinical ketosis, mastitis or lame DIM 1. A linear mixed model (MIXED procedure, SAS Institute, Inc., Cary, NC) was built to evaluate the association of prepartum lying behavior (time, bouts, bout duration) and health events. Cow was the experimental unit ( $n=287$ ). A repeated statement included day and cow as the subject and cow as a random statement. The structure of covariance (auto-regressive, unstructured, or compound symmetry) was chosen according to the Bayesian Akaike information criteria for

repeated measurements. Covariates examined for lying behavior included health status, parity (nulliparous, primiparous, and multiparous), day relative to calving, number of days in close-up pen, and average daily pen temperature.

The LOGISTIC procedure (SAS Institute) was used to perform a multivariate logistic regression and area under the curve (AUC) analysis. Time periods to find the highest AUC values on changes of lying behavior were determined independently for each health event. Variables included average lying behavior (time, number of bouts and bout duration), average day relative to calving, and parity. Receiver operating characteristic (ROC) analyses were performed using MedCalc (MedCalc Software, Mariakerke, Belgium) to evaluate daily lying behavior that resulted in the highest accuracy for predicting a health disorder. Sensitivity and specificity analysis was completed using MedCalc.

## RESULTS

Nulliparous cows tended to spend less time lying, had more lying bouts and shorter lying bout duration than primiparous and multiparous cows during the close-up prepartum period (Figure 1). Overall, primiparous cows spent  $13.6 \pm 0.2$  h/d more time lying than nulliparous ( $12.7 \pm 0.1$ ;  $P < 0.001$ ) and multiparous ( $13.0 \pm 0.2$ ;  $P = 0.010$ ) cows. Nulliparous and multiparous cows did not differ in daily lying time. As parity increased, the number of lying bouts per day decreased ( $P < 0.001$ ), from  $11.9 \pm 0.2$  for nulliparous,  $10.3 \pm 0.2$  for primiparous and  $9.4 \pm 0.2$  bouts/d for multiparous cows. As parity increased lying bout duration increased ( $P < 0.001$ ). Nulliparous cows had overall

bout duration of  $106.0 \pm 2.6$ , primiparous  $140.0 \pm 3.6$ , and multiparous cows  $158.5 \pm 3.9$  min/bout.

There were no associations of lying behavior (time, bouts, or bout duration) and RP, DA or mastitis up to 14 DIM. Cows with a DA (n=4) had large variations in lying behavior, leading to insufficient numbers for analysis. The incidence of health events are shown in Table 1.

### ***Lameness***

Two animals were observed lame at 1 DIM. Overall, cows that were lame spent  $2.7 \pm 1.0$  more h/d lying than non-lame cows (Figure 2). No differences in lying bouts or lying bout duration occurred until 1 wk prior to calving; lame cows had numerically fewer lying bouts ( $6.6 \pm 4.2$  vs.  $10.6 \pm 0.5$  bouts/d;  $P > 0.05$ ) and greater lying bout duration ( $238 \pm 36$  vs.  $166 \pm 5$  min/d;  $P = 0.047$ ) than healthy cows.

### ***Subclinical Ketosis***

The overall incidence of subclinical ketosis was 1.4% (n = 4) and all 4 animals were nulliparous cows. There were no differences in daily lying time up to 4 wk prior to calving. The number of lying bouts/d for healthy and SCK cows did not differ during wk -4, wk -3, or wk -2. During wk -1 SCK cows had more lying bouts/d than healthy nulliparous cows ( $21.3 \pm 2.4$  vs.  $16.1 \pm 0.5$ , respectively;  $P = 0.033$ ). Subclinical ketosis cows did not differ from healthy cows during wk -4 or wk -3 for lying bout duration; however, at wk -2 and wk -1 SCK cows had  $40.5 \pm 19.6$  ( $P = 0.040$ ) and  $35.4 \pm 14.8$  ( $P = 0.018$ ) min/bout shorter bout duration than healthy nulliparous cows, respectively.

The time period with the highest AUC values were from -5 to -1 d before calving. Receiver operator curve analysis was not significant for lying time and detecting the outcome of SCK. Within nulliparous cows lying bouts had an AUC value of 0.80, a critical threshold of 16.5 bouts/d and a sensitivity of 100% and specificity of 64%. Lying duration had an AUC value of 0.80, a critical threshold of less than 29.5 min/bout with a sensitivity of 100% and specificity of 78%.

### ***Metritis and Acute Metritis***

When all parities were combined for the analysis there were no differences in prepartum lying time among healthy and metritic and acute metritic cows; however, when parity was examined in three categories there were differences in lying times and health status within primiparous cows ( $P < 0.001$ ). No differences were observed between metritis status and lying behavior of multiparous or nulliparous cows. No differences were observed in lying time at wk -3 for primiparous cows, but at wk -2 and wk -1 metritic primiparous cows spent  $1.0 \pm 0.5$  ( $P = 0.048$ ) and  $1.2 \pm 0.5$  ( $P = 0.024$ ) fewer h/d lying than healthy primiparous cows. During wk -2 and wk -1 acute metritic primiparous cows spent  $1.4 \pm 0.6$  ( $P = 0.023$ ) and  $1.7 \pm 0.7$  ( $P = 0.011$ ) fewer h/d lying than healthy cows. The time period with the highest AUC values for metritis were from d -3 to -1 for primiparous cows. The AUC was 0.67 (0.52-0.81). There were no AUC values that were better than chance for detecting acute metritis.

There were no differences in the number of lying bouts per day by health status; however, when examining the time period from d - 3 to -1, nulliparous cows had an AUC

= 0.67 (0.52-0.81). There were no differences in lying bout duration and health status, nor were there any significant AUC values.

## DISCUSSION

The lying times for close-up prepartum Jersey cows (12.7 – 13.6 h/d) were similar to previously reported with cows housed in freestalls (11.4 – 13.9 h/d; Calderon and Cook, 2011). In the current study, primiparous cows spent more time lying than both nulliparous and multiparous cows; however, they were intermediate for the number of lying bouts and lying bout duration. After calving, first lactation animals had higher activity than older cows (Edwards and Tozer, 2004). Steensels et al. (2012) reported in a mixed lactation pen the older animals spent a greater portion of the day lying than the younger animals. In our study, nulliparous cows were housed separately pre- and post-calving from the older and larger animals, however primiparous cows were mixed with older animals. Body size and parity have been positively associated with social dominance (Phillips and Rind, 2002). Mixing of younger animals with older animals can result in increased standing time (Phillips and Rind, 2001). Typically smaller animals are lower ranked and may have reduced access to food, water, and resting area (Grant and Albright, 2001). Additionally, postpartum primiparous grazing dairy cows had more lying bouts/d (9.7 vs. 8.4 bouts/d) and spent less time lying (7.5 vs. 8.5 h/d) than multiparous cows (Sepúlveda-Varas et al., 2014) when mixed on pasture. These results were slightly lower than the results in the current study; however, our animals were prepartum cows in freestalls versus postpartum cows with mixed lactation on pasture.

Monitoring lying behavior during the prepartum period may be useful for identifying animals that are at risk for a transition health disorder. Although some health events, for example lameness and subclinical ketosis, had a low incidence there were still significant changes in lying behavior providing evidence that lying behavior is beneficial to monitor during the prepartum transition period. Health disorders such as RP, mastitis, and DA were not associated with altered lying behavior. To our knowledge this is the first study to observe differences in lying behavior and SCK and metritis. Other research groups have detected differences in lying behavior with cows that had subclinical hypocalcemia (Jawor et al., 2012) and primiparous cows with more than 2 clinical health disorders were observed to alter lying behavior (Sepúlveda-Varas et al., 2014). Lame cows were more likely to have greater BHBA concentrations than non-lame cows (Calderon and Cook, 2011) potentially putting the cows at risk for subclinical ketosis. Cyples et al. (2012) observed decreased lying time on the day of *E. coli* challenge. In another study with intramammary challenge of *E. coli*, cows spent more idle time standing, decreased feeding time, along with a decrease in self-grooming behavior (Fogsgaard et al., 2012). Lying behavior was not observed post calving and further research investigating changes in lying behavior post calving is warranted.

This is the first time prepartum lying behavior has been associated with metritis and acute metritis. Primiparous cows that developed metritis spent less time lying in the days before calving than non-metric primiparous cows. During the sickness response several changes in behavior and physiology occur to facilitate host survival during infection and tissue injury (Hart, 1988). Cows that became ill after calving tended to

spend more time lying after calving (Proudfoot et al., 2014). Most commonly behavioral patterns of sickness behavior related to infectious disease are lethargy, depression, anorexia, and reduction in grooming (Hart, 1988). It is interesting to note that primiparous cows reduced their lying time; however, it is unclear if the cows spent more time standing idle or altered feeding behavior. Cows that developed severe metritis spent less time feeding and consumed less feed compared with cows that were only mildly metritic (Huzzey et al., 2007).

Nulliparous cows with SCK increased the number of bouts/d and decreased the duration of these bouts approximately 1 week before calving. Post calving ketotic cows had lower activity than healthy cows up to 5 DIM and then were more active than healthy cows at 12 DIM (Edwards and Tozer, 2004). Cows that developed ketosis decreased their feed intake 3.6 d prior to diagnosis and decreased their daily feeding time by 45.5 min/d (González et al., 2008). For every 10-minute decrease in average daily time spent feeding during the week before calving the risk of SKC increased by 1.9 times (Goldhawk et al., 2009). Animal activity declined prior to milk yield decreases were detected (Edwards and Tozer, 2004).

Similar to the results reported by other researchers, lame cows spent more time lying and had longer lying bouts than non-lame herd mates. Watters et al. (2013) and Calderon and Cook (2011) had similar observations with lame cows more likely to have longer daily lying times and lying bout durations. In the current study, lame cows increased lying bout duration by 86 min/d compared to non-lame cows during the week before parturition. Ito et al. (2010) observed severely lame cows spent 12.8 h/d lying and

had a bout duration of 95.3 min/bout whereas non-lame cows spent 11.2 h/d lying and had a bout duration of 80.3 min/bout. Lame post-fresh multiparous cows on pasture had longer daily lying times (1.7 h/d) than non-lame multiparous cows (Sepúlveda-Varas et al, 2014). During the transition period moderate and severely lame cows had significantly longer lying times 3 d before and after calving (Calderon and Cook, 2011). It has been speculated that lame cows likely have an increased daily lying duration and have longer lying bouts in an effort to alleviate pain (Chapinal et al., 2009). In addition to changes in lying behavior, lame cows reduced feeding time and ruminating behaviors and increased self-grooming (Almeida et al., 2008). Lameness has been considered an important welfare concern for dairy cows and moderately lame cows are often missed by producers (Espejo et al., 2006). This information could be used to alert the producer to warrant an examination for treatment options.

## **CONCLUSIONS**

In conclusion, this study indicated changes in lying behavior can be observed in the prepartum for post-calving health disorders. Lame cows had longer lying times and longer bout duration during the days before calving. Nulliparous cows with subclinical ketosis had more lying bouts and shorter bout duration than healthy nulliparous cows. Primiparous cows with metritis and acute metritis had reductions in daily lying time with no differences in the number of lying bouts or bout duration. Lying behavior could potentially be used as an indicator of transition health disorders in dairy cows.

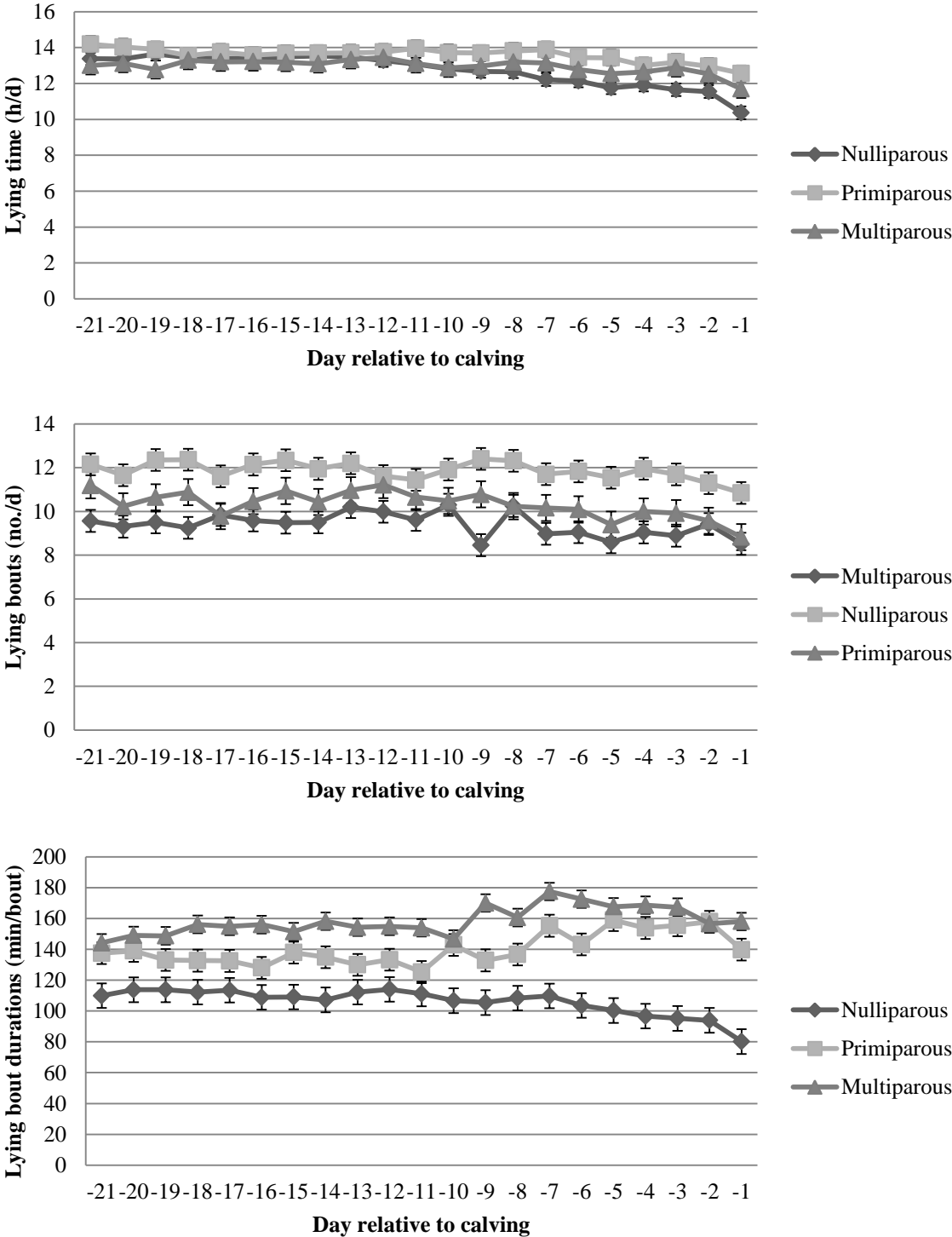
**Table 1.** Incidence of transition health events up to 60 days in milk in Jersey cows.

Health Event	Incidence (n)	%
Metritis	53	18.5
Acute Metritis	28	9.8
Retained fetal membrane	19	6.6
Displaced abomasum	4	1.4
Mastitis <sup>1</sup>	12	4.2
Lame at DIM 1	2	0.7
Subclinical ketosis <sup>2</sup>	4	1.4

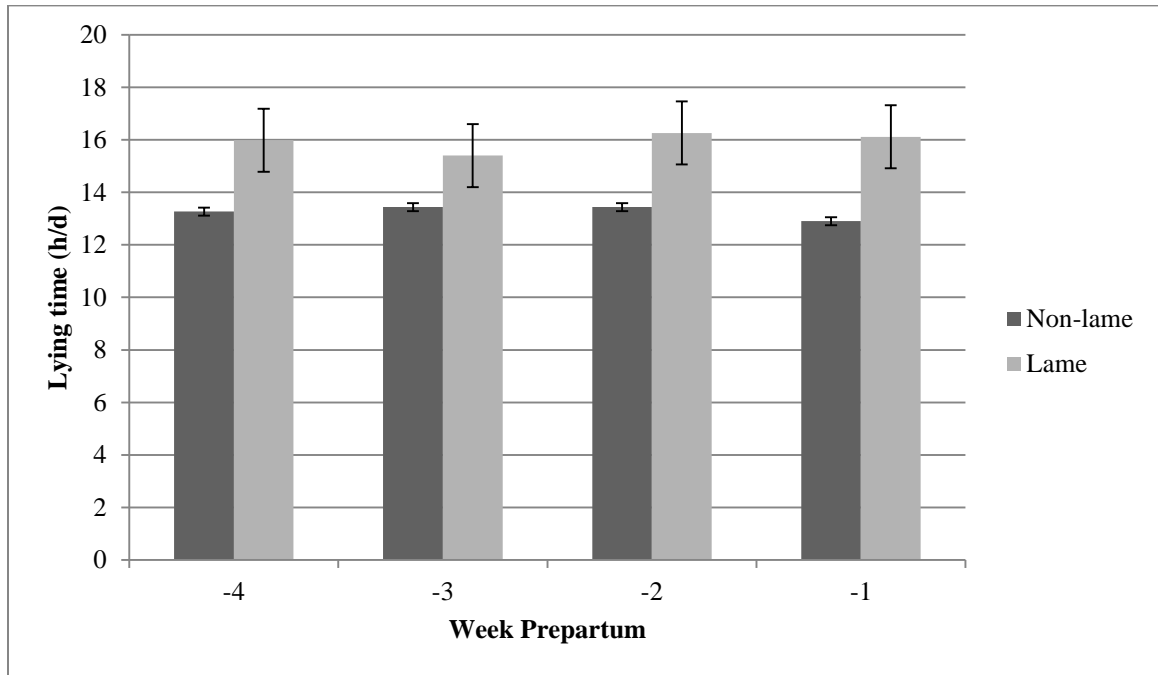
<sup>1</sup>Cases were evaluated up to 14 DIM

<sup>2</sup>Subclinical ketosis was declared when BHBA levels  $\leq$  1200  $\mu\text{mol/L}$

**Figure 1.** Daily lying time (h/d), lying bouts (no./d) and lying bout duration (min/bout) of nulliparous, primiparous and multiparous prepartum Jersey cows

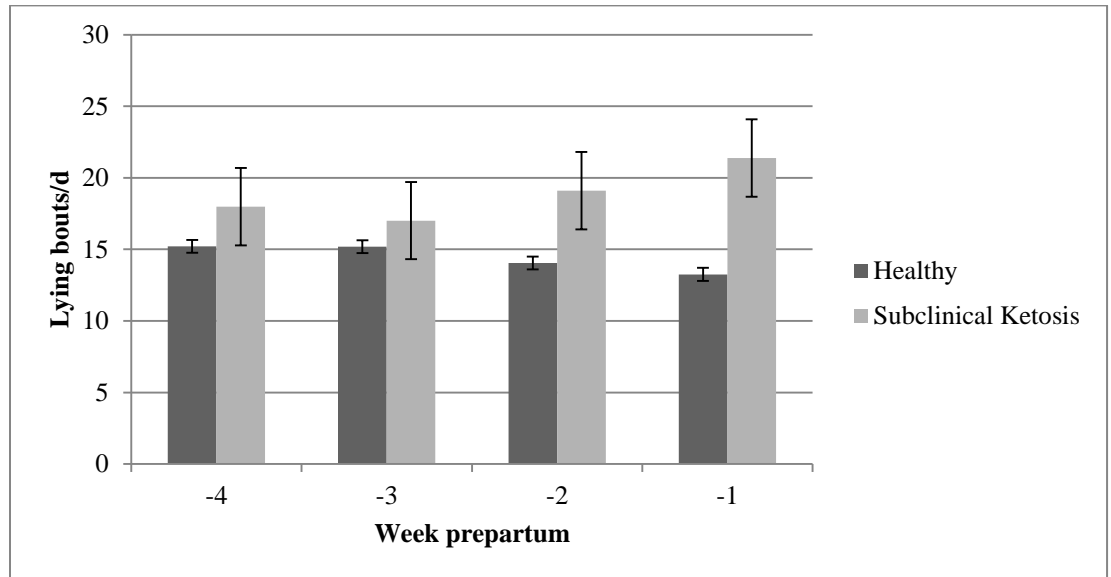


**Figure 2.** Daily lying time of prepartum Jersey cows (h/d) diagnosed lame at DIM 1 or healthy

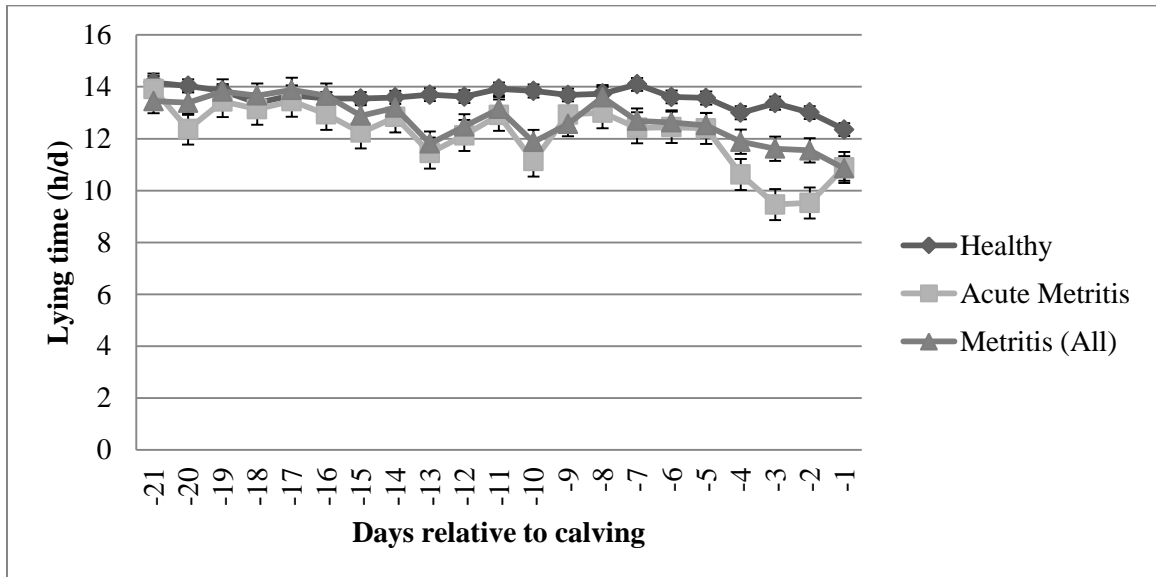


Lame cows spent significantly more time lying than healthy cows ( $P = 0.008$ ), Parity ( $P = 0.003$ ), Week prepartum ( $P = 0.74$ ), Health  $\times$  Time ( $P = 0.37$ ).

**Figure 3.** Daily lying bouts of prepartum Jersey cows (no. bouts/d) diagnosed lame at DIM 1 or healthy



**Figure 4.** Daily lying time of prepartum Jersey cows (h/d) diagnosed with metritic or healthy



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