Short communication: Relationship between competitive success during displacements at an overstocked feed bunk and measures of physiology and behavior in Holstein dairy cattle

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ABSTRACT

The objective of this study was to evaluate how behavioral and physiological parameters are affected based on a cow's level of success at displacing others at an overstocked feed bunk. Forty Holstein nonlactating, late-gestation dairy cattle were housed in an overstocked pen [5 stalls/10 cows and 0.34 m of linear feed bunk (FB) space/cow] in groups of 10 (4 heifers and 6 multiparous cows) for 14 d. Plasma nonesterified fatty acids, glucose, and fecal cortisol metabolites (11,17-dioxoandrostanes) were measured in blood and feces sampled every 2 d. A glucose tolerance test and an ACTH challenge were conducted on all cows on d 13 and 14, respectively to further explore the effects of competitive success on energy metabolism and stress physiology. Feeding behavior and displacements at the FB were recorded between d 7 to 10 of the observation period. A competition index (CInd) was calculated for each cow by dividing the number of times the cow displaced another at the FB by the total number of displacements the cow was involved in, either as an actor or reactor. Cows were then divided into 3 subgroups based on their CInd: high success (HS: CInd ≥ 0.6), medium success ($0.4 \leq \text{CInd} < 0.6$), and low success (LS: CInd < 0.4). Heifers accounted for 7, 36, and 79% of the total number of animals in the HS ($n = 15$), medium success ($n = 11$), and LS ($n = 14$) groups, respectively. No differences were observed in daily feeding time, total number of displacements, and time to approach the FB following fresh feed delivery between the 3 CInd groups; however, cows in the LS group had greater daily nonesterified fatty acid and 11,17-dioxoandrosterone concentrations relative to cows in the HS group. No differences existed in cortisol response to an ACTH stimulation test between CInd categories. During the glucose tolerance test, glucose response curves were the same between all 3 CInd categories;

however, the peak insulin response of LS cows was 130 $\mu\text{IU/mL}$ greater than the peak HS response, indicating that LS cows may have decreased tissue responses to insulin or increased pancreatic responses to glucose. In an overstocked environment, dairy cattle physiology is associated with a cow's level of success at displacing other individuals at the feed bunk.

Key words: overstocking, competition index, physiology, behavior

Short Communication

When cows are crowded at the feed bunk (FB), aggressive displacements increase as cows vie to gain access to feed (Huzzey et al., 2006); it is likely that some cattle are more successful than others during these interactions. Previous work has shown that level of success in agonistic interactions may be an important determinant of an animal's ability to cope with an aversive environment. A compromised ability to cope may be evidenced by a greater physiological stress response or increased risk for health disorders. For example, Mendl et al. (1992) showed that pigs who were aggressive but also displaced frequently during agonistic interactions (low success) had greater salivary cortisol concentrations, a greater cortisol response to an ACTH challenge test, and lower weight gains than individuals that were aggressive but successful at displacing others (high success). Galindo and Broom (2000) reported that cattle that were the least successful in agonistic interactions were at greater risk for lameness because they spent less time lying down and more time standing with 2 feet in the cubicle.

Competitive success during overstocking is associated with behavior in Holstein cattle. Cows that are displaced by a greater number of individuals at the FB than they can displace themselves (low success) eat faster (Proudfoot et al., 2009) and have a greater increase in feeding activity when more FB space is provided compared with cows with higher competitive success (DeVries et al., 2004). Val-Laillet et al. (2008) reported that cattle with low success in agonistic inter-

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actions at the FB spent a smaller percentage of their time at the feeder compared with high-success cattle. These behavioral differences may be associated with differences in physiological parameters; however, this has never been explored. The objective of this study was to evaluate how stress physiology and energy metabolism are affected based on a cow's level of success during competitive interactions at the FB.

Forty nonlactating, late-gestation Holstein dairy cows were housed in 4 groups of 10 cows (4 heifers and 6 multiparous cows per group) in a 2-row freestall barn and managed according to the guidelines set by the Cornell University Institutional Animal Care and Use Committee. Groups were formed when cows were between 61 to 81 d from their expected calving date, and cows were allowed 10 d to adapt to their respective groups before the feeding and resting space in the pens was modified to simulate conditions of overstocking. Access to the 4 freestalls facing (nearest) the FB and one additional freestall along the back wall of the pen were roped off to restrict resting space and access to the FB was restricted using plywood that was bolted across the feeding area. During the overstocked period, which lasted 14 d, each group of 10 animals had access to 5 freestalls and 0.34 m of linear post-and-rail FB space per animal; this represented a stocking rate 200% that of industry recommendations (NFACC, 2009).

Cows were fed a TMR once daily at approximately 0800 h, feed push-ups occurred at regular intervals throughout the day, and all cows had ad libitum access to water. The TMR consisted of wheat straw (24.6% of DM), corn silage (41.0% of DM), and dry cow grain (34.4% of DM) and wet chemistry analysis (Dairy One Cooperative Inc., Ithaca, NY) of a composite of weekly feed samples revealed the following TMR composition (% DM $\pm$ SD): CP = 14.5 ± 0.6 ; ADF = 31.4 ± 1.5 ; NDF = 49.8 ± 2.8 ; starch = 17.7 ± 0.7 ; Ca = 0.76 ± 0.07 ; P = 0.29 ± 0.01 ; Mg = 0.23 ± 0.01 ; K = 1.06 ± 0.04 ; and Na = 0.19 ± 0.03 .

Behaviors were monitored using video cameras (Sony CCD Digital ULTRA Pro Series, Hi-Resolution BW CCD Camera with Auto-Iris; Sony Corp., New York, NY) connected to a digital recording system (DiGiCam H.264, 120 and 240 FPS, DVR PC Version; Central Alarms Systems Inc., Littleton, CO). A camera was positioned directly above the feeding area to continuously record behavior at the FB. Hair dye was used to create unique alphanumeric symbols on the backs of the cows so that individuals could be identified on the video recordings. Daily feeding time and time to the FB after fresh feed delivery was estimated from 10-min time scans of the video recordings over 4 consecutive days (d 7 to 10 of the 14-d overstocked period). A cow was considered to be feeding when its neck collar was

visible beyond the top rail of the feed barrier on the feed alley side of the pen. To assess competitive behavior, 3 d of continuous (24-h; d 7 to 9) video recordings were reviewed and each successful competitive displacement that occurred at the FB was recorded. A displacement was recorded when a cow's head (actor) came in contact with a cow that was feeding (reactor), resulting in the reactor withdrawing its head from the FB. Unsuccessful displacements at the FB (i.e., a contact followed by a failure to displace the feeding individual) were not recorded in the present study because of the difficulty in establishing an objective and clear definition of what would constitute a contact that is an attempt to displace. Although unsuccessful displacement attempts likely occur in competitive environments, many physical contacts at the FB likely occur without the interacting animals intending to displace one another; distinguishing between these types of physical contacts is difficult to do accurately and, thus, a measure of unsuccessful displacements would be hard to interpret. Data collected on successful competitive displacements were used to calculate a competition index (**CInd**) for each cow. This index has previously been used in cattle (Val-Laillet et al., 2008; Galindo and Broom, 2000) and was calculated as follows:

$$\text{CInd} = \frac{\text{no. of times cow is the actor}}{\text{no. of times cow is the actor} + \text{no. of times cow is the reactor}}$$

For each cow, the CInd score could vary from 0 to 1; an index value of 0 would indicate that a cow was never successful at displacing another individual but was displaced themselves, whereas an index value of 1 would indicate that a cow could displace others but never be displaced themselves. These index values were used to categorize cows into 3 subgroups according to their level of success during competitive interactions at the FB (Galindo and Broom, 2000; Val-Laillet et al., 2008): low success (**LS**: CInd < 0.40), medium success (**MS**: $0.40 < \text{CInd} \leq 0.60$), and high success (**HS**: CInd > 0.60).

Blood and fecal samples were collected on d 1, 3, 5, 7, 9 and 11 of the 14-d overstocked period. Plasma concentrations of glucose and NEFA were measured by enzymatic analysis (glucose oxidase, P7119, Sigma Chemical Co., St. Louis, MO; NEFA-C: Wako Pure Chemical Industries, Osaka, Japan). The intra- and interassay coefficients of variation for the NEFA assay were 3.7 and 4.4%, respectively, and for the glucose assay were 2.9 and 6.1%, respectively. Fecal samples were collected fresh, sealed within plastic bags and placed immediately under ice. Steroids from the fecal samples were extracted using the wet extraction method de-

scribed by Palme and Möstl (1997). Concentrations of fecal cortisol metabolites (11,17-dioxoandrostanes, **11,17-DOA**) were measured using a competitive enzyme immunoassay developed by Palme and Möstl (1997) and validated for use in cattle (Palme et al., 1999). The intra- and interassay coefficient of variation for the 11,17-DOA assay were 3.2 and 3.4%, respectively. The 11,17-DOA concentrations determined by the assay were corrected for the DM content of the raw fecal samples.

A glucose tolerance test (**GTT**) and ACTH challenge were administered to each cow on d 13 and 14, respectively, of the overstocked period. Prior to these tests (d 11 and 12), all cows were fitted with a jugular catheter that was secured to the neck within a fabric pouch using a topical adhesive approved for use on animals. The cow's neck was wrapped with elastic bandages to prevent the cow from dislodging the catheter while residing in the group pen. Body weights were measured on the day of catheterization to determine glucose and ACTH doses for the GTT and ACTH challenge, respectively. Approximately 1 to 1.5 h before the start of the GTT and ACTH challenge, all cows were moved a short distance from their group pen to individual stanchions; this was done to facilitate sample collection during the procedures. Feed was removed from the cows 2 h before the start of the GTT. The GTT involved administering 0.25 g/kg of BW of glucose i.v. (dextrose 50% wt/vol.; #002460; Butler Animal Health Supply, Dublin, OH) and then collecting blood samples at -15, -5, 0, 5, 10, 15, 20, 25, 30, 45, 60, 75, 90, 120, 150, and 180 min relative to the administration of the glucose. The ACTH challenge was performed by administering 0.125 IU/kg of BW of ACTH i.v. (Porcine, A6303; Sigma Chemical Co.). Blood samples were collected at -60, -15, 0, 15, 30, 45, 60, 90, 120, 150, 180, and 240 min relative to the administration of ACTH. During both procedures blood samples were immediately centrifuged (for 15 min at $2,800 \times g$ and 4°C) and plasma collected and stored at -20°C for later determination of plasma glucose, NEFA, and insulin concentrations (GTT) or plasma cortisol concentration (ACTH challenge). Plasma insulin and cortisol were measured by RIA (Porcine Insulin RIA Kit #PI-12K, Millipore Corp., Billerica, MA; Coat-A-Count Cortisol RIA Kit, Siemens Medical Solutions Diagnostics, Los Angeles, CA). The intra- and interassay coefficient of variation for the insulin assay were 3.3 and 3.2%, respectively, and for the cortisol assay were 3.2 and 1.9%, respectively.

All statistical analyses were performed using SAS version 9.2 (SAS Institute, 2009). Cow was considered the experimental unit for all analyses. Measures that were sampled over multiple days during the observa-

tion period (e.g., plasma NEFA, glucose, 11,17-DOA, displacements, feeding time, and time to FB after fresh feed delivery) were averaged to generate one overall estimate of each measure per cow. These overall estimates were modeled as dependent variables using proc MIXED, with the following included as fixed effects: group, parity, CInd category, and the parity $\times$ CInd category interaction. In each of these models, the interaction term was not significant ($P \geq 0.23$) and so was removed from the models. The contrast statement in proc MIXED was used to describe the differences in the dependent variable between each of the 3 CInd categories. To analyze the NEFA, insulin, and glucose response to the GTT and the cortisol response to the ACTH challenge, the area under the curve (**AUC**) was estimated for each cow's response curves. The AUC was calculated using the trapezoidal method and sampled concentrations after discounting basal values. For the glucose, insulin, and cortisol response curves, only sampled concentrations that were above basal concentration were included in the AUC calculation, whereas for the NEFA response, only sampled concentrations that were below basal concentration were included in the AUC calculation (a negative estimate for NEFA). Natural logarithmic transformation (**Ln**) was required for all AUC data to comply with MIXED model assumptions and improve the model fit. The absolute value of the negative NEFA AUC estimate for each cow was used for the log transformation. The Ln AUC estimates for each response curve were modeled as dependent variables using the same MIXED model setup as described above.

The distribution of CInd scores for the 40 experimental cows is presented in Figure 1. The number of heifers, first-lactation, second-lactation, and third-lactation cows by CInd category were LS: 11, 2, 1, and 0; MS: 4, 5, 1, and 1; and HS: 1, 10, 3, and 1, respectively. The average ($\pm$ SD) BW of cows in the LS, MS, and HS groups were 603 ± 62 kg, 680 ± 67 kg, and 713 ± 89 kg, respectively.

Average daily feeding time per cow and time to approach the FB following fresh feed delivery was not different between CInd categories ($P \geq 0.28$; Figure 2). No difference was observed in the total number of displacements that cows in the LS, MS, and HS groups engaged in per day, either as an actor or reactor (28, 32, and 32 ± 3 displacements per day, respectively; $P \geq 0.41$).

The LS group had greater plasma NEFA and fecal 11,17-DOA concentrations during the overstocked observation period relative to the MS and HS groups ($P \leq 0.05$; Figure 2), whereas the average ($\pm$ SE) glucose concentration did not differ between CInd categories

Figure 1. Competition index (CInd) of 40 experimental cows that describes their level of success during competitive interactions at the feed bunk; 3 groups are distinguished: a high-success group (CInd ≥ 0.6), medium-success group ($0.4 \leq \text{CInd} < 0.6$), and low-success group (CInd < 0.4).

(LS: 65.9 ± 0.8 , MS: 67.2 ± 0.9 , and HS: 65.4 ± 0.9 mg/dL; $P \geq 0.14$).

No differences were observed in the plasma cortisol responses to an ACTH challenge between cows in the 3 CInd categories (Ln AUC of cortisol; LS: 9.78 ± 0.06 , MS: 9.74 ± 0.06 , HS: 9.83 ± 0.06 nmol/L $\times$ 240-min ACTH challenge; $P \geq 0.26$). The mean ($\pm$ SD) cortisol concentration for cows in all CInd categories before the ACTH challenge was 13.8 ± 2.8 nmol/L, with cortisol concentrations peaking at $t = 60$ min following ACTH administration at 173.8 ± 13.8 nmol/L. Glucose response curves did not differ between CInd categories following the GTT (Ln AUC of glucose; LS: 8.02 ± 0.06 , MS: 7.90 ± 0.06 , and HS: 7.91 ± 0.06 mEq/L $\times$ 180-min GTT; $P \geq 0.21$), suggesting that the glucose clearance rate was the same for all animals. The LS group had a greater insulin response to the GTT than the HS group (Ln AUC of insulin; LS: 8.73 ± 0.14 vs. HS: 8.14 ± 0.15 μ IU/L $\times$ 180-min GTT; $P = 0.01$) and tended to have a greater NEFA response than the HS group (Ln |AUC| of NEFA; LS: 8.82 ± 0.25 vs. HS: 8.02 ± 0.27 μ Eq/L $\times$ 180-min GTT; $P = 0.06$). The Ln AUC estimates for the insulin (8.42 ± 0.14 μ IU/L $\times$ 180-min GTT; $P > 0.14$) and NEFA (8.35 ± 0.27 μ Eq/L $\times$ 180-min GTT; $P > 0.23$) response curves of the MS group did not differ from the other 2 CInd cat-

egories. Figure 3 presents the nontransformed glucose, insulin, and NEFA response curves to the GTT for the 3 CInd categories.

These results suggest that an association exists between CInd and physiological status. Although no differences in the glucose response curves during the GTT between CInd categories were observed, the insulin response to the GTT among LS cows was greater than for HS cows; this may suggest that among LS cows, tissue sensitivity to insulin was decreased (more insulin was required to yield the same glucose response) or pancreatic sensitivity to glucose was greater, both of which may be indicators of insulin resistance. Insulin resistance can lead to a cascade of health problems that are analogous to type 2 diabetes in humans; most notably, rates of lipolysis are increased and plasma NEFA concentrations are high (Schinner et al., 2005). In the present study, greater plasma NEFA concentrations were observed among the LS cattle throughout the observation period and at the start of the GTT, which may support the hypothesis that LS cows are more insulin resistant than HS cows. Although increased NEFA concentrations during the 2-wk period before calving have been shown to be a risk factor for postpartum health disorders, including displaced abomasum, ketosis, retained placenta, and metritis (e.g.,

Figure 2. Least squares means ($\pm$ SE) plasma NEFA, fecal cortisol metabolite (11,17-DOA), daily feeding time, and time to approach the feed bunk (FB) following fresh feed (FF) delivery of cows grouped into 3 categories based on their competition index (CInd) score: high-success group (HS: CInd ≥ 0.6), medium-success group (MS: $0.4 \geq$ CInd < 0.06), and low-success group (LS: CInd < 0.4).

LeBlanc et al., 2005; Ospina et al., 2010), it is unclear whether higher NEFA concentrations during the dry period (before 3 wk prepartum) also are associated with increased disease risk. In the present study, average daily NEFA concentrations observed across all CInd groups were relatively low (0.97 to 0.13 mEq/L) compared with NEFA thresholds that previous researchers have identified as being a risk factor for disease (e.g., greater than 0.3 mEq/L during the 2-wk period before calving; Ospina et al., 2010). Overstocking during the dry period and the corresponding changes in energy metabolism related to insulin resistance may set cows up for additional physiological challenges as they approach the weeks leading up to calving; this hypothesis, however, requires further investigation to explore its validity.

Insulin resistance can arise from several different factors, including plane of nutrition and stress. Increased plasma NEFA concentrations have been shown to decrease insulin sensitivity through direct FFA inter-

actions with insulin receptor proteins; these interactions lead to dysfunctional insulin signaling and, thus, impaired translocation of insulin-dependent glucose transporters to cellular membranes (Schinner et al., 2005). Individual DMI was not able to be determined in the present study and so it is uncertain whether increased NEFA concentrations in LS were a function of decreased nutrient intake. No differences were detected in average daily feeding time between cows in the 3 CInd groups or in the time it took them to approach the FB following fresh feed delivery, suggesting that all animals had equal opportunity to consume a feed that was not over sorted and, thus, of high quality. Differences in feeding rate between animals can influence total DMI when feeding time is held constant; however, previous research showed no relationship between DMI and success at displacing others in an overstocked feeding environment (Proudfoot et al., 2009).

Increased stress, leading to a rise in plasma glucocorticoid concentration, has also been linked to insulin

resistance (reviewed in Andrews and Walker, 1999). A stressor can be any situation or event that threatens or is perceived by the animal to threaten overall fitness. Glucocorticoids, including cortisol, oppose the actions of insulin so that there can be increased substrate

(NEFA) for oxidative energy metabolism and this, in turn, helps the animal respond to the stressor. A variety of pathways by which glucocorticoids can contribute to insulin resistance have been suggested such as by reducing the translocation of glucose transporter type 4 (GLUT4) to the cell surface, increasing NEFA concentrations by promoting lipolysis, or upregulating enzymes such as glucose-6-phosphate and phosphoenolpyruvate carboxykinase (PEPCK) to increase hepatic gluconeogenesis (Andrews and Walker, 1999). In the present study, no differences in the plasma cortisol response to the ACTH challenge between CInd groups were detected, suggesting that adrenal capacity for cortisol secretion following acute stimulation was unaltered. This observation could be evidence that after a 14-d period of overstocking, a level of desensitization in the physiological stress response to overcrowding occurs at the level of the adrenal gland. Friend et al. (1979) observed a greater cortisol response to an ACTH challenge when cattle were exposed to the same level of crowding at the lying stalls as used in the present study (1 stall per 2 cows); however, this challenge was performed after only 7 d of exposure to the crowded environment.

Although no differences existed between CInd groups in the cortisol response to the ACTH challenge, higher concentrations of fecal cortisol metabolites (11,17-DOA) in the LS group suggest still higher cumulative cortisol secretion in this group relative to the other 2 CInd categories. Even with no difference in the amount of cortisol secreted from the adrenal gland upon stimulation, higher average 11,17-DOA concentrations could have been achieved if the adrenal gland was stimulated to secrete cortisol more frequently. Fecal 11,17-DOA concentrations are an integrated reflection of cumulative cortisol secretion about 10 to 12 h before fecal sample collection and, therefore, are also not confounded by the stress of sample collection (Palme et al., 1999). The results of the current study suggest that it is not necessarily the act of participating in displacements at the FB that determines stress response but rather the frequency of success during those interactions. All cows in the present study were involved in competitive displacements at the FB and the average number of displacements per day was not different between the 3 categories. The observation that the MS and HS groups did not differ in physiological responses to overstocking suggests that, to some degree, cattle can cope with some failure during competitive displacements at the FB; however, for those animals that are the least successful during displacements at an overstocked FB, their 11,17-DOA profile is indicative of a greater physiological stress response.

Figure 3. The glucose, insulin, and NEFA responses curves during a glucose tolerance test of cows grouped into 3 categories based on their competition index (CInd) score: high-success group (CInd ≥ 0.6), medium-success group ($0.4 \geq \text{CInd} < 0.06$), and low-success group (CInd < 0.4). The shaded portion of the figure highlights basal analyte concentrations at $t = 15$ and 5 min before glucose infusion.

Previous work has shown that cows will sacrifice feeding time to gain additional resting time when access to both resources is limited (Metz, 1985). Behavior at the lying stalls was not measured in the current study; however, if cows did maximize their occupancy of the lying stalls throughout the day, this may have decreased stocking pressure (cow numbers) at the FB and, thus, decreased the level of competition for feed. Previous work has shown that cows are highly motivated to feed during the period following fresh feed delivery (DeVries and von Keyserlingk, 2005). Although no difference was observed in the average time cows in each CInd category took to approach the FB following fresh feed delivery, these times averaged between 45 to 90 min (range approximately 0 min to 3 h); these lag times may be a reflection of some cows showing a preference for lying rather than feeding when these resources are both limited. If overstocking the lying stalls decreased competition pressure at the FB, it is possible that the physiological response to overstocking may differ depending upon which resource is overstocked and the magnitude of the stocking rate. These hypotheses require further investigation.

The proportion of heifers in the LS group was much higher (79% of group) than in the HS group (7% of the group) and this may have also been a confounding effect in the present study. During the weeks leading up to calving, heifers have greater NEFA concentrations than multiparous cows and appear to be able to withstand higher NEFA concentrations without developing health conditions such as fatty liver relative to multiparous cows (Vandehaar et al., 1999); however, it is unclear whether these relationships also exist during the far-off period. The extent to which innate physiological differences between heifers and cows explain the observed differences in NEFA profiles of LS and HS cows is unclear; however, insulin responses during the GTT likely cannot be explained by parity alone. The average BW of cows in the LS group was over 100 kg lower than the average BW of cows in the HS group; this is almost certainly explained by the higher proportion of growing heifers in the LS group. Insulin is an important regulator of skeletal muscle protein synthesis and insulin sensitivity is reported to be higher in growing animals (Davis and Fiorotto, 2005). If the observed differences in insulin response to the GTT were attributable to only an innate difference between heifers and older cows, higher tissue sensitivity to insulin would be expected in the younger animals; this was not observed in the current study.

In conclusion, this study shows that LS cows, which are displaced frequently from the FB and are not successful at displacing others, have a different physiological profile than HS cows. Evidence of a greater physi-

ological stress response exists, as evidenced by greater average daily fecal cortisol metabolite concentrations as well as changes in energy metabolism that is suggestive of insulin resistance. This study also found that cattle could cope with some failure during competitive displacements at the FB, as no physiological differences were found between MS and HS cows. Finding ways to decrease displacement frequency, such as by using a headlock feed barrier (Huzzey et al., 2006) or feeding partitions that extend from the FB into the pen (DeVries and von Keyserlingk, 2006), might be a strategy producers can use to improve the overall wellbeing of LS cows, possibly by making it more difficult for others to displace them.

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REFERENCES

- Andrews, R. C., and B. R. Walker. 1999. Glucocorticoids and insulin resistance: Old hormones, new targets. *Clin. Sci.* 96:513–523.
- Davis, T. A., and M. L. Fiorotto. 2005. Regulation of skeletal muscle protein metabolism in growing animals. Page 53 in *Biology of Metabolism in Growing Animals*, Vol. 3. D. G. Burrin and H. J. Mersmann, ed. Elsevier, New York, NY.
- DeVries, T. J., and M. A. G. von Keyserlingk. 2005. Time of feed delivery affects the feeding and lying patterns of dairy cows. *J. Dairy Sci.* 88:625–631.
- DeVries, T. J., and M. A. G. von Keyserlingk. 2006. Feed stalls affect the social and feeding behavior of lactating dairy cows. *J. Dairy Sci.* 89:3522–3531.
- DeVries, T. J., M. A. G. von Keyserlingk, and D. M. Weary. 2004. Effect of feeding space on the inter-cow distance, aggression, and feeding behavior of free-stall housed lactating dairy cows. *J. Dairy Sci.* 87:1432–1438.
- Friend, T. H., F. C. Gwazdauskas, and C. E. Polan. 1979. Change in adrenal response from free stall competition. *J. Dairy Sci.* 62:768–771.
- Galindo, F., and D. M. Broom. 2000. The relationships between social behavior of dairy cows and the occurrence of lameness in three herds. *Res. Vet. Sci.* 69:75–79.
- Huzzey, J. M., T. J. DeVries, P. Valois, and M. A. G. von Keyserlingk. 2006. Stocking density and feed barrier design affect the feeding and social behavior of dairy cattle. *J. Dairy Sci.* 89:126–133.
- LeBlanc, S. J., K. E. Leslie, and T. F. Duffield. 2005. Metabolic predictors of displaced abomasum in dairy cattle. *J. Dairy Sci.* 88:159–170.
- Mendl, M., A. J. Zanella, and D. M. Broom. 1992. Physiological and reproductive correlates of behavioural strategies in female domestic pigs. *Anim. Behav.* 44:1107–1121.

- Metz, J. H. M. 1985. The reaction of cows to a short-term deprivation of lying. *Appl. Anim. Behav. Sci.* 13:301–307.
- NFACC (National Farm Animal Care Council). 2009. Code of Practice for the Care and Handling of Dairy Cattle. National Farm Animal Care Council, Lacombe, AB, Canada. Accessed Aug. 30, 2011. <http://www.nfacc.ca/codes-of-practice/dairy-cattle>.
- Ospina, P. A., D. V. Nydam, T. Stokol, and T. R. Overton. 2010. Evaluation of nonesterified fatty acids and β -hydroxybutyrate in transition dairy cattle in the northeastern United States: Critical thresholds for prediction of clinical diseases. *J. Dairy Sci.* 93:546–554.
- Palme, R., and E. Möstl. 1997. Measurement of cortisol metabolites in faeces of sheep as a parameter of cortisol concentration in blood. *Int. J. Mammal. Biol.* 62(Suppl. II):192–197.
- Palme, R., C. Robia, S. Messmann, J. Hofer, and E. Möstl. 1999. Measurement of faecal cortisol metabolites in ruminants: A non-invasive parameter of adrenocortical function. *Wien. Tierarztl. Monatsschr.* 86:237–241.
- Proudfoot, K. L., D. M. Veira, D. M. Weary, and M. A. G. von Keyserlingk. 2009. Competition at the feed bunk changes the feeding, standing, and social behavior of transition dairy cows. *J. Dairy Sci.* 92:3116–3123.
- SAS Institute. 2009. SAS and STAT User's Guide, Release 9.2. SAS Institute Inc., Cary, NC.
- Schinner, S., W. A. Scherbaum, S. R. Bornstein, and A. Barthel. 2005. Molecular mechanisms of insulin resistance. *Diabet. Med.* 22:674–682.
- Val-Laillet, D., D. M. Veira, and M. A. G. von Keyserlingk. 2008. Short communication: Dominance in free-stall-housed dairy cattle is dependent upon resource. *J. Dairy Sci.* 91:3922–3926.
- Vandehaar, M. J., G. Yousif, B. K. Sharma, T. H. Herdt, R. S. Emery, M. S. Allen, and J. S. Liesman. 1999. Effect of energy and protein density of prepartum diets on fat and protein metabolism of dairy cattle in the periparturient period. *J. Dairy Sci.* 82:1282–1295.