THE EFFECTS OF DIETARY FORAGE, SOCIAL HIERARCHY, AND STOCKING DENSITY ON STRESS IN LACTATING COWS DURING RELOCATION

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(ABSTRACT)

The objective of these studies was to determine the effects of forage fiber, social hierarchy, and stocking density on stress in lactating cows during relocation to new dairy facilities. In experiment one, 23 lactating cows were fed a basal ration, or the basal ration plus grass or alfalfa hay at 10% of DM offered from 3 wk pre-move to 9 wk post-move. In experiment two, 17 lactating cows were housed together before and after relocation and evaluated for dominance rank. In experiment three, 44 cows were housed together before relocation, then in pens of varying stocking density (0.67, 0.83, 1.0, or 1.17 cows per stall) post-move. In these studies, the effects of treatment on MY, lameness, behaviors, plasma cortisol, cow cleanliness, and DMI were monitored. In experiment one, cows fed grass or alfalfa hay diets had higher plasma cortisol concentrations on the day of relocation than cows fed TMR, but there were no differences in DMI or MY. Cows fed alfalfa hay or TMR had increased lameness scores following relocation; cows fed grass hay did not have increased lameness scores. In experiment two, there were no differences in plasma cortisol or lameness scores between dominant and subordinate cows. Subordinate cows had lower MY following relocation compared to dominant cows. In experiment three, cows housed at a stocking rate of 1.17 had higher plasma cortisol than cows housed at a stocking rate of 0.67. All cows had higher lameness scores following relocation, but cows housed at a stocking rate of 0.67 tended to have higher lameness scores than cows housed at stocking rates of 0.83, 1.0, and 1.17. In summary, some management practices may decrease the negative effects of stress on lactating cows during relocation.

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REVIEW OF LITERATURE

1.1 INTRODUCTION

Animal welfare is a major concern faced by dairy producers today. Stress is the result of physical, physiologic, or emotional factors that induce an alteration in the animal's homeostasis or adaptive state (Kitchen et al., 1987). The changes that occur within the animal often suppress the immune system leading to a state of disease (Moberg, 1987). Multiple criteria, such as behavior, health, productivity, physiological and biochemical characteristics, and reproductive ability, can be used by the producer to assess welfare or well-being in cattle (Albright, 1987). Critical measures for evaluating the welfare of cattle include plasma cortisol, lameness, dry matter intake (DMI), abnormal behaviors or stereotypies, milk yield (MY), parlor behaviors, and cleanliness (Craig et al., 1986; Hultgren and Bergsten, 2001; Paranhos da Costa and Broom, 2001; Rushen, 2003). Diet, management, environment, and individual animal variation affect an animal's response to stressors (Moberg, 1987).

1.2 CRITICAL MEASURES

1.2.1 GENERAL BEHAVIORS AND STEREOTYPIES

The average dairy cow spends approximately 12 h/d lying down, 4.5 h/d feeding, 2.3 h/d standing and drinking in the alleys, 2.6-3.2 h/d milking, and 1.8-3.4 h/d standing in the stall (Cook et al., 2004). A study by Tucker and Weary (2004) found cows spent more time lying down in free stalls (1.2 x 2.7 m) with mattresses covered with 7.5 kg of sawdust compared to mattresses with no sawdust covering or covered with 1 kg of sawdust. Lame cows tend to spend a greater amount of time lying down (Hassell et al., 1993) and have a greater number of abnormal lying and sitting positions than non-lame cows (Singh et al., 1993). Lame cows chose stalls closer to the parlor when returning from milking compared to non-lame cows (Juarez et al., 2003).

Aland et al. (2002) found that cows have a significantly greater number of defecations per day than urinations. They found no correlation between number of eliminations per day and milk production or stage of lactation, but increased eliminations have been associated with stress in the milking parlor (Hemsworth et al., 2002).

Effect of management on general behaviors and stereotypies

Increased aggression and stereotypies such as tongue-rolling, head shaking, or vocalizations are used as indicators of frustration and decreased animal welfare. Sandem et al. (2002) found an increased occurrence of stereotypies in food deprived animals when compared to those animals that had access to feed. When animals are able to spend more time eating there is a decrease in stereotypies (Lindstrom and Redbo, 2000).

Animal density must be considered when determining well-being. When cows are overstocked, there may be changes in milk production, stress levels, and overall animal behavior. Cows that are unable to lie down spend an increased amount of time eating, idling, grooming and leaning. These responses are most likely displacement behaviors due to frustration stemming from deprivation of lying time. Cows experiencing social isolation increase self-grooming and abnormal behaviors, such as leaning and oral manipulations (Munksgaard and Simonsen, 1996).

When cows are handled with positive tactile interactions rather than negative tactile interactions, the flight distance to normal human interactions has been shown to decrease (Hemsworth et al., 2002). Stockpeople found more favorable responses from the animals when they spoke in gentler voices and reduced their harshness when moving cattle. Hemsworth et al. (2002) also found that cows handled positively had fewer defecations in the parlor than cows handled negatively. When cows are handled with either loud metal clanging of gates or loud shouting they were found to have higher heart rates than cows handled in a quieter setting, showing that they had an increased level of fear (Waynert et al., 1999). In general, cows are more likely to respond to stockpeople who avoid sudden movements, talk quietly, and touch animals on first approach (Rushen et al., 1999a).

Effect of social hierarchy on general behaviors and stereotypies

Social hierarchies in cattle can be determined by the number of wins or losses during aggressive interactions involving food competition (Phillips and Rind, 2002). Animals can either be paired for competition (Houpt and Wolski, 1980) or observed in barns or pastures (Phillips and Rind, 2002). Aggressive interactions include "forceful behavior", where the dominant animal swings its head in the direction of the subordinate animal, "bunting"; where the dominant animal uses its head to displace the subordinate animal; or "pushes", where the dominant animal uses its body rather than its head to displace the subordinate animal (Phillips and Rind, 2002).

Social hierarchy can determine which free stalls an animal occupies and its ability to hold a place at the feed bunk. Dominant cows occupied preferred free stalls at the barn entrance, while submissive cows occupied the less desirable innermost free stalls. Friend and Polan (1974) observed that most aggressive interactions occurred when cows returned from milking and when hay was offered. Dominant animals gained access to feed over submissive cows when limited feeding space was available. They concluded that dairy cattle offered feed in a competitive situation should be fed one complete ration to ensure the same nutrition is received by each animal.

1.2.2 CORTISOL

Cortisol is a glucocorticoid produced by the adrenal cortex. Glucocorticoids increase blood glucose concentration and aid the body in resisting stressors (Marieb, 2001). The release of glucocorticoids from the adrenal cortex is stimulated by adrenocorticotropic hormone (ACTH). Serum or plasma cortisol is frequently measured as an indicator of stress in animals. Negrao et al. (2004) found that saliva can also be used as a reliable indicator of cortisol concentration. Adrenal response testing (ART) can also be used to measure an animal's stress level. During ART, a cow is challenged with an injection of ACTH, and blood samples are collected and analyzed to determine glucocorticoid response (Friend et al., 1977a).

Plasma cortisol concentrations can be affected by diet, transport and handling, crowding, isolation, and individual animal variation (Willett and Erb, 1972; Friend et al., 1979; Galyean et al., 1981; Markus et al., 1998). An increase in plasma cortisol concentration is equated with an increase in animal stress (Craig et al., 1986) and therefore, decreased animal welfare. Plasma cortisol concentration cannot be used as a sole determinant of welfare, however, because of individual animal variation (Willett and Erb, 1972). Increased blood glucocorticoid concentration for a prolonged period of time can have a detrimental effect on an animal's immune (McEwen et al., 1997) and reproductive systems (Dobson and Smith, 1995). Varner et al. (1983) found that serum cortisol concentrations were increased for 3 d following herd relocation. Dairymen have reported an increase in health and reproductive problems after herd relocation (Varner et al., 1983).

There are diurnal plasma cortisol patterns in cattle, with lowest concentrations in the dark period and highest concentrations in the light period (Trenkle, 1978). Wagner and Oxenreider (1972) observed that mean plasma cortisol concentrations were significantly higher at 1600 h than at midnight.

Changes in temperature can lead to changes in plasma cortisol concentrations in cattle. Thompson et al. (1963) observed that cattle accustomed to temperatures of $3^{o}C$ to $18^{o}C$, then exposed to environmental temperatures of $24^{o}C$ to $35^{o}C$ experienced an increase in plasma cortisol. Cattle exposed to an acute raise in temperature showed the same increase in plasma cortisol concentrations (Christison and Johnson, 1972). Cattle under long term exposure to high temperatures have lower plasma cortisol concentrations than normal (Alvarez and Johnson, 1973).

Effect of nutrient consumption on plasma cortisol

Changing macronutrient composition in the diet alters responses to stressful situations. Humans that ate a diet rich in carbohydrates and low in protein had lower plasma cortisol concentrations following a stressful task than humans that ate a diet low in carbohydrates and high in protein (Markus et al., 1998). The increased carbohydrates led to an increase in tryptophan. Tryptophan is a precursor to serotonin synthesis in the brain, which is correlated with increased mood and performance ability (Markus et al., 1998). Similar to this human study, Tannenbaum et al. (1997) observed that by increasing the amount of fat in the diet of rats, ACTH and exogenous corticosterone increased with increased stress. Plasma glucocorticoids (cortisol and corticosterone) were elevated in Holstein heifers fed a high concentrate diet when compared with Holstein heifers fed a control ration low in concentrate (Mills and Jenny, 1979). Mills and Jenny (1979) concluded that the increased plasma glucocorticoids during high concentrate feeding most likely reflected an adaptation to the high concentrate feeding, so that the liver could better handle the influx of rumen metabolites.

Plasma glucocorticoid concentrations increase when feed intake is reduced in ruminants, especially during times of fasting, and when there is a greater demand for nutrients, such as during peak lactation (Trenkle, 1978). Plasma cortisol concentrations in calves are also generally higher during times of hunger because of an increase in corticotrophin-releasing factor (Gardy-Godillot et al., 1989). Cows introduced to a novel food after 12 h food deprivation showed no change in plasma cortisol concentration compared with cows not food deprived (Herskin et al., 2003). When dairy cows were fasted for 72 h there was an increased level of plasma glucocorticoids (Mills and Jenny, 1979; Herskin et al., 2003). No elevation in plasma cortisol was found when feedlot steers were fasted for 32 (Galyean et al., 1981) or 72 h (van der Walt et al., 1992). Galyean et al. (1981) had a wide spread of sampling times and van der Walt et al.

(1992) chose animals based on their docility, which could explain the lack of effect of fasting on plasma cortisol concentrations in these two studies.

A postprandial decrease in plasma cortisol concentration has been found in calves (Gardy-Godillot et al., 1989). Calves fed uncurdled milk had lower daily mean cortisol concentrations than calves fed curdled milk. Uncurdled milk leads to a higher rate of gastric emptying, increased feeling of satiety, and increased absorption of nutrients (Gardy-Godillot et al., 1989).

Effect of handling and transport on plasma cortisol

Improvements in a dairy facility that lead to better handling may create less stress on the animals. Restraint for jugular venipuncture led to an increase in plasma cortisol concentrations in sheep; the increase was less if the samples were collected while the animal was in familiar surroundings (Bassett and Hinks, 1969). Newer dairy facilities often have headlocks located in front of the feed bunk, making restraint less stressful than in facilities in which cows must be walked to an outside loading chute or pen for blood collection or other treatments. Animal care standards generally indicate that jugular venipuncture is an acceptable method for blood collection if it is completed within 60 s of restraint and if it is used to obtain a single sample (Trenkle, 1978).

In addition to handling, transporting animals may also cause an increase in stress. Warriss et al. (1995) observed that steers exposed to loading and transport had increased plasma cortisol concentrations during loading and the first part of the transport. Cortisol returned to normal concentrations before the transport was complete. In contrast, no increases in serum cortisol concentration were found in steers exposed to handling and loading followed by 32 h of transport on state highways when compared with a control group of steers that were not transported (Galyean et al., 1981). Differences in the results of the studies are most likely the result of sampling times used. In the Galyean et al. (1981) study the steers had 32 h to acclimate to transport and any differences in serum cortisol were probably undetected due to timing of samplings, since the first blood sample was not collected until 18 h after the start of transport. Increases in plasma cortisol concentrations were similar whether cows were walked or hauled to new facilities (Varner et al., 1983). There was no change in plasma ACTH concentration when animals were transported for only 30 min, showing that loading animals onto a trailer is not a major source of stress (Dixit et al., 2001).

Effect of management and crowding on plasma cortisol

Management practices, such as overcrowding or social isolation, can lead to increased stress in dairy cattle. Glucocorticoid response to ACTH during ART was found to increase with increased crowding of animals (Friend et al., 1979). Increasing animal density leads to a decrease in the amount of lying time per day (Friend et al., 1977b). Cows need to have the ability to lie down for extended periods of time to allow optimal rumination conditions and, therefore, increased production and well-being. It has been found that if stocking rate is increased to allow only 0.33 stalls per cow, lying time is reduced by more than half (Friend et al., 1977b).

Krohn and Konggaard (1987) found that more animals per pen lead to an increase in aggressiveness. Housing can be taken to the opposite extreme by housing animals in isolation. An increase in plasma cortisol concentration was found when heifers were kept in social isolation (Willett and Erb, 1972). When housing animals, they should be housed with other animals and stocking rates should allow cows the opportunity to lie down frequently so that stress levels are not increased.

1.2.3 LAMENESS

Lameness is the third most significant health related economic loss facing the dairy industry, making it an economic as well as an animal welfare concern (Juarez et al., 2003). Lameness leads to a decrease in reproductive ability and increased culling rate (Sprecher et al., 1997). High producing dairy cattle are at risk of lameness due to the high metabolic stresses placed on their bodies (Warnick et al., 2001). Lameness is more likely to occur in early lactation cows than in late lactation cows and in multiparous cows than in primiparous cows (Warnick et al., 2001). Animal behaviors such as decreased lying, increased standing on hard surfaces, or standing with the two front feet on the free stall platform and the two rear feet in the alley all lead to a higher lameness score (Galindo and Broom, 2000).

Lameness in cattle can be quantified by using a 1-5 scoring system, where 1 = normal, 2 = mildly lame, 3 = moderately lame, 4 = lame, and 5 = severely lame (Sprecher et al., 1997). Scores are assigned by observing the cow’s gait and back posture, without requiring the animal to be observed in transition from a lying to standing position as was necessary in previous scoring systems (Manson and Leaver, 1988). Lameness scores should be assessed on the entire lactating herd, while the cows are walking on a solid, flat, non-slip, and well-lit surface

(Nordlund et al., 2004).

Lameness in dairy cows can result from several different foot problems such as ***hard*** and ***soft feet, foot rot, heel erosions, digital dermatitis, laminitis, sole ulcers,*** and ***claw lesions*** (Ishler et. al, 1999). ***Soft feet*** normally occur in free stalls where cows are standing in manure and urine, while ***hard feet*** occur in stall-barns where sawdust is used for bedding. Both conditions can lead to cracks in the hoof leading to infections or abscesses. ***Foot rot*** is an infection between the claws of the foot caused by the bacterium, *Fusiformis necrophorus*. Cows with foot rot generally exhibit lameness in only one leg. ***Heel erosion*** usually starts at the bulb of the heel as pits on the surfaces develop into grooves that are susceptible to bacterial infection. The grooves cause the horn to separate and allow a new sole to develop with material packed between the layers. Heel erosion is usually found in the hind claws of dairy cows housed in wet, dirty lots. ***Digital dermatitis***, also known as heel warts, strawberry foot disease, digital papillomatosis, and Mortellaro disease, results in lesions on the foot. It is usually seen in primiparous cows leading to acute lameness and excessive lying time (Ishler et al., 1999).

Laminitis, or pododermatitis aseptic diffusa, is an inflammation of the dermal layers of the foot (Nocek, 1997). Laminitis leads to long, overgrown feet or toes with hemorrhages in the soles and walls of the feet. Foreign material enters the hoof through cracks where the wall and sole have separated causing infections, abscesses, or ulcers. The highest incidence of laminitis occurs during the first 100 d postpartum (Ishler et al., 1999). ***Sole ulcers*** are open sores located on the inside of the lateral claw, and are usually associated with laminitis. Laminitis should be suspected in the herd if more than 10% of the cows have been diagnosed with sole ulcers. Other causes of sole ulcers can include standing in moisture and manure, excessive wear, and poor hoof trimming.

Hernandez et al. (2001) found that 59% of lame cows observed had ***claw lesions***. Cows housed in environments that lead to softer claws are more susceptible to developing claw lesions than animals with hard claws (Borderas et al., 2004). In a smaller study, Galindo et al. (2000) found that 94% of lameness events involved hind foot claws. Lame cows with claw lesions were 0.52 times as likely to conceive, average time to conception was 40 days longer, and number of breedings per conception was higher than for non-lame cows (Hernandez et al., 2001). In a survey conducted by Russell and Hino (1985), approximately 62% of claw lesions could be associated with laminitis. Sprecher et al. (1997) found cows having a lameness score of > 2 had longer days open and were 8.4 times more likely to be culled than cows with a lameness score of = 2. Nordlund et al. (2004) state that if greater than 15% of the herd is identified as lame, the cause should be determined. Nutrition, housing and environment, and management each impact the lameness score in cattle; their effects are reviewed below.

Effect of diet on lameness

Nutritional management is considered a leading cause of laminitis in dairy cattle. Bovine acidosis occurs when there is a large increase in lactic acid in the rumen, resulting in increased VFA production and decreased ruminal pH. Diets high in carbohydrates, or forages low in effective fiber, or both lead to acidosis (Nocek, 1997). The highest incidence of acidosis in dairy cows occurs during the first 30 d postpartum and becomes virtually nonexistent 90 d postpartum (Grohn and Bruss, 1990).

Subacute ruminal acidosis (SARA) is more difficult to diagnose than acute acidosis because of its nonspecific symptoms (Nocek, 1997). Transition animals, high DMI animals, and animals offered highly variable rations and meal patterns, or poorly formulated diets are at risk of developing SARA (Stone, 2004). Decreased or irregular DMI in feedlot cattle is a reliable indicator of SARA (Owens et al., 1998). However, feeding protocols of most dairies make changes in the DMI of an individual cow difficult to document.

Four mechanistic phases lead up to acute, subclinical, or chronic laminitis. The drop in ruminal pH associated with acute acidosis (< 5.0) or SARA (< 5.5) is critical in phase one of the development of laminitis. Phase two results in local vascular damage, which leads to a decrease in nutrients and oxygen reaching the epidermal cells. During phase three, the epidermis breaks down due to the lack of nutrients and oxygen leading to corium degeneration and breakdown of the laminar region. The bone support structure breaks down during phase four leading to double sole, hemorrhage, ulcers, and diffuse lesions. Phase four results in acute, subclinical, or chronic laminitis (Nocek, 1997).

Effect of housing and environment on lameness

While nutrition is most frequently thought of as the cause of lameness in cattle, environmental factors also have an impact. The type of flooring used in a dairy facility greatly impacts a cow's hoof health and lameness score. Concrete flooring with a rough surface is frequently used, but after the surface is worn smooth it becomes slippery. Cows are able to take more confident steps on rougher surfaces, for example those with aggregate, because of a lower chance of slipping. Surfaces with aggregates may be used in areas where there is an increased risk of slipping, such as near the water area. If a surface-applied bauxite aggregate is used on the floor, the mean aggregate diameter should be 0.5-1.2 mm (Phillips et al., 1999). The coefficient of friction (?) of floors can be obtained by using the methods of Phillips et al. (1998, 1999), which use a weighted platform with four hooves beneath it to simulate a cow walking. Floors with a coefficient of friction less than 0.4 are considered to be low friction floors (Phillips and Morris, 2001). Slip frequency increases as the coefficient of friction decreases. Phillips and Morris (2001) found that the coefficient of friction for cattle floors is optimal between 0.4 and 0.5.

Differences in free stall surface and changes in ambient temperature can also impact lameness. Free stall surface types include sawdust, sand, rubber mats, or mattresses filled with rubber crumbs, air pockets, foam, or water. When comparing sand based free stalls with mattresses filled with rubber crumbs, cows housed in the sand based free stalls were significantly less lame. Cows housed on mattresses spent a greater amount of time standing per day than cows on the sand surface (Cook et al., 2004). As ambient temperatures increase, the amount of time cows spend standing in a day is increased (Schultz, 1984; Overton et al., 2002). The location of fans should be considered when planning a free stall barn, so animals are not forced to stand in alleyways to dissipate heat.

Effect of management on lameness

The frequency at which alleyways are cleaned can impact the cow's locomotion and can become a welfare concern. When alleyways are covered in slurry, walking speed is decreased and the walking pattern of cows is changed from that of cows walking on wet or dry concrete (Phillips and Morris, 2000). Cows walking on the slurry covered alleyways kept their legs less vertical at the beginning of a step and more vertical at the end of a step than cows walking on dry concrete. The amount of time a cow spends standing each day has an impact on lameness. The time cows are in the holding area before milking should be kept to a minimum. To determine the total time cows spend away from the free stalls per milking, the time the first cow leaves to the time the last cow returns should be recorded (Nordlund et al., 2004).

Proper hoof care and trimming techniques are essential for maintaining a herd's hoof health. The ideal times for hoof trimming are once at drying-off and again at 100 DIM (Ishler et al., 1999). Cows that appear lame between regularly scheduled trimmings should be looked at on an individual basis.

Effect of social status on lameness

Galindo et al. (2000) found that cows spending more time standing halfway in a free stall were more lame than cows that stood with their whole body either in the alley or in the free stall. The same study also showed that the cows standing halfway in the free stall were cows of lower social ranking than cows that stood in the alley or free stall. Social status of cows plays an important role in stall usage and amount of time spent lying or standing each day.

Lameness Summary

In order to reduce lameness in a herd, all possible causes of lameness must be investigated before forming a management strategy. A well-formulated diet is a good starting point, but is not the only consideration to be made. Ideally cows should be able to lie down for 10 to 14 h a day. Stall size, availability, and bedding should be optimized to ensure cows can meet this requirement. A regular hoof trimming schedule should be maintained to decrease loss of productivity due to lameness.

1.2.4 DRY MATTER INTAKE

Changes in DMI are an indication of health problems such as SARA, which can in turn lead to additional concerns such as laminitis (Owens et al., 1998; Ishler et al., 1999). Both health problems can greatly impact production leading to significant economic losses when considered across the entire herd. Fiber content of a diet is often increased during times of stress, to avoid occurrences of acidosis (Ishler et al., 1999).

Effect of forage fiber on intake and chewing

Changing the fiber content or form in a ruminant diet can affect DMI, time spent eating, saliva output, milk yield, rumination time, and pH. Adding long hay to the diet has been shown to increase the time a cow spends ruminating (Beauchemin and Buchanan-Smith, 1989). The same study found that increasing the amount of fiber in the diet led to increased total chewing time. The greatest changes were noted when the amount of fiber in the diet was increased from 17% ADF (acid detergent fiber) to 21% ADF. Benefits from long hay seem to be the greatest when the hay is fed one hour prior to feeding concentrates.

Increased time spent chewing has beneficial effects in dairy cows. By increasing chewing time, saliva output increases. Higher saliva production helps maintain rumen pH. A low rumen pH can result in reduced activity or number of cellulolytic microorganisms, or both (Russell and Hino, 1985). Increased chewing leads to an increased buffering capacity during rumination and increased cellulose breakdown (Beauchemin and Buchanan-Smith, 1989).

Cubing silage and hay leads to a reduction in rumination time and BW (Beauchemin et al., 1997). The decreased time spent ruminating is most likely caused by the easier breakdown of the fiber found in cubing rather than in longer forms. The rumen receives less stimulation from the reduced particle size. Thus, the fiber in the cubed hay and silage is most likely less effective than other forms.

Galyean et al. (1999) found that when calves are stressed they are less likely to eat low energy/high roughage diets than high energy/low roughage diets. As the proportion of dietary concentrate fed to calves increases so does sickness (Lofgreen, 1983). Sickness and death were lowest in calves fed an all hay diet during stressful periods, but at the cost of poor performance. Lofgreen (1983) concluded that the best compromise is to offer free choice hay as a supplement.

Effect of management on dry matter intake and chewing

Producers should consider the style of feed bunk that is being used for feeding cattle. Traditional feed bunks that are raised off the ground do not allow cows to eat in a natural position. When cows are fed at the fenceline their heads are in a natural grazing position, which increases salivary flow by as much as 17% (Albright, 1993).

Feed accessibility is often as important as the nutrients offered in the feed. Friend et al. (1974) found that feed intake did not decrease until less than 0.2 m of feed bunk space was available per cow. Feed intake is higher when cows are housed in groups rather than in isolation because of their social nature (Albright, 1993).

Devries and Von Keyserlingk (2005) found that if cows are offered fresh feed as they return from the milking parlor, they spend 26% more time eating in the hour after returning than cows not offered fresh feed. The same study found that if fresh feed was offered approximately 6 h after the cows returned from milking, they spent 82% more time eating in the hour after the feed was delivered than cows not offered fresh feed at the same time. Feeding additional feed 6 h after the cows returned from milking resulted in a total increase in eating time of 12.5% per day for those cows. This shows that the delivery of fresh feed has a greater impact on a cow's eating behavior than timing of feed offered (i.e., returning from the milking parlor).

1.2.5 MILK YIELD

Increases in stress, fear, or handling difficulty can all lead to a decrease in milk production (Rushen et al., 1999a,b). Mean milk yields for cows relocated into a new dairy facility were lower at the first milking, but returned to normal production levels at subsequent milkings (Varner et al., 1983).

Effect of forage fiber on milk yield

Beauchemin and Buchanan-Smith (1989) found that by substituting long hay for 15% of alfalfa silage dry matter fed, milk yield was increased by 4%. This was in disagreement with another study, which found no change in milk yield when long hay was substituted for alfalfa silage (Fischer et. al., 1994). Another study found that changing only silage length and not fiber content of the diet had no effect on milk yield (Kononoff et al., 2003). Cubing hay and silage decreased milk yield by 4% (Beauchemin et al., 1997). Various milk production responses have been noted with the addition of different types of forage or fiber length to diets, but a clear, best method has not been determined.

Effect of handling and management on milk yield

Human-animal interactions greatly impact the productivity of lactating dairy cows. In a study by Hemsworth et al. (2002), researchers measured the effects of handler behavior on milk production. Milk production of cows handled by stockpeople trained for improved handling attitude and behavior toward cows and cows handled by stockpeople with unchanged attitudes (control) were compared. The cows that were handled by stockpeople trained for improved handling had higher milk yields than cows handled by the control stockpeople.

In a study using different rearing methods of young heifers, the effects on milk yield of group housing, individual housing, and isolation were compared (Arave et al., 1985). Heifers raised in isolation from other calves had higher milk production at first calving than heifers raised in either group housing or individual housing. The heifers raised in isolation may have been better adapted to handling by stockpeople and spent less time in aggressive and social interactions with other cows once they joined the milking herd. Heifers raised in group housing had a higher number of defecations and urinations when they were separated from other cows, indicating that their stress level increased when they were no longer in a group.

The dominant cows in a group had higher milk yield than subordinate animals (Phillips and Rind, 2002). Friend and Polan (1974) found no such relationship between dominance rank and milk yield. The former study used 72 British Friesian cows which grazed on pasture, while the latter study used 21 Holstein cows housed in a conventional free stall system. The different housing and feeding methods of the two studies likely caused the conflict in results.

Juarez et al. (2003) found a decrease in milk production as lameness score increases. A possible explanation is that lame cows spend more time lying down and less time eating than non-lame cows (Galindo et al., 2000), so their nutritional requirements are not met. Green et al. (2002) found that lame cows lose approximately 350 kg of milk per 305 day lactation period compared to non-lame cows. The economic loss from this decrease in milk yield highlights the importance of proper hoof care.

Warnick et al. (2001) found that decreases in milk yield were greater in lame multiparous cows and cows with higher lameness scores when compared with lame primiparous cows and cows with less severe lameness scores. Rajala-Schultz et al. (1999) in contrast, found that lame primiparous cows had a greater milk loss than lame multiparous cows. Milk loss resulting from lameness ranged between 1.5 and 2.8 kg/d for the 2 wk following diagnosis.

1.2.6 CLEANLINESS

Cow cleanliness may have an impact on animal welfare because of its possible relationship with mastitis, as indicated by milk somatic cell count (SCC; Barkema et al., 1998). Cows with higher cleanliness scores (ie., dirtier cows) have higher SCC's than cows with lower cleanliness scores. Cleanliness can be determined using a hygiene scoring system developed by Cook (2002). The scoring system determines the level cleanliness on a four-point scale (where 1 = clean, 2 = slightly dirty, 3 = moderately dirty, and 4 = excessively dirty) in three zones; the udder, the lower legs, and the upper leg and flank zones. Cows that are given a hygiene score of 3 or 4 may have negative health effects and are considered "too dirty".

The cleanliness of cows in a herd is dependant on the housing and bedding types as well as the management of those systems. Hultgren and Bergsten (2001) found that cows kept on a rubber slatted floor that allowed feces and urine to drain between scrapings were cleaner than cows kept on a solid floor. Those cows housed in barns with rubber-slatted floors also showed fewer hoof diseases, such as foot dermatitis, heel horn erosion, and sole lesions, at the time of trimming than cows housed on solid floors. The decrease in hoof problems is most likely due to the avoidance of soft feet, which often result when cows spend most of their time standing in moist environments, allowing hooves to soak in water.

Proper management of stalls and alleyways is essential to keeping cows clean. If alleyways require scraping, it should be done regularly to prevent buildup of manure and excess bedding. It is also important to change bedding or at least add more bedding to free stalls on a regular basis. Cows housed in barns where free stalls and alleyways were not regularly maintained had much higher cleanliness scores than cows housed in barns with regular maintenance (Scott and Kelly, 1989).

Increased stocking density in free stalls with straw bedding leads to a decrease in cow cleanliness regardless of the addition of new straw (Scott and Kelly, 1989). Cows in slatted floor housing systems stocked at lower or higher rates have higher cleanliness scores than cows in slatted floor housing systems stocked at optimal rates. Slatted floor housing systems depend on a certain number of cows being present to walk the manure through the slats. When the stocking rate is too low in a pen, manure begins to build up because there is not enough cow traffic in the alleyways. In contrast, if the stocking rate is higher than optimal in slatted floor housing systems cows are unable to move around enough to clean out the alleyways.

1.2.7 PARLOR BEHAVIORS

Parlor behavior can be measured as parlor entry order, latency to enter the parlor, time spent fitting the milking cluster, cow reactivity during milking and number of urinations and defecations in the parlor. Cow reactivity is assessed during the pre-milking udder preparation using a 0-3 scale, where 0 = very quiet in the milking parlor, no leg movements, 1 = stands quietly, some slight leg movements, 2 = continual leg movements which are occasionally vigorous, but does not kick, and 3 = continual vigorous leg movements or kicking (Paranhos da Costa and Broom, 2001).

Lame cows tended to have a longer latency to enter the parlor and had more leg and feet movement while in the parlor than non-lame cows (Hassell et al., 1993). Lame cows took longer to return to the free stalls following milking than non-lame cows (Juarez et al., 2003). Phillips and Rind (2002) found that dominant cows were more likely to enter the parlor ahead of subordinate cows at milking.

Ceballos and Weary (2002) found that offering cows a small amount of concentrate as they entered the parlor reduced their time to enter the parlor. When two groups of cows were offered 0.3 or 0.6 kg of concentrate in the parlor and one group was given no concentrate, the cows offered concentrate moved ahead of those cows not receiving concentrate when entering the parlor. No difference was found in parlor behavior when the amount of concentrate offered was changed (0.3 or 0.6 kg). There was no significant change in numbers of defecations or urinations between groups of cows that received concentrate and those that did not. Both control cows and treatment cows required less pushing to move them into the parlor when treatment cows were offered concentrate in the parlor than when no concentrate was offered to any of the cows.

A cow's behavior in the milking parlor can be significantly affected by the temperament of the handler or the individual animal. Rushen et al. (1999a) found that cows had significantly increased movements per minute during milking when milked by an aversive handler rather than a gentle handler. Heart rate increased significantly when cows were handled by either an aversive or gentle handler in the milking parlor, but the increase was greater in cows handled by the aversive handler.

1.4 SUMMARY

An animal's age, gender, previous experience, and individual variation make it impossible to find one universal measure that is indicative of stress across all animals. For this reason, multiple measures must be taken when determining the level of stress and well-being in a group of animals. Typical indicators used to determine stress or welfare include heart rate, respiration rate, hormone levels, animal production, and changes in normal animal behavior. Diet, social hierarchy, and stocking density can all affect an animal's state of stress and welfare.

It is desirable for the producer to decrease the amount of stress with which an animal is faced. Improvements in handling and facilities can decrease the amount of stress that an animal feels. When management and facilities are improved, an increased level of production can be achieved. Handling and restraint times can be decreased with management and facility improvements resulting in less stress for the animal. Improving animal welfare on a farm can have economic benefits to the producer by reducing lameness and sickness and increasing milk production and reproductive abilities.

The objectives of this study were to determine the effects of diet, social hierarchy, and stocking density on lactating cows during relocation. The first hypothesis was that cows offered increased forage fiber would adapt to the new facilities with greater ease than cows offered the control diet. The second hypothesis was that cows classified as “dominant” would adapt to the new facilities with greater ease than cows classified as “subordinate”. The third hypothesis was that cows stocked at lower stocking rates (0.67) would adapt to the new facilities with greater ease than cows stocked at higher stocking rates (1.17). The adaptation of the cows was determined by the assessment of the critical measures.

CHAPTER 2

THE EFFECTS OF DIETARY FORAGE ON STRESS IN LACTATING COWS DURING RELOCATION

ABSTRACT

The objective of this study was to determine the effects of dietary forage amount and source on stress in lactating dairy cows during relocation to a new dairy facility. Twenty-three lactating Holsteins were assigned to one of three dietary treatments from 3 wk prior to relocation to 9 wk following the move. The three treatments were a basal total mixed ration (TMR) consisting of alfalfa and corn silage, ground corn and barley, soybean meal, and pressed brewer's grains (n=8); the TMR plus orchard grass hay as 10% of the DM offered (n=8); and the TMR plus alfalfa hay as 10% of DM offered (n=7). All cows were fed in Calan doors twice daily pre and post move. Plasma cortisol concentrations, lameness scores, DMI, and milk yield (MY) were monitored. Blood samples were collected between 1400 and 1600 h on d -7, -1, 0, 1, 2, 7, 14, and 21 relative to relocation. Lameness scores were assigned weekly between d -21 and 63. Dry matter intake and MY data were collected daily. Prior to relocation, cows were housed as one group in a barn with 31 free stalls (1.07 x 2.13 m) and milked twice daily in a six stall side-open parlor. Following relocation, cows were housed as one group in a barn with 24 free stalls (1.17 x 2.29 m) and milked twice daily in a double-eight herringbone parlor. Lameness scores, DMI, and MY data were analyzed in three periods; PD1 = pre-move (3 wk), PD2 = wk 1-4 following relocation, and PD3 = wk 5-9. The effects of diet, period and their interaction were evaluated with Proc MIXED of SAS. The interaction of diet by period was significant ($P < 0.01$) for plasma cortisol, lameness score, and DMI. Plasma cortisol concentrations were affected by diet on the day of relocation only. On that day, plasma cortisol was lower in cows offered the TMR than cows offered the orchard grass hay or alfalfa hay diets (6.7, 12.1, and 12.7 ng/mL). Lameness scores increased following relocation for cows on the TMR (PD1 = 1.5, PD2 = 1.6, PD3 = 2.0) and alfalfa hay (PD1 = 1.5, PD2 = 1.7, PD3 = 2.6) diets, but did not change in cows fed grass hay (PD1 = 1.5, PD2 = 1.6, PD3 = 1.8). Cows offered grass hay had lower DMI than cows offered alfalfa hay ($P < 0.03$) during PD1. No interaction of diet and period was observed

for MY. Offering lactating cows orchard grass hay during relocation may decrease lameness while cows are adapting to a new facility, but offering alfalfa hay did not improve production or measures of well-being.

Key words: relocation, plasma cortisol, lactating dairy cows

2.1 INTRODUCTION

Diet can impact an animal's response to stressful situations, influencing plasma cortisol, feed intake, MY, and lameness. Tannenbaum et al. (1997) found that rats fed a high fat diet (20% fat) had higher plasma cortisol concentrations when restrained for 120 min than rats fed a control diet (4% fat). Both groups of rats showed the same initial increases in plasma cortisol, but after 60 min the control group became acclimated to restraint while the rats fed high fat diets continued to increase in their plasma cortisol response. Humans consuming a diet high in protein and low in carbohydrates had higher plasma cortisol concentrations when faced with a stressful task than humans consuming a diet high in carbohydrates and low in protein (Markus et al., 1998).

Changing the amount of fiber in a cow's diet can affect chewing and rumination rates and milk yield. As NDF content of the diet was increased from 26 to 30 or 34%, the amount of time a cows spent chewing each day increased. An increase in chewing time leads to an increase in salivary output (Beauchemin and Buchanan-Smith, 1989). The increased salivary output results in greater ruminal buffering and improved ruminal pH maintenance. They also found that as dietary NDF increased, the total time cows spent ruminating each day was increased. Increased rumination time is also an indicator of improved ruminal health. Milk yield decreased linearly, however, as NDF in the cows' diets increased. The improved ruminal health implied by the increased chewing and rumination was offset by decreased energy intake on the high fiber diets.

Maintaining optimal rumen pH is one way to reduce the risk of lameness in a herd. If rumen pH drops to 5.6 or below, subacute or acute acidosis will result. The low pH allows endotoxins normally contained in the rumen to cross the ruminal epithelium and enter the body (Stone, 2004). The endotoxins lead to decreased circulation and can result in laminitis. Increasing NDF in the diet, decreasing dietary variations, and keeping meal patterns constant can reduce the chance of both ruminal acidosis and laminitis. Forage NDF is commonly increased by 1 to 3% at freshening in an attempt to minimize the incidence of SARA (Stone, 2004). The same increase can also be made if the animals may be exposed to a stressful situation, such as relocation.

Many studies have been carried out to determine the effects of dietary fiber on lactating dairy cows, but none have evaluated effects on cows during relocation to new facilities. The objective of this study was to determine the effects of dietary forage and amount on stress in lactating cows during relocation to a new dairy facility.

2.2 MATERIALS AND METHODS

Cows and Diets

Twenty-three lactating Holstein cows (6 primiparous; 175 DIM at the start of the study) were used to determine dietary effects on stress during relocation to new dairy facilities. Cows were assigned to groups balanced for parity and DIM, and assigned to one of three dietary treatments. Dietary treatments were imposed from 3 wk prior to relocation through 9 wk following the move. The three treatments consisted of a basal total mixed ration (TMR) consisting of alfalfa and corn silage, ground corn and barley, soybean meal, pressed brewer's grains, and minerals (Control; n=8); the TMR plus orchard grass hay as 10% of the DM offered (Grass; n=8); and the TMR plus alfalfa hay as 10% of the DM offered (Alfalfa; n=7). Dietary ingredient and nutrient composition are presented in Tables 1 and 2. All cows were fed in Calan doors throughout the study. Relocation did require cows to adapt to new Calan doors, but the cows were assigned to doors in the same order in the new facilities, and adaptation was rapid (< 6 h for most cows, < 24 h for all).

Cows were fed twice daily at 0900 h and 1900 h. All cows were fed two-thirds of the daily TMR portion at the morning feeding. The total daily portion of alfalfa or grass hay was placed on top of the morning TMR for those cows on hay supplemented diets. Cows received the remaining one-third of the TMR at the evening feeding.

Prior to relocation, cows were housed in a barn with 31 free stalls (1.07 x 2.13 m) bedded with sawdust (Figure 1). They had access to one water trough (0.61 x 0.61 m) and were milked twice daily in a six stall side-open parlor at 0100 and 1300 h. Following relocation, cows were housed in a barn with 24 free stalls (1.17 x 2.29 m) bedded with rubber mattresses and sawdust (Figure 2). They had access to two water troughs (0.5 x 2.4 m) and were milked twice daily in a double-eight herringbone parlor at 0100 and 1300 h.

Sample Collection

Weekly TMR, orchard grass hay, and alfalfa hay samples were collected, and orts were sampled and pooled by dietary treatment. Quantity of feed offered was adjusted daily to maintain greater than 5% and less than 20% feed refusals. Quantity of hay offered was adjusted daily to 10% of total DM offered.

Cows were assigned a locomotion score once weekly throughout the 12 wk trial, using the scoring system of Sprecher et al. (1997). Body condition scores (Elanco Animal Health, 1994) were assigned in alternating weeks. Both lameness scores and BCS were assigned by two trained investigators blinded to the treatments.

Blood was collected into tubes containing EDTA via jugular venipuncture on d -7, -1, 0, 1, 2, 7, 14, and 21 relative to relocation. Samples were collected immediately following the afternoon milking (approximately 5 h post-feeding). Samples were stored on ice until sampling was complete, then centrifuged at 2200 x g for 20 min at 4^oC to separate plasma. Plasma was stored frozen at -20^oC for later analysis.

Parlor behaviors were monitored on d -14, -3, 0, 1, 2, 5, 7, and 14 relative to the move. Time to fit the milking claw, cow reactivity, and number of urinations and defecations in the milking parlor were recorded using the methods of Paranhos da Costa and Broom (2001). Cow reactivity was scored on a scale of 0-3; where 0 = no movements during milking, 1 = some leg movements during milking, 2 = constant leg movements and occasional kicking during milking, 3 = vigorous leg movements and kicking during milking.

Sample Analysis

Feed and feed refusal samples were dried in a 55^oC oven and analyzed weekly for dry matter. Dried feed and feed refusal samples were ground through a 1-mm screen in a Wiley Mill (Arthur H. Thomas, Philadelphia, PA). Feed and feed refusals were analyzed in duplicate for P, Ca, TKN (AOAC, 1984), and NDF and ADF sequentially with ? -amylase (Van Soest et al., 1991). Ash content of feed and feed refusals was measured by incineration at 600^oC for 2 h in a muffle furnace. Net energy was calculated in the feed samples using the equation NE_L (mcal/kg) = 0.0245 x TDN (%) – 0.12 (Weiss, 1998). The TDN was calculated in the feed samples using the equation $TDN = 0.98 \times (100 - NDFn - CP - ash - EE) + e^{-0.012*ADIN} \times CP + 2.25 \times (EE - 1) + 0.75 \times (NDFn - Lig) \times [1 - (Lig / NDF)^{0.667}] - 7$ (Weiss, 1998).

Plasma cortisol concentration was determined on duplicate samples by radioimmunoassay (DiaSorin Inc., Stillwater, MN). A tracer-buffer reagent was added in 1 mL aliquots to each of the sample tubes. Standards and unknown samples were placed in rabbit anti-cortisol GammaCoat tubes and incubated for 45 min in a 37^{o}C water bath. Plasma cortisol was determined through competitive protein binding. The inter-assay variation was 11.4 % and the intra-assay variation was 13.5 %.

Statistical Analysis

The first 2 wk of treatment were used as an adjustment period and data collected during that time were not included in analysis. Lameness scores, DMI, and milk production were analyzed in three periods; PD1 = pre-move (1 wk), PD2 = wk 1-4 following relocation, and PD3 = wk 5-9 following relocation. Plasma cortisol concentrations and parlor behaviors were analyzed using four periods to detect changes immediately following relocation. Differences in plasma cortisol concentration between diets were analyzed in the baseline (d -7 and -1), day of relocation (d 0), 2 d following relocation (d 1 and 2), and long term (d 7, 14, and 21) samples. Changes in parlor behaviors between diets were assessed in the baseline (d -14 and -4), day of relocation (d 0), 2 d following relocation (d 1 and 2), and long term (d 7 and 14). The effects of diet, period and their interaction were evaluated with PROC Mixed of SAS (version 8.0) with the following model:

$$Y_{ijk} = ? + D_i + C_{ij} + P_k + DP_{ik} + e_{ijk}$$

Where

Y_{ijk} = Response of dependent variable;

? = Mean;

D_i = Fixed effect of dietary treatment;

C_{ij} = Random effect of cow within dietary treatment;

P_k = Fixed effect of period;

DP_{ik} = Interaction of diet and period; and

e_{ijk} = Residual error, assumed to be normally distributed.

The dependent variables included all measures of BCS, DMI, MY, plasma cortisol concentration, locomotion score, and parlor behaviors. Using pre-planned contrasts, cows fed alfalfa hay were compared to cows fed grass hay, and cows fed alfalfa hay and grass hay were compared to cows fed the control diet. For all statistical analyses, effects were declared significant at $P < 0.05$, and trends at $P < 0.10$. Data are presented as least square means.

2.3 RESULTS

Nutrient composition for each of the three diets is in Table 2. Total mineral content, NDF, ADF, CP, Ca, P, and net energy differed with diet. Nutrient intakes for each of the three groups are in Table 3. Intakes of CP, Ca, and P were higher for cows fed alfalfa hay compared to cows fed grass hay. Intakes of ADF tended to be higher in cows fed alfalfa hay compared to cows fed grass hay. Cows fed the alfalfa hay diet had higher intakes of net energy compared to cows fed the grass hay diet (36.7 vs. 31.0 mcal/d). Normally energy intake decreases as ADF intake increases, but that comparison is only valid within a forage type (Van Soest, 1994). Intakes of NDF, ADF, CP, and Ca were higher in cows fed hay diets compared to cows fed the control diet.

There was no effect of diet on BCS, MY, lameness score, plasma cortisol, or parlor behaviors (Table 4). Cows fed the alfalfa hay diet had higher DMI than cows fed the grass hay diet (25.6 vs. 21.9 kg/d).

There was no change in BCS across the three periods (mean = 2.9????? ilk yield decreased between PD1 and PD2 and remained constant for PD3 (29.9, 28.1, and 28.4 kg/d). There was no interaction of diet and period for either BCS or MY.

Dry matter intake decreased from PD1 to PD2 (24.7 vs. 22.3 kg/d), but returned to PD1 DMI in PD3 (24.0 kg/d). There was a significant effect of the diet by period interaction on DMI (Figure 3). Cows fed the grass hay diet had a lower DMI than cows fed the alfalfa hay diet during PD1 (21.1 vs. 28.0 kg/d). During PD2, cows fed the grass hay diet tended to have a lower DMI than cows fed the alfalfa hay diet (21.1 vs. 24.3 kg/d; $P < 0.06$). By PD3, there were no differences in DMI among the three diets (mean = 24.0 kg/d).

Lameness scores did not change between PD1 and PD2 (1.49 vs. 1.61), but increased during PD3 (2.14). There was a significant diet by period interaction on lameness score (Figure 4). During PD1 (mean = 1.49) and PD2 (mean = 1.62) lameness scores were not affected by diet. Cows fed the alfalfa hay and control diets increased in lameness score by PD3 (2.6 and 2.04), but cows fed the grass hay diet had no change in lameness score during the study (mean = 1.62).

There was a significant diet by period interaction on plasma cortisol (Figure 5). Baseline plasma cortisol concentrations did not differ between cows fed the grass hay, alfalfa hay, or control diets (6.6, 7.07, and 7.95 ng/mL). Plasma cortisol increased on the day of relocation for cows fed the grass or alfalfa hay diets, but not in cows fed the control diet (12.1, 12.69, and 6.71 ng/mL). By 2 d following relocation cortisol concentrations returned to baseline (3.01, 4.3, and 2.67 ng/mL). There were no effects of diet on plasma cortisol at d 7, 14, and 21.

There was a significant effect of period on cow reactivity in the parlor. There was no difference between cow reactivity scores between the baseline and the day of the move (0.58 vs. 0.69). There was a decrease in cow reactivity during the 2 d following relocation (0.26). There was no diet by period interaction on cow reactivity. There was no effect of period or diet by period interaction on fitting time (mean = 10.68 s) or number of defecations (mean = 0.1) and urinations (mean = 0.07) in the parlor.

2.4 DISCUSSION

Diet affected some measures of the cows' ability to adapt to relocation in this study. Prior to relocation, concrete flooring was smooth from years of cow traffic. Following relocation, concrete flooring had a grooved surface, which has been shown to decrease slipping and increase walking confidence (Phillips et al., 1998). The cows were probably able to recognize the differences in flooring between the two facilities, which led to a change in their walking pattern or stride length (Phillips and Morris, 2002). The changes in flooring and walking pattern or stride length in the new facilities likely led to the increase in lameness by wk 5 through 9 following the move.

Grass hay appeared to offer some protection against lameness, since those cows fed grass hay did not become more lame in wk 5 through 9 following the move. The increase in lameness score observed in the cows fed the alfalfa and control diets may lead to an increased risk of culling. Sprecher et al. (1997) found that cows having a lameness score > 2 had longer days open and were 8.4 times more likely to be culled than cows with a lameness score of = 2. Even though NDF intake was the same for cows fed both the grass and alfalfa hay diets, the amount of effective fiber was probably greater in the grass hay group. Legumes tend to break down into smaller particles, while grass tends to maintain a rougher composition in the rumen. More coarse particles found in grass hay when compared with alfalfa hay have been shown to stimulate chewing activity and saliva output (Beauchemin et al., 2003), suggesting that chewing and rumination rates may have been increased in cows on the grass hay diet in the current study. Beauchemin et al. (2003) found that increasing the physically effective fiber in a diet increased chewing activity and improved rumen pH. Cows fed diets low in fiber may develop subclinical ruminal acidosis. Cows fed alfalfa hay had higher energy intakes than cows fed grass hay, which may also explain the increased lameness in those cows. Higher energy diets usually result in lower rumination times because of the higher density of the diet and lower "scratch factor" (Van

Soest, 1994). The cow numbers used in this study were small and it is not possible to determine if these differences would have occurred if larger animal numbers had been used.

There was an increase in plasma cortisol in the cows fed grass or alfalfa hay on the day of relocation, but no change in cows fed the control diet. West et al. (1999) observed that cows on diets high in NDF had higher plasma cortisol concentrations when exposed to heat stress than cows on diets low in NDF. The cows on the grass and alfalfa hay diets had higher NDF intakes than cows on the control diet. There were no differences in temperature on the day of relocation relative to the pre-move period, but the move itself is likely responsible for the increased plasma cortisol seen in the cows on higher NDF diets. Rats fed high energy diets had higher ACTH and exogenous corticosterone during restraint than rats fed lower energy diets (Tannenbaum et al., 1997). Cows fed alfalfa hay had the highest energy intakes, which could be responsible for the spike in plasma cortisol concentration seen in those animals.

Cows maintained an average BCS of 2.90 throughout the 10 wk study, which is within the normal range for cows in mid to late lactation (Wildman et al., 1982.). Milk production decreased in PD2 and PD3, which is best explained by advancing stage of lactation rather than a result of the new facilities. Previous research has shown that a loss of milk production may occur during the first milking in new facilities, but is recovered at subsequent milkings (Varner et al., 1983).

In the current study, the cow reactivity score decreased during the 2 d following relocation, but the overall scores never reached 1. This indicates that cows were calm throughout milking in both the old facilities and in the new facilities, since a score of 1 indicates that cows had only slight leg movements during milking. There were no effects of diet in any of the other parlor behaviors recorded. Cows adapted quickly to the new parlor system and milking procedures.

Lameness, milker temperament, and social hierarchy have been found to affect cows' behavior during milking. Lame cows have a longer latency to enter the parlor, higher cow reactivity, and take longer to return to the free stalls following milking (Hassell et al., 1993; Juarez et al., 2003). Temperament of the milker also affects a cow's behavior during milking. When cows were milked by an aversive handler they had higher reactivity scores than cows milked by a gentle handler (Rushen et al., 1999a). Phillips and Rind (2002) found that dominant cows entered the parlor ahead of subordinate cows. No previous work has shown the effects of diet on parlor behaviors.

2.5 CONCLUSIONS

Adding chopped orchard grass hay to diets seemed to offer some protection against lameness. Increased plasma cortisol in cows fed grass or alfalfa hay was probably due to the additional NDF in those diets compared to the control diets. The higher energy intakes in cows fed alfalfa hay probably also contributed to the increased plasma cortisol concentration in those cows. Addition of grass hay may reduce lameness following relocation, but no other beneficial effects were observed. The addition of alfalfa hay did not improve the cows' ability to adapt to relocation.

2.5 IMPLICATIONS

Supplementing diets of lactating cows with grass hay may help alleviate increases in lameness typically seen when cows are placed on new flooring. In the long term supplementation may reduce problems typically associated with increased lameness, such as reduced reproductive health and production. Future studies should determine the effects of diet on stress in lactating cow diets with more extreme differences evaluated. To determine true differences and effects in lameness and plasma cortisol, greater cow numbers are needed.

Table 1. Ingredient composition of diets fed to 23 lactating cows.

	Control	Grass	Alfalfa
		---% of diet DM---	
Alfalfa silage	21.2	19.1	19.1
Corn silage	32.8	29.5	29.5
Ground corn	9.8	8.8	8.8
Ground barley	8.7	7.8	7.8
Soybean meal	8.1	7.3	7.3
Pressed brewer's grains	16.2	14.6	14.6
Mineral mix	2.2	2.0	2.0
Orchard grass hay	0	10.0	0
Alfalfa hay	0	0	10.0

Table 2. Nutrient composition of diets fed to 23 lactating Holsteins.

	Control	Grass	Alfalfa	SEm[1]	*P* <		
					Overall diet effect	Hay vs. no hay	Grass vs. alfalfa
	----*% of diet DM*----						
NDF	43.9	48.9	42.3	0.01	0.01	0.01	0.01
ADF	22.0	25.0	21.9	0.01	0.01	0.01	0.01
CP	15.0	12.8	14.2	0.01	0.01	0.01	0.01
Ca	1.34	1.26	1.53	0.001	0.01	0.01	0.01
P	0.49	0.45	0.45	0.002	0.01	0.01	0.01
Ash	8.62	9.10	8.40	0.004	0.01	0.01	0.01
NE_L[2], Mcal/kg	1.43	1.41	1.43	0.003	0.01	0.01	0.01

[1] n = 11 weekly samples

[2]TDN content calculated as TDN = 0.98 x (100 – NDFn – CP – ash – EE) + $e^{-0.012*ADIN}$ x CP + 2.25 x (EE – 1) + 0.75 x (NDFn – Lig) x [1 – (Lig / NDF) $^{0.667}$] – 7; NE_L content calculated as NE_L (mcal/kg) = 0.0245 x TDN (%) – 0.12 (Weiss, 1998)

Table 3. Effect of dietary hay inclusion and type on nutrient intake by 23 lactating Holsteins.

	Control	Grass	Alfalfa	SEm[1]	*P* <			
					Diet	Diet x Pd	Hay vs. no hay	Grass vs. alfalfa
n	8	8	7					
NDF, kg/d	9.84	10.3	10.7	0.18	0.01	0.01	0.01	0.14
ADF, kg/d	4.90	5.22	5.45	0.09	0.01	0.01	0.01	0.06
CP, kg/d	3.41	3.15	4.15	0.08	0.01	0.01	0.01	0.01
Ca, g/d	315	291	395	7.1	0.01	0.01	0.01	0.01
P, g/d	112	102	117	2.13	0.01	0.01	0.20	0.01
Ash, kg/d	2.00	2.04	2.10	0.04	0.12	0.01	0.08	0.24
NE_L[2], Mcal/d	33.0	31.0	36.7	0.66	0.01	0.01	0.28	0.01

[1]n = 7 cows

[2] TDN content calculated as TDN = 0.98 x (100 – NDFn – CP – ash – EE) + $e^{-0.012 \times ADIN}$ x CP + 2.25 x (EE – 1) + 0.75 x (NDFn – Lig) x [1 – (lig / NDF) $^{0.667}$] – 7; NE_L content calculated as NE_L (Mcal/kg) = 0.0245 x TDN (%) – 0.12 (Weiss, 1998)

Table 4. The effects of dietary hay inclusion and type on performance and stress measures in 23 lactating cows during relocation

	Control	Grass	Alfalfa	SEm	*P*<		
					Diet	Diet x Pd	Sig. Contr.[1]
Body condition score	2.98	2.86	2.87	0.49	0.12	0.78	NS
Milk yield, kg/d	27.1	28.4	30.9	1.67	0.27	0.42	NS
Dry matter intake, kg/d	23.5	21.9	25.6	0.79	0.02	0.03	G
Lameness[2]	1.71	1.62	1.92	0.27	0.71	0.01	NS
Cortisol, ng/mL	4.96	5.77	6.26	0.78	0.48	0.01	NS
Parlor behaviors							
Fitting time[3], s	10.25	10.06	11.75	3.57	0.88	0.72	NS
Cow reactivity[4]	0.49	0.51	0.41	0.14	0.64	0.94	NS
Defecations[5]	0.15	0.04	0.12	0.15	0.51	0.79	NS
Urinations[6]	0.06	0.06	0.09	0.09	0.91	0.13	NS

[1]Significant contrasts: H = Hays vs. control; G = Grass vs. Alfalfa; NS = No contrasts were significant

[2] Lameness score was determined on a 1 – 5 scale; 1 = normal, 2 = mildly lame, 3 = moderately lame, 4 = lame, 5 = severely lame (Sprecher et al., 1997)

[3]Time for milker to attach milking claw

[4]Cow reactivity was determined on a 0-3 scale; 0 = very quiet in the milking parlor, no leg movements, 1 = stands quietly, some slight leg movements, 2 = continual leg movements which are occasionally vigorous, but does not kick, and 3 = continual vigorous leg movements or kicking (Paranhos da Costa and Broom, 2001).

[5]Number of defecations per cow in the milking parlor

[6]Number of urinations per cow in the milking parlor

Figure 1. Housing for 23 lactating Holsteins in the old facilities.

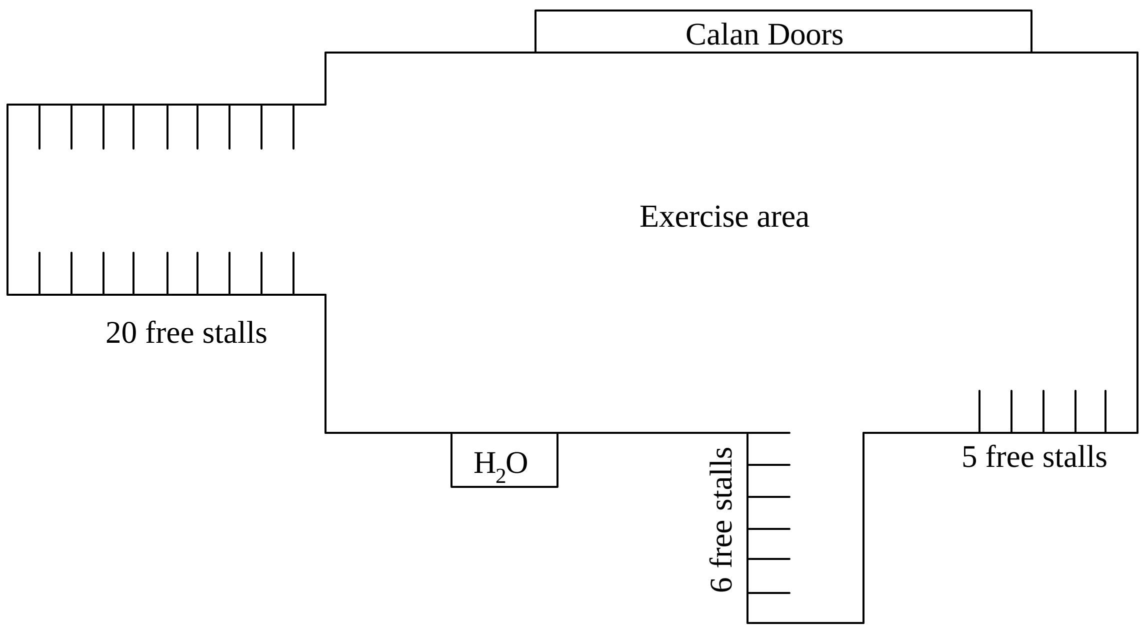


Figure 2. Housing for 23 lactating cows in the new facilities.

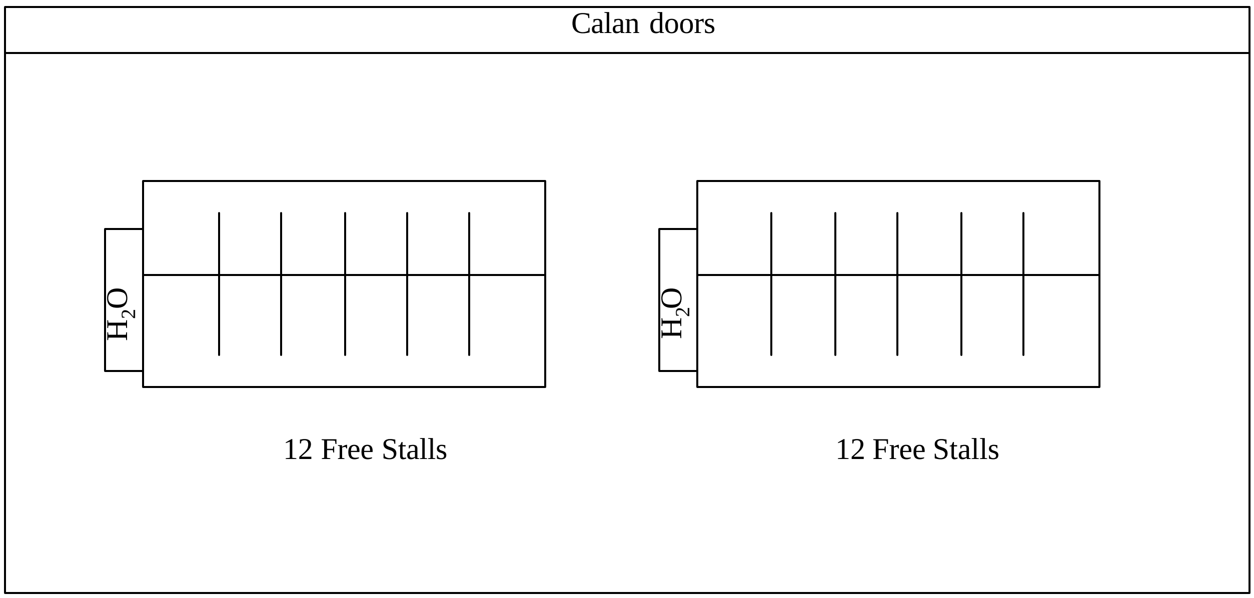
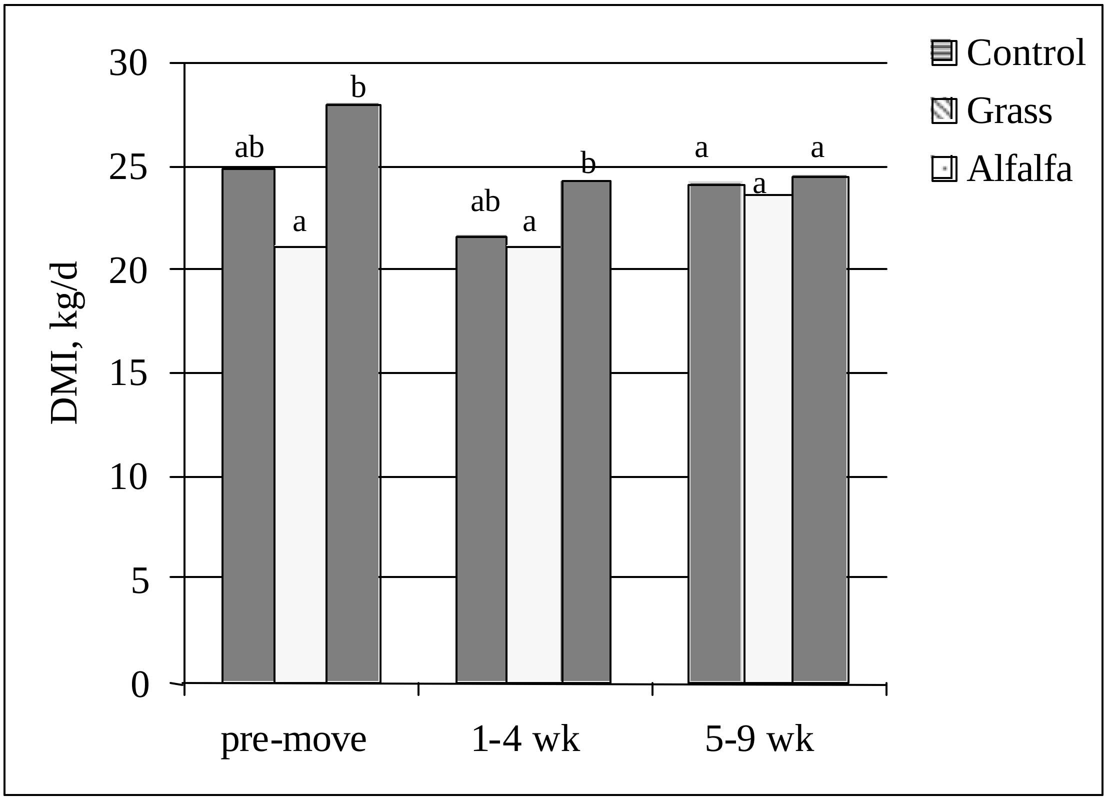
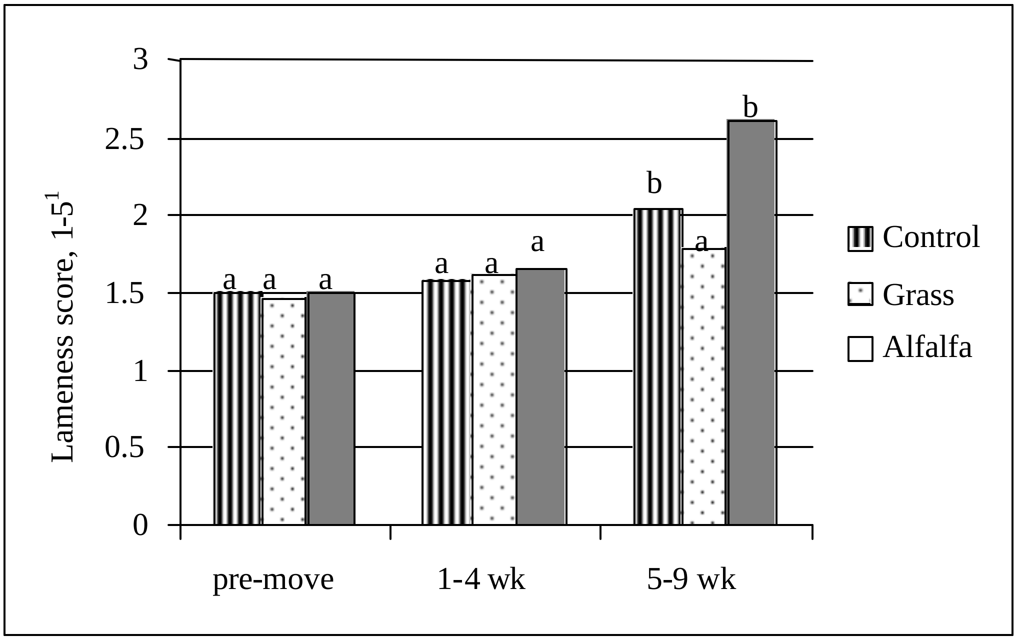


Figure 3. The effect of the interaction of diet and period on dry matter intake in 23 lactating Holsteins[1].



[1]Bars with different superscripts are significantly different.

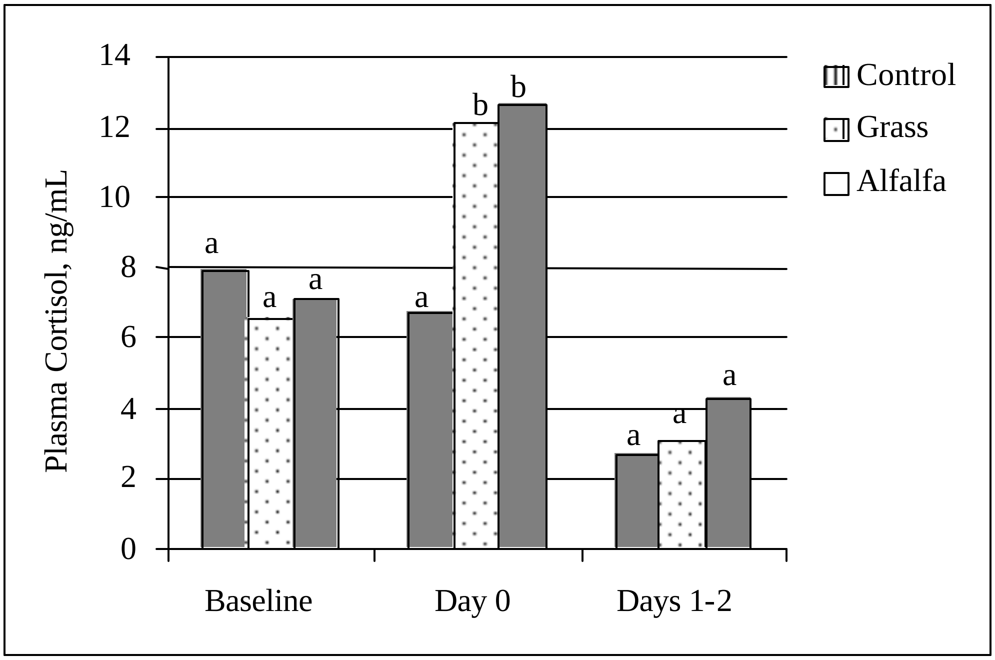
Figure 4. The effect of the interaction of diet and period on lameness[1] in 23 lactating Holsteins[2].



[1]Lameness score was determined on a 1 – 5 scale; 1 = normal, 2 = mildly lame, 3 = moderately lame, 4 = lame, 5 = severely lame (Sprecher et al., 1997)

[2]Bars with different superscripts are significantly different.

Figure 5. The effect of the interaction of diet and period on plasma cortisol concentration on 23 lactating Holsteins[1].



[1]Bars with different superscripts are significantly different.

CHAPTER 3

THE EFFECTS OF SOCIAL HIERARCHY ON STRESS IN LACTATING COWS DURING RELOCATION

ABSTRACT

The objective of this study was to determine the effect of social hierarchy on stress in lactating cows during relocation. Social hierarchy was determined by forced competition in 17 lactating Holstein cows (3 primiparous) 1 mo prior to relocation. Each of the cows was paired once with each of the other cows over a 4-d test period. The test period involved offering each pair of cows a bucket containing 0.5 kg of grain for 3 min. The total amount of time each cow gained access to the grain over the 4-d test period was analyzed to determine dominance rank. Prior to relocation, the 17 cows were housed in a barn with 20 free stalls (1.07 x 2.13 m) per pen, and milked twice daily in a six stall side-opening parlor. Following relocation, the same group of cows was housed in a barn with 20 free stalls (1.17 x 2.29 m) per pen, and milked twice daily in a double-eight herringbone parlor. Days in milk (DIM), BW, MY, and age were not different between dominant and subordinate animals. Blood samples were collected on d -7, -1, 0, 1, 2, 7, 14, and 21 relative to relocation. Milk production was analyzed in three periods; PD1 = pre-move, PD2 = wk 1-2 following relocation, and PD3 = wk 3-4 following relocation. Also, three 24 h observation periods (5 d prior to relocation, 1 d following relocation, and 12 d following relocation) were conducted to assess behavioral differences. Behaviors recorded included location, primary activity, urinations, defecations, and stereotypies. The effects of dominance rank, period and their interaction were evaluated with Proc MIXED of SAS. Plasma cortisol concentration (mean = 4.5 ng/ml) and observed behaviors were not influenced by dominance rank, period or the interaction. Milk yield was reduced following relocation in subordinate cows (PD1 = 26.3 kg/d, PD2 = 25.6 kg/d, PD3 = 21.6 kg/d), but not dominant cows (mean = 25.9 kg/d). Changes in post-move MY indicate that subordinate cows did not adapt to relocation as well as dominant cows. However, the lack of significant period effects or interaction of social rank and period for all other traits suggest that the stress was not extreme.

Key words: Social hierarchy, relocation, lactating dairy cows

3.1 INTRODUCTION

Social hierarchy can impact an animal's response to stressful situations, influencing plasma cortisol, MY, lameness, and behaviors. Social hierarchy often determines stall usage, amount of time spent lying or standing each day, and which cows will have access to the feed bunk. Friend and Polan (1974) observed that dominant cows occupied preferred free stalls at the barn entrance, while subordinate cows occupied the less desirable innermost free stalls. Most aggressive interactions occurred when cows returned from milking and when hay was offered. Dominant animals gained access to feed over subordinate cows when limited feeding space was available. These authors concluded that dairy cattle offered feed in a competitive situation should be fed one complete ration with adequate bunk space to ensure the same nutrition is received by each animal.

Galindo et al. (2000) observed that the cows standing halfway in the free stall were cows of lower social ranking than cows that stood in the alley or free stall. These authors also observed that lame cows spent more time standing halfway in a free stall and less time with their whole body either in the alley or in the free stall.

Friend and Polan (1974) found no relationship between dominance rank and milk yield. In contrast, the dominant cows had higher milk yield than subordinate animals (Phillips and Rind, 2002). The former study used 21 Holstein cows housed in a conventional free stall system, while the latter study used 72 British Friesian cows which grazed on pasture. The different housing and feeding methods are the likely caused the conflict in results. Phillips and Rind (2002) found that dominant cows were more likely to enter the parlor ahead of subordinate cows at milking.

Studies have been conducted to determine the effects of social hierarchy in lactating cows, but not during relocation. The objective of this study was to determine how social hierarchy affected a cow's ability to adapt to relocation.

3.2 MATERIALS AND METHODS

Cows and Testing Methods

Dominance rank was assessed in 17 Holstein cows (3 primiparous), using modifications of the methods of Houpt and Wolski (1980) and Phillips and Rind (2002). The cows averaged 190 DIM at the start of the study, and 250 DIM when the study was completed. The tests for dominance rank were conducted 52 to 55 d before the move (test period one) and 20 to 23 d after the move (test period two). An initial test was conducted 30 d prior to test period one to familiarize the cows with the test procedures and testing area. Feed was withheld for 3 h prior to testing. Each of the 17 cows was paired once with each of the other 16 cows over the 4 d test periods. No cow was tested more than five times per day. Each day, all competitions were finished within 3.5 h. Each pair of cows was lead into the testing area, and offered a bucket with 0.5 kg of grain for competition. The cows were allowed access to the bucket for 3 min. The percentage of time each cow maintained control of the pail was analyzed to determine dominance rank.

Prior to relocation, cows were housed in a barn with 20 free stalls (1.07 x 2.13 m) bedded with saw dust (Figure 1). They had access to one water trough (0.61 x 0.61 m) and were milked twice daily at 0100 and 1300 h in a six stall side-open parlor. Prior to relocation, cows were fed once daily at 0900 h. Following relocation, cows were housed in a barn with 20 free stalls (1.17 x 2.29 m) bedded with rubber mats and saw dust (Figure 2). They had access to two water troughs (0.5 x 2.4 m) and were milked twice daily at 0100 and 1300 h in a double-eight herringbone parlor. Following relocation, cows were fed twice daily at 1000 and 1400 h. All cows were fed the herd TMR throughout the study. Diet composition is listed in Table 1.

Data Collection

The amount of time each cow controlled access to the bucket of grain was analyzed to determine dominance rank. The fraction of time each cow was in control of the bucket for each pairing during test period two was averaged to obtain the mean percentage of time each cow was in control of the bucket. The eight cows that controlled the bucket for the highest percentage of time were classified as the dominant cows, while the nine cows that controlled the bucket for the lowest percentage of time were classified as the subordinate cows. The most dominant cow spent 72% of time in control of the bucket, while the most subordinate cow spent only 9% of time in control of the bucket over the 4-d test period.

Cows were assigned a locomotion score once weekly for 3 wk prior to relocation and 4 wk following relocation, using the scoring system of Sprecher et al. (1997). Body condition scores (Elanco Animal Health, 1994) were assigned on alternating weeks. Both lameness scores and BCS were assigned by two trained investigators blinded to the cows' dominance classifications.

Three 24-h behavioral observations were conducted on d -4, 2, and 14 relative to relocation. Primary location, posture, and activity were recorded for each cow at 20 min intervals and are presented as hours per day. Secondary activities (i.e., urinations and defecations), agonistic interactions (i.e., head-butts), and stereotypic behaviors (i.e., tongue-rolling, excessive licking, and grooming) were recorded as they were observed and are presented as occurrences per day. Behavioral activities were not recorded while cows were in the milking parlor.

Blood was collected into tubes containing EDTA via jugular venipuncture on days -7, -1, 0, 1, 2, 7, 14, and 21 relative to the move. Samples were collected immediately following the afternoon milking (approximately 5 h post-feeding). Samples were stored on ice until sampling was complete, then centrifuged at 2200 x g for 20 min at 4^{o}C to separate plasma. Plasma was stored frozen at -20^{o}C for later analysis.

Plasma cortisol concentration was determined on duplicate samples by radioimmunoassay (DiaSorin Inc., Stillwater, MN). A tracer-buffer reagent was added in 1 mL aliquots to each of the sample tubes. Standards and unknown samples were placed in rabbit anti-cortisol GammaCoat tubes and incubated for 45 min in a 37^{o}C water bath. Plasma cortisol was determined through competitive protein binding. The inter-assay variation was 11.4% and the intra-assay variation was 13.5%.

Parlor behaviors were monitored on d -14, -3, 0, 1, 2, 5, 7, and 14 relative to the move. Time to fit the milking claw, cow reactivity, and number of urinations and defecations while in the milking parlor were recorded (Paranhos da Costa and Broom, 2001). Cow reactivity was scored on a scale of 0-3; where 0 = no movements during milking, 1 = some leg movements during milking, 2 = constant leg movements and occasional kicking during milking, 3 = vigorous leg movements and kicking during milking.

Statistical Analysis

Lameness scores and milk production were analyzed in three periods: PD1 = pre-move (3 wk), PD2 = wk 1-2 following relocation, and PD3 = wk 3-4 following relocation. Plasma cortisol concentrations and parlor behaviors were analyzed using four periods to detect changes immediately following relocation. Differences in plasma cortisol concentration between dominant and subordinate cows were analyzed in the baseline (d -7 and -1), day of relocation (d 0), 2 d following relocation (d 1, 2), and long term (d 7, 14, 21) samples. Changes in parlor behaviors between dominant and subordinate cows were assessed in the baseline (d -7 and -1), day of relocation (d 0), 2 d following relocation (d 1, 2), and long term (7, 14, and 21). Changes in behavior over the three 24-h observational periods were determined. The effects of social hierarchy, period and their interaction were evaluated with PROC Mixed of SAS (version 8.0) using the following model:

Y_{ijk} = ? + S_i + C_{ij} + P_k + DP_{ik} + e_{ijk}

Where

Y_{ijk} = Response of dependent variable;

? = Mean;

S_i = Fixed effect of social class;

C_{ij} = Random effect of cow within social class;

P_k = Fixed effect of period;

SP_{ik} = Interaction of social class and period; and

e_{ijk} = Residual error, assumed to be normally distributed

The dependent variables included all measures of MY, plasma cortisol concentration, locomotion score, parlor behaviors, and general behaviors during three 24-h observation periods. Values for the group classified as dominant and values for the group classified as subordinate were compared. For all statistical analyses, effects were declared significant at $P < 0.05$. Data are presented as least square means.

3.3 RESULTS AND DISCUSSION

Previous studies have found dominant cows to be older and have higher body weights than subordinate cows (Dickson et al., 1969). In the current study, no correlations were found between age or body weight and social rank. There were no differences between dominant and subordinate cows in plasma cortisol concentration, parlor behaviors (Table 2), or general behaviors (Table 3). There was no increase in plasma cortisol concentration seen on the day of the move or the days immediately following the move. This indicates that neither the dominant or subordinate cows were stressed enough by the change in facilities to cause an adrenal response.

There was no effect of social hierarchy on BCS (? = 3.81), plasma cortisol concentration (mean = 4.71 ng/mL), fitting time (mean = 12.15 s), cow reactivity (mean = 0.55) in the parlor, or general behaviors (Tables 2 and 3). Dominant cows tended to defecate more in the parlor than subordinate cows (0.12 vs. 0.03 per milking), while subordinate cows tended to urinate more in the parlor than dominant cows (0.12 vs. 0.01 per milking).

Prior to relocation there were no differences in lameness scores between dominant and subordinate cows (mean = 1.78). Cows classified as dominant had higher lameness scores following relocation (mean = 2.10) than cows classified as subordinate (mean = 1.78). The higher lameness scores in the dominant cows are contradictory to findings in previous studies. Galindo et al. (2000) found that the subordinate cows in a herd are generally more lame than dominant cows because dominant cows are more likely to occupy the most desirable stalls. Following relocation no stalls were more desirable than others, since all the stalls were larger and cows could fit in the stall more easily, without their back legs in the alleyway. The cows classified as dominant did not seem to adapt to walking on the new barn floors as well as the cows classified as subordinate.

There was no overall effect of social hierarchy on MY, but subordinate cows had reduced MY by 3-4 wk following the move (26.0 vs. 21.6 kg/d) while the dominant cows had no change in MY (mean = 25.9 kg/d; Figure 3). Phillips and Rind (2002) found similar results with dominant cows in a group having higher milk yields than subordinate cows in a group.

Cow reactivity (0.31 vs. 0.67) increased when cows moved into the new facilities. The increase in cow reactivity was statistically significant, but the generally low scores do not indicate a major change in reactivity in the parlor. There was only a 0.36 increase in cow reactivity following the move. The overall cow reactivity score was less than 1.0 on a 0-3 scale, indicating that the cows had good temperaments in the milking parlor at both the old and new facilities.

Cows spent more time in free stalls (9 vs. 12 h) and less time walking (3.3 vs. 1 h) in alleys in the new facilities compared to the old facilities. The average dairy cow spends approximately 12 h/d lying down, so the increased free stall usage in the new facilities when compared to the old facilities indicates an improvement in animal welfare (Cook et al., 2004). There were no significant period by social rank interactions on any of the measures recorded.

3.4 CONCLUSIONS

Cows in this study showed few signs of increased stress during relocation. There were no differences in plasma cortisol concentration, parlor behaviors, or general behaviors between dominant and subordinate cows. Subordinate cows did not get more lame following relocation, while dominant cows did get more lame, which might indicate that subordinate cows adapted slightly better to the new facility. However, changes in post-move MY indicate that at this production level subordinate cows may not have adapted as well as dominant cows to the new facilities. The overall increase in free stall usage and decrease in walking indicates that the overall welfare of these animals was improved with the move to the new facilities.

3.5 IMPLICATIONS

Differences between dominant and subordinate cows may have been greater if the cows were placed under crowded conditions during the study. It does appear that subordinate cows may have lower milk production when compared to dominant animals. To ensure that these differences are kept to a minimum, adequate feed and feed bunk space and free stalls should be provided to allow all cows equal access to resources. In future studies to determine the effect of social hierarchy on milk yield cows in earlier lactation should be chosen. To determine true differences and effects in lameness greater cow numbers should be used.

Table 1. Ingredient composition of diet fed to 17 lactating cows.

	---% of diet DM---
Alfalfa silage	21.2
Corn silage	32.8
Ground corn	9.8
Ground barley	8.7
Soybean meal	8.1
Pressed brewer's grains	16.2
Mineral mix	2.2

Table 2. The effects of social hierarchy on performance and stress measures in 17 lactating cows during relocation

	Dominant	Subordinate	SEm	*P*<
n	8	9		
Body condition score	4.42	3.20	0.64	0.18
Milk yield, kg/d	25.9	24.5	1.98	0.62
Lameness[1]	2.10	1.78	0.10	0.03
Cortisol, ng/mL	5.04	4.37	0.83	0.57
Parlor behaviors				
Fitting time[2], s	11.5	12.8	1.43	0.53
Cow reactivity[3]	0.61	0.49	0.10	0.37
Defecations[4]	0.12	0.03	0.03	0.07
Urinations[5]	0.01	0.12	0.03	0.08

[1]Lameness score was determined on a 1 – 5 scale; 1 = normal, 2 = mildly lame, 3 = moderately lame, 4 = lame, 5 = severely lame (Sprecher et al., 1997)

[2]Time for milker to attach milking claw

[3]Cow reactivity was determined on a 0-3 scale; 0 = very quiet in the milking parlor, no leg movements, 1 = stands quietly, some slight leg movements, 2 = continual leg movements which are occasionally vigorous, but does not kick, and 3 = continual vigorous leg movements or kicking (Paranhos da Costa and Broom, 2001).

[4]Number of defecations per cow in the milking parlor per milking

[5]Number of urinations per cow in the milking parlor per milking

Table 3. The effects of social hierarchy on the general behaviors of 17 lactating cows during relocation.

	Dominant	Subordinate	SEm	*P*<
Free stall[1], h	12.2	10.6	3.32	0.47
Feed bunk[2], h	4.0	4.5	0.63	0.03
Walking[3], h	1.8	1.5	0.54	0.17
Standing[4], h	12	12.2	1.75	0.83
Drinking[5], h	1.6	1.8	0.71	0.27
Eating[6], h	4.0	4.2	0.72	0.64
Defecations[7]	13.1	12.0	1.07	0.39
Urinations[8]	5.71	4.78	0.87	0.45
Grooming[9]	10.5	10.4	2.54	0.96
Tongue-rolling[10]	1.53	1.67	0.67	0.89

[1] Number of hours cows spent in the free stalls in a 24 h period

[2] Number of hours cows spent standing at the feed bunk in 24 h

[3] Number of hours cows spent walking in 24 h

[4] Number of hours cows spent standing in 24 h

[5] Number of hours cows spent drinking in 24 h

[6] Number of hours cows spent eating in 24 h

[7] Number of times cows defecated in 24 h

[8] Number of times cows urinated in 24 h

[9] Number of times cows groomed themselves or each other in 24 h

[10] Number of times cows rolled their tongues in 24 h

Figure 1. Housing for 17 lactating Holsteins in the old facilities.

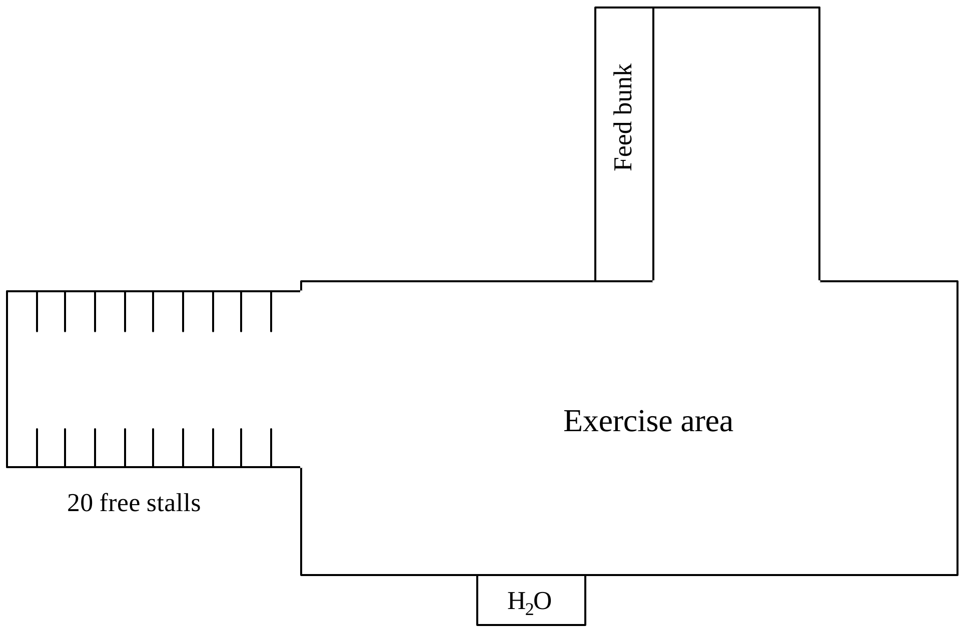


Figure 2. Housing for 17 lactating Holsteins in the new facilities.

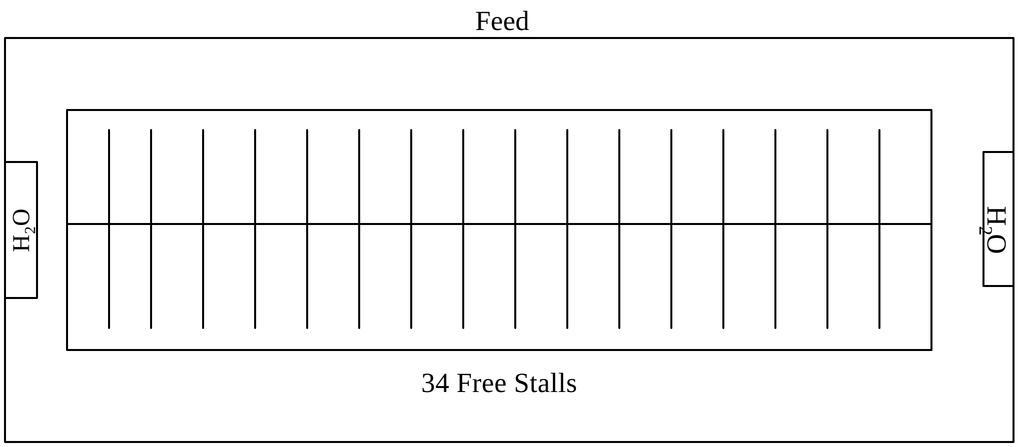
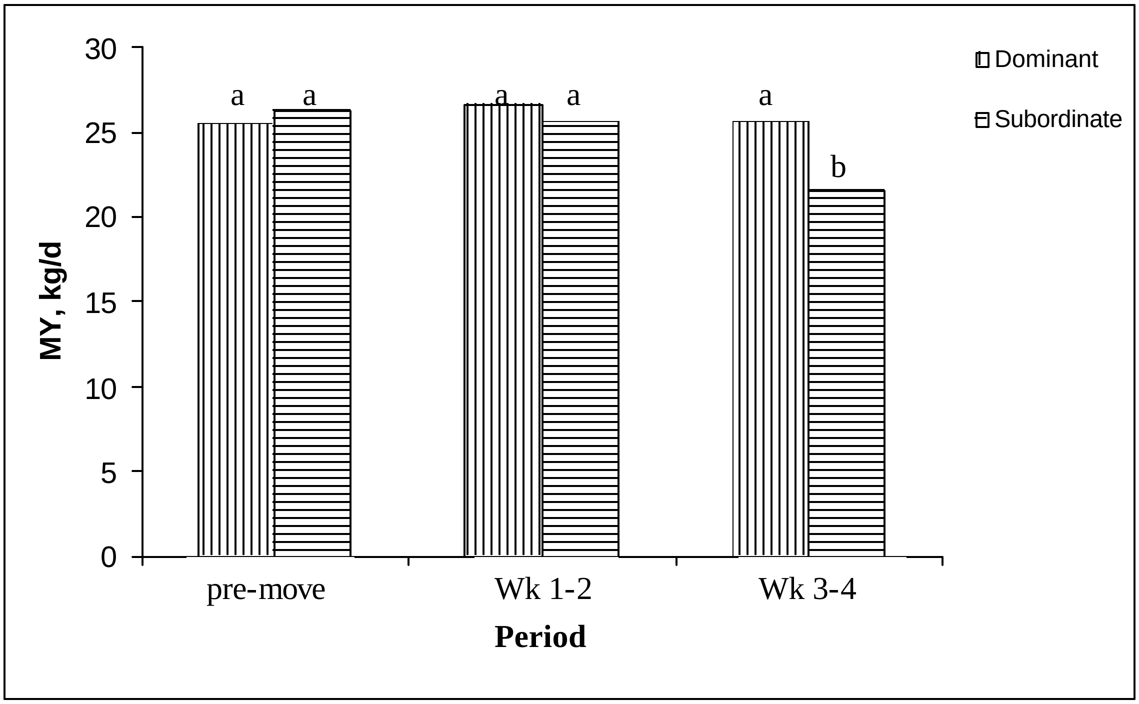


Figure 3. The effects of the interaction of social hierarchy and period on the interaction of milk yield on 17 lactating Holsteins[1].



[1]Bars with different superscripts are significantly different

CHAPTER 4

THE EFFECTS OF STOCKING DENSITY ON STRESS IN LACTATING COWS DURING RELOCATION

ABSTRACT

The objective of this study was to determine the effects of stocking density on stress in lactating cows during relocation to new facilities. Twenty-two Holstein and 22 Jersey cows (104 +/- 40 DIM) were randomly assigned to one of four stocking densities (0.67, 0.83, 1.0, or 1.17 cows per stall) in new dairy facilities. Prior to relocation, the 44 cows were housed together in a barn with 60 free stalls (1.07 x 2.13 m) per pen, and milked in a six stall side-opening parlor. Following relocation, the cows were housed in 4 pens with 12 free stalls (1.17 x 2.29 m) per pen in groups of 8, 10, 12, or 14, and were milked in a double-eight herringbone parlor. Blood samples were collected via jugular venipuncture on d -7, -1, 0, 1, 2, 7, 14, and 21 relative to relocation immediately following the afternoon milking. Milk production was analyzed in three periods; PD1 = pre-move, PD2 = wk 1-2 following relocation, and PD3 = wk 3-4 following relocation. Also, three 24-h observation periods (5 d prior to relocation, 1 d following relocation, and 12 d following relocation) were conducted to determine behavioral differences. Behaviors recorded included location, primary activity, urinations, defecations, and stereotypies. Lameness scores were evaluated weekly and BCS and cleanliness scores were assigned in alternating weeks. The effects of stocking density, period and their interaction were evaluated with Proc MIXED of SAS. Cows housed at a stocking rate of 1.17 had higher plasma cortisol concentrations than cows housed at a stocking rate of 0.67 (2.90 vs. 1.45 ng/mL). All cows had increased lameness scores following relocation, but cows housed at a stocking rate of 0.67 had higher lameness scores than cows housed at stocking rates of 0.83, 1.0, and 1.17 (1.97 vs. 1.51). Cow numbers were probably insufficient to determine if this was a biologically meaningful difference. Jerseys had lower plasma cortisol concentrations throughout the study than Holsteins (3.52 vs. 5.28 ng/mL). The change in plasma cortisol concentrations observed may indicate that overstocked cows did not adapt to relocation as well.

4.1 INTRODUCTION

Management practices such as overcrowding or social isolation can lead to increased stress in dairy cattle. When cows are overstocked, there may be changes in milk production, stress levels, and overall animal behavior. Housing cattle in overcrowded pens reduces the time that the cows are able to spend in free stalls and increase the amount of time they spend standing in the alleys. The average dairy cow spends approximately 12 h/d lying down, 4.5 h/d feeding, 2.3 h/d standing and drinking in the alleys, 2.6-3.2 h/d milking, and 1.8-3.4 h/d standing in the stall (Cook et al., 2004). If stocking density is increased to allow only 0.33 stalls per cow, lying time is reduced by more than half compared to cows that are each provided stalls (Friend et al., 1977b). Cows need to have the opportunity to lie down for extended periods of time to allow optimal rumination conditions and, therefore, increased production and well-being. Cows that are unable to lie down spend a greater amount of time eating, idling, grooming and leaning (Munksgaard and Simonsen, 1996). These responses are most likely displacement behaviors due to frustration stemming from deprivation of lying time (Munksgaard and Simonsen, 1996). Overcrowding can also lead to a change in aggressive behavior between animals. Krohn and Konggaard (1987) found that more animals per pen lead to an increase in aggressiveness. Animal behaviors such as decreased lying, increased standing on hard surfaces, or standing with the two front feet on the free stall platform and the two rear feet in the alley all lead to a higher lameness score (Galindo and Broom, 2000).

Stocking rate not only causes changes in behavior, but it can also change physiological measures of stress. Glucocorticoid response to ACTH during ART increased with increased crowding of cows (Friend et al., 1979). Housing can be taken to the opposite extreme by housing cows in isolation. Willett and Erb (1972) observed increased plasma cortisol concentration when growing heifers were kept in social isolation.

Stocking density may also affect cow cleanliness. Increased stocking density in free stalls with straw bedding leads to a decrease in cow cleanliness regardless of the addition of new straw. Slatted floor housing systems depend on a certain number of cows being present to walk the manure through the slats. Cows in slatted floor housing systems stocked at lower or higher rates have higher cleanliness scores than cows in slatted floor housing systems stock at optimal rates (Scott and Kelly, 1989). When the stocking rate is too low in a pen, manure begins to build up because there is not enough cow traffic in the alleyways. In contrast, if the stocking rate is higher than optimal in slatted floor housing systems cows are unable to move around enough to clean out the alleyways. Similarly, overstocking facilities with scraping or flush systems may decrease their effectiveness, leading to dirtier cows.

Studies have been done to determine the effects of overcrowding or isolating cattle, but none have been done during a time of relocation. Housing cattle in overcrowded conditions may decrease both animal welfare and production. The objective of the current study was to determine the effects of stocking density on stress in lactating cows during relocation.

4.2 MATERIALS AND METHODS

Cows and Data Collection

Twenty-two Holstein (107 +/- 56 DIM) and 22 Jersey cows (102 +/- 63 DIM) were randomly assigned to one of four stocking densities (0.67, 0.83, 1.0, or 1.17 cows per stall) in the new dairy facilities. An equal number of Holsteins and Jerseys was assigned to each of the four pens. The cows were housed in free stall pens designed for 12 Holsteins, in groups of 8, 10, 12, or 14. Sawdust placed on top of rubber mattresses was used for bedding the free stalls.

Prior to relocation the 44 cows were housed together in a barn with 60 free stalls (1.07 x 2.13 m) bedded with sawdust (Figure 1). They had access to two water troughs (0.61 x 0.61 m) and were milked in a six stall side-open parlor twice daily (0100 and 1300 h). Following relocation, cows were housed in groups of 8, 10, 12, or 14 in pens with 12 free stalls (1.17 x 2.29 m) bedded with rubber mattresses and sawdust (Figure 2). They had access to one water trough (0.5 x 2.4 m) and were milked in a double-eight herringbone parlor twice daily (0100 and 1300 h). All cows were fed the herd TMR throughout the study. Diet composition is listed in Table 1.

Cows were assigned a locomotion score once weekly for 3 wk prior to relocation and 6 wk following relocation, using the scoring system of Sprecher et al. (1997). Body condition (Elanco Animal Health, 1994) and cleanliness scores (Cook et al., 2004) were assigned on alternating weeks. The cleanliness scoring system determines the level cleanliness on a four-point scale (where 1 = clean, 2 = slightly dirty, 3 = moderately dirty, and 4 = excessively dirty) in three zones; the udder, the lower legs, and the upper leg and flank zones. The cows were washed with a power washer on d -14 and d 14 relative to the move to ensure that all cows were scored from a baseline cleanliness score. The first cleanliness score was assigned following the washing on d -14. Lameness, body condition, and cleanliness scores were assigned by two trained investigators blinded to the cows' classifications.

Three 24-h behavioral observations were conducted on d -4, 2, and 14 relative to relocation. Primary location, posture, and activity were recorded for each cow at 20 min intervals and are presented in hours per day. Secondary activities (i.e., urinations and defecations), agonistic interactions (i.e., head-butting), and stereotypic behaviors (i.e., tongue-rolling, excessive licking, and grooming) were recorded as they were observed and are presented as occurrences per day. Behavioral activities were not recorded while cows were in the milking parlor.

Blood was collected into tubes containing EDTA via jugular venipuncture on days -7, -1, 0, 1, 2, 7, 14, and 21 relative to the move. Samples were collected as cows returned to the free stalls following the afternoon milking. The blood was stored on ice until sampling was complete, then centrifuged at 2200 x g for 20 min at 4^{o}C to separate plasma. Plasma was stored frozen at -20^{o}C for later analysis.

Plasma cortisol concentration was determined on duplicate samples by radioimmunoassay (DiaSorin Inc., Stillwater, MN). A tracer-buffer reagent was added in 1 mL aliquots to each of the sample tubes. Standards and unknown samples were placed in rabbit anti-cortisol GammaCoat tubes and incubated for 45 min in a 37^{o}C water bath. Plasma cortisol was determined through competitive protein binding. The inter-assay variation was 11.4% and the intra-assay variation was 13.5%.

Parlor behaviors were monitored on d -14, -3, 0, 1, 2, 5, 7, and 14 relative to the move. Time to fit the milking claw, cow reactivity, and number of urinations and defecations in the milking parlor were recorded (Paranhos da Costa and Broom, 2001). Cow reactivity was scored on a scale of 0-3; where 0 = no movements during milking, 1 = some leg movements during milking, 2 = constant leg movements and occasional kicking during milking, 3 = vigorous leg movements and kicking during milking.

Statistical Analysis

Lameness scores and milk production were analyzed in three periods; PD1 = pre-move (3 wk), PD2 = wk 1-3 following relocation, and PD3 = wk 4-6 following relocation. Plasma cortisol concentrations and parlor behaviors were analyzed using four periods to detect changes immediately following relocation. Blood samples were analyzed for differences in plasma cortisol concentration among the four stocking densities in the baseline (d -7 and -1), day of relocation (d 0), 2 d following relocation (d 1, 2), and long term (d 7, 14, 21) samples. Changes in parlor behaviors among the four stocking densities in the baseline (d -7 and -1), day of relocation (d 0), 2 d following relocation (d 1, 2), and long term (7, 14, and 21) were analyzed. Changes in behavior over the three 24-h observational periods were determined. The effect of stocking density, period, and their interaction were analyzed in PROC Mixed of SAS (version 8.0) using the following model:

$$Y_{ijkl} = ? + S_i + C_{ij} + B_{ik} + P_l + DP_{il} + e_{ijkl}$$

Where

Y_{ijkl} = Response of dependent variable;

? = Mean;

S_i = Fixed effect of stocking density (0.67, 0.83, 1.0, or 1.17);

C_{ij} = Random effect of cow within stocking density;

B_{ik} = Fixed effect of breed within stocking density;

P_l = Period;

DP_{il} = Stocking density by period interaction; and

e_{ijkl} = Residual error, assumed to be normally distributed

Dependent variables included all measures of MY, plasma cortisol concentration, locomotion score, parlor behaviors, general behaviors during three 24-h observation periods, and cleanliness scores. In pre-planned contrasts, values for stocking rate 0.67 were compared with values for stocking rate 1.17, values for stocking rates 0.67, 0.83, 1.0 were compared with values for stocking rate 1.17, and values for stocking rate 0.67 were compared with values for stocking rates 0.83, 1.0, and 1.17. For all statistical analyses, effects were declared significant at $P < 0.05$. Trends were declared at $P < 0.10$. Data are presented as least square means.

4.3 RESULTS AND DISCUSSION

Breed Effects

There were some breed effects observed in the measures taken (Table 2). As expected, there was a significant breed effect on MY, with Holsteins producing more milk than Jerseys (29.5 vs. 23.1 kg/d). There was no effect of breed on BCS (mean = 2.85), any of the general behaviors measured, or lameness (mean = 1.51). There was a significant breed effect on plasma cortisol concentration. Holstein cows had higher plasma cortisol concentrations than Jersey cows throughout the study (5.28 vs. 3.52 ng/mL). There was a significant breed effect on cleanliness score. Holsteins had higher cleanliness scores than Jerseys (3.28 vs. 2.69). This is unexpected because the free stalls in the new facilities were made for Holsteins, allowing the Jerseys extra space in the stalls to urinate and defecate.

There were no effects of breed on cow reactivity or number of defecations and urinations. Jerseys had a longer fitting time than Holsteins (12.6 s vs. 10.3 s). Since the Jerseys did not have a higher cow reactivity score than Holsteins in the parlor, the 2 s time difference is most likely due to the structure of the parlor. Following relocation, it was difficult to adjust the brisket bar to hold both Jerseys and Holsteins in place in mixed groups because of the size differences in the animals. The extra space probably allowed more movement from the Jerseys, making it more difficult to fit them with the milking claw.

Stocking Density Effects

Stocking density affected some measures of cows' ability to adapt to relocation in this study (Table 3). There was no overall effect of stocking density on MY (mean = 26.3 kg/d), plasma cortisol concentration (mean??= 5.14 ng/mL), or cleanliness score (mean = 2.98). There was no effect of stocking density on BCS (mean = 2.85).

There was no effect of stocking density on any of the general behaviors measured except the amount of time cows spent grooming, and the amount of time spent they spent in the free stalls and alleys (Table 4). Cows housed at a stocking rate of 1.17 spent less time grooming than cows housed at a stocking rate of 0.67, 0.83, and 1.0 (1.2 vs. 2 h). Cows housed at a stocking rate of 1.17 tended to spend less time in the free stalls than cows housed at a stocking rate of 0.67, 0.83, and 1.0 (10.7 vs. 12.3 h). Cows housed at a stocking rate of 1.17 tended to spend more time in the alleys than cows housed at a stocking rate of 0.67, 0.83, and 1.0 (2.6 vs. 3.8 h). Overstocked cows would be expected to spend less total time in free stalls and more time in alleys than understocked cows or cows stocked at a rate of 1.0 because there were not enough free stalls available for the cows.

There was a significant period effect on lameness score. Prior to relocation, there were no differences in lameness scores among the four stocking rates (mean = 1.29). All cows showed a significant increase in lameness scores across each of the three periods (mean = 1.29, 1.48, and 1.76). The stocking density by period interaction on lameness score tended to be significant. Following relocation, cows housed at a stocking rate of 0.67 tended to have higher lameness scores than the average lameness scores of cows housed in stocking rates of 0.83, 1.0, and 1.17 (1.77 vs. 1.43). These results are unexpected since cows housed at a stocking rate of 0.67 spent the same amount of time in free stalls and alleys as cows housed at a stocking rate of 0.83 and 1.0 and more time in free stalls and less time in alleys than cows housed at a stocking rate of 1.17.

There was a significant effect of the stocking density by period interaction on plasma cortisol concentration (Figure 3). There was no change in plasma cortisol concentration on the day of relocation, but there was a decrease in plasma cortisol concentration for the 2 d following relocation compared to the baseline concentration (7.66 vs. 1.98 ng/mL). Plasma cortisol concentrations for cows housed in a pen with stocking density 0.67 tended to be lower than cows housed in a pen with stocking density 1.17 (1.45 vs. 2.90 ng/mL) during the 2 d following relocation. The lower plasma cortisol concentration in the cows housed in a less crowded pen indicates that those cows may have been less stressed during the 2 d following relocation than the cows housed in an overcrowded pen. Friend et al. (1979) observed that as cow density increased glucocorticoid response to ACTH also increased during adrenal response testing. However, in this study, cows housed in both stocking densities had significantly lower plasma cortisol concentrations than the pre-move baseline, which indicates that none of the cows was particularly stressed. The contrast between the current study and the study conducted by Friend et al. (1979) is most likely due to the differences used in stocking densities between the two studies. The latter study used pens that were much more overstocked than the former study. Plasma cortisol concentration was lower than the pre-move baseline for all four stocking rates for each of the subsequent samplings on d 7, 14, and 21 (mean = 3.53, 2.70, and 5.20 ng/mL).

There was a significant period effect on cleanliness scores. Cows had lower cleanliness scores following relocation (3.10 vs. 2.87). Cows were expected to have lower cleanliness scores following relocation, since the overall cleanliness of the facilities was increased. If cows had been more overstocked there would likely have been differences seen in cleanliness scores among the different stocking rates. There was no effect of stocking density by period interaction on cleanliness score.

There were no effects of stocking density or the interaction of stocking density with period on any of the parlor behaviors measured; entry order, latency to enter, cow reactivity, fitting time, and number of defecations and urinations.

4.4 CONCLUSIONS

Overstocking cows led to expected differences in the amount of time cows were able to spend in free stalls, since there were too few stalls available to cows at high stocking density. The higher plasma cortisol concentrations observed in Holsteins compared to Jerseys indicates that Jerseys may have adapted to relocation better than Holsteins. The higher plasma cortisol concentrations seen in cows stocked at a rate of 1.17 indicate that they may have been more stressed following the move than cows stocked at a rate of 0.67, 0.83, and 1.0.

The increase in lameness in all cows in the 6 wk following relocation was expected due to the changes in flooring. Cows housed at a stocking rate of 0.67 tended to have higher lameness scores than cows stocked at a rate of 0.83, 1.0, or 1.17, but greater cow numbers are needed to determine the biological significance of this observation.

4.5 IMPLICATIONS

Overcrowding cows can cause a decrease in animal welfare, since cows are forced to spend more time in the alleys rather than in free stalls. Lameness scores were not affected by this difference, but had cow numbers been greater or the had the study lasted longer greater differences in lameness scores may have been seen. Differences in the different stocking rates of 0.67, 0.83, 1.0, and 1.17 may have been greater if the “overcrowded” cows had been housed at a higher stocking rate. The cows housed at a stocking rate of 1.17 only had two fewer free stalls than were needed for the number of cows in that pen.

Table 1. Ingredient composition of diet fed to 44 lactating cows.

	---*% of diet DM*---
Alfalfa silage	21.2
Corn silage	32.8
Ground corn	9.8
Ground barley	8.7
Soybean meal	8.1
Pressed brewer's grains	16.2
Mineral mix	2.2

Table 2. The effects of breed on performance and stress measures in 44 lactating Holsteins and Jerseys during relocation.

	Holstein	Jersey	SEm	*P*<
n	22	22		
Milk yield, kg/d	29.5	23.1	0.56	0.01
Body condition score	2.87	2.82	0.04	0.43
Lameness score[1]	1.48	1.55	0.10	0.66
Plasma cortisol, ng/mL	5.28	3.52	0.54	0.03
Cleanliness score[2]	3.28	2.69	0.12	0.01
Parlor behaviors				
Fitting time[4], s	10.3	12.6	0.08	0.02
Cow reactivity[5]	0.57	0.55	0.25	0.86
Defecations[6]	0.08	0.15	0.03	0.07
Urinations[7]	0.09	0.07	0.11	0.66

[1]Lameness score was determined on a 1 – 5 scale; 1 = normal, 2 = mildly lame, 3 = moderately lame, 4 = lame, 5 = severely lame (Sprecher et al., 1997)

[2]Cleanliness scored on a 1-4 scale; 1 = clean, 2 = slightly dirty, 3 = moderately dirty, and 4 = excessively dirty (Cook, 2004)

[3]Time elapsed as cow moved from holding area to milking stall

[4]Time for milker to attach milking claw

[5]Cow reactivity was determined on a 0-3 scale; 0 = very quiet in the milking parlor, no leg movements, 1 = stands quietly, some slight leg movements, 2 = continual leg movements which are occasionally vigorous, but does not kick, and 3 = continual vigorous leg movements or kicking (Paranhos da Costa and Broom, 2001).

[6]Number of defecations per cow in the milking parlor per milking

[7]Number of urinations per cow in the milking parlor per milking

Table 3. The effects of stocking density on performance and stress measures in 44 lactating Holsteins and Jerseys during relocation.

	Cows per stall					
	0.67	0.83	1.0	1.17	SEm	*P* <
n	8	10	12	14		
Body condition score	2.80	2.81	2.91	2.85	0.64	0.63
Milk yield, kg/d	26.4	26.3	25.9	26.6	0.98	0.99
Lameness[1]	1.77	1.46	1.29	1.54	0.17	0.19
Cortisol, ng/mL	3.27	4.90	3.96	5.47	0.90	0.20
Parlor behaviors						
Fitting time[3], s	11.7	11.0	10.6	11.7	0.78	0.76
Cow reactivity[4]	0.46	0.77	0.58	0.45	0.21	0.12
Defecations[5]	0.10	0.16	0.10	0.10	0.04	0.58
Urinations[6]	0.04	0.08	0.10	0.10	0.03	0.50

[1]Lameness score was determined on a 1 – 5 scale; 1 = normal, 2 = mildly lame, 3 = moderately lame, 4 = lame, 5 = severely lame (Sprecher et al., 1997)

[2]Time elapsed as cow moved from holding area to milking stall

[3]Time for milker to attach milking claw

[4]Cow reactivity was determined on a 0-3 scale; 0 = very quiet in the milking parlor, no leg movements, 1 = stands quietly, some slight leg movements, 2 = continual leg movements which are occasionally vigorous, but does not kick, and 3 = continual vigorous leg movements or kicking (Paranhos da Costa and Broom, 2001).

[5]Number of defecations per cow in the milking parlor per milking

[6]Number of urinations per cow in the milking parlor per milking

Table 4. The effects of stocking density on the general behaviors of 44 lactating Holsteins and Jerseys during relocation.

	Cows per stall					
	0.67	0.83	1.0	1.17	SEm	*P*<
Free stall[1], h	11.8	13.1	11.8	10.7	2.01	0.06
Feed bunk[2], h	5.15	5.13	5.34	5.35	0.82	0.96
Alley[3], h	2.90	2.00	2.80	3.80	1.58	0.08
Walking[4], h	0.81	0.77	0.92	0.71	0.37	0.45
Standing[5], h	11.9	10.7	11.4	11.9	2.01	0.50
Drinking[6], h	2.06	2.14	1.96	2.06	0.68	0.95
Eating[7], h	5.00	5.30	5.27	5.27	0.70	0.76
Defecations[8]	9.58	9.47	8.42	8.67	0.66	0.47
Urinations[9]	5.63	5.73	5.80	5.02	0.50	0.50
Grooming[10], h	1.96	2.12	1.67	1.19	0.72	0.02

[1] Number of hours cows spent in the free stalls in 24 h

[2] Number of hours cows spent standing at the feed bunk in 24 h

[3] Number of hours cows spent in the alley in 24 h

[4] Number of hours cows spent walking in 24 h

[5] Number of hours cows spent standing in 24 h

[6] Number of hours cows spent drinking in 24 h

[7] Number of hours cows spent eating in 24 h

[8] Number of times cows defecated in 24 h

[9] Number of times cows urinated in 24 h

[10] Number of hours cows groomed themselves or each other in 24 h

Figure 1. Housing for 44 lactating Holsteins and Jerseys prior to relocation.

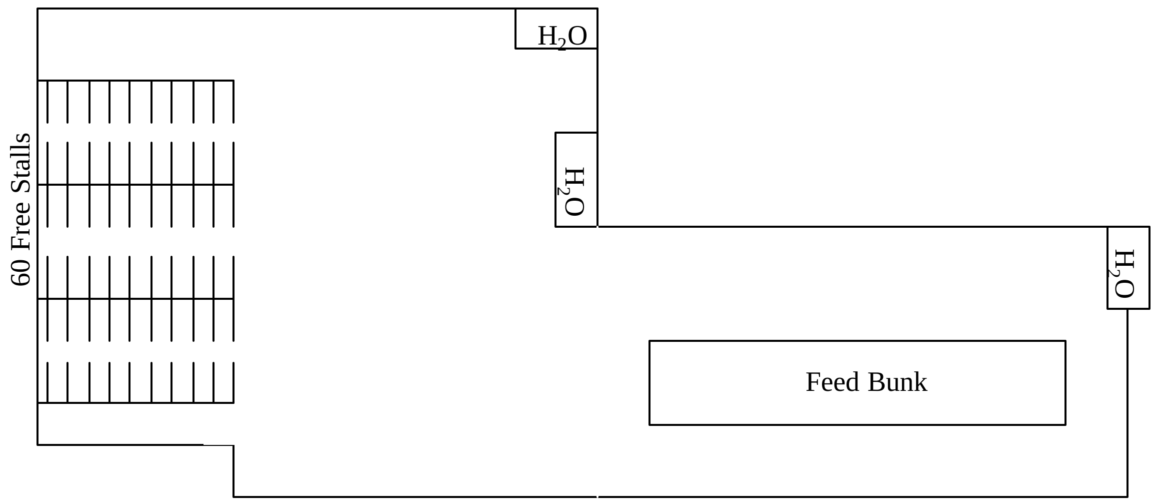


Figure 2. Housing for 44 lactating Holsteins and Jerseys following relocation.

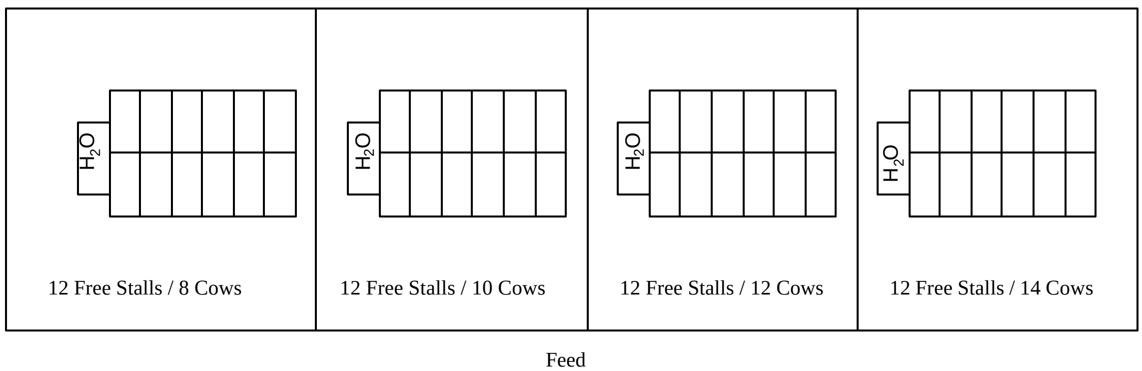
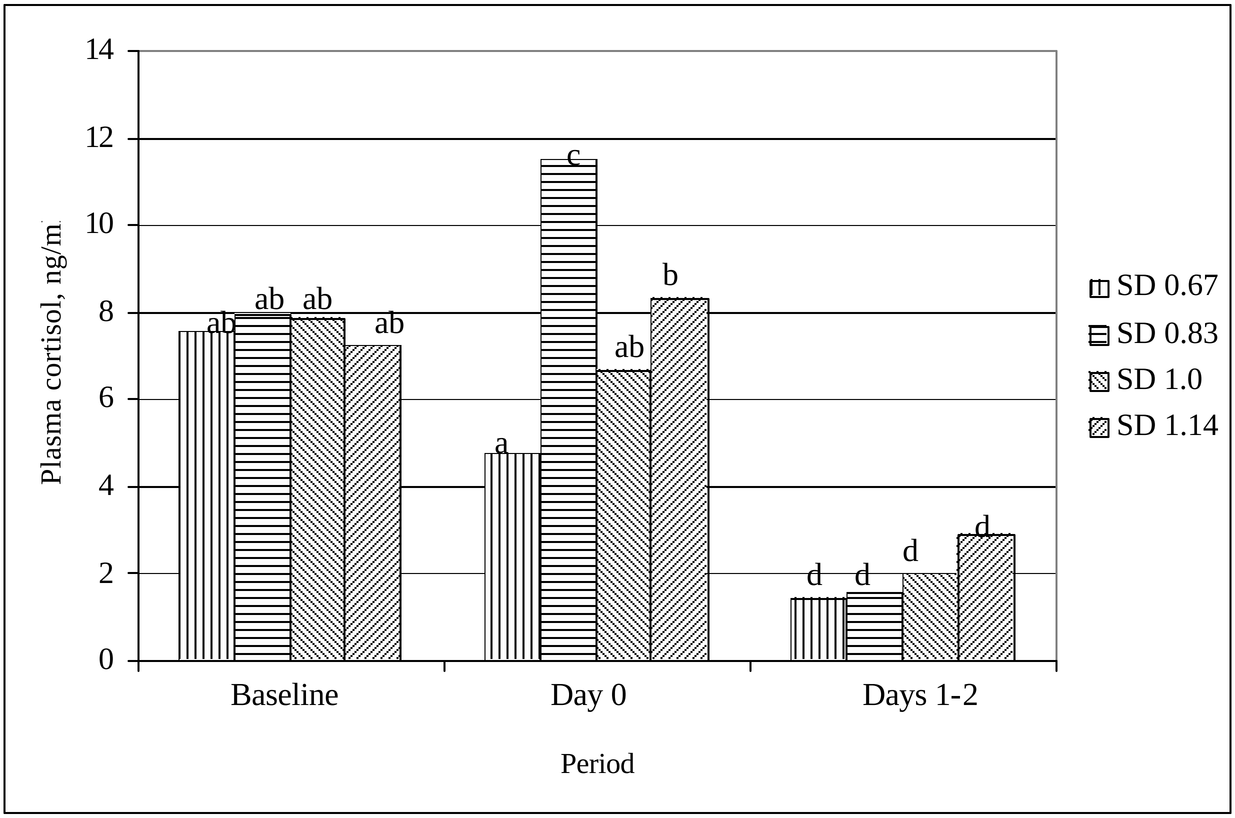


Figure 3. The effect of the interaction of diet and period on plasma cortisol concentration in 44 lactating Holsteins and Jerseys.



CHAPTER 5

CONCLUSIONS

In these studies, multiple measures were taken to indicate a possible effect of diet or management on animal welfare in lactating cows during relocation to new dairy facilities. In each of the three experiments conducted there was not a clearcut response that indicated with certainty that one group of animals was responding to the relocation differently than another group.

In experiment one, the addition of forage fiber to the diet did not result in changes in MY or DMI. Decreases in either measure are typically considered by the dairy producer as an indicator of decreased health or welfare in the herd. The increased plasma cortisol concentration observed in cows fed the alfalfa or grass hay diets may indicate that they were more stressed during relocation than cows fed the TMR diet, but cortisol concentrations were within the normal range for cows at all samplings.

Cows fed both the TMR only and the TMR plus alfalfa hay had increased lameness scores following relocation. Since there were no changes in lameness in cows fed the diet containing grass hay, offering lactating cows orchard grass hay during relocation may decrease the incidence of lameness while cows are adapting to new facility. Offering alfalfa hay to lactating cows during relocation did not improve production or measures of well-being.

In experiment two, there were no differences between dominant and subordinate cows in most of the measurements taken. Plasma cortisol concentration, lameness scores, parlor behaviors, and overall general behaviors were not different between the two classes of cows. Changes in post-move MY may indicate that subordinate cows did not adapt to relocation as well as dominant cows. Dry matter intake was not recorded in this experiment, but may have explained the lower MY in subordinate cows.

In experiment three, the increase in plasma cortisol concentration observed in overstocked cows during relocation indicated that those cows were more stressed than the understocked cows, but cortisol concentrations were within the normal range for cows at all samplings. However, the overstocked cows were probably more stressed with the decreased availability of free stalls than the actual move itself. The return to baseline plasma cortisol concentrations indicated that they adapted quickly.

As expected, cows that were overstocked spent more time standing and less time in free stalls than cows that were understocked, which made the increase in lameness scores observed in understocked cows compared to overstocked cows interesting, but difficult to explain.

In these studies cows were not particularly stressed with relocation and so it was difficult to determine effects of forage fiber, social hierarchy, and stocking density on stress. The differences in lameness scores may not have been of biological significance because of the low cow numbers used in this study. If there is the potential for lactating cows to be in a stressful situation, such as relocating to new facilities, supplementing their feed with chopped orchard grass hay may help alleviate lameness during the adjustment period. More research is needed to see if grouping dominant and subordinate cows separately minimizes effects on MY. Moving cows to overcrowded housing may cause an increase in stress, so all cows in a herd should always have equal access to free stalls. Minor changes in management and increased awareness of cow behavior can help producers maintain a high level of animal welfare and avoid losses in production.

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Table 1. Effects of forage fiber on performance and stress measures in 23 lactating cows during relocation across three periods.

		Period			Effect of period		Effect of treatment			
		1	2	3	SEM[1]	*P* <	TRT	Hay	Grass vs. Alfalfa	TRT*PD
DMI, kg/d2										
	Alfalfa	28.0	24.3	24.5						
	Grass	21.1	21.1	23.5						
	Control	24.9	21.6	24.1	1.08	0.01	0.02	0.82	0.01	0.03
Milk yield2, kg/d										
	Alfalfa	33.0	30.4	29.3						
	Grass	29.5	27.4	28.2						
	Control	27.0	26.5	27.8	1.91	0.05	0.27	0.21	0.29	0.42
Lameness score2[3]										
	Alfalfa	1.50	1.65	2.60						
	Grass	1.47	1.60	1.78						
	Control	1.50	1.58	2.04	0.29	0.01	0.71	0.85	0.42	0.01
Plasma cortisol[4], ng/ml										
	Alfalfa	7.07	12.7	4.30						
	Grass	6.56	12.1	3.01						
	Control	7.95	6.71	2.67	1.56	0.01	0.23	0.11	0.54	0.04
Cow reactivity[4,5]										
	Alfalfa	0.42	0.60	0.21						
	Grass	0.69	0.67	0.38						
	Control	0.63	0.80	0.19	0.35	0.02	0.64	0.78	0.38	0.94

[1]Due to unequal n, largest SEm (n=7) reported.

[2]Period 1 = 1 wk (pre-move), Period 2 = wk 1 – 4 post-move, Period 3 = wk 5 – 9 post-move

[3]Lameness score was determined on a 1 – 5 scale; 1 = normal, 2 = mildly lame, 3 = moderately lame, 4 = lame, 5 = severely lame (Sprecher et al., 1997)

[4]Period 1 = baseline pre-move, Period 2 = day of relocation, Period 3 = d 1-2 post-move

[5]Cow reactivity score was determined on a 0 – 3 scale; 0 = very quiet in the milking parlor, no leg movements, 1 = stands quietly, some slight leg movements, 2 = continual leg movements which are occasionally vigorous, but no kicking, 3 = continual vigorous leg movements and kicking (Paranhos do Costa and Broom, 2001)

Table 2. Effects of stocking density on performance and stress measures in 44 lactating Holsteins and Jerseys during relocation across three periods.

	Period			Effect of period		Effect of treatment				
	1	2	3	SEM	*P* <	TRT *P* <	SD0.67 vs SD1.17	SD0.83, 1.0, and 1.17 vs 0.67	SD0.67, 0.83, and 1.0 vs 1.17	TRT*PD
Milk yield[1], kg/d										
SD 0.67	24.8	25.9	26.9							
SD 0.83	27.9	26.5	26.1							
SD 1.00	26.1	25.7	26.1							
SD 1.17	28.0	26.6	26.6	1.67	0.16	0.90	0.57	0.68	0.59	0.01
Lameness score[1,2]										
SD 0.67	1.38	2.00	1.94							
SD 0.83	1.33	1.23	1.80							
SD 1.00	1.11	1.25	1.50							
SD 1.17	1.31	1.45	1.85	0.21	0.01	0.19	0.28	0.08	0.85	0.06
Plasma cortisol[3], ng/mL										
SD 0.67	7.58	4.76	1.50							
SD 0.83	7.97	11.53	1.57							
SD 1.00	7.86	6.65	2.00							
SD 1.17	7.30	8.37	2.91	1.55	0.01	0.29	0.21	0.14	0.60	0.04
Cleanliness[4,5]										
SD 0.67	2.93	2.67	---							
SD 0.83	3.25	2.87	---							
SD 1.00	3.08	3.06	---							
SD 1.17	3.13	2.87	---	0.17	0.01	0.47	0.18	0.49	0.13	0.45

Cow reactivity[3,6]											
	SD 0.67	0.56	0.22	0.50							
	SD 0.83	0.46	0.80	0.60							
	SD 1.00	0.87	0.36	0.38							
	SD 1.17	0.36	0.53	0.27	0.39	0.46	0.52	0.32	0.25	0.61	0.20

[1]Period 1 = 3 wk pre-move, Period 2 = wk 1-2 post-move, Period 3 = wk 3-5 post-move

[2]Lameness score was determined on a 1-5 scale; 1 = normal, 2 = mildly lame, 3 = moderately lame, 4 = lame, 5 = severely lame (Sprecher et al., 1997)

[3]Period 1 = baseline pre-move, Period 2 = day of relocation, Period 3 = d 1-2 post-move

[4]Period 1 = 2 wk pre-move, Period 2 = 2 wk post-move

[5]Cleanliness score was determined on a 1-4 scale; 1 = clean, 2 = slightly dirty, 3 = moderately dirty, 4 = excessively dirty (Cook, 2002)

[6]Cow reactivity was determined on a 0-3 scale; 0 = very quiet, 1 = stands quiet, some leg movements, 2 = continual leg movements which are occasionally vigorous, 3 = continual leg movements and kicking (Paranhos da Costa and Broom, 2001)

VITA

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