IMPROVING THE WELFARE OF DAIRY COWS AND CALVES: THE IMPORTANCE OF THE ENVIRONMENT

DISSERTATION

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By

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Abstract

The objective of this dissertation was to address animal welfare as a continuous state as it pertains to dairy cattle and their environment. Chapter 1 reviews the concept of animal welfare and how to assess it scientifically, based on the three critical components of welfare proposed by animal welfare scientists: 1) the animal’s health and biological functioning, 2) the affective state of the animal, and 3) the animal’s ability to display innate behavior. Chapter 2 thoroughly reviews the literature pertaining to Chapters 3, 4, 5, and 6, beginning with the welfare of the dairy calf in utero, continuing through the pre-weaning phase for young heifer calves in relation to the benefits of social companionship, and concluding with the importance of the environment to the welfare of the mature dairy cow. Chapter 3 acknowledges that animal welfare science thus far has primarily considered the homeostatic challenges production animals may encounter after birth; however, it emphasizes that the prenatal period is also of critical importance to mammalian species, as this period of development may significantly influence and predetermine the capability of offspring to respond and adapt to their future environment. Chapter 3 specifically investigates the prenatal period in relation to maternal social stress experienced by overstocking the feeding area for multiparous cows during late gestation.
and how this may affect the postnatal growth of the offspring. The results of this first experiment indicate that the experimental conditions of overstocking imposed did not compromise the postnatal growth of the offspring through weaning. Chapter 4 continues to examine the effect of pair housing on the behavior and performance of Jersey heifer calves during the milk-feeding phase; the majority of studies have been conducted with Holstein calves, and it is currently unknown if Jersey calves behave the same as Holstein calves when pair-housed. Calves housed in pairs performed better than calves housed individually, especially during the weaning period. However, cross-sucking behavior was prevalent, as calves were fed milk via bucket. Future research should aim to reduce cross-sucking behavior within the Jersey breed through alternative feeding systems or environmental enrichment. Lastly, Chapters 5 and 6 examine the effect of overstocking the feed bunk during the dry period on dairy cow metabolic health, stress, productivity, and indicators of cow temperament. Although the overstocking conditions imposed did not compromise metabolic health or productivity, overstocking the feed bunk made cows less approachable by an approaching experimenter.
This dissertation is dedicated to 11912.
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Chapter 1: Introduction

The welfare of production animals remains an issue in animal agriculture and is one of growing social salience. Federal and state governments are continuously being challenged with questions about the quality of life of livestock in the United States, and legislation is being proposed and enacted to safeguard and improve farm animal welfare. However, the long-term implications of such legislative initiatives remain unknown and proposed standards vary widely from state-to-state. Thus, it is the role of science to inform this process and provide the framework for which animal welfare can be considered by individuals in an informed manner to address broadening concern.

Assessing animal welfare has proven to be a challenge for scientists, as they sometimes disagree about which approach is the most appropriate form of assessment; some may place emphasis solely on the affective state on an animal, while others do not believe such an approach can be objectively measured. Although approaches to assessment may vary, it is widely accepted that there is no single measure of animal welfare (Dawkins, 2004). In addition, such conceptual uncertainty may lead to further complication, as policymakers are also likely to disagree about which housing and management practices best promote farm animal welfare (Barnett and Hemsworth, 2009).
Thus, assessing and safeguarding animal welfare is not without difficulty, and the use of a multidisciplinary approach that is able to incorporate a variety of measures, both input- and output-based, is ideal to mitigate such uncertainties. Despite the challenges confronting animal welfare scientists and policymakers alike, the United States continues to progress with the development of standards to promote the welfare of more than 9 billion animals raised for food per year across the nation (Appleby, 2007).

1.1. Animal welfare

The term ‘animal welfare’ is not a modern concept; producers and veterinarians have long considered the well-being of the animals under their care. This mutualistic or two-way relationship in which both humans and animals have gained benefit through the process of domestication has been in existence for tens of thousands of years (Rollin, 1995). However, animal welfare as a science is still relatively new, and scientists, ethicists, veterinarians, and others in the field approach the study of animal welfare using very different concepts and semantics.

Animal welfare is a complex science, yet oftentimes, individuals tend to use the term ‘animal welfare’ loosely in context. In addition, individuals have strong opinions about how animals should (or should not) be raised, which has led to a variety of proposed definitions for animal welfare. As the list is long in length, the chapters of this dissertation will only introduce and consider the two concepts of animal welfare that were used to assess the quality of life of dairy cows and calves used in the following experiments presented in this dissertation.
One commonly used and well-accepted definition of animal welfare is described as an animal’s “state as regards its attempts to cope with its environment” (Broom, 1991, p. 4168). Additionally, animal welfare involves how much has to be done by the animal to cope with its environment – this may be in a wild or a captive setting – and how well or how poorly such coping attempts succeed (Broom, 1991). In this sense, it is also important to define coping, as this term, like animal welfare, may be defined in a variety of ways. The term coping refers to the ability of the animal to maintain both mental and bodily control, or in other words, it refers to the ability of the animal to maintain homeostasis (Broom and Johnson, 1993). It is also important to recognize that welfare is not something that is given to an animal; this term refers to a characteristic of an individual animal as it experiences the environment in which it lives (Keeling et al., 2011).

A second and seemingly all-encompassing definition of animal welfare for domesticated animals is as follows:

Animal welfare means how an animal is coping with the conditions in which it lives. An animal is in a good state of welfare if (as indicated by scientific evidence) it is healthy, comfortable, well-nourished, safe, able to express innate behaviour, and if it is not suffering from unpleasant states such as pain, fear, and distress. Good animal welfare requires disease prevention and veterinary treatment, appropriate shelter, management, nutrition, humane handling and humane slaughter/killing (OIE, 2008).
Because this second definition is rather broad, it takes into account the three critical components of animal welfare that have been proposed by scientists: 1) the animal’s health and biological functioning, 2) the affective or mental state of the animal, and 3) the animal’s ability to display innate behavior (Fraser et al., 1997).

1.2. Animal welfare assessment

1.2.1. Health and biological functioning

Animal welfare scientists place different, or oftentimes, sole emphasis on different components of animal welfare; some scientists argue that it is only the health of the animal that will determine its welfare, while others argue that it is only the ability or inability of the animal to display natural, species-specific behaviors that they are highly motivated to perform that will be the determinant of either good or poor welfare (Fraser, 2008). To exemplify the health and biological functioning approach used to assess animal welfare, Sainsbury (1986) believed that “good health is the birthright of every animal that we rear, whether intensively or otherwise. If it becomes diseased we have failed in our duty to the animal and subjected it to a degree of suffering that cannot be readily estimated.” Thus, according to this approach, and to Sainsbury, if an animal is in good health and free from disease, then it must have good welfare and vice versa.

Those in support of this approach also place emphasis on the animal’s level of productivity, efficiency of gain, rate of reproduction, survivability, etc., and the role chronic stress as it pertains to poor biological functioning; a high level of agricultural productivity is enough to ensure good animal welfare, according to this argument (Fraser,
2008). For example, if a Holstein cow maintains an average milk production level of 40 kg/day of milk, then those solely in support of the health and biological functioning approach may conclude that this particular cow has a high level of welfare.

In support of the health and biological functioning approach, it should be apparent that a reduction in productivity, growth, reproduction, and/or survival reflects a disturbance to homeostasis and the normal biological functioning of the animal. However, other factors, such as genetic selection, must be considered under modern production systems, as they may cause a degree of uncoupling between animal welfare and animal productivity (Fraser, 2008).

1.2.2. Affective states

A second component of animal welfare is the animal’s ability to experience positive affective states (i.e., internal emotional states), or lack of experiencing negative states. The recent prominence given to the affective state of animals is often attributed to the writings of Marian Dawkins and Ian Duncan (Appleby et al., 2011). Both Duncan and Dawkins (1983) argued that animal feelings play the most important role in animal welfare and that animals should be void of suffering and strong, negative subjective states (i.e., pain, fear, frustration, deprivation, and boredom). The literature to date has primarily focused on the absence of such negative affective states, yet although an underdeveloped area of scientific research, the scientific study of promoting positive affective states continues to expand (Fraser, 2008).

In recent years, animal welfare scientists have developed various methods that may be used to identify and quantify the subjective experiences of animals, as they have
measurable consequences or correlates (Fraser, 2008). The two main approaches will be
discussed in this section in further detail, and they involve providing the animal with
some form of control over its environment and simply observing the choices it makes.
This may be accomplished using preference or ‘choice’ tests and/or motivation tests;
feelings are linked to both motivation and preference and associated with obtaining
desired resources (Kirkden and Pajor, 2006).

1.2.2.1. Preference and motivation tests

Preference tests are able to provide insight into the environmental conditions that
animals may prefer by simply giving them more than one option to choose and
subsequently measuring the duration of time spent with each. For example, Falk et al.
(2012) evaluated dairy cow preference for pasture versus indoor freestall housing with
variable stocking densities at the freestalls. The authors discovered that stocking density
had no effect on the time spent outside, but time spent on pasture decreased with
increasing temperature-humidity index (THI) during the day. In contrast to this form of
testing, which is longer in duration, researchers may also use more instantaneous testing
procedures, such as T- or Y- mazes, in which an animal is required to make a series of
discrete choices between alternative environments or resources (Kirkden and Pajor,
2006). Although preference tests provide insight into to which environment is viewed
more positively or negatively by the animal, this form of testing does not evaluate
specific feelings or emotions. In addition, preference tests cannot measure the
importance of the resource to the animal or provide indication of the animal’s long-term
priorities (Duncan and Fraser, 1997).
Unlike preference tests, motivation tests provide scientists the ability to quantify how important a particular resource or environment is to an animal; such tests incorporate ‘instrumental’ tasks, such as pushing a lever for a particular resource or food reward (Fraser, 2008). For example, the importance of social contact to young dairy calves has since been examined and quantified scientifically using motivation tests. For example, Holm et al. (2002) reported that young calves are willing to work by pressing a lever to obtain access to a social partner, which is indicative of their behavioral need for social contact. Calves were also prepared to work harder in order to obtain full social access to the companion calf, as opposed to head-to-head contact through the metal bars. Thus, calves may find full social contact more valuable than partial. Holm et al. (2002) concluded that the deprivation of social contact, i.e. through individual housing, may compromise animal welfare.

1.2.3. Natural behavior

Rollin (1993) defines animal welfare in terms of the animal’s natures with respect to the expression of natural behavior, as he suggests, “animals, too, have natures - the pigness of the pig, the cowness of the cow . . . - which are as essential to their welfare as speech and assembly are to us.” Intensive housing systems often prevent farm animals from performing certain types of species-specific behaviors that may often be observed in a less restrictive environment. However, as scientists continued to incorporate such natures into their assessment of animal welfare and within the constraints of modern production systems, a number of issues were encountered (Fraser, 2008). For example, what is the definition of ‘natural’? Farm animals have been under the care of humans for
millennia, so this may be incredibly difficult term to define and concretely assess. In addition, is it feasible to transform current rearing conditions for farm animals into something that is more ‘natural’ for them if creating the definition alone is such a grand task? Lastly, do ‘natural’ settings really provide the best welfare for the animal?

1.2.4. An integrative model of animal welfare

Animal welfare scientists and others in the field have responded to the number of views on animal welfare by trying to develop a scientific concept that is most amenable to scientific investigation. However, the emphasis assigned to the different components of animal welfare by different scientists will continue to reflect their personal values; science alone cannot impose a ‘correct’ definition (Fraser, 2008). It is imperative to recognize that three different approaches to animal welfare can and do overlap, as shown by Figure 1.1.

![Figure 1.1. The overlapping components of animal welfare (Adapted from Fraser et al., 1997)](image-url)
One example of an integrated model of animal welfare will be provided, as there are potentially an infinite number. If one were to consider a dairy cow suffering from lameness, they should reach the following conclusion: 1) the cow is most likely to have lower milk production and reproduction potential; her health and biological functioning is compromised, 2) the cow is in pain; her affective state is compromised, and 3) she has reduced mobility; her ability to display natural behavior is compromised (von Keyserlingk et al., 2009). It is ideal for animal welfare scientists to take a multidisciplinary approach in order to assess and safeguard animal welfare; investigating only one component of animal welfare and neglecting the other two may continually lead one to the wrong conclusion/decision. Thus, the overall objective of this dissertation is to improve the welfare of dairy animals using an integrated model of animal welfare.
Chapter 2: Review of Literature

2.1. Introduction

Dairy cows and their calves experience a number of stressors throughout their lives, but more research is needed to understand how the social and physical environment of these animals affects their welfare. Thus, this research focused on three time periods in the life of the dairy cow: 1) prenatal, 2) early life, and 3) transition (or the period around calving). These periods are also the periods when dairy cows and calves are most susceptible to disease; thus, they warrant further investigation to continuously improve the welfare of dairy animals.

Animal welfare science thus far has primarily considered the homeostatic challenges production animals may encounter after birth; however, the prenatal period is also of critical importance to mammalian species, as this period of development may significantly influence and predetermine the capability of offspring to respond and adapt to their future environment. Thus, maternal stressors experienced during gestation, especially late-gestation, may negatively affect the welfare of the offspring even prior to parturition (Arnott et al., 2012).
In the U.S., dairy heifer calves are separated from the dam within 24 h of life and in general, housed in individual hutches or pens that ensure that calves do not have direct contact with other calves until they are weaned from milk or milk replacer (USDA, 2010a). A second form of housing is group (or pair) housing, which provides calves the opportunity for direct contact and social interaction. Young dairy heifer calves (and ungulates, in general) are gregarious and highly social in nature; social interactions of animals are now recognized as conscious choices and represent their desire for social companionship (Rushen et al., 2010). However, cross-sucking behavior or non-nutritive sucking directed toward another calf’s ears, mouth, navel, scrotum, prepuce, or other body parts (de Wilt, 1985) is also observed in pair or group housing systems, and this behavior is anecdotally higher within the Jersey breed but has yet to be investigated. As there are behavioral differences among breeds of other species, it may be inappropriate to make the assumption that breeds of dairy calves behave in the same manner, and therefore benefit from similar management practices, such as group housing. Thus, there is opportunity for science to advance current knowledge with regard to alternative housing systems for Jersey heifer calves, as social interactions with conspecifics or members of the same species may enhance their well-being and increase measures of performance.

2.2. Effect of the prenatal environment on dairy calf welfare

As animal welfare is a continuous state that may be impacted in utero, it is imperative to understand the stages of development and production, especially for dairy
cattle. Modern production systems result in dairy cattle generally lactating for 10 to 11 months per year, which is 4 months beyond what may be observed in a more natural setting (Smith, 1959). In addition, dairy cows are also pregnant for 7 of the 10 to 11 months of lactation, which requires a great deal of energy expenditure as the animals simultaneously support the current pregnancy as well as lactation. Approximately 2 months (60 d) prior to the expected calving date, dairy cattle enter a non-lactating phase upon cessation of milking. This is commonly referred to as the dry period. This period is very important for the welfare of the cow as she prepares for an impending parturition and the upcoming period of lactation; the mammary glands recover from the previous lactation by replacing senescent epithelial cells and reorganizing tissue architecture. During the dry period, 60% of fetal growth also occurs (Dingwell et al., 2001). This period is of critical importance to promote the production potential of the cow’s next lactation, but it must too promote the development of the calf. Thus, the dry period must be void of unwarranted environmental stressors in order for these aspects to be accomplished.

2.2.1. A general model of stress

In accordance with the promotion of good health and fitness, the health and biological functioning approach to animal welfare also considers the biology and physiology of the animal’s stress-response system (Duncan, 2005). As depicted by Figure 2.1, the stress-response system is often divided into three separate components: 1) the animal’s recognition or perception of the stressor, 2) the animal’s subsequent biological defense against the stressor, and 3) the potential long-term consequences
incurred by the animal due to the elicited stress response; the third and final stage, if the animal is suffering from distress, will negatively impact its level of welfare (Moberg, 1985).

**Figure 2.1.** A model for the biological response of animals to stress (Moberg, 1985)

In the majority of mammalian experimental stress studies, the hypothalamic-pituitary-adrenal (HPA) stress-response system, also referred to as the general adaptation syndrome, has been the primary neuroendocrine system monitored (Moberg, 2000). Thus, the remaining portion of this section will focus on the HPA stress-response system in relation to chronic stress and animal welfare.

The biological response of an animal to a stressor requires a progression of events beginning with the animal sensing, and thus, signaling various biological mechanisms
that a potential threat is present. The two primary physiological systems that respond to stress are the sympathetic nervous system (SNS) and the HPA axis. The SNS is often referred to as the fight-flight response and is characterized by a chain of neural and humoral events that prepare the animal for an impending emergency (Ewing et al., 1999). Noted effects of SNS activation are: increased metabolic rate, increased heart rate and blood output, increased blood flow to the heart, brain, and muscles, and conversion of glycogen to glucose. (Ewing et al., 1999). The HPA stress-response system, however, represents a longer-term, sustained stress response in contrast to the rapid response generated by the SNS. In this system, the hypothalamus, following the initial stimulus, releases corticotropin-releasing hormone (CRH), which stimulates the anterior pituitary gland to release adrenocorticotropic hormone (ACTH) into the blood (Figure 1.2). ACTH then activates the adrenal cortex to increase its output of steroid hormones called glucocorticoids (Fraser, 2008).
Figure 2.2. Schematic representation of the hypothalamic-pituitary-adrenal (HPA) axis (Adapted from Smith and Vale, 2006)

**Glucocorticoids**

A number of hormones (i.e., ACTH, glucocorticoids, catecholamines, etc.) are involved in the stress-response system, but only one mediator of the stress-response system, glucocorticoid (i.e., cortisol), and its role in severe chronic stress will be considered in this section of this dissertation. Although stress is not inherently bad severe and/or chronic stress or prolonged periods of high circulating cortisol concentrations (compared to baseline values) may lead to behavioral (i.e., stereotypies)
disorders (Burnett et al., 2014). In dairy cattle, prolonged activation of the HPA axis has been linked to immunosuppression and reduced fertility, and the animals may become more susceptible to disease (Hemsworth, 2003).

Potential sampling concerns (i.e., handling and restraint, time of day, etc.) and the lack of a reliable, non-invasive method for evaluating chronic stress in farm animals has led to an increased interest in the development of new techniques designed to monitor adrenocorticol activity. Measuring fecal cortisol analytes (rather than blood measures) have recently been recommended for identification of individuals or herds that are experiencing chronic stress and are at increased risk for disease (Tucker et al., 2007; Huzzey et al., 2011). A main advantage of fecal cortisol is its usefulness as an indicator of chronic stress, without the confounding effect that handling has on alternative measures such as plasma cortisol (Huzzey et al., 2011).

Measuring cortisol metabolites in the feces is advantageous as fecal samples may be collected without stressing the animal, thus providing a feedback free method of sampling. To provide validation, Palme et al. (1999) reported a correlation between fecal cortisol metabolites (11, 17-dioxoandrostanes) and plasma cortisol in ruminants through the administration of ACTH and dexamethasone tests; fecal cortisol metabolites were reported to parallel plasma cortisol with a delay time of approximately 10 to 12 hr, depending on the passage rate of the species. In addition, circulating hormones are integrated over a period of time and represent a cumulative secretion of hormones as opposed to point estimates that may be obtained from blood sampling techniques; fecal samples are not subject to short episodic fluctuations (Palme, 2012). Therefore, fecal
collection, a non-invasive measure that does not impose additional stress on the animal or require additional personnel for sample collection, was the chosen approach in the present study for both cows and calves.

**Fetal programming of hypothalamo-pituitary-adrenal function**

Stress activation of the HPA axis also influences fetal development (Lay et al., 1997). Prenatal stress and maternal exposure to exogenous glucocorticoids can permanently modify HPA function and stress related behavior, which in turn, leads to increased fetal exposure to glucocorticoids (Kapoor et al., 2006). Glucocorticoids are essential for many aspects of normal fetal brain development; however, continued exposure of the fetal brain to an excess of glucocorticoids may have long-term or potentially life-long effects on neuroendocrine function for many species, including primates, guinea pigs, sheep, cattle, goats, pigs, rats and mice (Kapoor et al., 2006).

In addition, it is important to note that the timing of maturation of the HPA axis relative to birth is largely species specific. For example, in species that give birth to relatively mature young, such as sheep and primates (Owen and Matthews, 2003), a large proportion of neuroendocrine maturation occurs *in utero*. However, in contrast, in species that give birth to litters of relatively immature young, such as rats and mice (Sapolsky, 1996), the majority of neuroendocrine maturation occurs postnatailly. Thus, neuroendocrine development with regard to fetal manipulation will impact different species at different stages.

Although female livestock species may encounter a wide variety of physical and physiological stressors concurrent with pregnancy, prenatal stress with regard to livestock
species has received little attention. Such stressors may include but are not limited to thermal stressors, malnutrition, social stressors related to re-grouping, overcrowding, isolation, etc., transport stressors, and stressors related to animal handling (Roussel et al., 2004). As the majority of the literature with regard to fetal hypothalamo-pituitary-adrenal function with regard to livestock species involves sheep and beef cattle, these species will also be included in the discussion of this section.

Similar to cattle, sheep are gregarious in nature and extremely sensitive to isolation; isolation has been found to have a larger effect on cortisol release than handling or restraint (Parrot, 1990). To examine the effects of repeated stress in pregnant ewes on the behavioral and physiological reactivity of their lambs, Roussel et al. (2004) exposed ewes at 2.5 months of pregnancy to isolation twice per wk during the last 5 wk of gestation. The authors reported that prenatally stressed lambs had higher basal cortisol concentrations at 25 d of age than control lambs that were not exposed to prenatal stress. However, this effect was not observed when lambs were re-tested at 8 mo of age. This finding was thus attributed to the high concentration of maternal cortisol secretion during the stress treatment of the dam.

In addition, maternal nutritional status directly influences nutrient partitioning and fetal growth, development, and function of the major organ systems (Godfrey and Barker, 2000). Maternal nutrition during gestation is also one of the extrinsic factors linked to calf health; Corah et al. (1975) reported increased morbidity and mortality rates in calves born from primiparous heifer receiving only 65% of their dietary energy requirement during the last 90 d of gestation compared to calves born from primiparous heifers.
receiving 100% of their dietary energy requirement. Calves born to nutrient restricted dams were also lighter at birth, which may contribute to the observed increased in morbidity and mortality. Greenwood et al. (2004) also demonstrated that steers born from cows nutritionally restricted during gestation had lower BW and carcass weights at 30 mo of age compared with steers born from cows fed adequately.

Cows may also experience potentially stressful management practices and environmental variables, such as temperature, humidity, and photoperiod, during gestation, which may consequently affect their fetal offspring (Arnott et al., 2012). One environmental stressor that dramatically affects the dairy industry is heat stress or hyperthermia; cattle generally experience moderate hyperthermia in humid environments when ambient temperatures rise above 26.7°C, and severe hyperthermia may develop as ambient temperatures reach or exceed 32.2°C (Wolfenson, 2009). An alternative approach to evaluating cooling needs in cattle is to use the temperature humidity index (THI), a combined measure of both ambient temperature and relative humidity. This has been shown to be more effective in evaluating environmental effects on lactating cattle than temperature alone. For instance, Collier et al. (2012) reported the THI threshold for lactating dairy cows producing more than 35 kg of milk per day is 68 (i.e., 27.0°C with 0% humidity; 22.0°C with 50% humidity). Cows exposed to such conditions during lactation showed a significant decrease in milk production and reproductive performance, as well as an increased incidence of disease (West, 2003). In recent years, however, such investigation has shifted focus from cows during lactation to those in late gestation during the nonlactating period (West, 2003), as heat stress may negatively affect immune
function during the transition period from 3 wk before to 3 wk after calving and milk production during the subsequent lactation (Tao et al., 2011).

Extended periods of high ambient temperature coupled with high relative humidity also negatively affects the welfare of the dam’s offspring. For example, Collier et al. (1982) investigated the effects of maternal heat stress during the last trimester of gestation on calf birth weight and reported significantly lower birth weights of calves born from dams that were denied access to shade during this critical period of fetal development. More recently, Tao et al. (2012) evaluated the effect of heat stress during the last 45 d of gestation on subsequent growth and immune function of dairy calves. Cows were assigned to 1 of 2 treatment groups, heat stress (HT) or cooling (CL), and were housed in a freestall barn; sprinklers and fans were installed in the stall areas assigned to the CL group. The authors reported a significant 4 d reduction in the length of gestation in the HT group, and calves born from dams under such conditions weighed significantly less at birth, which continued through weaning. In addition, calves from environmentally stressed dams also were born with higher circulating cortisol levels, a biological indication that such animals may have been stressed in utero and their welfare negatively influenced prior to parturition.

2.3. Effect of the postnatal environment on dairy calf welfare

2.3.1. Health and growth performance

Although individual housing systems are often recommended as a means of reducing disease transmission among calves, research studies that have been conducted to
evaluate the health of calves housed individually or in groups have shown calf health to be similar among calves housed individually and in small groups with proper management (Kung et al., 1997; Chua et al., 2002).

Early epidemiological studies of veal calf group housing systems have indicated that diseases, such as respiratory disease, tend to develop among calves housed in groups, indicating that calf-to-calf contact may advance the proliferation of the disease (Miller et al., 1980). Correspondingly, Webster et al. (1985b) examined the effect of different calf-rearing systems on the incidence of disease, cleanliness, and injury across multiple farms; calves were either home-reared or bought-in for veal production and housed in individual wooden crates or straw-bedded pens in groups. Calves were monitored for 16 wk, and mortality rate was found to be lower for calves housed individually (1.7%) compared to those housed in groups (3.8%). The proportion of group-housed veal calves treated for enteric disease, especially during the first 2 wk of life, was significantly greater than those housed individually in crates. However, this study did not report specific group size. Svensson and Liberg (2006) and Svensson et al. (2006) found that rearing young calves in large groups (6 to 30 animals) with automated feeders was associated with earlier onset and more severe cases of scours and increased risk of respiratory disease; such problems were not observed with groups of smaller animals (3 to 8 animals) with manual milk feeding. Together, these results suggest that health problems associated with group housing systems may be specific to individual farm management practices and dependent on the size of the group and specific method of milk delivery.
Although there is clear evidence that risk for health problems may increase for large groups, more recent studies have reported a good status of health and similar or even improved growth rate among calves reared in smaller group housing systems. For instance, a national survey of 1,685 United States dairy operations revealed a positive correlation between group size and mortality; calves housed in groups of 7 or more had a higher incidence of mortality compared to calves housed in smaller groups or individually, which were equivalent (Losinger and Heinrichs, 1996). Kung et al. (1997) also reported that calves housed individually in hutches required 19 d of medication, whereas calves housed in smaller group settings only required 11 d.

Comparisons of different housing systems by use of large-scale epidemiological studies challenge the former claim that individual housing of pre-weaned calves is most advantageous for their health. Smaller-scale studies have recently made an attempt to isolate the potential confounding effects of group housing by controlling for various management practices. For instance, Chua et al. (2002) examined the health and performance of heifer calves (n = 30) that were housed individually or in pairs through 8 wk of age; each animal was fed and managed identically. This study revealed that pair-housed calves remained healthy, and there were no differences between housing treatments with regard to diarrhea. Before and after weaning, calves in both treatment groups gained weight rapidly, and there were no significant differences in gains, except during wk 6. During this week of weaning, calves housed in pairs continued to gain weight at pre-weaning levels (approximately 1 kg/d), yet calves housed individually gained weight at half this rate.
Similar studies have also investigated the potential beneficial effects of group housing on growth performance (Pempek et al., 2013; Xiccato et al., 2001). Pempek et al. (2013) reported that pair-housed calves ingested more starter grain during the pre-weaning period than did individually housed calves, which contributed to this treatment group also having a greater total DMI. Xiccato et al. (2001) also observed that calves reared in groups of 4 from 2 to 4 mo of age had a greater final body weight (BW) and significantly higher feed efficiency compared to calves reared individually. Thus, these controlled studies seem to reinforce the larger epidemiological studies by showing that young calves can be reared in small groups without it being a detriment to their health, if housing, feeding, and management practices are appropriate.

2.3.2. Behavior of dairy calves

The primary disadvantages of individual housing systems, with regard to behavior as a component of calf welfare, are the inability of the calves to engage in social interactions and the lack of space provided for exercise. However, housing calves individually can reduce cross-sucking and aggressive behaviors and decrease competition among calves for food resources (Rushen et al., 2010).

Locomotion/locomotor play

The amount of locomotion that young calves will display is largely dependent upon the total area of space they are allotted. Generally, even though the amount of space provided per calf may be similar, calves housed in group pens commonly have access to a greater total amount of available space (Rushen et al., 2010). This, in turn, will shape the form and frequency of locomotor behaviors exhibited.
It is important to closely examine how each study monitored and scored various movements, as this can distort and lead to the misinterpretation of the results presented. For example, apart from whether calves were housed individually or in groups, Webster et al. (1985a) concluded that veal calves spent between 3 and 7% of their time engaged in locomotion. However, specific types of locomotor behaviors were not noted; pacing forwards and backwards by veal calves was scored as the equivalent of play behavior exhibited by group-housed calves. This is certainly not the case, and it is unlikely that these behaviors are of equal importance to the animals.

Conversely, other experiments, which employed a more detailed ethological approach to measure locomotion, have reported that calves reared in group settings moved more than calves reared alone. Chua et al. (2002) compared the behavior of calves housed in pairs to those housed individually and determined that pair-housed calves had twice as many movements as did the individually housed calves (1.43 versus 0.64% of the day). Jensen et al. (1998) reported similar findings in a study that aimed to investigate the effect of social contact and space allowance on play behavior in dairy calves kept in pens. In this experiment, 48 heifer calves were assigned to 1 of 4 housing types: 1) small individual pen (0.9 x 1.5 m); 2) large individual pen (1.8 x 3.0 m); 3) small group pen (1.8 x 3.0 m; housing 4 calves); and 4) large group pen (3.0 x 5.4 m; housing 4 calves). As the authors expected, space availability influenced the quality and quantity of locomotor behaviors exhibited. They reported a significant increase in locomotor play displayed by calves housed in the larger pen treatment groups compared to calves housed in smaller pens. In addition, elements of locomotor play that require
elevation of the hind legs, such as galloping, leaping, buck-kicking, and high bucks, were either entirely absent or rarely observed when calves were housed in small individual pens. Thus, the results of the latter studies seem to indicate that sufficient space is essential for the expression of certain locomotor behaviors and that a more spatial environment may even stimulate such behaviors.

**Social behavior**

The majority of domesticated animal species are social animals, but contact or rearing with conspecifics is often prohibited in captive settings (Chua et al., 2002). Individual rearing systems prevent calves from making physical contact with one another, and raising calves in total isolation prohibits physical and visual contact; both forms of housing may impede social development (Bøe and Færevik, 2003). Social development is of critical importance to dairy animals, as they form and maintain complex social groups. As defined by Bøe and Færevik (2003), a *group* is a combination of animals that are of the same species and are able to remain relatively stable over time. The establishment of a group encompasses a social hierarchy within a given group of animals that is influenced by not only by genetic predisposition, but also by previous positive and negative interactions experienced by the animal (Kondo and Hurnik, 1990). Thus, it is important for calves to learn how to interact socially with conspecifics or members of the same species, as this may have indirect effects later in life.

The grouping of unfamiliar animals with one another has been found to increase aggression and social stress (Nakanishi et al., 1993; Hasegawa et al., 1997). It may also have negative effects on production traits, such as feed intake (Nakanishi et al., 1993) and
milk yield (Hasegawa et al., 1997). With regard to social grouping and re-grouping, cows are the focus of the majority of the literature, and very few studies have been conducted to investigate group stability among young calves. Kondo et al. (1984) investigated the social stability of 12 Holstein steers housed individually from birth to 5 mo of age. Calves were then divided into 2 groups of 6 calves, referred to as Groups A and B, and Group B was further divided into 3 groups of 2 calves each. Paired calves were re-grouped every 3 d in order to combine and expose all calves in Group B to one another. All calves from Groups A and B were then combined together and observed at 15 min intervals for 153 h. During the first 24 h, the number of aggressive interactions observed was significantly greater for calves from Group B compared to Group A (53 versus 23 bouts of aggression, respectively; \( P < 0.05 \)). Thus, calves originally from Group A appeared to establish social stability at a faster rate compared to those that were continually re-grouped.

With regard to animal welfare, it has been suggested that if animals are willing to work in order to gain access to a specific resource (i.e., food), their welfare is likely improved if they are further allowed access to that resource (Broom, 1988). Holm et al. (2002) examined the motivation of dairy calves for 2 different forms of social contact with a known companion calf, with either head-to-head contact through metal bars or full social contact without restrictions, using operant conditioning methods. Calves were reared individually, but they were trained to press a panel located within their pen to open a gate that allowed them to enter into another pen housing the companion calf. As the authors predicted, the calves were motivated to work to gain access to the companion
calf. When required to press the panel only 6 times in order to obtain social contact, they did so a total of 10 times, with sessions lasting a minimum of 20 min and a maximum of 50 min. In addition, calves were prepared to work harder in order to obtain full social access to the companion calf as opposed to head-to-head contact through the metal bars, indicating that calves find full social contact more valuable than partial. It was also observed that the calves were socially active throughout 8.3% of the social period when tested for head contact and 54.1% when tested for full social contact. Thus, the type or quality of the social interaction seems to be of great importance. The authors ultimately concluded that “calves are willing to work to get access to a conspecific, which points to a behavioral need for social contact . . . as a consequence, calves’ welfare may be threatened if they are not allowed to perform social behaviors” (Holm et al., 2002).

Chua et al. (2002) recorded the behavior of calves reared individually or in pairs once a week for 24 h over 7 wk. Results showed that pair-housed calves engaged in social contact for approximately 2% of the day. Another study also categorized play as a form of social behavior, and found that play was only displayed by calves housed in small groups compared to those housed individually (Babu et al., 2004); these results were consistent across both pre- and post-milk feeding periods through 14 wk of age. Dannemann et al. (1985) also reported that calves housed in small groups of 5 engaged in social play for a total of 6.75 min in the period from 800 to 1600 h, which corresponds to 3.8% of the active time for social play.

One potential consequence of denying young calves the opportunity for social contact is that they may not develop the social skills necessary to adjust and cope with
group housing situations later in life, as they may be either more fearful of conspecifics (Rushen et al., 2010). Several studies have also reported higher weight gains for group-housed calves compared to calves housed individually during the milk-feeding period, which is often attributed to social facilitation or an improvement in performance produced by the presence of others (Chua et al., 2002; Xiccato et al., 2002). The studies discussed thus far have measured social behavior during the pre-weaning phase of life, but it is just as important to evaluate calf welfare and behavior upon being introduced to larger groups of calves post-weaning. Unfortunately, this dissertation only examines the milk-feeding and weaning periods. Ideally, future studies should try to extend beyond the milk-feeding period and continue to follow the calves post-weaning and through first lactation.

Veisser et al. (1994) examined the effects of rearing young calves individually or in a group of 4 on subsequent social behavior. After re-grouping the calves at 14 wk of age, more agonistic and fewer affiliative behaviors (positive social behaviors such as playing or allogrooming) were observed in groups of calves that were previously individually housed. Complementary research studies, although scarce in quantity, have investigated the longer-term effects associated with the absence of social behavior in individually reared calves (Jensen et al., 1999). For the first 3 mo of life, calves were housed either in: 1) single pens with open sides; 2) single pens with closed sides (physically and visually isolated); 3) groups of 5 calves; or 4) groups of 5 cow-calf pairs. After weaning, all experimental calves were housed in similar tie-stalls, and at 26 wk of age, they were subjected to an open-field test by introducing them into a novel area containing an
unfamiliar heifer. Previously isolated calves housed in pens with closed sides had a longer latency to enter the open-field test arena. Additionally, group-reared calves (both groups of 5 calves and groups of 5 cow-calf pairs) spent more time engaged in mock fighting (interpreted as play behavior) and sniffed and mounted the unfamiliar heifer more than calves housed in single pens (both single pens with open sides and single pens with closed sides). Thus, individual rearing may reduce the calf’s ability to cope with unfamiliar animals during initial encounters (Rushen et al., 2010). More research in this area is needed to fully understand the long-term effects that individual housing systems may impose on social behavior and the welfare of young calves.

**Cross-sucking**

From the results of the previous studies, cross-sucking occurs immediately after meals of milk in contrast to other times throughout the day (de Passillé, 2001), and very little sucking occurs after the calves are weaned from milk (Lidfors, 1993). Thus, the focus of this review will remain only on the milk-feeding period.

Few studies have reported cross-sucking as being a problem or injurious to calf health. However, a large majority of the experiments that did find this to be a concern only offered milk to calves in a bucket or trough; neither bottles nor buckets fitted with a teat were provided as a method for comparison nor were artificial teats present to manage sucking behavior (Margerison et al., 2003). According to Rushen et al. (2010), cross-sucking may be controlled by the utilization of proper management practices and the adoption of a suitable feeding program. For instance, when calves were provided the same amount of milk and only the method of delivery varied, calves fed with a bucket
spent significantly more time cross-sucking than teat-fed calves (1.91 versus 0.16 min/30 min observations) (Jensen and Budde, 2006). The cross-sucking that did occur was directed toward the head and around the muzzle, which was smeared with milk after completing a meal.

In addition to the use of individual bottles, automated milk feeders also promote sucking behavior and may be employed as a method to reduce cross-sucking. For example, in a study where the milk was delivered slowly in a bucket and calves were allowed to suck on a floating teat placed within, calves were observed to perform significantly less cross-sucking compared to other calves that did not have access to a floating teat (Loberg and Lidfors, 2001). The authors attributed this to the combination of low milk flow rate and the opportunity for young calves to exhibit sucking behavior. Access to a dry, artificial teat coupled with milk feeding in open buckets can also significantly reduce the occurrence of cross-sucking behavior in young calves housed in small groups (de Passillé and Caza, 1997).

However, the use of automated milk-feeding systems is not invariably associated with a decline in cross-sucking behavior. A study conducted by Veissier et al. (2002) reported an increase in cross-sucking in group-housed calves that were fed with an automatic device fitted with a teat compared to bucket-fed calves. The authors postulated that this might have been due in part to the fact that each treatment was only observed once throughout the experimental period; more observation periods may have been needed. In addition, Rushen and de Passillé (1995) reported that calves’ the motivation to suck is stimulated at every milk feeding irrespective of how much milk the calf is
offered. These results suggest that in addition to feeding method, feeding management practices are of great importance in order to reduce cross-sucking behavior.

2.4. Effect of the environment on dairy cow welfare

2.4.1. Overstocking the feed bunk

In addition to the importance of the pre- and postnatal environment to the welfare of dairy animals, the environment of the mature dairy cow should too be considered. Dairy cows may be subjected to unintentional social stressors, such as overstocking, during the dry period. The optimal number of animals per group is a function of 1) competition for space, 2) competition for feed or water, and 3) availability of comfortable, useable free stalls (Grant and Albright, 2001). Current industry-recommend best practices with regard to space allowance for dairy cows housed in a freestall barn is to provide 1 freestall per cow within the group and at least 0.6 m of linear feeding space per animal (NFACC, 2009). However, even with the provision of such recommendations, overstocking remains very common; 43% of farms provide less than the recommended lying stall availability and 58% provide less than the recommended 0.6 m of feeding space per cow (USDA, 2010).

Overstocking has been shown to have profound behavioral and physiological consequences, both of which may negatively affect the welfare of the cow. For instance, Fregonesi et al. (2007) reported that cows spent on average 12.9 h/d lying when 1 stall was available per cow, but that this time significantly decreased to 11.2 h/d when cows were overstocked to 150% and cows spent an increased amount of time standing idle. In
addition, cows housed in overstocked conditions competed indirectly for stall usage, as they were observed to lie down more quickly after returning from the parlor as opposed to eating. In such competition situations, decreased lying time is also associated with increased plasma cortisol concentrations (Gonzalez et al., 2003), which may be attributed to increased aggressive displacements (i.e., physically displacing one another from the feed bunk or freestalls) often observed at the overstocked feed bunk or freestalls (Huzzey et al., 2006; Fregonesi et al., 2007).

In addition, Huzzey et al. (2012) examined the relationship between competitive success during displacements and measures of physiology and behavior in Holstein dairy cattle; each group of 10 cows had access to 5 freestalls and 0.34 m of linear post-and-rail feed bunk space per cow. Behavior data were collected on the number of successful competitive displacements (i.e., an instigated displacement resulting in the complete withdrawal of another animal from the feeding area) and then used to calculate a competitive index (CInd) value for each cow. The CInd was then used to categorize cows as having: low success (LS), medium success (MS), or high success (HS). The authors reported no difference observed in the total number of successful displacements that cows in the LS, MS, or HS groups engaged in per day. However, the LS group had greater plasma nonesterified fatty acid (NEFA) and fecal cortisol metabolite concentrations during periods of overstocking compared to the MS and HS groups.

Proudfoot et al. (2009) also examined competition at the feed bunk and discovered that transition cows with lower displacement indices (i.e., were more likely to be displaced from the feeding area than to do the displacing) also ate more rapidly, particularly 2 wk
postpartum. The authors confirmed that overstocking the feed bunk alters the feeding behavior of dairy cows around the transition period, potentially increasing the risk of illness after calving. Therefore, the negative behavioral and physiological consequences of overstocking dairy cattle have been well documented; overstocking may impart unwarranted social stress. However, it is entirely unknown as to whether overstocking during the dry period may also affect the growing fetus during the final weeks of gestation.

2.5. Specific research questions of this dissertation

The research presented in this dissertation addresses some of the gaps outlined in the preceding sections with regard to the importance of the environment in relation to dairy cow welfare. Specifically, the hypothesis of this dissertation is that environmental manipulations influence the welfare of dairy cattle during the prenatal, early life, and transition (or the period around calving) stages. The following research questions were used to address this hypothesis:

1) Chapter 3: Does stocking density at the feed bunk during late gestation affect the growth of heifer calves?

2) Chapter 4: Do paired housing systems affect the behavior and performance of Jersey heifer calves?

3) Chapter 5: Does increased stocking density at the feed bunk during different stages of the dry period affect the metabolic health and productivity of dairy cows?

4) Chapter 6: Does overstocking the feed bunk affect dairy cow temperament?
Chapter 3: The Effect of Stocking Density at the Feed Bunk During Late Gestation on the Growth of Heifer Calves

3.1. Abstract

Overstocking the feed bunk has been shown to have behavioral and physiological consequences, both of which negatively affect the health and welfare of the cow. However, it is unknown if overstocking the feed bunk during late-gestation also affects the welfare of the calf. The goal of this study was to investigate some potential negative effects of overstocking the feed bunk during different stages of the dry period might have on the postnatal growth of their offspring. One hundred twenty non-lactating dairy cows were blocked and assigned to one of four experimental groups with different stocking density conditions at the feed bunk (Overstocked (OS): 0.88 headlocks/cow; Understocked (US): 1.17 headlocks/cow). The experimental groups were as follows: a) OS from 60 to 1 (OS), b) OS from 60 to 26; US from 25 to 1 (OS-US), c) US from 60 to 26; OS from 25 to 1 (US-OS), and d) US from 60 to 1 (US) d prior to calving. The heifer calves’ treatment reflected the treatment assignment of their dam (n = 13, 18, 16, and 11, respectively). Body weight (BW) was measured once weekly for heifer calves through
weaning at 5 wk of age, and wither height (\textbf{WH}) and hip height (\textbf{HH}) measurements were obtained at birth, 3 wk, and 5 wk of age. Gestation length was similar ($P > 0.05$) among treatments. With initial BW as a covariate, calf BW was similar among treatment groups ($P > 0.05$), and there was no treatment by week interaction. Overstocking the feed bunk of the dam during the dry period also did not affect calf growth in terms of stature ($P > 0.05$ for all measurements). In conclusion, overstocking cows at the feed bunk during the dry period did not compromise postnatal calf growth in our study.

Further research is encouraged to determine the potential consequences of more severe overstocking treatments or the effect of overstocking both the feed bunk and lying stalls on fetal development and postnatal calf growth and welfare.

3.2. Introduction

Animal welfare science thus far has primarily considered the challenges production animals may encounter after birth; however, the prenatal period is also of critical importance to mammalian species, as this period of development can influence and predetermine the capability of offspring to respond and adapt to their future environment (Arnott et al., 2012). Thus, maternal stressors experienced during gestation, especially late-gestation, may negatively affect the welfare of the offspring prior to parturition.

Stress activation of the HPA axis influences fetal development (Lay et al., 1997). Prenatal stress and maternal exposure to exogenous glucocorticoids can permanently modify hypothalamic-pituitary-adrenal (HPA) function and stress related behavior, which
in turn, leads to increased fetal exposure to glucocorticoids (Kapoor et al., 2006). Glucocorticoids are essential for many aspects of normal fetal brain development; however, continued exposure of the fetal brain to an excess of glucocorticoids may have long-term or potentially life-long effects on neuroendocrine function and coping capabilities of offspring to future stress events for many species, including primates, guinea pigs, sheep, cattle, goats, pigs, rats and mice (Kapoor et al., 2006).

Induced stress during gestation for bovine species has been shown to have detrimental effects on offspring health (Corah et al., 1975) and productivity (Collier et al., 1982; Tao et al., 2012). Maternal nutrition during gestation is also one of the extrinsic factors linked to calf health; Corah et al. (1975) reported increased morbidity and mortality rates in calves born from primiparous heifers that received only 65% of their dietary energy requirement during the last 90 d of gestation compared to calves born from primiparous heifers that received 100% of their dietary energy requirement. Calves born to nutrient restricted dams were also lighter at birth, which may contribute to the observed increased in morbidity and mortality. Greenwood et al. (2004) also demonstrated that steers born from cows nutritionally restricted during gestation had lower BW and carcass weights at 30 mo of age compared with steers born from cows fed adequately. In addition to maternal nutrition, environmental factors, such as heat stress, may also be of detriment to the offspring. For example, offspring from dairy cows exposed to late-gestation heat stress were significantly lighter at birth compared to offspring from cows exposed to cooling (Tao et al., 2012). Tao et al. (2012) also reported lower plasma protein and total serum IgG during the first 28 d of age for calves exposed
to heat stress in utero, which may suggest that IgG transfer from colostrum to the circulation was greater in calves exposed to cooling compared with those exposed to heat stress. However, no studies have examined the maternal social environment or housing conditions in relation to prenatal development and the postnatal growth and health of offspring.

Dairy cows may be subjected to unintentional social stressors, such as overstocking the feed bunk, during the dry period. Current industry-recommended best practices with regard to feeding space allowance for dairy cows housed in a freestall barn is to provide at least 0.6 m of linear feeding space per animal (NFACC, 2009). However, even with the provision of such recommendations, overstocking the feed bunk remains very common; 58% of farms provide less than the recommended 0.6 m of linear feeding space per cow (USDA, 2010). Increasing the number of animals per feeding space induces competition at the feed bunk, which can cause changes in feeding behavior (Huzzey et al., 2006; Proudfoot et al., 2009). Huzzey et al. (2006) reported a significant curvilinear decrease in daily feeding time and increase in the number of displacements from the feeding area with increased stocking density (0.81, 0.61, 0.41, and 0.21 m/cow) at the feed bunk. Therefore, the negative behavioral and physiological consequences of overstocking dairy cattle have been well documented; overstocking may impart unwarranted social stress. However, it is unknown as to whether a social stressor during the dry period may also affect the growing fetus during the final weeks of gestation.

The objective of the present study was to determine whether increased stocking density at the feed bunk for the dam during different stages of the dry period (i.e., far-off
versus close-up period) affects the postnatal performance and health of their heifer calves. We hypothesized that calves born from dams overstocked during the entire 60 d dry period would have the lowest birth and pre-weaning BW due to a shorter period of gestation, as well as the lowest blood IgG concentrations. It was also hypothesized that overstocking the dams only during the close-up period (last 30 d of the dry period) would have a more deleterious effect on the offspring compared to only overstocking the dams during the far-off period (first 30 d of the dry period).

3.3. Materials and methods

The present study was conducted from June to December 2014 at Catapadale Dairy located in Marshallville, Ohio, in accordance with the guidelines set by the Institutional Animal Care and Use Committee of The Ohio State University (Protocol No. 2014A00000063).

3.3.1. Cows, housing, and diet

One hundred twenty multiparous Holstein cows were dried-off approximately 60 d prior to their expected calving date (60 ± 8 d before actual calving date) and balanced by expected calving date, lactation number, previous 305-d mature-equivalent milk yield, and sire identification, respectively. Using a randomized complete block design (RCBD), cows were blocked and allocated to 1 of 4 treatment groups (n = 30 cows/treatment) with different stocking densities at the feed bunk during different stages of the dry period (Overstocked (OS): 0.88 headlocks/cow; Understocked (US): 1.17 headlocks/cow). All cows were housed in a two-row freestall barn throughout the dry period and had access to
at least one deep-bedded sand freestall per cow. Cows were provided feed and water ad libitum, with fresh feed delivery twice daily and diets were formulated according to recommendations provided by the National Research Council (NRC, 2001).

3.3.2. Experimental design and treatments

The 4 experimental treatment groups were as follows: a) OS from 60 to 1 (OS), b) OS from 60 to 26; US from 25 to 1 (OS-US), c) US from 60 to 26; OS from 25 to 1 (US-OS), and d) US from 60 to 1 d (US) prior to calving. The two-row freestall barn was partitioned and divided into 2 adjacent experimental pens; OS-US and US-OS treatment groups switched experimental pens as they entered the close-up period approximately 26 d prior to calving. The conditions of overstocking at the feed bunk were simulated and adjusted in the OS pen by attaching wire hog panels to the headlocks by cable ties in order to restrict access to the feeding area. Feed was only placed in front of accessible headlocks. As cows began to show signs of impending parturition (i.e., milk let-down, relaxation of the tail ligament, udder enlargement, and swelling of the vulva), they were moved to a straw-bedded group maternity pen where they remained until calving.

The calves’ treatment reflected the treatment assignment of their dam. Except for birth weight, only heifer calves (OS: n = 13; OS-US: n = 18; US-OS: n = 16; US: n = 11) were used in the current experiment. Newborn heifer calves were separated from their dams immediately after birth. All calves were fed 3.8 L of pooled colostrum, using an esophageal tube feeder, within 1 h after birth. All calves were housed in individual pens (Calf-Tell Indoor Calf Pen, Germantown, WI) and provided with sawdust bedding through weaning.
3.3.3. Serum IgG

Blood samples were collected in 5-mL Vacutainer serum collection tubes (BD Vacutainer Plus Blood Clot Collection Tubes, Franklin Lakes, NJ) via jugular venipuncture within 48 h after calves were fed colostrum. The blood samples were immediately placed on ice after collection and transported to the laboratory within 1 h. Samples were centrifuged at 3,500 RPM (1,180 x g) a 4°C for 15 min. Calf serum IgG concentration was analyzed using an optical, hand-held Brix refractometer (Jorgesen Laboratories, Inc., Loveland, CO).

3.3.4. Feed

Calves were fed whole milk twice daily via bucket at approximately 0530 and 1500 h at a rate of 0.95, 1.89, 2.84, 3.79, and 2.84 L/feeding during wk 1, 2, 3, 4, and 5, respectively, according to farm protocols. Gradual weaning began at the beginning of wk 4, as calves were decreased to one milk-feeding (morning only) per day. All calves were weaned by 5 wk of age. Calves had ad libitum access to a texturized starter grain (Purina Animal Nutrition, Gray Summit, MO) and water throughout the experiment.

3.3.5. Performance and health

All calves (OS: n = 32; OS-US: n = 31; US-OS: n = 33; US: n = 30) were weighed at birth and heifer calves weekly thereafter. In addition, hip height (HH) and wither height (WH) measurements were taken at birth and 3 and 5 wk of age. Rectal body temperature was recorded for the first 6 d of life, and fecal scores (Diaz, et al., 2001) were recorded daily throughout the experimental period.
3.3.6. Fecal collection and analysis

Fecal samples were collected from each calf during week 1 of age. Fecal samples were collected fresh, sealed within Whirl-Pak® bags (18-oz, Nasco, Fort Atkinson, WI), and immediately placed on ice after collection. Fecal samples were stored at -20°C until frozen contents were transferred to aluminum pans and freeze-dried. The DM percentage (20.0% DM, on average across treatment and sampling wk) of each fecal sample was obtained by weighing the sample before and after drying. Steroids were then extracted from the dried fecal samples. Briefly, 2 g of each fecal sample was weighed and mixed with 3 mL of 90% ethanol for 30 min. Samples were then centrifuged at 8,400 RPM (4000 x g) for 15 min, and 1 mL of supernatant was transferred to a clean tube and evaporated to dryness under nitrogen; dried, extracted samples were stored at -20°C in a desiccator. The concentration of cortisol was measured using an enzyme immunoassay validated for use in cattle (Appendix A; DetectX® Cortisol Enzyme Immunoassay Kit, Arbor Assays, Ann Arbor, MI).

3.4. Statistical analysis

The effect of treatment on gestation length, birth weight, IgG concentration, and fecal cortisol concentration at 1 wk of age were analyzed using an ANOVA (PROC GLM procedure of SAS 9.3, 2012); least squares means ± standard errors of the mean (LSM ± pooled SEM) are reported. The model included the fixed effect of treatment, with calf within treatment included as a random effect. The effect of treatment on repeated measures (change in BW, body temperature, HH, and WH) were assessed using a
repeated measures ANOVA (PROC MIXED); LSM ± pooled SEM are presented. Birth measurements were used for covariate adjustment of data. The covariance structure of error for the repeated measures in time was selected based on the lowest Bayesian information criteria (BIC), and the selected covariance structure of error was the compound symmetry (CS) structure. The model included the fixed effects of treatment, time, and a treatment by time interaction, with calf within treatment included as a random effect. Significant differences were declared at $P \leq 0.05$ and a trend at $0.05 > P \leq 0.10$.

### 3.5. Results

The gestation length of dams was similar among treatments (OS: 276, OS-US: 274, US-OS: 275, and US: 276 ± 1 d). There was no effect of treatment on calf serum IgG concentrations (Table 3.1). All calves received adequate colostrum and had success of passive transfer; serum IgG concentrations were well above the 10 mg/dL threshold. Birth weight was also similar across treatments for both bull and heifer calves. Heifer calves’ BW increased at a similar rate throughout the experimental period regardless of treatment, and there was no treatment by time interaction (Figure 3.1), thus, there was no difference in overall ADG. Body temperature through 6 d of age was also similar among treatments. Heifer calves were also of similar stature with regard to both wither and hip height, and there no significant difference observed by wk of age. Fecal cortisol metabolite concentrations were similar among treatments (OS: 356, OS-US: 357, US-OS: 358, and US: 308 ± 212 ng/g of fecal DM). There was also no correlation between
prepartum fecal cortisol metabolite concentrations of the dam and postpartum fecal cortisol metabolite concentrations of the offspring ($r = -0.26; P = 0.19$).

### 3.6. Discussion

The objective of this study was to determine the effect of a social stressor imposed on the dam during various stages of late gestation on the growth and performance of their young calves. The overstocking treatment had no effect on any of our measures, suggesting that moderately overstocking the feedbunk did not have a major effect of calves *in utero.*

All calves in the present study had success of passive transfer, and serum IgG concentrations were well above the 10 mg/dl threshold. Calves were fed with a pooled colstrum, which may explain this lack of difference. However, Stott (1980) reported late-gestation heat stress to also decrease the IgG concentration in neonatal calves after the ingestion of pooled colostrum. Because this is the first study to our knowledge to investigate maternal social stress in relation to passive immunity in calves, it is difficult to directly compare the model of stress employed in the present study to others. Thus, further investigation is warranted in relation to the maternal social environment during late-gestation with regard to offspring passive immune transfer.

Overstocking the feed bunk did not affect calf birth weight or growth through weaning; calves born from OS, OS-US, US-OS, and US dams had similar birth weights. Many factors may contribute to compromised fetal growth in late gestation, including a shorter gestation length. For example, both Aidin et al. (2009) and Tao et al. (2012)
reported a 4-d shorter gestation length for cows under heat stress during the dry period compared to cows that were cooled. However, OS, OS-US, US-OS, and US dams had similar gestation lengths, which were not affected by the potential stress of overstocking the feed bunk as was initially hypothesized. Again, this difference between our study and others may be due to the type, duration, and strength of the stressor used. Overstocking dairy cows at the feedbunk and not the lying stalls may be less of a severe stressor.

Heifer calves born from OS, OS-US, US-OS, and US dams were also of similar height at birth, 3 and 5 wk of age, and there was no treatment by week interaction. Similar to our results, Tao et al. (2012) reported that heifer calves born from heat stressed dams were of similar height at the withers after weaning at 2 mo of age compared to heifer calves born from cows that were cooled during the dry period.

Increased stocking density at the feed bunk during late gestation did not affect the stress level of heifer calves through wk 1 of age, as fecal cortisol metabolite concentrations were similar among treatments. In contrast to our results, Jarvis et al. (2005) reported an increased and more prolonged salivary cortisol response to an acute social stressor at 10 wk of age for prenatally stressed female pigs compared with controls, indicating enhanced stress reactivity in prenatally stressed offspring. Brunton and Russell (2010) also reported greater HPA axis responses (i.e., greater ACTH and corticosterone responses) to acute stress for rodents born to mothers exposed to social stress during pregnancy. It is hypothesized that the model of social stress used in the current study did not impose stress on the dam, and therefore, this did not impose stress on the offspring. In other words, it is possible that overstocking the feed bunk at the
stocking densities employed in the current study did not increase competition (i.e., the number of times cow were displaced from the feeding area) and thus, maternal social stress. Future research is encouraged to investigate increased stocking densities at the feed bunk beyond what was used in this study.

In addition, there was also no correlation between prepartum fecal cortisol metabolite concentrations of the dam and postpartum fecal cortisol metabolite concentrations of the offspring. This is in contrast to others who reported chronic maternal stress during late gestation in primates (Clarke et al., 1994) and rats (Brunton and Russell, 2010) permanently alters HPA function in the offspring. For instance, offspring of prenatally stressed rhesus monkeys were noted to have higher circulating ACTH and cortisol levels at birth compared to offspring that were not prenatally stressed (Clark et al., 1994). It is possible that at 1 wk of age, a number of other factors within the environment of the calf (i.e., dehorning, morbidity, etc.) have contributed to the animal’s level of stress, and these external factors may have a larger influence on the stress level of the offspring than the maternal hormone levels at this point in time. For instance, calves were dehorned via caustic paste at 3 d of age, which may have confounded the results of our study. Thus, future research should consider obtaining meconium or fresh fecal samples prior to 1 wk of age.

3.7. Conclusions

Overstocking cows at the feed bunk during the dry period did not compromise postnatal calf growth or health in the present study. In a commercial setting, however,
stocking densities often exceed those employed in the current experiment, especially during months with high calving frequency. Thus, increased stocking densities at the feed bunk beyond what was used in this study should be investigated. In addition, further research should be conducted to determine the potential consequences of overstocking both the feed bunk and lying stalls on fetal development and postnatal calf growth and welfare.
Table 3.1. Gestation length and data for calf serum IgG concentration, birth weight, body temperature through 6 d of age, overall average daily gain (ADG), wither height (WH), hip height (HH), and fecal cortisol metabolite (FCORT) concentrations for dams housed under various stocking densities during late gestation

<table>
<thead>
<tr>
<th></th>
<th>OS</th>
<th>OS-US</th>
<th>US-OS</th>
<th>US</th>
<th>SEM</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gestation length of dams (d)</td>
<td>276</td>
<td>274</td>
<td>275</td>
<td>275</td>
<td>1</td>
<td>0.74</td>
</tr>
<tr>
<td>IgG (mg/mL)²</td>
<td>14.7</td>
<td>15.1</td>
<td>14.8</td>
<td>15.3</td>
<td>1.0</td>
<td>0.56</td>
</tr>
<tr>
<td>Birth weight (kg)</td>
<td>41.7</td>
<td>40.4</td>
<td>42.1</td>
<td>43.0</td>
<td>6.0</td>
<td>0.41</td>
</tr>
<tr>
<td>Body temperature (ºC)</td>
<td>39.0</td>
<td>38.9</td>
<td>38.9</td>
<td>38.9</td>
<td>0.1</td>
<td>0.81</td>
</tr>
<tr>
<td>ADG (kg/d)</td>
<td>0.56</td>
<td>0.53</td>
<td>0.56</td>
<td>0.57</td>
<td>0.03</td>
<td>0.80</td>
</tr>
<tr>
<td>WH (cm)</td>
<td>79.5</td>
<td>79.2</td>
<td>79.0</td>
<td>79.2</td>
<td>0.5</td>
<td>0.68</td>
</tr>
<tr>
<td>HH (cm)</td>
<td>83.1</td>
<td>83.1</td>
<td>82.8</td>
<td>82.8</td>
<td>0.5</td>
<td>0.94</td>
</tr>
<tr>
<td>FCORT (ng/g of fecal DM)</td>
<td>356</td>
<td>357</td>
<td>308</td>
<td>358</td>
<td>212</td>
<td>0.98</td>
</tr>
</tbody>
</table>

¹ OS: Overstocked from 60 to 1, b) OS-US: Overstocked from 60 to 26; Understocked from 25 to 1, c) US-OS: Understocked from 60 to 26; OS from 25 to 1 (US-OS), and d) US from 60 to 1 d (US) prior to calving; n = 13, 18, 16, 11, respectively
² ≥10 mg/ml = Success of passive transfer
Figure 3.1. Calf body weight (BW) (+ SEM) during the milk feeding period for dams housed under various stocking densities during late gestation.

1OS: Overstocked from 60 to 1, b) OS-US: Overstocked from 60 to 26; Understocked from 25 to 1, c) US-OS: Understocked from 60 to 26; OS from 25 to 1 (US-OS), and d) US from 60 to 1 d (US) prior to calving.
Chapter 4: Housing System May Affect Behavior and Performance of Jersey Heifer Calves

4.1. Abstract

There is increasing social pressure to adopt alternative housing and management practices that allow farm animals more opportunity to exercise and demonstrate social behavior. The present study investigated the effect of pair housing on the behavior and performance of Jersey heifer calves. Forty female Jersey calves were allocated to individual or pair housing at birth and monitored for 9 wk. Calves were provided with a single hutch, and those allocated to the pair housing treatment were provided a pen enclosure twice the size of individually housed calves and only one hutch was provided per pair. All calves were fed milk replacer via bucket twice per day (1.9 L/feeding first 7 d; 2.27 L/feeding until weaned) and had ad libitum access to calf starter and water. Gradual weaning commenced on day 49 by reducing the calves’ milk allowance to one feeding per day and weaning occurred on day 56. Grain consumption was monitored daily and calves were weighed weekly. Direct behavioral observations were conducted twice per week. Calves housed in pairs tended to have greater average daily gain (ADG)
compared with calves housed individually (0.63 versus 0.59 kg/d; respectively). Pair housing also increased final body weight (BW) compared with individual housing (64.9 versus 61.7 kg, respectively). During observation periods, calves housed individually spent more time engaging in nonnutritive sucking than calves housed in pairs (21.5 versus 8.15%). Calves housed in pairs were observed cross-sucking 13.5% of the time during observational periods. Although housing Jersey calves in pairs may increase measures of performance, future research should aim to reduce cross-sucking behavior within the Jersey breed through alternative feeding systems or environmental enrichment.

*A version of Chapter 4 has been submitted for publication: Pempek, J. A., M. L. Eastridge, S. Swartzwelder, K. M. Daniels, and T. T. Yohe. Housing system may affect behavior and growth performance of Jersey heifer calves. J. Dairy. Sci.*

### 4.2. Introduction

Modern dairy production is sometimes criticized for on-farm procedures including early separation (< 24 h after birth) of the calf from the dam and individually housing pre-weaned heifer calves (as opposed to paired or in groups) (Rushen et al., 2010). In a recent survey (USDA, 2012), 78.9% of respondents reported that they housed pre-weaned heifer calves individually, with 42.1% of the population being housed outside, 10.5% housed inside with external heat, and 26.3% housed inside without external heat. This is in contrast to 15.9% of survey respondents that reported housing pre-weaned animals in any kind of group facilities. Although common, individual housing has been criticized due to restricted space and social isolation from other
animals, and state and federal governments are increasingly being pressured to move towards alternative housing standards (Rollin, 1995; Croney and Millman, 2007).

Historically, the dairy industry favored housing pre-weaned calves individually in order to reduce disease transmission (Gulliksen et al., 2009). However, recent experiments conducted to evaluate calf health status when housed individually or in groups have challenged this traditional claim (Kung et al., 1997; Chua et al., 2002). For example, Chua et al. (2002) examined the health status of pre-weaned heifer calves housed individually or in pairs and reported no differences in health status between individual and pair-housed calves with all calves remaining healthy with no incidence of diarrhea. Similarly, Kung et al. (1997) reported fewer days of medication were provided to calves housed in small groups compared with those housed individually in hutches, suggesting that grouping calves does not increase the likelihood of disease transmission or increases in frequency or duration of treatments.

From a behavioral standpoint, individual housing systems prevent calves from making physical contact with conspecifics, thus impeding social development which can result in increases in fearful and aggressive behaviors towards novel conspecifics after grouping (Bøe and Færevik, 2003; Rushen et al., 2010). Because of the natural complex hierarchies established by dairy cattle, it is important for calves to learn how to interact socially with conspecifics (Jensen et al., 1999). Gaillard et al. (2014) recently reported that individual rearing (as opposed to group rearing) results in cognitive impairments in young dairy calves as assessed by conducting a reversal learning task. Gaillard et al. (2014) trained calves to associate a white or black colored stimulus with a food reward,
and once calves reached the appropriate learning criterion, the colors were reversed, i.e. calves that were initially trained to associate the white stimuli with the reward then had the reward paired with the black stimulus and vice versa. Pair-housed calves were better able to adapt and modify their behavior to obtain the food reward after the stimuli were reversed, yet individually housed calves continued to choose the incorrect stimuli. In addition, de Paula Vieira et al. (2010) demonstrated that calves that are group-housed prior to weaning are also better able to learn how to use automated feeding equipment after weaning, as they visit the feeder more often and ingest more concentrate than calves that were previously housed individually (de Paula Vieira et al., 2010). Thus, individual rearing during the pre-weaning period may reduce behavioral flexibility and limit the calves’ ability to cope with novel situations or changes within their environment later in life.

In contrast, social interactions may result in poor welfare for the individual calf as calves are able to express undesirable behaviors such as cross-sucking on one another. Cross-sucking is defined as an abnormal behavior wherein non-nutritive sucking directed toward another calf’s ears, mouth, navel, scrotum, prepuce, or other body parts occurs (de Wilt, 1985), and this behavior stems from redirection of the calf’s innate desire to suckle (Jensen, 2003). One reason dairy producers are reluctant to adopt modern group-housing systems is because this behavior may cause hair loss, inflammation, or infection of the body part exposed to cross-sucking (Lidfors, 1993). Jersey cattle are an important breed to evaluate in a group setting, as the Jersey breed has been identified to have heightened cross-sucking behavior and are more frequently observed performing oral stereotypic
behaviors, such as tongue-rolling and intersucking, more often than other breeds (Lidfors and Isberg, 2003). However, to date, the majority of studies have been conducted with Holstein calves, and it is currently unknown if Jersey calves will behave the same as Holstein calves when pair-housed. The duration and/or frequency of cross-sucking behavior also have yet to be quantified for Jersey calves. As there are behavioral differences among breeds of other species, such as aggression in pigs, (Breurer et al., 2003), it is inappropriate to make the assumption that all breeds of dairy calves behave in the same manner when housed similarly.

The objective of this experiment was to compare the behavior and performance of Jersey heifer calves housed individually or in pairs. We hypothesized that cross-sucking behavior would occur in pair-housed calves, as the Jersey breed appears to have a higher frequency of performing this behavior. In addition, we hypothesized that pair-housed calves would have increased measures of performance compared with individually housed calves in part due to social facilitation. Lastly, we hypothesized that the provision of a social partner would increase the temperature within the calf hutch, thus reducing the calves’ susceptibility to cold stress during cool weather.

4.3. Materials and methods

This study was conducted at The Ohio State University’s Waterman Dairy Center, located in Columbus, Ohio, in accordance with guidelines set by the Institutional Animal Care and Use Committee (Protocol No. 2012A0000099). Forty female Jersey calves born between August 2012 and February 2013 were used in this study. Calves were
blocked by date of birth and weight and allocated to one of two treatments; Treatment one: individual housing; Treatment two: pair housing. At birth, calves were housed by designated treatment and monitored for 9 wk (63 d); pair-housed calves were within 4 d of age. All calves were housed in hutches (non-tethered, wire pen enclosure) placed on loose gravel. Both individually (n = 20 calves) and pair-housed (n = 20 calves) calves were provided with one hutch, and those allocated to the pair housing treatment were provided a pen enclosure twice the size of individually housed calves (Individual housing: 1.22 × 1.17 m (1.43 m²/calf); Pair housing: 1.22 × 2.39 m (1.46 m²/calf)). Only one hutch was provided to pair-housed calves due to the calves’ tendency to remain in the same hutch over 80% of the time when 2 hutches are provided (J. Pempek, unpublished data). Hutches were bedded with straw.

All calves received 1.9 L of maternal colostrum via bottle from Johne’s negative dams as soon as possible after birth and again within 12 h of the first colostrum feeding per regular herd standard operating procedures. If good-quality maternal colostrum was not readily available for use, replacement colostrum (bovine IgG, colostrum replacement; Land O’Lakes Animal Milk Products, St. Paul, MN) was fed to the calf.

4.3.1. Total serum protein

Blood samples were collected in 5-mL Vacutainer serum collection tubes (BD Vacutainer Plus Blood Clot Collection Tubes, Franklin Lakes, NJ) via jugular venipuncture within 48 h after calves were fed colostrum. The blood samples were immediately placed on ice after collection and transported to the laboratory within 1 h. Samples were centrifuged at 3,500 RPM (1,180 x g) a 4°C for 15 min. Total serum
protein was analyzed using a JorVet clinical hand-held refractometer (Jorgensen Laboratories, Inc., Loveland, CO).

4.3.2. Feed

Calves were fed milk replacer (Cow’s Match Jersey Blend; 28% crude protein (CP) and 25% fat, as-fed basis; Land O’ Lakes Animal Milk Products, Shoreview, MN) twice daily at approximately 0600 and 1700 h in buckets. The buckets were removed as soon as the calves completed their milk meal, and it was ensured that calves housed in pairs had access to the milk replacer simultaneously. During the first 7 d of life, calves were fed 1.9 L of milk per feeding which was increased to 2.27 L of milk per feeding thereafter. Gradual weaning began on d 49, as calves were decreased to one milk feeding (morning only) per day, and all calves were weaned on d 56. Calves had ad libitum access to a texturized starter grain (22% CP; AMPLI-Calf 22 Jersey R40, Land O’ Lakes Purina Feed, LLC, Shoreview, MN) medicated with 44 g/t of monensin (Rumensin; Elanco Animal Health, Greenfield, IN) and water throughout the experiment.

4.3.3. Behavior observations

Calf behavior was recorded by direct observation using instantaneous scan-sampling with 60 s intervals. Observation periods were conducted twice per week (1 h session duration) and were centered around one morning and one evening milk-feeding period. Scan-sampling began 30 min prior to the delivery of milk and ended 30 min after milk delivery to calves. The scan sample period length for each animal was approximately 5 s, and only the initial posture (standing or lying) and behavioral state (non-nutritive sucking, locomotor play, object play, self-grooming, ingesting starter,
water or milk, cross-sucking, allogrooming, social play, or other) of the calf were recorded. The recorded behaviors are listed and defined in Table 4.1.

**4.3.4. Performance and health**

All calves were weighed at birth and weekly thereafter. Grain consumption was recorded daily by the collection of feed refusals prior to the evening milk feeding. Feed refusals for pair-housed calves were averaged, as it was not possible to monitor individual feed intake. In addition, hip height (HH), wither height (WH) and body length measurements were taken at birth and 3, 6, and 9 wk of age.

Fecal scores (Diaz et al., 2001) and rectal body temperature were recorded daily at 1500 h each day. When calves were diagnosed as ill or having a fecal score of 3 or greater, they were treated per veterinarian recommendations using an oral electrolyte solution (Entrolyte H.E.; Pfizer Animal Health, New York, NY) and antibiotics. If a calf’s body temperature was ≥39.4°C, 2 mL of Flu-nix (Agri Laboratories Ltd., St. Joseph, MO) was administered intravenously. When a calf’s fever did not readily reduce in response to the Flu-nix treatment treatment, the calf received 2 mL of Excenel (Pharmacia & Upjohn Co., Pfizer Inc., New York, NY) intramuscularly. Both type and duration of treatment were recorded.

To examine the accuracy of a wireless data logger as a noninvasive alternative to monitoring core body temperature, wireless data loggers (Thermochron iButton DS1922T, Maxim Integrated, San Jose, CA) were adhered to the underside of calves’ tails with medical tape and further secured with vet wrap (n = 8 calves due to the cost of data loggers). Each iButton was set to record the calf’s temperature once every 15 min in
order to observe daily temperature variation throughout the experiment. The rectal body temperature was then matched to the 1500 h recorded skin temperature.

4.3.5. Environmental factors

AcuRite Wireless Digital Thermometers (Lake Geneva, Wisconsin) were secured within suet wire baskets (KAYTEE Cake Feeder Station, Chilton, WI) for protection and mounted directly above the straw bedding in the back of each hutch in order to monitor daily interior hutch temperature (maximum and minimum). In addition, weather data were collected from the National Oceanic and Atmospheric Administration’s National Weather Service (Columbus, Ohio) for all days of the experimental period.

4.4. Statistical analysis

Data were analyzed as a randomized complete block design with repeated measures in time using the MIXED procedure of SAS (2004). One pair was separated after wk 3 of the experiment due to an aural hematoma; these data were still included as a pair for the analysis with missing data points after wk 3. The covariance structures of error for behavior and performance and health repeated measures were selected based on the lowest Bayesian information criteria (BIC). Least squares means and standard errors were determined using the LSMEANS statement in the MIXED procedure. Significant differences were declared at $P \leq 0.05$ and a trend at $0.05 > P \leq 0.10$.

4.4.1. Behavior analysis

Because the main effect of treatment did not vary across experimental week, these data were combined to provide one morning and one evening behavior observation period.
per calf for statistical analyses. However, only overall amounts of time engaged in behaviors are reported. The model included the fixed effects of treatment (1 df), observation period (1 df), treatment x observation period interaction (1 df), and the random effect of block (9 df). Calf within treatment by block was used as the experimental unit. To obtain normality, the mean proportion of the behaviors displayed by all calves, independent of housing treatment, was transformed using the arcsin transformation (Snedecor and Cochran, 1967), and all transformed data were back-transformed for reporting. The selected covariance structure of error was the banded main diagonal (UN(1)) structure.

**4.4.2. Performance and health analysis**

The model included the fixed effects of treatment (1 df), week of experiment (8 df), treatment by week interaction (8 df), and the random effect of block (9 df). Calf within treatment by block was used as the experimental unit. Birth measurements were used for covariate adjustment of data. The selected covariance structure of error was the first-order autoregressive (AR(1)) structure. The equations used to calculate body surface area were $0.14 \times W^{0.57}$ (Brody, 1945) and $0.09 \times W^{0.67}$ (Mitchell, 1928). The correlations among body surface area and performance variables were determined using PROC CORR (SAS Institute, 2004). Due to the low level of occurrence, morbidity data were summarized descriptively.

The REG procedure of SAS (2012) was used to conduct a regression analysis to determine if tail skin temperature could be used as an accurate predictor of calf rectal
temperature. The final data set used 430 paired observations and included tail skin temperature as the regressor variable and rectal temperature as the outcome variable.

4.4.3. Environmental factors analysis

The effects of housing treatment on average internal hutch temperature (below 10°C) and d below 10°C were also compared. The model included the fixed effects of treatment (1 df) and the random effect of block (9 df).

4.5. Results

4.5.1. Behavior

Behavior results revealed that the posture of calves housed in pairs was similar to the posture of calves housed individually (Table 4.2). During periods of observation, calves housed individually spent more time engaged in non-nutritive sucking compared with calves housed in pairs (21.5 versus 8.15% of total observations). However, calves housed in pairs were observed cross-sucking (13.5% of total observations), which occurred predominantly after the completion of their milk meal. Locomotor play, object play, and self-grooming behaviors were observed less frequently than non-nutritive sucking, yet calves housed individually were observed performing object play and self-grooming behaviors more often than calves housed in pairs (Table 4.2). In addition, calves housed in pairs consumed their milk meal faster than calves housed individually (4.20 versus 4.86% of total observations). However, no differences were observed between the amount of time calves spent consuming calf-starter and water (Table 4.2). Lastly, affiliative behaviors, such as allogrooming and social play, were rarely observed
among calves housed in pairs during periods of observation (0.30 and 0.06% of total observations, respectively).

4.5.2. Performance and health

Although housing Jersey heifer calves in pairs did not significantly increase overall mean body weight (BW) (Table 4.3), a treatment by time interaction ($P = 0.05$) revealed that calves housed in pairs tended to weigh more than individually housed calves during wk 7 and 8 (Figure 4.1), and calves housed in pairs completed the experiment with a greater final BW compared with calves housed individually (64.9 versus 61.7 kg). In addition, ADG tended to be higher for pair-housed calves compared with calves housed individually (Table 4.3). Overall grain DMI did not differ between treatments, yet a treatment by time interaction (Figure 4.2) revealed that calves housed in pairs consumed significantly more calf-starter during wk 9 than calves housed individually (2.36 versus 2.12 kg/d).

Calves housed in pairs were taller at the withers compared with calves housed individually (74.7 versus 74.1 cm). However, the hip heights of calves were similar between both treatments (Table 4.3). Body length measurements also did not differ among treatments, yet there was an approaching tendency for pair-housed calves to grow more from the withers to the pins than individually housed calves (56.1 versus 55.2 cm; $P = 0.11$).

The equations used to calculate body surface area derived by Brody (1945) and Mitchell (1928) were highly correlated with one another (Table 4.4). As expected, calf body weight had the strongest relationship with body surface area ($r = 0.998; P < 0.01$).
0.0001). Also, both wither and hip heights shared a strong, positive relationship with body surface area (Table 4.4). Lastly, although both body length measurements were highly correlated with body surface area, the measurement from the shoulders to the pins resulted in a slightly higher correlation with body surface area ($r = 0.93; P < 0.0001$) compared to the withers to the pins ($r = 0.90; P < 0.0001$).

All calves had a total serum protein concentration $> 5.5$ g/dl, which did not differ by treatment (Table 4.5). Calf fecal scores were not affected by housing treatment (Table 4.5). However, there was a significant week effect, as fecal score increased with age from 1.33 during wk 1 to 2.98 during wk 9. Rectal body temperature also did not differ by treatment (Table 4.5), yet there was a significant wk effect; calves’ rectal body temperature decreased slightly with age.

Rectal temperature ($^\circ$C) was best predicted as $37.6 \pm 0.75 + (0.03 \pm 0.02 \times \text{Thermochron iButton temperature})$ (°C). This equation had an $R^2$ value of 0.01 and RMSE of 0.37, indicating that tail skin temperature is not an accurate predictor of calf core body temperature ($P = 0.10$).

**3.5.3. Environmental factors**

The mean ambient high and low temperatures throughout the duration of the experiment are listed in Table 4.6; average high temperatures ranged from 3.53 to 30.4$^\circ$C, and the average low temperatures ranged from -4.48 to 16.35$^\circ$C. When the internal hutch temperature fell below 10$^\circ$C, the average environmental temperature did not differ by housing treatment; calves housed in pairs and calves housed individually experienced similar thermal conditions when the temperature fell below the
thermoneutral zone (3.58 versus 3.59°C). In addition, the average number of days in which calves may have been exposed to cold-stress conditions did not differ by housing treatment; calves housed in pairs experienced approximately 39.4 ± 2.97 d below thermoneutral temperatures, whereas calves housed individually experienced 41.0 ± 2.97 d below thermoneutral temperatures.

4.6. Discussion

4.6.1. Behavior

In the current study, calves housed individually were observed to engage in non-nutritive sucking significantly more often than calves housed in pairs. Non-nutritive sucking may be observed under natural conditions, yet it more commonly occurs within artificial rearing systems (Jensen, 2003). Previous research suggests that this behavior may be detrimental to calf health and performance, as the consumption of non-feed particles (soil, metal oxides, hair, etc.) can have a direct effect on stomach upset, blockage, and/or the absorption of nutrients (Broom, 1991). Although non-nutritive sucking was observed more often among individually housed calves, calves housed in pairs appeared to redirect this behavior to their companion calf as cross-sucking. In our study, non-nutritive sucking and cross-sucking were analyzed as separate behavioral variables. Yet, if such variables were combined and compared numerically, the behavioral occurrence is nearly identical. It is also important to note that milk was provided via bucket in this study, which may have contributed to the heightened expression of non-nutritive sucking and cross-sucking. The young calf’s motivation to
suckle is inherently strong (de Passillé, 2001), and the inability to perform such behaviors that are intrinsic in nature may directly and indirectly affect animal welfare. A further consequence of the inability to suckle may be the development of stereotypical oral behaviors as an attempt to satisfy behavioral needs (Bergeron et al., 2006).

In the United States, bucket feeding is by far the predominant feeding method and is widely used for its convenience in the dairy industry. For instance, a recent survey conducted by the United States Department of Agriculture (USDA, 2012) reported 61.5% of feeding management systems employed the use of an open bucket for milk delivery, whereas only 26.9% of systems employed the use of a bottle fitted with a teat. Transitioning to a teat system for feeding could reduce non-nutritive sucking behaviors as demonstrated by Lodberg and Lidfors (2001) and Jensen and Budde (2006). Further studies evaluating the difference in non-nutritive sucking behaviors among Jersey calves with a teat system should be conducted.

Calves housed in pairs engaged in cross-sucking behavior 13.5%, which was predominantly directed toward the navel and the ears of the companion calf. In the current study, one pair had to be permanently separated as a consequence of cross sucking, resulting in an aural hematoma. In addition to this incident, frost bite, inflammation of navels, and one ear infection (*Mycoplasma bovis*) were also observed. Our study demonstrates the significant impact of cross-sucking may have on the health and welfare of calves. In contrast to our results, few studies conducted with Holstein calves have reported cross-sucking as being injurious to calf health (deWilt, 1985; Chua et al., 2002; Babu et al., 2004). In those studies that did report negative impacts to the
health of the calves, the feeding system used was a bucket or trough (Margerison et al., 2003), similar to the feeding management system employed in this research study. These results suggest that to reduce the potential detrimental effects of cross-sucking behavior, offering milk via bottle fitted with a teat is recommended.

In conclusion, regardless of facility, calves spent a significant period of time performing non-nutritive sucking focusing on object within the pen, the calf itself, and other calves. Future experiments should aim to reduce cross-sucking and non-nutritive sucking behavior through alternative milk-feeding systems and the potential implementation of environmental enrichment devices. In addition, it may be of interest to investigate a potential association between cross-sucking behavior and the stereotypic tongue-rolling behavior, as Jersey cattle predominantly exhibit this oral stereotypic behavior when mature.

4.6.2. Performance and health

In the current experiment, disease prevalence, other than diarrhea, was minimal and did not differ between housing treatments. Average daily gain for calves regardless of treatment was comparable to Jersey calves in other studies, as well; Jensen (2006) reported an ADG of 0.594 kg/d. Housing Jersey heifer calves in pairs improved measures of performance with ADG tending to be higher for calves housed in pairs compared with calves housed individually and calves housed in pairs consuming more calf-starter during the week after weaning. Also, calves housed in pairs tended to have a higher BW during the weaning period, which significantly increased during the week after weaning. Our results agree with previous studies that also have reported increased
weight gains for group-housed calves (Xiccato et al., 2001; Chua et al., 2002). Such improvements may be attributed to social facilitation to promote eating as group activity and early social interactions allow calves to learn at a faster pace than those reared individually or in isolation (Babu et al., 2004; Gailliard et al., 2014).

In addition, the weaning period is one of the most stressful periods in the young calves’ life (Weary et al., 2008). Thus, social companionship may reduce the level of stress calves experience during this period and also minimize the often-observed slowed growth via social buffering (de Paula Vieira et al., 2010). For instance, de Paula Vieira et al. (2010) reported that pair-housed calves spent more time at the feeder, visited the feeder more often, and ingested more concentrate. The authors also reported that pair-housed calves showed a reduced vocal response to weaning compared with individually housed calves. Although the calves’ behavioral response to weaning was not quantified in the present study, calves housed individually did not perform as well as pair-housed calves; calves housed individually consumed less grain directly following weaning and had a lower body weight throughout and after weaning. Thus, this research supports the aforementioned studies conducted with Holstein heifer calves and suggests that housing Jersey calves in pairs also mitigates the stressors associated with weaning as young calves transition from milk to a solid diet.

4.6.2. Environmental factors

The thermoneutral zone for young dairy calves is between 10 to 20°C (Scibilia et al., 1987), and when environmental temperatures drop below this threshold, calves must consume more nutrients for body maintenance (NRC, 2001). Environmental temperature
below this range is considered one of the most commonly experienced stressors (Litherland et al., 2014). It was expected that when the internal hutch temperature fell below 10°C, the lower range of the young calf’s thermoneutral zone, the average temperature for calves housed in pairs would be higher than the temperature for calves housed individually. However, this initial hypothesis was incorrect, as our results indicated that the temperature within the hutch remained the same independent of treatment during potential periods of cold stress. In addition, although there was no difference observed by treatment, calves experienced chilling or cold-stress over two-thirds of the experimental duration. This is an important management consideration, as the majority of Jersey heifer calves in this experiment experienced cold-stress.

4.7. Conclusions

Housing Jersey heifer calves in pairs or in small groups allows for early social interactions and may increase measures of performance pre- and post-weaning. Future research should aim to compare Holstein and Jersey breeds behaviorally and reduce cross-sucking and non-nutritive sucking behavior specifically by using alternative feeding systems or environmental enrichment. In addition, a noninvasive proxy for core body temperature in pre-weaned calves is still needed, as tail skin temperature is not a viable alternative.
### Table 4.1. Ethogram of the recorded behaviors and their description

<table>
<thead>
<tr>
<th>Behavior</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lying</td>
<td>The calf is resting on the ground; head may be supported or unsupported by the neck</td>
</tr>
<tr>
<td>Standing</td>
<td>The calf is standing with all 4 legs on the ground</td>
</tr>
<tr>
<td>Other</td>
<td>The calf is ruminating, urinating, defecating, or performing another behavior not described</td>
</tr>
<tr>
<td>Non-nutritive sucking</td>
<td>The calf’s tongue is out of its mouth and is in contact with or biting any fixtures of the pen; may include bucket if milk is not available at the time of observation</td>
</tr>
<tr>
<td>Locomotor play</td>
<td>The calf is engaged in a gallop, leap, buck-low, buck-high, buck-kick, or turn</td>
</tr>
<tr>
<td>Object play</td>
<td>The calf is standing; butting head against milk or water buckets or hutch in a playful manner</td>
</tr>
<tr>
<td>Self-grooming</td>
<td>The calf’s tongue is out of its mouth and in contact with its own body</td>
</tr>
<tr>
<td>Ingesting starter</td>
<td>The calf is consuming calf-starter from a bucket</td>
</tr>
<tr>
<td>Ingesting water</td>
<td>The calf is ingesting water by drinking from a bucket</td>
</tr>
<tr>
<td>Ingesting milk</td>
<td>The calf is ingesting milk by drinking from a bucket</td>
</tr>
<tr>
<td>Cross-sucking</td>
<td>Pair-housed calves only - The calf is sucking on the body of another calf; the sucking movements are performed with the body part in the mouth</td>
</tr>
<tr>
<td>Allogrooming</td>
<td>Pair-housed calves only - The calf’s tongue is out of its mouth and in contact with the head, neck, or body of the companion calf</td>
</tr>
<tr>
<td>Social play</td>
<td>Pair-housed calves only - The calves are standing front-to-front; butting head against head/neck in a playful manner</td>
</tr>
</tbody>
</table>
Table 4.2. Least squares means for percentage of time calves engaged in each of the behaviors measured during the 1 h observation period centered around milk-feeding

<table>
<thead>
<tr>
<th>Behavior (%)</th>
<th>Individual</th>
<th>Pair</th>
<th>SEM</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lying</td>
<td>24.7</td>
<td>25.6</td>
<td>0.03</td>
<td>0.73</td>
</tr>
<tr>
<td>Standing</td>
<td>70.5</td>
<td>74.2</td>
<td>0.10</td>
<td>0.37</td>
</tr>
<tr>
<td>Idle</td>
<td>57.1</td>
<td>60.7</td>
<td>0.03</td>
<td>0.17</td>
</tr>
<tr>
<td>Other</td>
<td>0.37</td>
<td>0.28</td>
<td>0.004</td>
<td>0.36</td>
</tr>
<tr>
<td>Non-nutritive sucking</td>
<td>21.5</td>
<td>8.15</td>
<td>0.03</td>
<td>&lt; 0.0001</td>
</tr>
<tr>
<td>Locomotor play</td>
<td>1.02</td>
<td>0.66</td>
<td>0.01</td>
<td>0.11</td>
</tr>
<tr>
<td>Object play</td>
<td>1.36</td>
<td>0.21</td>
<td>0.01</td>
<td>&lt; 0.0001</td>
</tr>
<tr>
<td>Self-grooming</td>
<td>1.94</td>
<td>0.67</td>
<td>0.01</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>Ingesting starter</td>
<td>4.14</td>
<td>4.63</td>
<td>0.01</td>
<td>0.39</td>
</tr>
<tr>
<td>Ingesting water</td>
<td>0.76</td>
<td>0.55</td>
<td>0.003</td>
<td>0.11</td>
</tr>
<tr>
<td>Ingesting milk</td>
<td>4.86</td>
<td>4.20</td>
<td>0.002</td>
<td>0.01</td>
</tr>
<tr>
<td>Cross-sucking</td>
<td>--</td>
<td>13.5</td>
<td>0.02</td>
<td>--</td>
</tr>
<tr>
<td>Allogrooming</td>
<td>--</td>
<td>0.30</td>
<td>0.010</td>
<td>--</td>
</tr>
<tr>
<td>Social play</td>
<td>--</td>
<td>0.06</td>
<td>0.003</td>
<td>--</td>
</tr>
</tbody>
</table>
Table 4.3. Least squares means of body weight, average daily gain, grain dry matter intake, wither height, hip height, and body length measurements for calves housed individually or in pairs during the milk feeding and weaning periods (63 d)

<table>
<thead>
<tr>
<th>Variable</th>
<th>Individual</th>
<th>Pair</th>
<th>SEM</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>BW (kg)</td>
<td>41.3</td>
<td>41.9</td>
<td>0.53</td>
<td>0.39</td>
</tr>
<tr>
<td>ADG (kg/d)</td>
<td>0.59</td>
<td>0.63</td>
<td>0.02</td>
<td>0.09</td>
</tr>
<tr>
<td>Grain DMI (kg/d)</td>
<td>0.68</td>
<td>0.72</td>
<td>0.05</td>
<td>0.44</td>
</tr>
<tr>
<td>Withers height (cm)</td>
<td>74.1</td>
<td>74.7</td>
<td>0.23</td>
<td>0.02</td>
</tr>
<tr>
<td>Hip height (cm)</td>
<td>76.9</td>
<td>76.9</td>
<td>0.23</td>
<td>0.85</td>
</tr>
<tr>
<td>Shoulders to pins (cm)</td>
<td>65.3</td>
<td>65.2</td>
<td>0.32</td>
<td>0.88</td>
</tr>
<tr>
<td>Withers to pins (cm)</td>
<td>55.2</td>
<td>56.1</td>
<td>0.37</td>
<td>0.11</td>
</tr>
</tbody>
</table>
### Table 4.4

Coefficients of simple correlations between surface area (SA) using two different equations, body weight, average daily gain, wither height (WH), hip height (HH), and body length (BL) using two different measurements

<table>
<thead>
<tr>
<th></th>
<th>SA1</th>
<th>SA2</th>
<th>BW</th>
<th>ADG</th>
<th>WH</th>
<th>HH</th>
<th>BL1</th>
<th>BL2</th>
</tr>
</thead>
<tbody>
<tr>
<td>SA1</td>
<td>1.00</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>SA2</td>
<td></td>
<td>&lt;0.0001</td>
<td>1.00</td>
<td>&lt;0.0001</td>
<td>&lt;0.0001</td>
<td>&lt;0.0001</td>
<td></td>
<td></td>
</tr>
<tr>
<td>BW</td>
<td>1.00</td>
<td>1.00</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>ADG</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>WH</td>
<td>0.95</td>
<td>0.94</td>
<td>0.94</td>
<td>0.45</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>HH</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>BL1 (shoulders to pins)</td>
<td>0.93</td>
<td>0.93</td>
<td>0.93</td>
<td>0.43</td>
<td>0.90</td>
<td>0.89</td>
<td></td>
<td>1.00</td>
</tr>
<tr>
<td>BL2 (withers to pins)</td>
<td>0.90</td>
<td>0.90</td>
<td>0.90</td>
<td>0.37</td>
<td>0.88</td>
<td>0.87</td>
<td>0.91</td>
<td>1.00</td>
</tr>
</tbody>
</table>

1. \( SA1 = 0.14W^{0.57}\) (Brody, 1945)
2. \( SA2 = 0.09W^{0.67}\) (Mitchell, 1928)
Table 4.5. Least squares means (± SEM) of total serum protein within 48 h of birth and average fecal score (4-point scale) and body temperature for calves housed individually or in pairs during the milk feeding and weaning periods

<table>
<thead>
<tr>
<th>Variable</th>
<th>Individual</th>
<th>Pair</th>
<th>SEM</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total serum protein (g/dL)</td>
<td>7.22</td>
<td>7.02</td>
<td>0.21</td>
<td>0.35</td>
</tr>
<tr>
<td>Fecal score¹</td>
<td>1.98</td>
<td>2.08</td>
<td>0.09</td>
<td>0.28</td>
</tr>
<tr>
<td>Body temperature (°C)</td>
<td>38.8</td>
<td>38.8</td>
<td>0.03</td>
<td>0.27</td>
</tr>
</tbody>
</table>

¹Diaz et al., 2001 (1 = firm, well-formed (not hard); 2 = soft, pudding-like; 3 = runny, pancake batter; and 4 = liquid, splatters)
Table 4.6. Mean ambient temperature by month throughout the experimental period

<table>
<thead>
<tr>
<th>Month</th>
<th>High Temperature (°C)</th>
<th>Low Temperature (°C)</th>
</tr>
</thead>
<tbody>
<tr>
<td>August 2012¹</td>
<td>30.4</td>
<td>16.4</td>
</tr>
<tr>
<td>September 2012</td>
<td>24.7</td>
<td>12.8</td>
</tr>
<tr>
<td>October 2012</td>
<td>17.2</td>
<td>7.38</td>
</tr>
<tr>
<td>November 2012</td>
<td>11.0</td>
<td>0.24</td>
</tr>
<tr>
<td>December 2012</td>
<td>7.47</td>
<td>0.79</td>
</tr>
<tr>
<td>January 2013</td>
<td>4.32</td>
<td>-4.23</td>
</tr>
<tr>
<td>February 2013</td>
<td>3.53</td>
<td>-4.48</td>
</tr>
<tr>
<td>March 2013</td>
<td>7.01</td>
<td>-1.13</td>
</tr>
<tr>
<td>April 2013¹</td>
<td>18.4</td>
<td>6.00</td>
</tr>
</tbody>
</table>

¹Mean temperatures include the days for which calves were on trial
Figure 4.1. Body weight (BW) (± SEM) for calves housed in pairs (n = 20 calves) or individually (n = 20 calves) during the milk feeding and weaning periods.

*Means within housing treatment were different (P < 0.05).
†Means within housing treatment tended to differ (P < 0.10).
**Figure 4.2.** Grain dry matter intake (DMI) (± SEM) for calves housed in pairs (n = 20 calves) or individually (n = 20 calves) during the milk feeding and weaning periods. *Means within housing treatment were different (P < 0.05)
Chapter 5: The Effect of Stocking Density During Different Stages of the Dry Period on the Metabolic Health and Productivity of Dairy Cows

5.1. Abstract

The aim of this study was to investigate the effect of increased stocking density at the feed bunk during different stages of the dry period on metabolic health and productivity of dairy cows. One hundred twenty nonlactating Holstein dairy cows were blocked and assigned to 1 of 4 treatment groups with different stocking densities at the feed bunk (Overstocked (OS): 0.88 headlocks/cow; Understocked (US): 1.17 headlocks/cow). The 4 treatments included: OS from 60 to 1 d (OS), OS from 60 to 26 d and US from 25 to 1 d (OS-US), US from 60 to 26 d and OS from 25 to 1 d (US-OS), and US from 60 to 1 d (US) before calving. Blood samples were obtained from the cow at −60, −30, −14, −7, and +7 d relative to calving to determine concentrations of nonesterified fatty acids (NEFA). Colostrum quantity and quality were recorded from the cow’s first milking. Daily milk yield was recorded manually by the research staff once per wk through 90 DIM, with weekly recordings began during wk 3 of lactation. NEFA concentrations were similar among treatment groups across periods ($P > 0.05$).
There was an approaching tendency for colostrum quantity to differ among treatments \( (P = 0.11) \), primarily due to the difference between OS–US and US treatment groups. However, there was no difference in colostrum quality (weighted Brix value of 25.8%; \( P > 0.05 \)). Daily milk yield did not differ by treatment, with cows producing an average of 47.2 kg/d \( (P > 0.05) \). In conclusion, moderate increases in stocking density at the feed bunk did not appear to compromise the productivity or metabolic status of dairy cows.

5.2. Introduction

“The transition from the pregnant, nonlactating state to the nonpregnant, lactating state is too often a disastrous experience for the cow…The well-being and profitability of the cow could be greatly enhanced by understanding those factors that account for the high disease incidence in periparturient cows” (Goff and Horst, 1997).

Previous research has focused on dividing the dry period into two stages: 1) the early or “far-off” period including the first 4 to 6 wk and 2) the “close-up” period including the final 3 wk before expected parturition (Dann et al., 2006). Both stages of the dry period are of critical importance for the welfare of the dairy cow as she prepares for parturition and the upcoming period of lactation; approximately 60% of fetal growth occurs during this stage, and the mammary gland simultaneously recovers from the previous lactation by replacing damaged mammary tissue and epithelial cells (Dingwell et al., 2001). Thus, the dry period should be void of stressors to promote growth and reduce the risk of disease incidence in periparturient cows.
Dairy cows may be subjected to unintentional social stressors, such as overstocking the feed bunk, during the dry period. The feeding area has been well documented as a potential area of competition and agonistic interaction, both of which affect dairy cow feeding behavior (Huzzey et al., 2006; Proudfoot et al., 2009). Huzzey et al. (2006) reported a significant curvilinear decrease in daily feeding time and increase in the number of displacements from the feeding area with increased stocking density (0.81, 0.61, 0.41, and 0.21 m/cow) at the feed bunk. This effect became more pronounced with each increase in stocking density. Similarly, Batchelder (2000) observed reduced daily DMI and significantly fewer cows feeding during both the hour following milking and following delivery of fresh feed at 30% overcrowding of headlocks (1.3 cows per headlock). Proudfoot et al. (2009) also examined competition at the feed bunk (2 cows per 1 feed bin) and discovered that transition cows with lower displacement success ate more rapidly, particularly 2 wk postpartum; high feeding rates may lead to complications associated with slug feeding, such as acidosis, particularly if combined with poorly formulated TMR (DeVries et al., 2008). Cows with lower social status in overstocked conditions have also been reported to have greater plasma NEFA and fecal cortisol metabolite concentrations (Huzzey et al., 2012).

During the periparturient period, elevated NEFA concentrations are observed when dietary energy intake is insufficient to support energy requirements; therefore, as negative energy balance increases, more NEFA are mobilized from adipose tissue and the concentration of NEFA in the blood increases (Drackley et al., 2005). Increased NEFA concentrations during the periparturient period (i.e., ≥ 0.4 mEq/L during the 2-wk period
before calving) have also been shown to be a risk factor for postpartum health disorders, such as displaced abomasum, retained placenta, ketosis, and metritis (LeBlanc et al., 2005), and compromised reproductive performance (Opsina et al., 2010). In addition, for multiparous cows, every 0.15 mEq/L increase in plasma NEFA concentration during the 3 wk before calving nearly doubles the odds of developing more than one disorder or dying within 30 DIM more (Huzzey et al., 2011).

In addition to altering feeding behavior and physiology, overstocking the feed bunk may also influence milk production, but studies estimating this effect are variable. For example, Nordland et al. (2006) demonstrated a 0.7 kg/d decrease in milk yield for primiparous cows when housed with multiparous cows when pre-fresh pen stocking densities exceeded 80%; this decrease remained consistent with every 10% increase in stocking density of headlocks. However, Proudfoot et al. (2009) reported no differences in milk production in competitive (2 cows per 1 feed bin) and noncompetitive (1 cow per 1 feed bin) conditions for primiparous or multiparous transition cows. Thus, the effect of increased stocking density at the feed bunk on milk production warrants further investigation.

Few studies have examined overstocking the feed bunk during the dry period in conditions similar to a commercial dairy setting (Nordland et al., 2006; Lobeck-Luchterhand et al., 2015). To our knowledge, this is the first study to examine the effects of increased stocking density, specifically at the feed bunk, during different stages of the prepartum period, i.e. altered stocking densities at the feed bunk during the far-off and close-up periods. It was hypothesized that overstocking the feed bunk during the entire
dry period would most negatively effect the metabolic health and productivity of non-lactating dairy cows, followed by only overstocking the feed bunk during the close-up period, and to an even lesser extent, overstocking the feed bunk during the far-off period.

5.3. Materials and methods

The present study was conducted from June to December 2014 at Catapadale Dairy located in Marshallville, Ohio, in accordance with the guidelines set by the Institutional Animal Care and Use Committee of The Ohio State University (Protocol No. 2014A00000063).

5.3.1. Animals, housing, and diet

One hundred twenty multiparous Holstein cows were dried-off approximately 60 d prior to their expected calving date (60 ± 8 d before actual calving date) and balanced by expected calving date, lactation number, previous 305-d mature-equivalent milk yield, and sire identification, respectively. Using a randomized complete block design (RCBD), cows were blocked and allocated to 1 of 4 treatment groups (n = 30 cows/treatment) with different stocking densities at the feed bunk during different stages of the dry period (Overstocked (OS): 0.88 headlocks/cow; Understocked (US): 1.17 headlocks/cow). All cows were housed in a two-row freestall barn throughout the dry period and had access to at least one deep-bedded sand freestall per cow.

Cows were provided feed and water ad libitum, with fresh feed delivery twice daily and provided with diets formulated according to recommendations provided by the National Research Council (NRC, 2001; Table 5.1). Representative samples of the
prepartum TMR were taken once weekly from each experimental pen to determine DM content; these two samples were then pooled weekly for nutrient composition analysis. Samples were dried at 55°C for 48 h to determine DM.

5.3.2. Experimental design and treatments

The 4 experimental treatment groups were as follows: a) OS from 60 to 1 (OS), b) OS from 60 to 26; US from 25 to 1 (OS-US), c) US from 60 to 26; OS from 25 to 1 (US-OS), and d) US from 60 to 1 d (US) prior to calving. The two-row freestall barn was partitioned and divided into 2 adjacent experimental pens (Figure 5.1); OS-US and US-OS treatment groups switched experimental pens as they entered the close-up period approximately 26 d prior to calving. Stocking density based on the number of headlocks available per pen throughout the experiment is represented in Figure 5.2. The conditions of overstocking at the feed bunk were simulated and adjusted in the OS pen by attaching wire hog panels to the headlocks by cable ties in order to restrict access to the feeding area. Feed was only placed in front of accessible headlocks. As cows began to show signs of impending parturition (i.e., milk let-down, relaxation of the tail ligament, udder enlargement, and swelling of the vulva), they were moved to a straw-bedded group maternity pen where they remained until successful calving.

5.3.3. Productivity

Colostrum quantity and quality were recorded after each cow’s first milking; colostrum quality was analyzed using a digital Brix refractometer (MISCO PA202X-400-005 Palm Abbe Digital Fluid Refractometer, Aero Specialties, Boise ID). Cows were milked 3 times daily (0400, 1200, and 2000 h), and daily milk yield was recorded.
manually by the research staff once per wk through 90 DIM, with weekly recordings began during wk 3 of lactation. Daily as-fed intakes were obtained throughout the dry period by the weighing of feed refusals prior to the morning feeding; DM intake measures were obtained by correcting as-fed intakes for the DM content of the feed. Because this experiment was conducted on a commercial dairy farm, it was not possible to obtain individual cow intake. Clinical health problems after calving were monitored and recorded by farm personnel.

5.3.4. Blood and fecal collection and analysis

Blood and fecal samples were collected from each cow on d -60, -30, -7, and +7 relative to calving. Blood samples were obtained from the cow via coccygeal venipuncture and collected in 5-mL Vacutainer serum collection tubes (BD Vacutainer Plus Blood Clot Collection Tubes, Franklin Lakes, NJ). The blood samples were immediately placed on ice after collection. Samples were allowed approximately 45 min to thaw and were then centrifuged at 3,500 RPM (1,180 x g) a 4°C for 15 min. Plasma was harvested after centrifugation and stored at -20°C until further laboratory analysis. Plasma concentrations of NEFA were measured via enzymatic colorimetric method assay (Appendix B; HR Series NEFA-HR (2)), Wako Pure Chemical Industries, Osaka, Japan); all samples were analyzed in duplicate. The concentration of NEFA were calculated using the absorbance values obtained from spectrophotometric measurements via a microplate reader (Molecular Devices, Sunnyvale, CA).

Fecal samples were collected fresh, sealed within Whirl-Pak® bags (18-oz, Nasco, Fort Atkinson, WI), and immediately placed on ice after collection. Fecal samples were
stored at -20°C until frozen contents were transferred to aluminum pans and freeze-dried. The DM percentage (17.0% DM, on average across treatment and sampling day) of each fecal sample was obtained by weighing the sample before and after drying. Steroids were then extracted from the dried fecal samples. Briefly, 2 g of each fecal sample was weighed and mixed with 3 mL of 90% ethanol for 30 min. Samples were then centrifuged at 8,400 RPM (4000 x g) for 15 min, and 1 mL of supernatant was transferred to a clean tube and evaporated to dryness under nitrogen; dried, extracted samples were stored at -20°C in a desiccator. The concentration of cortisol was measured using enzyme immunoassay validated for use in cattle (Appendix A; DetectX® Cortisol Enzyme Immunoassay Kit, Arbor Assays, Ann Arbor, MI).

5.4. Statistical analysis

Statistical analyses were conducted using ANOVAs (PROC GLM and MIXED procedures) in SAS (2012) using the cow as the experimental unit; least squares means ± standard errors of the mean (LSM ± pooled SEM) are reported. The effect of treatment on gestation length and colostrum quality and quantity were analyzed using PROC GLM procedure, whereas repeated measurements (milk production and NEFA and fecal cortisol concentrations) were analyzed using the PROC MIXED procedure. The models included the fixed effects of treatment, time, and a treatment by time interaction, with block included as a random effect. The covariance structure of error for repeated measures in time was selected based on the lowest Bayesian information criteria (BIC),
and the selected covariance structure of error was the first-order autoregressive structure. Significant differences were declared at \( P \leq 0.05 \) and a trend at \( 0.05 > P \leq 0.10 \).

5.5. Results

5.5.1. Productivity

There was an approaching tendency for colostrum quantity to differ among treatment groups \( (P = 0.11; \text{Table 5.2}) \), primarily due to the difference between OS–US and US treatment groups. However, there was no difference in colostrum quality (weighted Brix value of 25.8%). Milk production data for wk 3 through 12 of lactation are provided in Table 5.2 and Figure 5.4, respectively. Daily milk yield did not differ by treatment, with cows producing an average of 47.2 kg/d. As expected, a significant week difference by DIM was observed for daily milk yield \( (P < 0.05) \) (Figure 5.3); however, there was no treatment by week interaction for daily milk yield for OS, OS-US, US-OS, or US cows \( (P > 0.05) \). Diets averaged 42.9% DM. Based on pen measurements of feed consumed, DM intake averaged 14.1 kg/d during the dry period. In addition, as expected, DM intake decreased relative to calving (Figure 5.4). All health events are shown in Table 5.3; the incidence of monitored health events was low across treatments and there were no incidence of displaced abomasum.

5.5.2. Blood and fecal samples

The prepartum NEFA concentrations did not differ among treatment groups, and all cows were below the critical NEFA concentration threshold of 0.4 mEq/L (LeBlanc et al., 2005) used to predict cows more at risk for contracting metabolic disease (Figure
Postpartum NEFA concentrations increased over two-fold compared to the wk prior to calving ($P < 0.0001$), yet there was no difference observed among OS, OS-US, US-OS, or US treatment groups. In addition, the majority of cows were again below the critical postpartum NEFA concentration threshold of 0.7 mEq/L (LeBlanc et al., 2005) (Figure 5.5).

There was no difference observed among OS, OS-US, US-OS, or US treatment groups with regard to fecal cortisol metabolite concentrations (Figure 5.6). However, fecal cortisol metabolite concentrations differed by day, and there was a significant treatment by day interaction. Fecal cortisol metabolite concentrations differed from -60 to -30 d relative to calving for OS, OS-US, and US treatment groups, from -60 to -7 d relative to calving for OS, OS-US, US-OS, and US treatment groups, and -60 to +7 d relative to calving for OS and US-OS treatment groups. In addition, fecal cortisol metabolite concentrations differed from -30 to -7 d relative to calving and -30 to +7 d relative to calving for all treatment groups. Lastly, there was a tendency for fecal cortisol metabolite concentrations to differ from -7 to +7 d relative to calving for OS-US and US treatment groups.

5.6. Discussion

5.6.1. Productivity

The objective of this study was to determine if overstocking the feeding area at different times during the dry period affected physiology and performance of dairy cows. Although there were no differences observed with regard to colostrum quality, quality
across treatment groups was above the recommended break point for good-quality colostrum, with a weighted Brix value of 25.8%. According to Quigley et al. (2013), 21% Brix be considered the break point for high-quality (>50 g of IgG/L) maternal colostrum. In addition, the results of the present study indicated an approaching tendency for colostrum yield to differ among treatments, primarily due to the difference between OS–US and US treatment groups. Studies related to effects of stress on mammary gland growth and development are limited, and this is the first study to our knowledge to examine this relationship with regard to overstocking the feed bunk during the dry period.

The process of mammary gland involution occurs during the dairy cow’s transition from a lactating to a nonlactating state. Completion of the functional changes occurring in the mammary gland during the process of involution may be required for the gland to redevelop fully for maximal milk yield in the subsequent lactation (Hurley, 1988). It is possible that overstocking the feed bunk during the far-off period compromised the process of involution, and thus the process of mammary gland redevelopment. However, the difference observed remains unclear as to whether this effect occurred as a result of altering the stocking density at the feed bunk during different stages of the dry period or from the potential stress imposed by overstocking, as colostrum quantity was not reduced in OS cows. Thus, future studies of bovine epithelial cell numbers are needed to quantify the extent of actual cell loss during involution of the bovine mammary gland and how such losses may be exacerbated under stressful conditions (Hurley, 1988).
Increased stocking density at the feed bunk before calving did not compromise milk production after calving, consistent with the results from other studies (Bach et al., 2008; Proudfoot et al., 2009). However, Nordland et al. (2006) demonstrated a decrease in milk yield for primiparous cows when housed with multiparous cows when prefresh pen stocking densities exceeded 80%; first lactation cows in mixed groups are generally subordinate. Yet, even if first lactation dairy cows are managed separately from mature cows, the authors hypothesized that one-third of each group will remain subordinate and show reduced productivity when feed bunk space is limited. The failure to consistently show reductions in milk yield when feeding space is restricted may likely be a contributing factor to the continued practice of overstocking. However, according to Huzzey et al. (2013), “… the maintenance of milk production alone should not be used to justify a management practice as the relationship between milk production and animal welfare is complex.”

5.6.2. Blood and fecal samples

The results of the present study suggest that overstocking the feed bunk does not alter physiological parameters associated with energy metabolism during the far-off or close-up period. The concentration of NEFA typically begins to increase as the cow prepares for impending parturition and may range from 0.2 to 0.3 mEq/L during the week prior to calving. Such values increase sharply directly before calving and generally peak on the day of calving due to natural hormonal changes and the stress of calving (LeBlanc, 2010). There were no differences observed by treatment; both pre- and postpartum NEFA levels followed this tendency and were within a similar range reported by others
(LeBlanc et al., 2005). Thus, these data provide evidence that cows on our trial did not suffer from severe negative energy balance. The majority of the literature pertains to the consequences of increased NEFA concentrations during the close-up period; however, future studies should aim to investigate increased NEFA concentrations during the far-off period in direct relation to the potential consequences on dairy cow health and performance postpartum, as this was not directly quantified in this study.

In contrast to the results of the current study, Huzzey et al. (2012) reported greater plasma NEFA concentrations during periods of overstocking (1 lying stall/2 cows and 0.34 m of linear feed bunk space/cow) compared to periods of control (1 lying stall/cow and 0.67 m of linear feed bunk space/cow) approximately 3 wk prepartum (0.11 vs. 0.09 ± 0.006 mEq/L, respectively). However, it is important to note that cows were also overstocked at the freestalls in that study, which did not occur in the present study. In addition, the reported NEFA concentrations observed by Huzzey et al. (2012) during both treatment periods were low compared with those observed in the present experiment. Thus, such a significant increase during the overstocking period may not be of biological significance.

The results of this study suggest that overstocking the feed bunk during different stages of the dry period does not increase the stress level of cows during the far-off or close-up period. This is in contrast to Huzzey et al. (2012) who reported a tendency for fecal cortisol metabolite concentrations to be greater during the overstocking period when feeding space was restricted to 0.34 m/cow compared to 0.67 m/cow. Thus, it is possible that the stocking densities employed were not severe enough to increase competitive
behavior and increase social stress among cows. In addition, cows did not have to compete for access to freestalls, which is often an additive stressor within an overcrowded environment.

5.7. Conclusions

In conclusion, moderate increases in prepartum stocking density at the feed bunk did not compromise the productivity or metabolic status of dairy cows. In addition, there was no difference observed between OS-US and US-OS treatment groups; cows responded similarly to altering the stocking density at the feed bunk between the far-off and close-up periods. Although it is a variable relationship and dependent on barn design, overstocking at the feed bunk typically tends to overstock the freestalls. Thus, further research is encouraged to investigate the potential consequences of overstocking both the feed bunk and lying stalls in relation to the different stages of the dry period in a commercial farm setting.
Table 5.1. Ingredients of the far-off and close-up dry cow TMR (% of DM)

<table>
<thead>
<tr>
<th>Item</th>
<th>Far-off</th>
<th>Close-up</th>
</tr>
</thead>
<tbody>
<tr>
<td>Grass hay</td>
<td>13.6</td>
<td>11.5</td>
</tr>
<tr>
<td>Grass haylage</td>
<td>57.2</td>
<td>N/A</td>
</tr>
<tr>
<td>Ground wheat straw</td>
<td>N/A</td>
<td>11.6</td>
</tr>
<tr>
<td>Corn silage</td>
<td>27.8</td>
<td>41.2</td>
</tr>
<tr>
<td>Brewers grain</td>
<td>N/A</td>
<td>10.6</td>
</tr>
<tr>
<td>Soybean meal, 48% CP</td>
<td>N/A</td>
<td>11.5</td>
</tr>
<tr>
<td>Canola meal</td>
<td>N/A</td>
<td>N/A</td>
</tr>
<tr>
<td>Animate&lt;sup&gt;1&lt;/sup&gt;</td>
<td>N/A</td>
<td>3.8</td>
</tr>
<tr>
<td>Protein/mineral pre-mix</td>
<td>1.4</td>
<td>9.8</td>
</tr>
</tbody>
</table>

<sup>1</sup>Prince Agri Products, Inc., Quincy, IL
Table 5.2. Mean gestation length, colostrum yield, colostrum quality, milk yield, and dry matter intake (DMI) for cows housed at various stocking densities during the dry period\(^1\)

<table>
<thead>
<tr>
<th></th>
<th>OS</th>
<th>OS-US</th>
<th>US-OS</th>
<th>US</th>
<th>SEM</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gestation length (d)</td>
<td>276</td>
<td>274</td>
<td>275</td>
<td>276</td>
<td>1</td>
<td>0.74</td>
</tr>
<tr>
<td>Colostrum yield (kg)</td>
<td>7.72</td>
<td>5.70</td>
<td>7.03</td>
<td>8.76</td>
<td>0.86</td>
<td>0.11</td>
</tr>
<tr>
<td>Colostrum quality (%Brix)</td>
<td>25.2</td>
<td>26.2</td>
<td>26.6</td>
<td>25.3</td>
<td>0.8</td>
<td>0.55</td>
</tr>
<tr>
<td>Milk yield (kg/d)</td>
<td>47.1</td>
<td>46.8</td>
<td>47.4</td>
<td>47.4</td>
<td>1.21</td>
<td>0.99</td>
</tr>
<tr>
<td>DMI (kg/d)(^2)</td>
<td>13.9</td>
<td>14.1</td>
<td>14.2</td>
<td>14.3</td>
<td>--</td>
<td>--</td>
</tr>
</tbody>
</table>

\(^1\)OS: Overstocked from 60 to 1, b) OS-US: Overstocked from 60 to 26; Understocked from 25 to 1, c) US-OS: Understocked from 60 to 26; OS from 25 to 1 (US-OS), and d) US from 60 to 1 d (US) prior to calving

\(^2\)DMI based on pen average
Table 5.3. Incidence of health events for cows housed at various stocking densities during the dry period\(^1\)

<table>
<thead>
<tr>
<th></th>
<th>OS</th>
<th>OS-US</th>
<th>US-OS</th>
<th>US</th>
</tr>
</thead>
<tbody>
<tr>
<td>Retained placenta</td>
<td>3</td>
<td>4</td>
<td>3</td>
<td>4</td>
</tr>
<tr>
<td>Metritis</td>
<td>3</td>
<td>5</td>
<td>5</td>
<td>2</td>
</tr>
<tr>
<td>Ketosis</td>
<td>2</td>
<td>3</td>
<td>3</td>
<td>2</td>
</tr>
<tr>
<td>Milk fever</td>
<td>0</td>
<td>0</td>
<td>1</td>
<td>0</td>
</tr>
</tbody>
</table>

\(^1\)OS: Overstocked from 60 to 1, b) OS-US: Overstocked from 60 to 26; Understocked from 25 to 1, c) US-OS: Understocked from 60 to 26; OS from 25 to 1 (US-OS), and d) US from 60 to 1 d (US) prior to calving
Figure 5.1. Experimental pen design within the two-row freestall barn
Figure 5.2. Stocking density (%) based on the number of headlocks available per pen
Figure 5.3. Dry matter (DM) intake for OS and US pens
Figure 5.4. Milk production from wk 3 to 12 of lactation for cows housed at various stocking densities during the dry period

1OS: Overstocked from 60 to 1, b) OS-US: Overstocked from 60 to 26; Understocked from 25 to 1, c) US-OS: Understocked from 60 to 26; OS from 25 to 1 (US-OS), and d) US from 60 to 1 d (US) prior to calving
Figure 5.5. Plasma non-esterified fatty acid (NEFA) concentrations (+ SEM) for cows housed at various stocking densities during the dry period at -60, -30, -7, and +7 d relative to calving\textsuperscript{1,2}

\[ \begin{align*}
\text{NEFA Concentrations (mEq/L)}
\end{align*} \]

\[ \begin{align*}
\text{Days Relative to Calving}
\end{align*} \]

\[ \begin{align*}
\text{OS} & \quad \text{OS-US} & \quad \text{US-OS} & \quad \text{US}
\end{align*} \]

\[ \begin{align*}
\text{-60} & \quad \text{-30} & \quad \text{-7} & \quad \text{+7}
\end{align*} \]

\textsuperscript{1}OS: Overstocked from 60 to 1, b) OS-US: Overstocked from 60 to 26; Understocked from 25 to 1, c) US-OS: Understocked from 60 to 26; OS from 25 to 1 (US-OS), and d) US from 60 to 1 d (US) prior to calving

\textsuperscript{2}Thresholds of pre- and postpartum NEFA concentrations used to predict cows more at risk for contracting metabolic disease (0.4 and 0.7 mEq/L, respectively) (LeBlanc et al., 2005)
**Figure 5.5.** Fecal cortisol metabolite (FCORT) concentrations (± SEM) for cows housed at various stocking densities during the dry period at -60, -30, -7, and +7 d relative to calving\(^1\)

\(^1\)OS: Overstocked from 60 to 1, b) OS-US: Overstocked from 60 to 26; Understocked from 25 to 1, c) US-OS: Understocked from 60 to 26; OS from 25 to 1 (US-OS), and d) US from 60 to 1 d (US) prior to calving
Chapter 6: Effect of Overstocking at the Feed Bunk on Indicators of Cow Temperament

6.1 Abstract

Our objective was to investigate the effect of overstocking the feed bunk on dairy cow behavioral responses to human approach and reactivity to blood sampling. One hundred and twenty dry Holstein cows were allocated to 1 of 2 treatment groups with different stocking densities (Overstocked (OS): 0.88 headlocks/cow; Understocked (US): 1.17 headlocks/cow) at the feed bunk. Over 2 testing periods (7 d apart), flight response was assessed using a human-approach test with a 5-point ordinal scale defining the distance at which the cow stepped away from the approaching experimenter (0 = not approachable from 3 m to 4 = cow moves away when experimenter is 0 m from the cow). A qualitative assessment was also made of the cow’s response to the experimenter using a visual analogue scale (VAS) that included the terms: relaxed, nervous, alert, shy, aggressive, social, and curious. Reactivity to blood sampling via the coccygeal vein was also assessed in the pen using a 4-point scale (0 = least reactive to 3 = most reactive). Data were analyzed through a mixed model analysis, using treatment, time, and their
interaction. The relationship between qualitative measures was assessed using a Pearson correlation. Treatment did not affect the cow’s flight response; however, there was a significant treatment by time interaction whereby flight response scores decreased with time in OS cows and increased with time in US cows ($P = 0.02$). Reactivity to blood sampling did not differ by treatment ($P = 0.47$), and there was also no treatment by time interaction ($P = 0.88$). The overall correlation between qualitative terms was low. However, the terms ‘relaxed’ and ‘nervous’ showed a significant negative correlation ($P < 0.0001$). In conclusion, overstocking the feed bunk affected the animal’s response to an approaching human. Cows in the OS treatment became less approachable over time, which may indicate fear, stress, or an increase in arousal. Future research should investigate the effect overstocking the feed bunk may have on cow temperament for a longer duration, as this may further decrease approachability.

6.2. Introduction

Management of dairy cows during late gestation is a growing area of interest, as many factors within their environment may influence their welfare, behavior, and production potential. During the dry period, the welfare of the cow is paramount, as she quickly recovers from the previous lactation, prepares for upcoming parturition, and the subsequent transition to lactation. A potential source of social stress often encountered during the dry period is overstocking the feed bunk. Current industry-recommended best practices are to provide at least 0.6 m of linear feeding space per cow when they are housed in a freestall barn (NFACC, 2009). However, even with such recommendations,
overstocking remains common with 58% of farms in the United States reportedly providing less than the recommended feeding space per cow (USDA, 2010).

The negative behavioral and physiological effects of overstocking dairy cows have been well documented. For example, Fregonesi et al. (2007) reported that cows spent, on average, 12.9 h/d lying when 1 stall was available per cow, but this time was significantly decreased to 11.2 h/d when cows were overstocked at the lying stalls to 150%. Additionally, cows competed indirectly for stall usage; they were observed lying down more quickly after returning from the milking parlor as opposed to standing and feeding. Huzzey et al. (2006) also reported that cows were displaced more often from the feed bunk when stocking density increased from 0.67 to 0.33 headlocks/cow, and therefore, cows spent more time standing idle in the feed alley. Under such conditions, increased standing time is also associated with increased plasma cortisol concentrations (Gonzalez et al., 2003), which may be attributed to the stress imposed by the increased number of displacements observed at the overstocked feeding area or freestalls (Huzzey et al., 2006; Fregonesi et al., 2007). Thus, overstocking may place social stress on the animal.

In addition to the role of the physical environment, the human-animal relationship is also of great importance to the welfare of dairy cattle; producers and farm staff members interact with their animals regularly on a day-to-day basis. However, this relationship can be negatively affected by unwarranted stress imposed on the animal. For example, stress due to negative interactions (shouting, quick movements, hits, slaps, etc.) with humans has been shown to have a negative impact on the human-animal
relationship. In addition, if animals are fearful of humans, this may also reduce animal productivity (Hemsworth et al., 2000). Much of the research pertaining to the human-cow relationship has focused on the development of a practical, on-farm assessment tool that may be used to evaluate the temperament and welfare of the animal. Previous research has investigated approach and avoidance behavior of dairy cows towards humans when the animals are in three different locations within a freestall barn: 1) standing in the passageway, 2) lying in a freestall, or 3) standing at the feed bunk (Waiblinger et al., 2003; Rousing and Waiblinger, 2004; Winckler et al., 2007; Windschnurer et al., 2008). Of these settings, it was observed that approaching the cows while standing in the passageway showed the most consistency over time with regard to individual responses across subtest repeats. In addition to approach-avoidance testing, the reaction of dairy cows to routine veterinary procedures has also been used to examine the effect of previous handling experience on cow behavior (Waiblinger et al., 2003).

In addition to quantitatively assessing the human-animal relationship, the reactivity of dairy cows to humans has also been assessed qualitatively (Gibbons et al., 2009). This type of assessment allows experimenters to capture subtle fluctuations in behavioral expressions, such as changes in posture or even slight movements, which can give a better assessment of the animal’s temperament. Quantitative approach-avoidance tests, as well as qualitative assessments have been validated as applicable methods of evaluating the human-animal relationship as part of on-farm animal welfare assessments. However, it is currently unknown as to whether social stress from overstocking the feed bunk during the dry period may affect the approach-avoidance behavior of dairy cows.
To date, overstocking the feed bunk has yet to be investigated in relation to the human-cow relationship and indicators of cow temperament. Thus, the aim of the present study was to investigate the effect of overstocking the feed bunk on dairy cow behavioral responses to human approach and blood sampling procedures. It was hypothesized that the stress imparted from overstocking the feed bunk would increase the flight response and reactivity of cows to humans. It also was hypothesized that the stress imparted from overstocking the feed bunk would increase the reactivity of cows to blood sampling procedures.

6.3. Materials and methods

This study was conducted in July 2014 at Catalpadale Dairy located in Marshallville, Ohio, in accordance with the guidelines set by the Institutional Animal Care and Use Committee of The Ohio State University (Protocol No. 2014A00000063). One hundred twenty multiparous Holstein cows were dried-off approximately 60 d before their expected calving date. Using a randomized complete block design, cows were assigned to 1 of 2 treatment groups and balanced by expected calving date, lactation number, previous 305-d mature-equivalent milk yield, and sire identification, respectively. The stocking densities of the 2 treatment groups were as follows: 1) Understocked (US): stocking density of approximately 88% at the feed bunk; n = 60; 2) Overstocked (OS): stocking density of approximately 117% at the feed bunk; n = 60.

All cows were housed in a freestall barn divided into 2 experimental pens. The conditions of overstocking at the feed bunk were simulated in the OS pen by attaching
hog panels to the headlocks by cable ties in order to restrict access to the feeding area (US: 1.17 headlocks/cow; OS: 0.88 headlocks/cow). All cows were allowed ad libitum access to feed; a total mixed ration (TMR) diet was formulated according to the recommendations of the National Research Council, and fresh feed was provided twice per day (NRC, 2001).

### 6.3.1. Human-animal interaction assessment

Flight response was assessed by a single experimenter over 2 testing periods (7 d apart) during the far-off period (-60 to -26 d prior to expected calving date) using a human-approach test with a 5-point ordinal scale adapted from Gibbons et al. (2009). This scale defined the distance at which a cow stepped away from an approaching experimenter (Table 6.1; 0 = Not approachable from 3.1 m to 4 = Cow moved away when the experimenter was 0 m away). Focal cows that were facing the experimenter while standing in the passageway with room to step away from the experimenter were approached starting at a distance of 3.1 m away from the animal. From this standardized distance, the experimenter took approximately 0.46 m long steps at a diagonal towards the shoulder of the animal while avoiding eye contact and keeping arms at the sides. After each step, the experimenter remained motionless for 3 s to allow the cow to respond. The test was considered complete when the cow stepped away from the experimenter.

A qualitative assessment was also made of the cow’s response to the approaching experimenter by a second identically dressed experimenter. This assessment was done using a visual analogue scale (VAS), adapted from Gibbons et al. (2009), which included...
the terms: relaxed, nervous, alert, shy, aggressive, social, and curious (Table 6.2). The VAS consisted of a 69 mm horizontal line with 2 vertical lines marking the extreme points of the scale (Figure 6.1; 0 mm = Term absent to 69 mm = Term present throughout the test). Scores for each term were measured as the distance in mm from the 0-point.

6.3.2. Reactivity to blood-sampling assessment

Behavioral reactivity of the cows to blood sampling from the coccygeal vein was also assessed in the pen at dry-off and -45 d prior to calving while cows were headlocked. This was done using an original 4-point numerical rating scale where 0 = Not reactive and 3 = Very reactive (Table 6.3).

6.4. Statistical analysis

Data were analyzed using the MIXED procedure of SAS (2012), using treatment, time, and their interaction as fixed effects; block was included in the model as a random effect. The relationship between qualitative measures was assessed using the PROC CORR procedure of SAS (2012). Significant differences were considered as $P < 0.05$ and a trend as $P < 0.10$.

6.5. Results

6.5.1. Human-animal interaction

There was no main effect of treatment with relationship to the animal’s flight response score (Figure 6.2). However, there was a significant treatment by time interaction in which flight response score decreased with time among the OS cows and
increased with time among the US cows (Figure 6.2; OS: 1.65 to 1.47, US: 1.33 to 1.68; \( P = 0.02 \)). This indicates that the OS cows were becoming less approachable with time, while the US cows were becoming more approachable.

The overall correlation between qualitative behavioral terms was low (Table 6.4). However, the terms ‘relaxed’ and ‘nervous’ showed significant negative correlation across both assessment days (\( r = -0.76; P < 0.0001 \)); the more nervous the cow was, the less relaxed she seemed to be. ‘Nervous’ behavior was also correlated with ‘alert’ behavior (\( r = 0.56; P < 0.0001 \)); an ‘alert’ cow was identified as a ‘nervous’ cow. ‘Alert’ behavior also was negatively correlated with ‘relaxed’ behavior (\( r = -0.46; P < 0.0001 \)); an alert cow seemed to be less relaxed. The results also revealed that ‘relaxed’ behavior was negatively correlated with ‘shy’ behavior (\( r = -0.41; P < 0.0001 \)); a more relaxed cow seemed to be less shy. Additionally, ‘shy’ behavior was also positively correlated with ‘nervous’ behavior (\( r = 0.39; P < 0.0001 \)); a shy cow was also a more nervous cow.

Correlation between the qualitative behavioral terms, flight response score, and blood sampling reactivity score was low (Table 6.4). However, there was a significant positive, but low, correlation between ‘relaxed’ behavior and flight response score (\( r = 0.13; P = 0.04 \)); a ‘relaxed’ cow was more approachable. Additionally, there was a positive correlation between ‘curious’ behavior and flight response score (\( r = 0.39; P < 0.0001 \)); a more ‘curious’ cow was more approachable. In addition, there was a trend between ‘nervous’ behavior and the blood sampling reactivity score (\( r = -0.11; P = 0.09 \)); the more nervous a cow, the less reactive she tended to be to blood sampling. This may indicate a tendency for nervous cows to halt when having their blood sampled.
6.5.2. Reactivity to blood sampling

Reactivity of the cows to blood sampling did not differ by treatment (Figure 6.3; US: 1.00, OS: 1.14; \(P = 0.24\)). The reactivity of the cows to blood sampling also did not differ by testing day (Day 1: 1.10, Day 2: 1.04; \(P = 0.66\)). There also was no significant treatment by time interaction with respect to the cow’s reactivity to blood sampling (Figure 6.3; OS: 1.17 to 1.11, US: 1.01 to 0.98; \(P = 0.88\)).

6.6. Discussion

The results of the human approach test indicated that the OS cows became less approachable with time, while the US cows became more approachable with time. Although a limitation of the current study is the lack of replication with regard to experimental testing periods, this finding may have significant implications. Future research is encouraged to investigate the effect overstocking the feed bunk may have on the human-animal relationship for longer than was investigated in the current study, as overstocking for a longer duration may further decrease approachability.

It has been suggested that certain interactions with humans affect avoidance behavior. For example, Schmied et al. (2008) reported that positive interactions, such as stroking the animal, decreases avoidance distance. However, contrastingly, there is also evidence that negative attitudes and behaviors of humans toward animals increases avoidance distance; negative attitudes shape negative behaviors, and when negative behaviors are practiced, this may subsequently increase the animal’s level of fear of humans (Breuer et al., 2000; Hemsworth et al., 2000). It is also possible that if an animal
is already stressed, it will experience hypersensitivity, a factor that further affects the animal’s individual response to environmental stimuli (Broom and Johnson, 1993). With reference to the present study, the potential stress imposed by overstocking the feed bunk may have resulted in a hypersensitive response elicited by the animal to the approaching experimenter. This may be one explanation as to why OS cows became less approachable with time.

Hypersensitivity may have severe animal welfare consequences, and the result is that a given stimulus, such as an approaching human, will elicit a greater response than expected in such an animal (Broom and Johnson, 1993). In circumstances of increased or repeated stimulation, intolerable response levels are reached much more rapidly in a hypersensitive animal. As indicated by previous research, overstocking the feed bunk imparts unwarranted social stress on dairy cows (Huzzey et al., 2012), and if they are also fearful of humans, the human-cow relationship may induce a hypersensitive response. The nature of the human-cow relationship requires daily interaction, and the animal’s response to humans may increase with each interaction; the animal may become more and more sensitive despite the constancy of the stimulus (Broom and Johnson, 1993). This is not only a concern for animal welfare, but increased fear of humans may also lead to production and economic losses, as well (Hemsworth et al., 2000).

In addition, overall correlation between the qualitative behavioral terms, flight response score, and blood sampling reactivity score was low. However, there were positive correlations between the flight response score and the terms ‘relaxed’, ‘curious’, and ‘social’; cows that were identified as such were more approachable. Our results are
in accordance with Gibbons et al. (2008) who reported positive correlations between the flight response score at ‘at ease’, ‘passive’ and ‘social’ and a negative correlation between flight response score and ‘nervous’. In addition, our results showed a trend between ‘nervous’ behavior and the blood sampling reactivity score; cows that were identified as ‘nervous’ tended to be less reactive to blood sampling procedures. This may indicate a tendency for ‘nervous’ cows to halt or freeze when having their blood sampled.

To our knowledge, this is the first study to investigate the reactivity of dairy cows to blood sampling; however, the reactivity and behavioral responses of cows to other veterinary procedures has previously been explored (Waiblinger et al., 2004). Although it is difficult to directly compare the results of the current study to others, it may be possible to summarize them based on ‘positive environmental stimuli’ and ‘negative environmental stimuli’. Overstocking the feed bunk (i.e. negative environmental stimuli) did not affect the cows’ reactivity to blood sampling as initially hypothesized, and there was not a change over time. Waiblinger et al. (2004) investigated the effect of previous positive handling experience, i.e. positive environmental stimuli, on behavior and heart rate of dairy cows during rectal palpation and sham insemination; behavior was assessed using a 3-point scale: 0 = No; 1 = Partly; 2 = Yes. In contrast to the results of the current study, the authors reported that positively handled animals had lower heart rate during the veterinary procedure, kicked less when alone, and tended to show less restless behavior during testing.
6.7. Conclusions

Overstocking the feed bunk affected the animal’s response to an approaching human. OS cows became less approachable over time, while US cows became more approachable. These results may indicate fear, stress, or an overall increase in the arousal of the animal. An animal that becomes more fearful of humans will experience added stress through their daily interactions with humans. This additional stress may negatively affect the animal’s welfare and could lead to a decrease in milk production. Additional stress on the animal could also lead to potential negative implications for workers on the farm as ease of handling will decrease if an animal is stressed or fearful; the animals may bunch-up or attempt to flee, making them difficult to manage. The farm may also potentially be affected by a loss of profit due to the potential decrease in production from imparted stress.

One potential limitation of the present study, as mentioned previously, is that the animal’s response to an approaching human and reactivity to blood sampling were only evaluated twice. Future research should investigate the effect that overstocking the feed bunk may have on the human-animal relationship for longer than was investigated in the current study, as overstocking the feed bunk for a longer duration may further decrease approachability.
Table 6.1. The scoring system used to score the cow’s flight response to the approach test\(^1\)

<table>
<thead>
<tr>
<th>Score</th>
<th>Behavioral response</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>Cow moves away when experimenter was &gt; 3.1 m away</td>
</tr>
<tr>
<td>1</td>
<td>Cow moves away when experimenter is between 2.1 and 3.1 m away (~1-2 steps)</td>
</tr>
<tr>
<td>2</td>
<td>Cow moves away when experimenter is between 1.2 and 2.1 m away (~3-4 steps)</td>
</tr>
<tr>
<td>3</td>
<td>Cow moves away when experimenter is between 0.3 and 1.2 m away (~5-6 steps)</td>
</tr>
<tr>
<td>4</td>
<td>Cow moves away when experimenter is 0 m away</td>
</tr>
</tbody>
</table>

\(^1\)Adapted from Gibbons et al. (2009)
<table>
<thead>
<tr>
<th>Term</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>Relaxed</td>
<td>A calm animal showing no sign of tension</td>
</tr>
<tr>
<td>Nervous</td>
<td>An animal that is quite restless/wary/uneasy as the experimenter approaches. May avoid experimenter, or quiver/flinch when a hand is placed on her, whites in eyes visible</td>
</tr>
<tr>
<td>Alert</td>
<td>Animal is very alert and attentive to the experimenter approaching and/or other events happening around her, ears may be pointed toward experimenter</td>
</tr>
<tr>
<td>Shy/submissive</td>
<td>Animal appears hesitant but not nervous, could show signs of submission like lowered head or freezing</td>
</tr>
<tr>
<td>Aggressive</td>
<td>An animal that appears agitated/irritated or annoyed as experimenter approaches. A dominant animal which may attempt to kick or to butt the experimenter by lowering her head to swing/lunge towards the experimenter.</td>
</tr>
<tr>
<td>Social</td>
<td>An animal that interacts positively with the experimenter, maybe try to sniff/lick/rub against experimenter.</td>
</tr>
<tr>
<td>Curious</td>
<td>Animal appears inquisitive, protrudes muzzle to sniff/investigate experimenter</td>
</tr>
</tbody>
</table>

\(^1\)Adapted from Gibbons et al. (2009)
**Table 6.3.** The scoring system used to score the cow’s reactivity to blood sampling

<table>
<thead>
<tr>
<th>Score</th>
<th>Reactivity</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>Not reactive</td>
<td>Cow does not show any movement when sampled</td>
</tr>
<tr>
<td>1</td>
<td>Somewhat reactive</td>
<td>Cow shows some gentle movement upon initial handling, shifts weight back and forth and slightly sways</td>
</tr>
<tr>
<td>2</td>
<td>Reactive</td>
<td>Cow shows active movement upon initial handling, including movement and swaying, but stops movement after a few seconds</td>
</tr>
<tr>
<td>3</td>
<td>Very reactive</td>
<td>Cow is active for the duration of sampling period; aggressively sways and moves around and may kick</td>
</tr>
</tbody>
</table>
### Table 6.4. Coefficients of simple correlations between qualitative behavioral terms, flight response score, and blood sampling reactivity score

<table>
<thead>
<tr>
<th></th>
<th>Relaxed</th>
<th>Nervous</th>
<th>Alert</th>
<th>Shy</th>
<th>Aggressive</th>
<th>Social</th>
<th>Curious</th>
<th>Flight Score</th>
<th>Blood Score</th>
</tr>
</thead>
<tbody>
<tr>
<td>Relaxed</td>
<td>1.00</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Nervous</td>
<td>-0.76 &lt;0.0001</td>
<td>1.00</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Alert</td>
<td>-0.46 &lt;0.0001</td>
<td>0.56 &lt;0.0001</td>
<td>1.00</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Shy</td>
<td>-0.41 &lt;0.0001</td>
<td>0.38 &lt;0.0001</td>
<td>0.22</td>
<td>1.00</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Aggressive</td>
<td>0.09</td>
<td>-0.09</td>
<td>0.07</td>
<td>-0.02</td>
<td>1.00</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Social</td>
<td>0.22 0.001</td>
<td>-0.16</td>
<td>0.003</td>
<td>-0.11</td>
<td>0.02</td>
<td>0.02</td>
<td>1.00</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Curious</td>
<td>0.16 0.019</td>
<td>-0.29</td>
<td>-0.01</td>
<td>-0.059</td>
<td>0.133</td>
<td>0.35</td>
<td>1.00</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Flight Score</td>
<td>0.13</td>
<td>-0.04</td>
<td>0.14</td>
<td>0.053</td>
<td>0.05</td>
<td>0.20</td>
<td>0.39</td>
<td>1.00</td>
<td></td>
</tr>
<tr>
<td>Blood Score</td>
<td>0.043</td>
<td>0.511</td>
<td>0.040</td>
<td>0.429</td>
<td>0.455</td>
<td>0.003</td>
<td>&lt;0.0001</td>
<td>&lt;0.0001</td>
<td></td>
</tr>
<tr>
<td></td>
<td>0.10</td>
<td>-0.11</td>
<td>-0.04</td>
<td>-0.05</td>
<td>0.11</td>
<td>-0.01</td>
<td>-0.03</td>
<td>-0.02</td>
<td>0.757</td>
</tr>
<tr>
<td></td>
<td>0.138</td>
<td>0.087</td>
<td>0.597</td>
<td>0.500</td>
<td>0.084</td>
<td>0.861</td>
<td>0.666</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
**Figure 6.1.** Visual analogue scale for qualitative behavior assessment

<table>
<thead>
<tr>
<th>Date</th>
<th>Cow</th>
<th>Term</th>
<th>0</th>
<th>69</th>
<th>Visual Analog Scale</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Relaxed</td>
<td></td>
<td></td>
<td>---------------------</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Nervous</td>
<td></td>
<td></td>
<td>---------------------</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Alert</td>
<td></td>
<td></td>
<td>---------------------</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Shy/submissive</td>
<td></td>
<td></td>
<td>---------------------</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Aggressive</td>
<td></td>
<td></td>
<td>---------------------</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Social</td>
<td></td>
<td></td>
<td>---------------------</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Curious</td>
<td></td>
<td></td>
<td>---------------------</td>
</tr>
</tbody>
</table>

1Adapted from Gibbons et al. (2009)
Figure 6.2. Least square means (± SEM) of flight response score for cows exposed to understocked or overstocked feed bunk conditions

*Significant treatment by time interaction (P < 0.05)
Figure 6.3. Least square means (± SEM) of blood sampling reactivity score for cows exposed to understocked or overstocked feed bunk conditions.
Chapter 7: Conclusions and Future Directions

The objective of this dissertation was to address animal welfare as a continuous state with regard to the improvement of dairy calf and cow welfare. This dissertation began with a review of the concept of animal welfare and how to assess it scientifically based on the three components of animal welfare. A review of the existing literature emphasizing animal welfare as a continuous state subsequently discussed the following: 1) the welfare of the dairy calf in utero, 2) young heifer calves in relation to the benefits of social companionship, and 3) the importance of the environment to the welfare of the mature dairy cow. Gaps in the existing scientific literature were identified throughout the chapters of this dissertation, which aimed to address such gaps by using the data acquired through the subsequent research chapters.

The study presented in Chapter 3 aimed to determine whether increased stocking density at the feed bunk for the dam during different stages of the dry period (i.e., far-off versus close-up period) affected the postnatal growth and health of their heifer calves. The results of this study indicated that overstocking the feed bunk of the dam during different stages of the dry period did not affect calf growth (i.e., body weight and hip and
wither heights). In addition, maternal social stress experienced via overstocking the feed bunk did not compromise passive immunity in calves. This study was the first to examine aspects of the maternal social environment in relation to the prenatal development and postnatal growth of offspring. In addition, it also highlighted the prenatal period and its importance to the welfare of the developing fetus, which will optimistically prompt other animal welfare scientists to include the prenatal period of development in future studies.

In addition to the prenatal environment, it is imperative to consider the welfare of the neonatal calf, and there is increasing social pressure being placed on dairy producers to adopt alternative housing and management practices that allow animals more opportunity to exercise and demonstrate social behavior. Thus, Chapter 4 addressed such concerns pertaining to pre-weaned dairy heifer calves; this experiment investigated the effect of pair versus individual housing on the behavior and growth performance of Jersey heifer calves. The results of this experiment indicated that pair-housed calves tended to have greater average daily gain compared to calves housed individually. Pair housing also increased BW post-weaning at 9 wk of age compared with individual housing. These results provide evidence that housing Jersey heifer calves in pairs increases measures of growth performance, especially during the weaning period. However, cross-sucking behavior was prevalent, as calves were fed with a bucket and did not have access to an artificial nipple. Chapter 4 presented one of the first experiments to investigate alternative housing systems with regard to Jersey calves, and it is hypothesized that potential behavioral differences (i.e., cross-sucking and non-nutritive
behavior) may exist between calves of Holstein and Jersey breeds. Thus, future research should aim to quantify and directly compare the behavior of Holstein and Jersey calves. In addition, future research is encouraged to investigate means of reducing cross-sucking and non-nutritive sucking behavior, specifically within the Jersey breed through the use of alternative feeding systems or environmental enrichment.

Lastly, Chapters 5 and 6 examined the environment of the mature dairy cow with regard to the potential negative effects of overstocking the feed bunk during the dry period in conditions similar to a commercial dairy farm; few studies have examined overstocking in a modern production setting. Chapter 5 discussed the first study to examine the effects of increased stocking density at the feed bunk during different stages of the prepartum period, i.e. altered stocking densities at the feed bunk during the far-off and close-up periods. However, in contrast to our initial hypotheses, the results of this study indicated that overstocking the feed bunk did not affect the metabolic health or productivity (i.e., colostrum yield and quality and milk production through 90 DIM) of dairy cows during different stages of the dry period. Future research should aim to investigate the potential consequences of overstocking both the feed bunk and lying stalls in relation to the different stages of the dry period in a commercial farm setting. In accordance with Chapter 5, the objective of Chapter 6 was to investigate the effect of overstocking the feed bunk on dairy cow behavioral responses to human approach and reactivity to blood sampling procedures. A novel and main finding of this Chapter was that overstocking the feed bunk affected the animal’s response to an approaching human; overstocked cows became less approachable over time, while understocked cows became
more approachable over time. These results may indicate fear, stress, or an overall increase in the arousal of the animal. An animal that becomes more fearful of humans will experience added stress through their daily interactions with humans; this additional stress may negatively affect the animal’s welfare and could have negative implications for workers on the farm, as ease of handling will decrease if an animal is stressed or fearful. As discussed within Chapter 6, the main limitation of this study was the lack of replication. Future research should investigate the effect that overstocking the feed bunk may have on the human-animal relationship for longer than was investigated in the current study, as overstocking the feed bunk for a longer duration may further decrease approachability.

Collectively, the experimental studies conducted to support this dissertation address animal welfare as a continuous state as it pertains to dairy animals and their environment. It is encouraged for both current and future animal welfare scientists to begin placing emphasis on the welfare of the prenatal dairy calf, in addition to the continued consideration of the welfare of growing and mature animals.
List of References


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Appendix A: DetectX® Cortisol Enzyme Immunoassay Protocol

1. Freeze-dry all fecal samples before beginning extraction process.
2. Weigh out ≥ 0.2 g of dried fecal solid into a tube.
3. Add 3 mL of ethanol (or ethyl acetate) to 0.2 g of fecal solid.
4. Shake vigorously for at least 30 minutes.
5. Centrifuge samples at 4,000 x g for 15 minutes. Transfer 1 mL of supernatant to a clean tube for evaporation.
6. Evaporate supernatant solution to dryness under nitrogen (process takes approximately 3 d). Keep dried extracted samples frozen < -20°C in a desiccator.
7. Dissolve extracted sample with 100µL ethanol, followed by at least 400µL assay buffer (AB). Vortex well and allow to sit 5 minutes at room temperature. Vortex and sit for 5 minutes twice more to ensure complete steroid solubility.
8. Run reconstituted diluted samples in assay immediately.

Note: In step 5, if only a portion of the organic solvent is being evaporated, ensure final amounts of measured steroid per gm solid accounts for volume of solution evaporated.

Reagent Preparation:
Allow the kit reagents to come to room temperature for approximately 30 minutes. All standards and samples must be run in duplicate to allow the end user to accurately determine cortisol concentrations. Ensure that all samples have reached room temperature and have been diluted as appropriate prior to running them in the kit.
**Assay Buffer:**
Dilute Assay Buffer Concentrate 1:5 by adding one part of the concentrate to four parts of deionized water. Once diluted, this is stable for 3 months at 4°C.

**Wash Buffer:**
Dilute Wash Buffer Concentrate 1:20 by adding one part of the concentrate to 19 parts of deionized water. Once diluted, this is stable at room temperature for 3 months at room temperature.

**Standard Preparation:**
Label six test tubes as #1 through #6. Pipet 450 µL of Assay Buffer into tube #1 and 250 µL into tubes #2 through #6. The cortisol stock solution contains an organic solvent. Pre-rinse the pipet tip several times to ensure accurate delivery. Carefully add 50 µL of the cortisol stock solution to tube #1 and vortex completely. Take 250 µL of the cortisol solution in tube #1 and add it to tube #2 and vortex completely. Repeat the serial dilutions for tubes #3 through #6. The concentration of cortisol in tubes 1 through 6 will be 3200, 1600, 800, 400, 200, and 100 pg/mL.

**Use all standards within 2 hour of preparation.**

<table>
<thead>
<tr>
<th></th>
<th>Std 1</th>
<th>Std 2</th>
<th>Std 3</th>
<th>Std 4</th>
<th>Std 5</th>
<th>Std 6</th>
</tr>
</thead>
<tbody>
<tr>
<td>Assay Buffer Volume (µL)</td>
<td><strong>450</strong></td>
<td>250</td>
<td>250</td>
<td>250</td>
<td>250</td>
<td>250</td>
</tr>
<tr>
<td>Addition</td>
<td>Stock</td>
<td>Std 1</td>
<td>Std 2</td>
<td>Std 3</td>
<td>Std 4</td>
<td>Std 5</td>
</tr>
<tr>
<td>Volume of Addition (µL)</td>
<td><strong>50</strong></td>
<td>250</td>
<td>250</td>
<td>250</td>
<td>250</td>
<td>250</td>
</tr>
<tr>
<td>Final Concentration (pg/mL)</td>
<td>3,200</td>
<td>1,600</td>
<td>800</td>
<td>400</td>
<td>200</td>
<td>100</td>
</tr>
</tbody>
</table>
**Assay Protocol:**

1. Use a 96-well plate layout sheet to aid in proper sample and standard identification.
2. If you are using the 1 by 8 well strip plate version of the kit determine the number of wells to be used and return unused wells to foil pouch with desiccant. Seal the Ziploc plate bag and store at 4°C. Pipet standards or samples down the plate strip columns (A to H) to ensure maximum use of the strip wells. The use of any wells in the whole plate versions of the kit will not allow use of unused parts of that plate in a later assay.
3. Pipet 50 µL of samples or standards into wells in the plate.
4. Pipet 75 µL of Assay Buffer into the non-specific binding (NSB) wells; these wells serve as a blank.
5. Pipet 50 µL of Assay Buffer into wells to act as maximum binding wells (Bo or 0 pg/mL).
6. Add 25 µL of the DetectX® Cortisol Conjugate to each well.
7. Add 25 µL of the DetectX® Cortisol Antibody to each well, except the NSB wells.
8. Gently tap the sides of the plate to ensure adequate mixing of the reagents. Cover the plate with the plate sealer and shake at room temperature for 1 hour.
9. Aspirate the plate and wash each well 4 times with 300 µL wash buffer. Use different pipette tips for each well to omit contamination errors. Tap the plate dry on clean absorbent towels.
10. Add 100 µL of the TMB (3,3’,5,5’-Tetramethylbenzidine) Substrate to each well.
11. Incubate the plate at room temperature for 30 minutes without shaking.
12. Add 50 µL of the Stop Solution to each well.
13. Read the optical density generated from each well in a plate reader capable of reading at 450 nm.
Appendix B: NEFA Protocol Using 96 Well Spectrophotometer and WAKO Solutions

1. If serum samples are frozen, set out to thaw completely.
2. Turn on the spectrophotometer, make sure the wavelength is set at 550 nm and allow approximately 15 minutes to warm up and self-calibrate.
3. Open the software (SoftMax) on the computer.
4. Within the software, set the wavelength to 550 nm by going to ‘Settings’.
5. Once the machine has warmed up, obtain a 96 well plate, making sure it does not sit directly on the counter (use a paper towel) and is not touched without wearing gloves.
6. Place it in the spectrophotometer to measure the absorbance of the blank plate (Pre-Reading).
7. Click the ‘Read’ button in the software program to initiate the reading.
8. With the current plate highlighted in the software, save the data and then copy the data into an Excel file and save it.
9. Prepare the Color Reagent Solutions A and B according to the package insert instructions. Once mixed, these solutions are stable for 30 days. The NEFA Standard Solution is supplied ready for use.
10. Before beginning, calibrate all pipettes. Reverse-pipetting is highly recommended with such soapy solutions to avoid bubbles; thus, calibrate using reverse-pipetting.
11. Samples should be assayed in duplicate.
12. For each plate, a standard curve must be created, which should be at least duplicated (triplicate is recommended), as well.

13. The values for the standard curve are as follows:

<table>
<thead>
<tr>
<th>Calibrator #</th>
<th>Sample Volume</th>
<th>Color Reagent A Volume (µL)</th>
<th>Color Reagent B Volume (µL)</th>
<th>Total Volume (µL)</th>
<th>Concentration (Theoretical) (mEq/L)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>5 µL D.I. Water</td>
<td>200</td>
<td>100</td>
<td>305</td>
<td>0</td>
</tr>
<tr>
<td>2</td>
<td>2.5 µL D.I. Water and 2.5 µL Standard Solution</td>
<td>200</td>
<td>100</td>
<td>305</td>
<td>0.5</td>
</tr>
<tr>
<td>3</td>
<td>5 µL Standard Solution</td>
<td>200</td>
<td>100</td>
<td>305</td>
<td>1.0</td>
</tr>
<tr>
<td>4</td>
<td>10 µL Standard Solution</td>
<td>200</td>
<td>100</td>
<td>310</td>
<td>1.97</td>
</tr>
</tbody>
</table>

- The final reaction volume is slightly increased due to the fact that twice the normal volume of sample was aliquoted into this well. Therefore, the actual concentration of Calibrator #4 is 1.97 mEq/L when corrected for volume:
  \[ (2.0 \text{ mEq/L}) \times (305 \mu\text{L}) / (310 \mu\text{L}) = 1.97 \text{ mEq/L} \]

14. Invert the serum tubes and vortex individually for 3 to 5 seconds before pipetting.

15. Accurately pipet 5 µL of each serum sample into each well in duplicate. For the wells being used to create a standard curve, the sample volumes listed in the above table should be added to each well.

16. Add 200 µL of Color Reagent A Solution to each well by reverse-pipetting.

17. Mix well and incubate at 37°C for 5 minutes.

18. Set the spectrophotometer to ‘Shake’ (Settings → Shake) for 10 s prior to obtaining the reading (both A and B). Measure the absorbance at 550 nm ((Reading A) following steps 6 through 8 above.

19. Add 100 µL of Color Reagent B Solution to each well by reverse-pipetting.

20. Mix well and incubate for 5 minutes.

21. ‘Shake’ and measure the absorbance at 550 nm again (Reading B).

22. After all data has been saved and copied into Excel, calculate the actual sample absorbance (Reading B – Reading A – Pre-Reading)
24. Using those calculated values for the standard curve wells, prepare a standard curve in Excel.

25. Use the calculated sample absorbance values and the equation from the standard curve to calculate the NEFA concentrations.

26. Co-variance (CV) can also be calculated for each sample’s concentrations ((Standard Deviation of samples / Average of the samples) * 100). A CV of < 10% between samples is preferred.