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Alternative Approaches to Improving the Welfare of Transition Dairy Cows and their Calves: Use of Exercise

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I am submitting herewith a dissertation written by Randi Alyson Black entitled "Alternative Approaches to Improving the Welfare of Transition Dairy Cows and their Calves: Use of Exercise." I have examined the final electronic copy of this dissertation for form and content and recommend that it be accepted in partial fulfillment of the requirements for the degree of Doctor of Philosophy, with a major in Animal Science.

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Alternative Approaches to Improving the Welfare of Transition Dairy Cows and their Calves: Use of Exercise

A Dissertation Presented for the
Doctor of Philosophy
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DEDICATION

I dedicate this dissertation to all the incredible cows that have made my journey not only possible, but also an incredible experience.

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As a young girl, I only knew two things in life. First, I had an incredible network of family and friends that I could rely on, no matter what bank I robbed, and second, animals were my deepest passion in life and I would never be fulfilled if I did not pursue a future with them in it. Never did I ever see myself pursuing a PhD, but here I am, and I wouldn't change anything. Not one day, decision (ok, maybe one), tear, or cow.

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This PhD was fueled by Taco Bell.

ABSTRACT

Transition cows are the cows most susceptible to disease and prevalence has not changed over the past decade. However, increased physical activity during late gestation may represent a management option to improve transition. Therefore, the objectives of this study were to determine the effect of exercise, pasture turnout, or total confinement on 1.) physical fitness and cortisol concentrations during the dry period, 2.) neutrophil function and behavior during the dry period, 3.) horn growth and wear and sole thickness during the dry period 4.) calving behavior and cortisol concentrations at parturition, and 5.) calf performance, behavior, and cortisol concentrations at disbudding and weaning. Pasture turnout tended to reduce anaerobic metabolism 60 min after exercise and exercise and pasture turnout resulted in less variable heart rate during and after exercise compared with confined cows. Physical activity during late gestation may allow cows to maintain a certain level of fitness. Physical activity did not alter behavior or neutrophil function during the dry period. Exercise cows experienced greater hind hoof horn wear than confined and pasture cows but had more equal rates of horn growth and wear. Sole thickness was not altered with exercise or pasture turnout but tended to increase for cows in total confinement. Physical activity did not affect time for different periods within stage II labor; however, confined cows stood for longer periods during the days surrounding calving, which may be related to discomfort experienced when standing or lying. Cortisol did not differ between groups at calving or 3 days later. Maternal treatment did not affect calves' ability to cope with the stress of dehorning, as calves displayed similar performance, behavioral, and physiological responses. However, calves from pasture cows displayed shorter lying time than calves from control and exercise cows while calves from exercise cows displayed more frequent lying bouts, potentially highlighting increased stress from weaning. Future research should

investigate the impact of pasture turnout during periods cows are more active to increase the level of physical activity.

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CHAPTER I

NOVEL APPROACHES TO ANIMAL WELFARE RESEARCH: A REVIEW

INTRODUCTION

Animal welfare has been a topic of research for many years, beginning with the general science of animal behavior (Hafez, 1962) and evolving to research questioning motivations for behaviors (Dellmeier, 1989, Dawkins, 1990). Legislators look to researchers in animal welfare to identify situations within animal production that are preferable over others when writing legislation (Rushen, 2003), placing a large weight on the shoulders of researchers to perform meaningful and well thought-out science. However, animal welfare is hard to define due to a limited range of measures and its multivariate nature (Rushen, 2003). Further, animal welfare research contains issues, including the removal of animal’s feeling, emotions, or consciousness, too much focus on ethology and limited focus on biological functioning, and large amounts of variability when comparing welfare across systems (Rushen, 2003). Therefore, it is the task of animal welfare researchers to determine methods for evaluating the welfare of animals through traditional means, such as behavior, while including methods that will evaluate the animal’s feelings, or emotional state, determine the level of biological functioning and health, and focus on critical measures within animal welfare. In this review, I intend to illustrate novel approaches to animal welfare research.

WHAT IS ANIMAL WELFARE?

Welfare, as a general definition, is described as the health, happiness, and fortunes of a group, and it is often stated that one must look out for their own welfare. However, in production animals, the animal’s welfare becomes the responsibility of the producer. The definition of animal welfare can vary amongst individuals and researchers due to the inherent nature that it relies on one’s values (Fraser et al., 1997). While farm animals are described as sentient beings who should not be treated purely as commodities, the free market is a fact of life (Webster, 2001b) and finding ways to raise animals humanely is the key objective of most farm animal welfare scientists and the producers that raise them. Different definitions of animal welfare have been proposed, leading to various methods and interpretations of findings (Duncan and Fraser, 1997). However, Fraser et al. (1997) proposed a bridging definition that connects the concepts of welfare and centers around the quality of life of the animal as an assessment of its welfare.

First, the ability of the animal to live a natural-life must be considered (Fraser et al., 1997). The freedom to express normal behavior by providing space, proper facilities, and company of the animal's own kind is considered important in animal welfare. Rollin (1993) proposed that each species has a genetically encoded nature ('telos') and animals must be allowed to live according to that telos to have good welfare. This is further affirmed when animals perform abnormal behaviors when not provided an opportunity to perform normal behaviors, which can be a potential indicator of suffering (Mason and Latham, 2004). In dairy cattle, natural living is often assumed to be outdoors on pasture. However, researchers have considered this perspective as naïve due to exposure to extreme climates, disease, predators, and parasites, all which impair animal welfare (Špinka, 2006, von Keyserlingk et al., 2009). Instead, providing animals an environment that offers positive experiences and stimulates behavioral development may better promote good welfare (Špinka, 2006).

Second, promoting positive affective states (feelings, emotions) in animals should be considered (Fraser et al., 1997). Positive affect states are often associated with positive feelings or emotions while negative affective states are associated with negative feelings and emotions. The freedom from suffering and the promotion of comfort can lead to good welfare (Fraser et al., 1997). Classical research can often ignore consciousness and affective states in animals, focusing on biological dysfunction or behavior separately (Barnard and Hurst, 1996); however, recent research defends that consciousness and feelings may be more measurable than previously considered (Griffin, 2013, Proctor et al., 2013). Studies were often performed for the purpose of animal welfare and animal behavior, with affective states being a secondary or subsequent reason for studies and investigated the impact of negative affective states over positive ones (Proctor et al., 2013). This highlights the importance for additional studies, not only to study affective states, but to examine positive affective states for a better understanding of what these positive states are and how to better promote them in animal production systems.

The last concept of animal welfare is biological functioning (Fraser et al., 1997). This concept is typically the one considered by producers, veterinarians, and animal care personnel, and thought of as normal functioning of the animal's biological system (Fraser et al., 1997). Physiologists, immunologists, endocrinologists, and other animal scientists tend to examine the effect of different systems on biological functioning while ethologists tend to focus strictly on animal behavior (Rushen, 2003). Combining these perspectives can offer a more robust assessment of animal welfare by understanding how different systems or situations affect the function, behavior, and feelings of the animal to give an overall understanding of animal welfare while not just focusing on the smaller concern.

The five freedoms (www.fawc.org.uk/freedoms.htm) have been central in determining animal fitness and mental suffering within an animal system (Webster, 2001b). These include the freedoms 1.) from hunger and thirst, 2.) from discomfort, 3.) from pain, injury, or disease, 4.) to express normal behaviors, and 5.) from fear and distress. By combining the five freedoms as a guide for determining animal needs and the three concepts of animal welfare (Fraser et al., 1997) for determining whether science is inclusive of all aspects of animal welfare, novel approaches to animal welfare science can be identified.

NOVEL RESEARCH METHODS

Freedom from discomfort

Animals should be free from discomfort by providing shelter and a comfortable resting space. However, some elements within modern dairy farming systems can limit comfort, particularly through the means of shelter provided and the comfort of the resting space if not properly managed. This can have particularly negative effects on hoof health and calving behavior.

Hoof health. Lameness continues to be a major concern within the dairy industry, with high lameness prevalence throughout North America (California: 31%; Northeastern US: 55%; von Keyserlingk et al., 2012). Lameness is widely considered a welfare concern (von Keyserlingk et al., 2009), as it causes pain (Whay et al., 1998, Shearer et al., 2013), and is a performance concern due to loss of milk production (Green et al., 2002, Bicalho et al., 2008) and reduced longevity (Kossaibati and Esslemont, 1997, Booth et al., 2004). Lameness occurs from a number of causes including infectious disease (i.e. digital dermatitis, foot rot), claw horn disruptions (i.e. white line separation, ulcers, hemorrhage), or management factors (i.e. concrete flooring, uncomfortable stalls) and all increase the risk of lameness (Cook and Nordlund, 2009).

The majority of US dairy cattle are housed in tie stalls, stanchions, or freestall barns with no access to pasture (58.9%; USDA, 2016) and concrete is the predominate flooring type (concrete: 55.6%; dirt: 20%; rubber: 13.9%; pasture: 5.1%; USDA, 2010). Concrete flooring increases prevalence of claw disorders over pasture (48.5% vs. 28.2% digital dermatitis prevalence; Wells et al., 1999) and straw yards (88 to 81% vs 57.5%; Somers et al., 2003), and can often result in unequal hoof horn growth and wear and heel erosion (Hahn et al., 1986, Vanegas et al., 2006, van Amstel et al., 2016). Further, standing and walking on hard surfaces

(Greenough and Vermunt, 1991, Singh et al., 1993) and walking along rough cow tracks (Chesterton et al., 1989) can negatively impact hoof health.

Many welfare studies examining lameness assess social and lying behavioral changes (Galindo and Broom, 2002, Juarez et al., 2003, Ito et al., 2010) and lameness is often categorized using a behavioral index (Manson and Leaver, 1988, Sprecher et al., 1997, Flower and Weary, 2006); however, hoof pathology may not be connected with behavioral changes. Separately, hoof pathology and behavior can give indication of what a problem is or how a general problem changes behavior. However, used together, behavior and hoof pathology can determine how a particular problem alters natural behavior and to what degree. This information can then be used to determine what hoof ailments may be more important in influencing cow behavior. Many different strategies exist to qualify hoof diseases and two that can present novel information linking welfare and biological function are sole thickness and horn growth and wear.

Walking on concrete has been previously associated with thin soles (van Amstel et al., 2006). Soles provide protection to the claw capsule (Toussaint Raven, 1989) and thin soles are more prone to injury and contusion, particularly in environments with hard or irregular surfaces (Greenough, 1987, Toussaint Raven, 1989). Sole thickness, measured through ultrasonography (van Amstel et al., 2004), gives indication of environmental moisture (van Amstel et al., 2004), risk of sole ulceration (Greenough, 1987), and hoof wear (Toussaint Raven, 1989, Van Amstel et al., 2002).

Horn growth rates have been shown to be seasonal, particularly growing faster in the spring-summer period (Vermunt and Greenough, 1995), greater with higher energy diets (Greenough et al., 1990), lower with reduced insulin production or sensitivity (Tomlinson et al., 2004), and greater in young animals than in older animals (Vermunt and Greenough, 1995).

Higher rates of hoof wear were associated with concrete flooring, overstocking, poor cow comfort, claw horn moisture, poor stockmanship, and poor horn quality (Van Amstel et al., 2002). However, normal claws are characterized by equal rates of growth and wear (Vermunt and Greenough, 1995) and an imbalance can cause horn lesions (Bazeley and Pinsent, 1984, Greenough and Vermunt, 1991, Cook et al., 2004). Therefore, combining hoof growth and wear measurements with behavior can help determine when particular environments and management plans are more prone to poor hoof growth and wear rates and may negatively influence cow behavior due to pain and discomfort.

Freedom from pain, injury, and disease

Animals should be free from pain, injury, and disease by prevention or rapid diagnosis and treatment. One key method of prevention is by either boosting immune function or reducing the level of immune dysfunction.

Immune function. Transition cows, or cows three weeks prepartum and three postpartum, are the cows most susceptible to disease in the herd, making them a key cow group to manage, with the majority of all diseases occurring in the first ten days postpartum (Ingvartsen et al., 2003). Goff and Horst (1997) hypothesized that transition diseases developed from a combination of negative energy balance, immune dysfunction, and hypocalcemia around parturition. The stress of calving, coupled with increased cortisol concentrations brought on from disease, suppresses the immune system (Roth and Kaeberle, 1982). Further, at the time of calving, cows experience decreased serum immunoglobulin concentration (Kehrli et al., 1989, Detilleux et al., 1995), diminished lymphocyte responsiveness (Kashiwazaki et al., 1985, Ishikawa, 1987, Kehrli et al., 1989, Saad et al., 1989), and impaired neutrophil function (Guidry et al., 1976, Newbould, 1976), all leading to increased susceptibility to disease. Measuring immune function can help welfare researchers understand how the environment impairs or improves the cow's ability to deal with different situations.

Reactive oxygen species are free radicals produced by neutrophils after phagocytizing bacteria and used as an antimicrobial mechanism (Bayr, 2005). Neutrophils can be primed to increase killing ability and resistance to infection (Smith et al., 1990). Priming of neutrophils allows dormant neutrophils to acquire a state of preactivation, enabling them to provide a more powerful response (Smith, 1994). However, over generation of free radicals can cause oxidative stress and tissue damage (Smith, 1994). Neutrophilia can also occur (Rossdale et al., 1982, Korhonen et al., 2000, Quindry et al., 2003) with immature and less active neutrophils released from bone marrow, limiting the effectiveness and reaction of neutrophils (Rossdale et al., 1982, Simon, 1991, Iversen et al., 1994). Therefore, neutrophil function through reactive oxygen species generation can help determine whether different environments help cows fight infection through a priming condition of neutrophils or inhibit a cow's ability to fight infection through neutrophilia or excess free radicals.

Freedom to express normal behavior

Animals should be free to express normal behavior by providing sufficient space, proper facilities and company of the animal's own kind.

Automated behavioral monitoring. Automated behavioral monitoring exists for lying behavior in cows (Ledgerwood et al., 2010) and calves (Trénel et al., 2009), estrus detection (Nebel et al., 2000), lameness (Chapinal et al., 2009, Chapinal et al., 2010a), feeding behavior (Beauchemin et al., 1989, Bach et al., 2004), and rumination (Schirmann et al., 2009). However, alone, these systems do not give a big picture of the welfare of animals. Feeding time decrease for cows in estrus (Reith and Hoy, 2012, Pahl et al., 2015) and at calving (Schirmann et al., 2013, Büchel and Sundrum, 2014); however, similar decreases occur for lame cows (González et al., 2008). Combining feeding behavior with activity, which increases for cows in estrus (Arney et al., 1994) and at calving (Jensen, 2012) but decreases for lame cows (Walker et al., 2008), gives a more complete picture of cow welfare.

Studies have attempted to associate different behaviors with health outcomes (Weary et al., 2009). However, as many behavioral changes occur with multiple illnesses, diagnosis is not feasible. However, behavioral changes can currently be used as indicators of vigor and need and predictors of illness (Weary et al., 2009). This will signal producers to assess the health of the animal and make a diagnosis based on more physiological indicators. However, as more technology becomes available, such as biosensors within milking equipment to detect ketones, urea, hormones and enzymes (Mottram et al., 2002), paired behavioral and physiological data may make technological diagnosis of disease and illness possible. Using these technologies together can improve welfare research.

Calving. Parturition is considered painful (Mainau and Manteca, 2011) and leads to inflammation (Turk et al., 2005, Bionaz et al., 2007). Further, difficulty during calving was rated one of the most painful conditions in cattle by cattle practitioners in the UK (Huxley and Whay, 2006) and can cause subsequent reduction in performance (Dematawena and Berger, 1997). Inadequate expulsive forces (Noakes et al., 2001c, Jackson, 2004), feto-pelvic disproportion (Bellows et al., 1971, Johnson et al., 1988, Noakes et al., 2001b), and malpresentation (Meijering, 1984, Noakes et al., 2001a) are the primary reasons for difficult calvings.

Cow behavior changes as parturition approaches, characterized by reduced lying time, increased lying bout frequency, increased activity, and reduced feed intake (Huzzey et al., 2005, Miedema et al., 2011, Jensen, 2012). The process of calving is typically separated into three stages: cervical dilation and uterine contractions (stage I), expulsion of the calf (stage II), and expulsion of the fetal membranes (stage III; USDA, 2010). Visual indications of stage I labor (uterine contractions, nest-building behavior, tail raising, olfactory ground checks, grooming, vocalization, restlessness, tail raising, and defecation) can be subjective, vary amongst cow and parity, and may extend across stages (Wehrend et al., 2006). However, visual indicators of stage II labor (appearance of amniotic sac, appearance of the calf, and expulsion of the calf) can be objectively determined, do not extend across stages, and periods within this stage can be objectively assessed (USDA, 2010b, Schuenemann et al., 2011). These alterations of behavior can be used to monitor the progression and imminence of calving. Welfare scientists often look to behavior changes at calving as a means of assessing differences within systems and situations on welfare.

Freedom from fear and distress

Animals should be free from fear and distress by ensuring conditions and treatment which avoid mental suffering. Monitoring stress hormones, such as cortisol, is a broadly used technique to determine whether different situations cause or alleviate stress. Combining these methods at different life stages and combining these measures with behavioral changes can create a more robust assessment of the situation and the level of welfare.

Stress. Increased concentration of corticosteroids before calving signals luteolysis and signals for termination of pregnancy (Adams and Wagner, 1970, Hoffmann et al., 1973). Corticosteroid concentrations return to basal concentrations 3 to 7 d postpartum (Adams and Wagner, 1970). However, difficult calvings can intensify this stress response (Civelek et al., 2008). Monitoring cortisol changes simultaneously to behavioral changes, such as changes in lying behavior and labor stage behavior, can better define how cows react to different environments at calving.

The maternal environment plays a tremendous role in fetal growth and development during gestation, and manipulations during this time can either improve or impair calf performance. Prenatal heat stress can alter endocrine dynamics, reduce immune function, reduce calf birth and weaning weight, and potentially reduce future milk yield potential of calves (Collier et al., 1982a, Tao et al., 2012, Strong et al., 2015). Prenatal stress during cow transport can reduce cortisol clearance during stressful events, altering the physiological response to stress (Lay et al., 1997). Further, undernutrition of cows during the first trimester resulted in calves with potentially suboptimal fertility, enlarged aortic trunk size, and increased blood pressure (Mossa et al., 2013). Therefore, stress during pregnancy can likely cause impaired performance of calves early in life and potentially into their productive lives. Measuring the stress response of calves at important life events, such as weaning and dehorning, may give insight into the impact of stressful cow environments while pregnant and present solutions to improve cow and neonatal welfare.

CONCLUSIONS

Welfare research should consider affective states, natural behavior, and biological function while using the five freedoms as a guide to determine animal needs. This review demonstrated novel methods for animal welfare research. Evaluating sole thickness and horn growth and wear demonstrates the impact of the environment on hoof health. Neutrophil function, assessed using reactive oxygen species, indicates the ability of a cow to fight infection in a given environment during stressful life events. Automated behavioral monitoring gives producers additional information when determining cows that need additional attention.

Combining behavioral and physiological gives more robust information to potentially diagnose health disorders. Monitoring calving behavior assists in identification of difficulties and can be used to associate changes in management on calving ease and behavior. Stress hormones, such as cortisol, indicate when environments and management impose more stress on cows than alternatives. This can also be used to evaluate how stress impacts neonatal calves at important life events, such as dehorning and weaning. Using novel methods, included and outside of this review, can increase the robustness of welfare research and improve recommendations welfare researchers can make regarding management.

CHAPTER II

EFFECT OF PREPARTUM PHYSICAL ACTIVITY DURING THE DRY PERIOD ON PHYSICAL FITNESS OF DAIRY COWS

ABSTRACT

The objective of this study was to determine the effect of daily exercise or pasture turnout on physical fitness and cortisol concentrations during physical exertion during the dry period. Fifty-eight Holstein and two Jersey-Holstein crossbred, pregnant, non-lactating dairy cows were assigned to control (n = 20), exercise (n = 20), or pasture (n = 20) treatments using rolling enrollment from Jan to Nov 2015 at dry-off. Cows were balanced on parity (1.8 ± 0.9), projected ME FCM (13,831 ± 2,028 kg per lactation), and projected calving date. Cows were housed in a naturally ventilated, 4-row freestall barn at the University of Tennessee's Little River Animal and Environmental Unit (Walland, TN).Exercise was done over 5 consecutive days per week over 1.4 ± 0.1 h, at a pace of 1.88 ± 0.58 km/h until calving. Pasture turnout occurred on a grassy paddock five consecutive days per wk for 1.8 ± 0.3 h/d until calving. Control cows remained in the home pen throughout the dry period. Exercise challenge days occurred at dry-off and 42 d following where cows were walked 1.4 ± 0.1 h at a pace of 2.16 ± 0.45 km/h. Cows were fitted with a wireless electrocardiogram monitor to monitor heart rate 10 min prior, during, and 60 min following exercise challenge. Blood collection occurred 10 min before and 0, 3, 6, 9, 12, 15, and 60 min following exercise challenge to assess L-lactate concentration using a handheld meter and 10 min before and 0, and 60 min following exercise to determine plasma cortisol concentration using a commercially available kit. A mixed model was used to determine the effect of treatment, exercise challenge day, time, exercise pace, and their interactions on heart rate, L-lactate concentration, and plasma cortisol concentration. Cow within treatment and exercise challenge day were considered random. Lower L-lactate concentrations for pasture cows occurred immediately after exercise challenge compared with exercise cows. Concentrations 60 min after exercise challenge were also lower for pasture cows compared with control and

exercise cows. Pasture and exercise cows displayed less variable heart rate than confined cows during and following exercise. Cortisol did not differ by treatment but was lower 42 d after dry-off compared with dry-off. Physical activity during the dry period may help cows maintain a minimum level of physical ability during a relatively sedentary period of life.

INTRODUCTION

Consumers are increasingly concerned about how their food is raised and produced (Frewer et al., 2005) and indicate a willingness to pay more for products raised with a more "natural" life, particularly with access to pasture for dairy cattle (Olynk and Ortega, 2013). This is further noted through an increase in United States organic dairy operations from 2007 to 2014 (1.7 to 7.4%; USDA, 2010, 2016). The National Organic Program, under the Agricultural Marketing Service and USDA, requires 30% of DMI to come from grazing, with cows grazing at least 120 d per year (AMS, 2010). While access to pasture may provide a more natural life to cattle, additional benefits to increased physical activity may exist.

Increasing physical activity, specifically through the use of exercise, or planned, structured, and repetitive physical activity (Caspersen et al., 1985), previously resulted in improved physical fitness and performance. Barker et al. (1975) exercised prepartum heifers 4-to-8-wk a distance of 1.6 km at 5.5 km/h. Exercise improved ease of parturition, placental release, and feed efficiency in these heifers. Davidson and Beede (2009) exercised late-gestation, non-lactating dairy cows using a mechanical walker, noting exercise cows demonstrated reduced heart rate (**HR**) during an exercise challenge on a treadmill, faster return to resting HR, and lower L-lactate concentrations during and following exercise challenge compared with non-exercised paired controls. While an exercise routine can improve physical fitness, the application of exercise via mechanical walker in a production system is not practical on commercial dairy farms due to required labor. Alternatives that encourage physical activity, such as pasture access, offer a more realistic management practice for dairy producers.

Allowing pasture access to tie-stall housed cows daily reduced disease treatments by veterinarians, including bloat, paresis, retained placenta, leg disorders, and laminitis, and reduced culling and occurrences of subclinical mastitis during the first two weeks postpartum (Gustafson, 1993). Gustafson (1993) walked cows out to pasture 1 km per d, allowing cows to walk back to the barn over the following 2 h, and, while physical activity was not intense, cows still experienced benefits of low to moderate activity. Further, providing outdoor access to tie-stall cows resulted in improved welfare, denoted by fewer hock lesions, fewer lame cows, and lower mastitis prevalence (Popescu et al., 2013). The opportunity to move outdoors may impose similar improvements of physical fitness as noted with an exercise routine, potentially increased with the level of novelty the outdoor area holds. Cows were more explorative and active when given access 1 h access to an outdoor paddock once or twice per week compared with seven days per week (Loberg et al., 2004). However, cumulatively, daily access resulted in more overall activity.

Pasture access may be a more viable management decision, resulting in similar health benefits to an exercise routine through improved physical fitness; however, a direct comparison of exercise and pasture access has not yet been studied. Therefore, the objective of this study was to determine the effect of daily exercise, pasture access, or total confinement on physical fitness and cortisol concentrations during physical exertion during the dry period. We hypothesized that daily access to both exercise training and pasture would improve physical fitness and reduce cortisol concentrations over time.

MATERIALS AND METHODS

Animals, Housing, and Management

Twenty-nine primiparous and 31 multiparous, pregnant, non-lactating Holstein (n = 58) and Jersey-Holstein crossbred (n = 2) dairy cows were assigned to either control (n = 20), exercise (n = 20), or pasture (n = 20) treatments at dry-off using rolling enrollment from January to November 2015. Cows were balanced on parity (1.8 ± 0.9), projected ME FCM (13,831 ± 2,028 kg per lactation), and projected calving date. Cows were managed with a 60-d dry period (58.5 ± 5.4 d) divided into far-off (dry-off to 2 weeks before parturition) and close-up periods (two weeks before projected parturition).

Cows were housed in a naturally ventilated, 4-row, head-to-head freestall barn with drive-through feed bunk at the University of Tennessee's Little River Animal and Environmental Unit (Walland, TN). Deep-bedded sand freestalls were 2.4 m long and 1.2 m wide with a 1.2 m high neck rail positioned 1.7 m from the curb and a 0.6 m high PVC tube brisket board placed 1.7 m from the curb. Fresh sand was added once per week with manure removed from stalls twice daily before milking the lactating herd (0730 and 1730 h). Fans turned on automatically when temperatures rose above 23 °C. Throughout the study period, study cows were housed in either pen 1, 2, or 6 (Figure 1), with pens measuring 12.1 m wide and 19.4 m long, enclosing 24 freestalls and 26 0.6 m wide headlocks, and containing 2 waterers, one on each end. Study cows were comingled unless the pen was split into far-off and close-up groups, leaving 12 freestalls and 13 headlocks for each group. Cows were maintained below 80% stocking density, based headlock and freestall availability.

Cows were fed twice daily at 0730 and 1530 h. Far-off cows were fed a TMR from dry-off to two weeks before projected parturition consisting of 4.5 kg ryegrass hay, 3.4 kg orchardgrass hay, 2.3 kg corn silage, and 2.7 kg dry cow grain per cow per day. Close-up cows were fed a TMR up to parturition consisting of 3.6 kg orchardgrass hay, 1.8 kg clover, 11.3 kg corn silage, and 3.0 kg dry cow grain per cow per day. All cows had ad lib access to water, except exercise treatment cows during exercise.

Experimental Treatments

Before enrollment, all cows had been housed in the same freestall barn with no previous experience with exercise, aside from pasture access during the dry period before the previous calving. Cows were enrolled into treatments on the day of dry-off. Cows assigned to control remained in the pen at all times, except for general management reasons (i.e. cleaning, rebedding stalls) when cows were moved to an adjacent lane for a maximum of 30 min. Cows were permitted to eat, drink, and move around the pen during exercise times. Cows assigned to exercise were removed from the pen 5× per week, Monday through Friday, and walked for at targeted 1.5 h at 3.25 km/h beginning at 1200 h along the path denoted by a dashed black line in Figure 1, measuring 250 m for each lap. Cows were walked in a group using the cows' flight zones and implements (i.e. rattle paddle) to encourage walking. Exercise pace was calculated by the total exercise time divided by the distance walked. During periods of high heat load, determined subjectively through cow heat stress behavior (i.e. increased respiration rate, panting) and exerciser comfort, cows were offered water at the point where the walking path met the entrance to the milking parlor (Figure 1) from a 19 L bucket. Cows did not have access to feed during the exercise period.

Cows on pasture were moved into a 2.11 hectare pasture (Pasture 1) from January to April 2015 and a 0.42 hectare pasture (Pasture 2) from April to December 2015 5× per week, Monday to Friday. Pasture 1 was 330 m from the barn to the pasture gate while Pasture 2 was 15 m from the barn to the pasture gate. Pasture 1 had rolling hills and a 0.75 hectare wooded area while pasture 2 had a shade structure and trees around one side of the fence line. Both pastures were seeded with orchardgrass and KY-31 fescue and managed by the farm manager for a height of 0.3 to 0.5 m. Cows were put on pasture before and returned to the barn after exercising cows from the exercise treatment group. Cows were put on pasture for a target of 1.5 h, excluding travel time to and from the paddock, beginning at 1200 h. Both pastures had access to water and grass.

To assess fitness, all cows were subjected to an exercise challenge at dry-off and 42 d after dry-off. During the exercise challenge, challenged cows and exercise treatment cows were exercised simultaneously.

Blood Sampling

On d 0 and 42, cows were moved into a palpation chute and fitted with an indwelling jugular catheter the morning before exercise challenge. Cows were released back into their pen until 10 min prior to exercise where they were either restrained in the headlocks in the pen or moved to the palpation chute for blood collection. After exercise, cows were moved back into the palpation chutes for blood collection. Ten minutes prior to exercise, immediately after, and 60 min after exercise, 8 mL of blood were collected into 20 mL syringes and immediately transferred to a 6 mL sodium heparin blood tube (BD Vacutainer, BD, Franklin Lakes, NJ) and 2 mL potassium oxalate/sodium fluoride blood tube (BD Vacutainer, BD, Franklin Lakes, NJ). An additional 2 mL of blood was collected into 20 mL syringes 3, 6, 9, 12, and 15 min following exercise and immediately transferred into a 2 mL potassium oxalate/sodium fluoride blood tube.

After collection, sodium heparin blood tubes were centrifuged, plasma separated into microcentrifuge tubes, and tubes frozen at -80 °C. Plasma total cortisol concentration was determined by a radioimmunoassay procedure using a commercially available kit (ImmunChem Cortisol 125 | RIA Kit, BP Biomedical, LLC, Orangeburg, NY). Inter- and intra-assay CV for the low control (7 ng/mL) was 42.9% and 47.6%, respectively, and 13.7% and 13.8%, respectively, for the high control (25 ng/mL). A 0.2 mL whole blood sample from potassium oxalate/sodium fluoride blood tubes was used to determine L-lactate concentration using the Lactate Scout (range: 0.5 to 25 mmol/L; EKF Diagnostics GmbH, Mannheim, Germany) (Burfeind and Heuwieser, 2012). The meter would not read below 0.5 mmol/L, therefore, all samples that read as low (<0.5 mmol/L) were removed (68.2% of all recorded data).

Heart Rate

On d 0 and 42, cows were fitted with a wireless electrocardiogram monitor (Polar V800, Polar Electro, Port Washington, NY) after catheter insertion to monitor HR. Hair was clipped from the left wither down to the left elbow, approximately 7.5 cm wide, and the area drenched with water to allow increased contact between the skin and monitor electrodes. Heart rate was recorded every 1 s to a watch attached to the band at the right wither. Data were recorded for 10 minutes preceding exercise challenge, the entire length of exercise challenge, and the following 60 min.

Statistical Analysis

Mean, max, and min HR were determined using PROC MEANS of SAS (SAS 9.4, SAS Inst., Cary, NC), with results reported as means ± SD. The observational and experimental unit of this study was the cow. Data were analyzed using PROC MIXED of SAS. Cow within treatment and exercise challenge day was considered random in all models. Explanatory variables included treatment (control, exercise, pasture), exercise challenge day (d 0 and 42), time (cortisol: -10, 0, and 60 min; lactate: -10, 0, 3, 6, 9, 12, 15, and 60 min), and exercise pace.

Explanatory variables and all interactions between explanatory variables were tested ($P < 0.05$) using backward elimination. Resulting values are reported as least squares means ± SE.

RESULTS

Treatments

Exercise cows walked for 1.4 ± 0.1 h at 1.88 ± 0.58 km/h. Exercise periods began, on average, at 12:18:50 h, ranging from 10:12 to 14:39 h, and, on average, ended at 13:43:11, ranging from 11:16 to 16:03 h. Pasture cows spent a mean of 2.0 ± 0.3 h on Pasture 1, entering the pasture, on average, at 12:55:58 h, ranging from 11:24 to 14:41 h, and, on average, exiting the pasture at 14:56:17 h, ranging from 13:56 to 17:12 h. Cows spent a mean of 1.7 ± 0.3 h on Pasture 2, entering the pasture, on average, at 11:58:40 h, ranging from 10:04 to 14:32 h, and, on average, exiting the pasture at 13:40:01, ranging from 11:21 to 16:07 h. On exercise challenge days, cows were walked 3.1 ±0.7 km over 1.4 ± 0.1 h at a pace of 2.16 ± 0.45 km/h.

Exercise Challenge

L-lactate concentrations did not differ by treatment (control: 0.92 ± 0.09 mmol/L; exercise: 0.98 ± 0.08 mmol/L; pasture: 0.84 ± 0.10 mmol/L; $P = 0.54$), day (d 0: 0.92 ± 0.08 mmol/L; d 42: 0.91 ± 0.07 mmol/L; $P = 0.90$), time (-10 min: 0.99 ± 0.09 mmol/L; 0 min: 0.88 ± 0.10 mmol/L; 3 min: 0.88 ± 0.11 mmol/L; 6 min: 0.89 ± 0.12 mmol/L; 9 min: 0.71 ± 0.13 mmol/L; 12 min: 0.90 ± 0.13 mmol/L; 15 min: 0.98 ± 0.09 mmol/L; 60 min: 1.08 ± 0.10 mmol/L; $P = 0.45$), or pace ($P = 0.50$). Cortisol concentrations were not affected by treatment (control: 4.28 ± 0.48 ng/mL; exercise: 4.03 ± 0.48 ng/mL; pasture: 4.78 ± 0.48 ng/mL; $P = 0.70$), time (-10 min: 4.63 ± 0.47 ng/mL; 0 min: 4.82 ± 0.48 ng/mL; 60 min: 3.67 ± 0.46 ng/mL; $P = 0.25$), or pace ($P = 0.55$). Cortisol concentrations tended to be lower on d 42 at -10, 0, and 60 min compared with d 0 and concentrations were lower at 60 min post-exercise on d 42 compared with all other time points (P = 0.07; Figure 2).

Due to technical issues, data available from the heart rate monitors were limited, with data available on d 0 (n = 12) and d 42 (n = 12) for control (n = 7), exercise (n = 7), and pasture (n = 8) treatments. Pre-exercise, exercise, and post-exercise max, min, and mean HR are shown in Table 1. Heart rate prior to and during exercise was affected by time × treatment × challenge day (F*P* < 0.0001), time × pace × treatment (*P* < 0.0001), and time × time × time (P < 0.0001). All cows experienced a rise in HR at the initiation of exercise and again at the end of exercise; however, HR was lowest for control and highest for pasture on d 0 and lowest for exercise and highest for control on d 42 (Figure 3). On d 0 and 42, control cows increased HR more with increasing pace, compared with pasture and exercise cows; however, pasture cows consistently had higher initial HR at a low exercise pace (Figure 4).

Heart rate following exercise challenge was affected by time × pace × challenge day (P < 0.0001) and time × pace × treatment (P = 0.03). On d 42, cows had a more consistent reduction in HR post exercise than on d 0 and cows did not produce a spike in HR at the final blood sample on d 42 compared with d 0 (

Figure 5). Similarly, pasture and exercise cows displayed a more consistent reduction in HR post-exercise than control cows and exercise cows had a more consistent HR across paces after exercise compared with control and pasture cows (Figure 6).

DISCUSSION

This is the first study to examine the effect of an applied form of physical activity (pasture turnout) on physical fitness in late gestation dairy cows. L-lactate concentrations did not differ by treatment or day. All cows had lower cortisol concentrations on d 42 compared with d

0. Heart rate decreased less variably post exercise on d 42 than d 0 and for exercise and pasture cows compared with control cows.

Physical activity did not alter L-lactate concentrations and no groups increased over a mean concentration of 1.0 mmol/L, which may suggest that no group entered a period anaerobic metabolism. Davidson and Beede (2003) noted a marked increase of 3.25 mmol/L in L-lactate from the start to end of a 1 h treadmill exercise test, changing from 0.68 to 3.94 mmol/L in non-pregnant, non-lactating dairy cows. Pregnant, non-lactating dairy cows exercised 3.25 km/h for 1.25 to 1.5 h every other day for 70 d experienced a change in response to exercise with an increase in lactate of 3.3 mmol/L on d 0 to an increase of 1.7 mmol/L on d 60 (Davidson and Beede, 2009). Simmental oxen worked 1 h three times a week doing draft work experienced an increase in lactate from 0.81 to 3.60 mmol/L during exercise (Zanzinger and Becker, 1992). In the current study, cows walked considerably slower (2.16 ± 0.45 km/h) than previously noted in cattle exercise (Anderson et al., 1979, Blake et al., 1982, Davidson and Beede, 2003, Davidson and Beede, 2009), which did not likely demand enough work load to transition cows to anaerobic metabolism. Using a low stress method of exercise execution prevented cows from experiencing chronic stress, which can cause hyper-reactivity of the adrenal cortex to other stressors (Broom, 1988), exaggerating issues during periods of immune dysfunction, such as calving (Aleri et al., 2016). While this method may have prevented cows from experiencing anaerobic metabolism, it also prevented cows from experiencing negative impacts of chronic stress.

The impact of low stress exercise can be noted in the reduction of cortisol from d 0 to d 42. Cows with experience in handling had lower cortisol concentrations (Hemsworth et al., 1989). Further, familiarity with repeated blood sampling reduces the effect of handling stress (Hopster et al., 1999). Though the study cows were part of a research herd, cow experience with handling and blood sampling was unknown. Therefore, it could be hypothesized that the difference in cortisol from d 0 to d 42 to due to an acclimation to the handler, to the routine of blood sampling, and to the routine of exercise. Dry-off also occurred on the first exercise challenge and, although cows were milked before, cows received an intramammary antibiotic infusion, which may have led to a stress response. As the overall routine was different and more novel at dry-off, adjusting to the routine and experiencing fewer novel situations likely led to a reduced cortisol response 42 d after dry-off.

Mean HR during exercise stayed similar from d 0 to d 42 for exercise and pasture cows but increased for control cows. This may allude to maintenance of cardiac capacity in pasture and exercise compared with control. However, mean HR did not increase meaningfully from the pre-exercise to exercise periods, indicating cows did not increase their workload enough to initiate a strong cardiac response. In the current study, mean HR during exercise are lower than those previously reported during an exercise challenge (Davidson and Beede, 2003, Davidson and Beede, 2009), ranging between 170 to 182 bpm. During those studies, cows were subjected to greater workloads with walking speeds of 5 km/h using a treadmill with incline. In the current study, cows did not experience similar workloads to induce a similar cardiac change.

Leading up to exercise challenge and during the first 20 min, cows increased their HR. This could occur from moving and sorting from the pen to the exercise course and initiation of exercise. Further, cows displayed more energy during the initial minutes of exercise challenge, possibly due to novelty of the walking course and routine. Davidson and Beede (2009) reported a similar spike in HR at the start of exercise, followed by a less steep increase after 3 min. The assumed increase in comfort with the activity and reluctance to walk may have caused a reduction in HR further into exercise challenge, which was not previously noted in exercise studies (Davidson and Beede, 2003, Davidson and Beede, 2009). However, those studies were able to implement exercise using a treadmill, which removes the issue of reluctance to walk and reduction of the flight zone. A final increase in HR was likely attributed to moving cows to the chutes, which was a change in routine, but was similar to previous studies (Davidson and Beede, 2003, Davidson and Beede, 2009). A similar pattern was followed for each of the treatment groups on both D0 and D42. However, while exercise and pasture cows maintained similar HR from D0 to D42, control cows displayed an increase in HR during exercise challenge from D0 to D42, which was similar to the response of cows previous exercised and challenged (Davidson and Beede, 2009).

Cows also experienced a rise in HR with increasing pace. Previous studies have not examined the impact of pace on HR; however, studies that exercise cows at a faster pace than the current study noted higher exercise HR (Davidson and Beede, 2003, Davidson and Beede, 2009). Control cows increased HR at a greater rate with increasing pace than pasture and exercise cows. This was consistent on both d 0 and 42, as slope increased the same amount for all three treatments and may indicate cow variation more than treatment differences across exercise challenge days.

Similarities can be noted following exercise. Though cows moving at a faster pace displayed similar HR changes, control cows exhibited a higher HR 60 min following exercise challenge compared with pasture and exercise with greater variability during that time when moving at slower paces. Additionally, cows displayed more variability in HR reduction following exercise on D0 than on D42, potentially alluding to the concept that cows become more accustomed to regular physical activity. One hypothesis for differences in HR variability may be that control cows actually lose fitness ability due to physical inactivity while pasture and exercise cows maintain it. While exercise and pasture cows did not improve fitness level, they may have maintained a level of physical ability through daily physical activity during the dry period. In contrast, control cows developed a relatively sedentary routine during the dry period, which may have negatively impacted their ability to perform physical activity after 42 days.

Physical inactivity in humans can lead to heart disease (Fletcher et al., 1996), hypertension (Fagard, 1999), stroke (Goldstein et al., 2001), intermittent claudication (Gardner and Poehlman, 1995), higher platelet adhesion and aggregation (Rauramaa et al., 2001), and type II diabetes (Knowler et al., 2002) and has been considered one of the most important public health concerns of the 21st century (Blair, 2009). Further, increased physical activity has been associated with improved emotional well-being (Galper et al., 2006) and reduced physical frailty during old age (Spirduso and Cronin, 2001). While these diseases do not necessarily directly relate to dairy cattle, cows may suffer similar consequences when going from a routine of physical activity, such as moving to the milking parlor twice or thrice daily and different pen resources, to just moving to pen resources. This is of greater concern for dry cows as the need for certain resources changes from lactation. Dry matter intake and water requirement decreases considerably from lactation to the dry period (NRC, 1989), reducing the necessity to travel to the feedbunk. This leaves more time for cows to lie down and stand and become more physically inactive.

Though this study gives interesting and novel insight into the importance of physical activity over inactivity during the dry period, limitations within the study are not to be overlooked. As previously mentioned, only a small subset of data were used for analysis since the HR monitors did not work reliably. With more animals, variation may be reduced and some differences between groups and days may become more or less apparent. However, the number of data points collected from the few cows used was high and warranted investigation. Also, the exercise pace targeted in both the exercise challenge and during regular exercise was not met during this study as cows became reluctant to walk and more accustomed to the routine. Due to a lack of high workload, cow physical fitness did not improve and biologically meaningful changes in L-lactate concentration and HR did not occur. However, a different and novel idea of physical inactivity came from this research, offering another important perspective.

CONCLUSION

L-lactate concentrations did not differ by treatment, day, or time and low overall concentrations were not likely enough to indicate cows passed the aerobic-anaerobic threshold or improved overall fitness throughout the study. Heart rates of pasture and exercise cows remained relatively similar from d 0 to d 42; however, HR increased from d 0 to d 42 in control cows. Cows had a reduction in cortisol from d 0 to d 42, which may be due to habituation to handling and blood sampling. Physical activity during the dry period may help cows maintain a minimum level of physical ability during a relatively sedentary period of life. Further research into the consequence of physical inactivity on cow performance and health and how pasture turnout may help alleviate inactivity should be pursued.

CHAPTER III

EFFECT OF PREPARTUM PHYSICAL ACTIVITY DURING THE DRY PERIOD ON THE BEHAVIOR AND NEUTROPHIL FUNCTION OF DAIRY COWS

ABSTRACT

The objective of this study was to determine the effect of prepartum exercise, pasture turnout, or total confinement on activity and neutrophil function of dairy cows during the dry period. Fifty-eight Holstein and two Jersey-Holstein crossbred, pregnant, non-lactating dairy cows were assigned to control (n = 20), exercise (n = 20), or pasture (n = 20) treatments using rolling enrollment from Jan to Nov 2015 at dry-off. Cows were balanced on parity (1.8 ± 0.9), projected ME FCM (13,831 ± 2,028 kg per lactation) and projected due date. Cows were housed in a naturally ventilated, 4-row deep-bedded sand freestall barn at the University of Tennessee's Research Unit (Walland, TN). Fitted 3 d before dry-off, accelerometers determined daily lying time (h/d), daily lying bouts (n/d), lying bout duration (min/bout), and daily steps (n/d) at 1-min intervals. Data were summed by four periods relative to calving: -58 to -15 d (FO), -14 to -1 d (CU), d 0 (CA), and 1 to 14 d (PP). Exercise was done on five consecutive days per wk for 1.4 ± 0.1 h/d (targeted 1.5 h/d), at a pace of 1.88 ± 0.58 km/h until calving. Pasture turnout occurred on a grassy paddock five consecutive days per wk for 1.8 ± 0.3 h/d (targeted 1.5 h/d) until calving. Control cows remained in the home pen throughout the dry period. Blood was sampled on d -3 and 42, relative to dry-off to assess neutrophil function via reactive oxygen species generation using PMA. A mixed model determined the effects of treatment, period, and treatment × period on daily lying behavior and steps and the effect of treatment, day, PMA concentration, and their interactions on reactive oxygen species generation. Cow within treatment was the random variable. Exercise and pasture turnout increased daily activity over control but did not alter lying behaviors. Reactive oxygen species production was not affected by treatment. Although more active while standing, physical activity did not alter lying time budgets Furthermore, physical activity did not alter neutrophil function.

INTRODUCTION

Transition cows, or cows three weeks prepartum to three postpartum, are the cows most susceptible to disease in the herd, making them a key cow group to manage with the majority of all diseases occurring in the first ten days postpartum (Ingvartsen et al., 2003). Transition diseases, including subclinical and clinical ketosis, displaced abomasum, dystocia, retained placenta, and metritis, can cost $5,368, $1,409, $3,222, $4,504, $2,094, and $7,448, respectively, in milk loss alone over a 305-d lactation on a 100 cow dairy (assuming $20/cwt. milk; King, 1979, Dohoo and Martin, 1984, Deluyker et al., 1991, Østergaard and Gröhn, 1999, Dubuc et al., 2011). Goff and Horst (1997) hypothesized that transition diseases developed from a combination of negative energy balance, immune dysfunction, and hypocalcemia around parturition. Understanding how these physiological changes occur and ways to prevent them may be beneficial to minimizing costs and improving performance.

The stress of calving, coupled with increased cortisol concentrations brought on from disease, has a suppressive effect on the immune system (Roth and Kaeberle, 1982). Further, at the time of calving, cows experience decreased serum immunoglobulin concentration (Kehrli et al., 1989, Detilleux et al., 1995), diminished lymphocyte responsiveness (Kashiwazaki et al., 1985, Ishikawa, 1987, Kehrli et al., 1989, Saad et al., 1989), and impaired neutrophil function (Guidry et al., 1976, Newbould, 1976), all leading to increased susceptibility to disease. Mastitis is of particular concern since, with local protective factors impaired, such as neutrophils, infection is more likely. Cows housed in confinement had 1.8 times more cases of mastitis than those cows housed on pasture (Washburn et al., 2002). The authors speculated that bacteria exposure may be less on pasture compared with confinement housing; however, increased physical activity may also play a positive role. Immune dysfunction, negative energy balance, and hypocalcemia set transition cows up for susceptibility to disease and disorders. However, even with dietary intervention, disease incidence does not appear to be improving in the United States (USDA, 2008). Investigating novel non-dietary intervention methods may assist current practices in reducing predisposition and incidence of transitional disorders. Improved physical fitness through exercise may be a means to accomplish this reduction.

Physical activity, or exercise, may have beneficial influences on cow health and well-being. However, research investigating the benefit and direct impact in dairy cattle is minimal. Davidson and Beede (2009) investigated the impact of exercising cows during the dry period and at calving. Exercise cows exhibited an ability to walk for 22% longer periods of time on a treadmill with a reduced heart rate and faster recovery time on day 60 than those cows not exercised. Barker et al. (1975) exercised heifers 4 to 8 weeks prepartum a distance of 1.6 km at 5.5 km/h. Exercised heifers displayed improved ease of parturition, faster placental release, and increased feed efficiency, with reduced feed consumption but similar milk production compared with controls. However, when exercise continued two weeks postpartum, cows experienced a 2.5 kg/d milk loss, indicating exercise may negatively impact milk production through an increased requirement for energy.

Improved health may offset losses in milk. Gustafson (1993) determined exercising tie-stall housed cows 0.5 to 3 km daily reduced disease treatments by veterinarians, including bloat, paresis, retained placenta, non-infectious leg disorders, and laminitis, reduced culling, and reduced occurrences of subclinical mastitis during the first two weeks postpartum. Further, Popescu et al. (2013) determined providing exercise to tie-stall cows resulted in improved welfare, denoted by fewer hock lesions, fewer lame cows, and lower mastitis prevalence. Similarly, cows housed in a covered outdoor pen bedded with woodchips displayed lower odds of high locomotion scores and hock lesions than those housed in freestall barns (O'Driscoll et al., 2009). Methods of health improvement have not been investigated in dairy cattle, although reduced immune dysfunction may be a viable explanation.

Reducing immune dysfunction, thereby minimizing the level of immune dysfunction transition cows typically experience, may be a component of exercise. Horses exercised for 20 min at a slow trot of 3.5 m/s experienced an improvement in neutrophil phagocytosis and oxidative burst (Raidal et al., 2000). These changes in neutrophil function may impact the ability of the body to fight off infection. Mice exercise at 10, 25, and 20 cm/s for 5 min periods seven days per week experienced increased survival and reduced oxidative stress (Navarro et al., 2004), further illustrating the positive impact of exercise on the immune system through an reduction in immune dysfunction.

Reduced immune dysfunction and improved health and well-being may be related to an increase in physical activity in dairy cows. Understanding the dynamic of exercise in dairy cows and its relationship to physiology in regard to immunity and health is important to understanding the impact of total confinement housing and potentially creating recommendations to enhance cow health through supplemental physical activity. However, no studies have investigated the direct cause of improved health from increased physical activity. Therefore, the objective of this study was to determine the effect of exercise or pasture turnout on behavior and neutrophil function of dairy cattle.

MATERIALS AND METHODS

Animals, Housing, and Management

Twenty-nine primiparous and 31 multiparous, pregnant, non-lactating Holstein (n = 58) and Jersey-Holstein crossbred (n = 2) dairy cows were assigned to either control (n = 20), exercise (n = 20), or pasture (n = 20) treatments at dry-off using rolling enrollment from January to November 2015. Cows were balanced on parity (1.8 ± 0.9), projected ME FCM (13,831 ± 2,028 kg per lactation), and projected calving date. Cows were managed with a 60-d dry period (58.5 ± 5.4 d) divided into far-off (dry-off to 2 weeks before parturition) and close-up periods (two weeks before projected parturition).

Cows were housed in a naturally ventilated, 4-row head-to-head freestall barn with drive-through feed bunk at the University of Tennessee's Little River Animal and Environmental Unit (Walland, TN). Sand-bedded freestalls were 2.4 m long and 1.2 m wide with a 1.2 m high neck rail positioned 1.7 m from the curb and a 0.6 m high PVC tube brisket board placed 1.7 m from the curb. Fresh sand was added once per week with manure removed from stalls twice daily before milking the lactating herd (0730 and 1730 h). Fans turned on automatically when temperatures rose above 23 °C. Throughout the study period, study cows were housed in either pen 1, 2, or 6 (Figure 1), with pens measuring 12.1 m wide and 19.4 m long, enclosing 24 freestalls and 26 0.6 m wide headlocks, and containing 2 waterers, one on each end. Study cows were comingled unless the pen was split into far-off and close-up groups, leaving 12 freestalls and 13 headlocks for each group. Cows were maintained below 80% stocking density, based headlock and freestall availability.

Cows were fed twice daily at 0730 and 1530 h. Far-off cows were fed a TMR from dry-off to two weeks before projected parturition consisting of 4.5 kg ryegrass hay, 3.4 kg orchardgrass hay, 2.3 kg corn silage, and 2.7 kg dry cow grain per cow per day. Close-up cows were fed a TMR up to parturition consisting of 3.6 kg orchardgrass hay, 1.8 kg clover, 11.3 kg corn silage, and 3.0 kg dry cow grain per cow per day. All cows had ad lib access to water, except exercise treatment cows during exercise.

Experimental Treatments

Before enrollment, all cows had been housed in the same freestall barn with no previous experience with exercise, aside from pasture access during the dry period before the previous calving. Cows were enrolled into treatments on the day of dry-off. Cows assigned to control remained in the pen at all times, except for general management reasons (i.e. cleaning, rebedding stalls) when cows were moved to an adjacent lane for a maximum of 30 min. Cows were permitted to eat, drink, and move around the pen during exercise times. Cows assigned to exercise were removed from the pen 5× per week, Monday through Friday, and walked for at targeted 1.5 h at 3.25 km/h beginning at 1200 h along the path denoted by a dashed black line in Figure 1, measuring 250 m for each lap. Cows were walked in a group using the cows' flight zones and implements (i.e. rattle paddle) to encourage walking. Exercise pace was calculated by the total exercise time divided by the distance walked. During periods of high heat load, determined subjectively through cow heat stress behavior (i.e. increased respiration rate, panting) and exerciser comfort, cows were offered water at the point where the walking path met the entrance to the milking parlor (Figure 1) from a 19 L bucket. Cows did not have access to feed during the exercise period.

Cows on pasture were moved into a 2.11 hectare pasture (Pasture 1) from January to April 2015 and a 0.42 hectare pasture (Pasture 2) from April to December 2015 5× per week, Monday to Friday. Pasture 1 was 330 m from the barn to the pasture gate while Pasture 2 was 15 m from the barn to the pasture gate. Pasture 1 had rolling hills and a 0.75 hectare wooded area while pasture 2 had a shade structure and trees around one side of the fence line. Both pastures were seeded with orchardgrass and KY-31 fescue and managed by the farm manager for a height of 0.3 to 0.5 m. Cows were put on pasture before and returned to the barn after exercising cows from the exercise treatment group. Cows were put on pasture for a target of 1.5 h, excluding travel time to and from the paddock, beginning at 1200 h. Both pastures had access to water and grass.

Behavior

Cows were fitted with accelerometers (IceTag, IceRobotics, Edinburgh, Scotland) 3 d prior to dry-off. Activity was summarized by day from dry-off to the day prior to calving into lying time (h/d), lying bout frequency (bouts/d), lying bout duration (min/bout), and steps (n/d). All lying bouts under 2 min were removed (Endres and Barberg, 2007)

Reactive Oxygen Species

Blood was collected on d -3 and 43 relative to dry-off in 6 mL sodium heparin tubes (BD Vacutainer, BD, Franklin Lakes, NJ) between 0900 and 1000 h. Samples were immediately placed on ice and processed within 3 h of collection. Blood was analyzed for white blood cell count using an automated hematology analyzer (scil Vet abc, scil animal care company, Gurnee, IL). Neutrophils were isolated as previously described by Rambeaud and Pighetti (2005). The resulting 3 mL cell suspension was loaded with 0.6 μL of 1 μM dihydrorhodamine and incubated for 5 min at 37 °C. A 0.5 mL aliquot was added to 0.5 mL of each negative control Hank's Balanced Salt Solution (Corning, Tewksbury, MA), 20 nM phorbol myristate acetate (**PMA**) and 200 nM PMA to induce respiratory burst. Samples were incubated for 15 min at 37 °C and immediately placed on ice. Samples were immediately run on a flow cytometer (CyFlow SL, Partec, Münster, Germany) to determine fluorescence from reactive oxygen species (**ROS**) generation. Flow cytometry data were further analyzed using FlowJo (FlowJo, LLC, Ashland, OR) to determine percentage of cells that were neutrophils and generated ROS.

Animal Assessments

Cows were assigned a BCS using the system described by Edmonson et al. (1989) on d 0 and 42 relative to dry-off and d 0, 7, 14, 28, and 60 relative to calving by a single observer.

Statistical Analyses

The experimental and observational units of this study were the cow. Data were analyzed using the MIXED procedure of SAS (SAS 9.4, SAS Inst., Cary, NC). Cow within treatment was considered a random variable. Explanatory variables included day (d 0 to 58 relative to dry-off), treatment (control, exercise, pasture), and their interaction to analyze lying behaviors (lying time, lying bout frequency, lying bout duration, steps). White blood cell count was analyzed using treatment, day (d -3 and 43 relative to dry-off), and their interaction as explanatory variables. Reactive oxygen species generation was analyzed using treatment, PMA concentration (0, 10nM, 100nM), day (d -3 and 43 relative to dry-off), and their interactions as explanatory variables. Finally, explanatory variables included treatment and day (d 0 and 42 relative to dry-off, d 0, 7, 14, 28, and 60 relative to calving) and their interaction to analyze their effect on BCS and gait score.

RESULTS

Treatments

Exercise cows walked for 1.4 ± 0.1 h at 1.88 ± 0.58 km/h. Exercise periods began, on average, at 12:18:50 h, ranging from 10:12 to 14:39 h, and, on average, ended at 13:43:11, ranging from 11:16 to 16:03 h. Pasture cows spent a mean of 2.0 ± 0.3 h on Pasture 1, entering the pasture, on average, at 12:55:58 h, ranging from 11:24 to 14:41 h, and, on average, exiting the pasture at 14:56:17 h, ranging from 13:56 to 17:12 h. Cows spent a mean of 1.7 ± 0.3 h on

Pasture 2, entering the pasture, on average, at 11:58:40 h, ranging from 10:04 to 14:32 h, and, on average, exiting the pasture at 13:40:01, ranging from 11:21 to 16:07 h.

Behavior

Treatment and treatment × day did not affect lying time ($P \geq 0.12$; Figure 7) or lying bout frequency ($P \geq 0.12$; Figure 8) though both were affected by day ($P < 0.0001$). Cows laid down for the least amount of time and had the fewest lying bouts on d 0 and 58, relative to dry-off. A treatment × day effect existed for lying bout duration ($P = 0.01$; Figure 9) and steps ($P < 0.0001$; Figure 10). Exercise cows took the most steps during exercise days ($P < 0.0001$), compared with pasture and control, except for d 0 and 42 where all cows were exercised. Pasture cows took more steps than control cows on turnout days ($P < 0.10$). All cows took a similar number of steps during the 2 d when treatments were not applied (Saturday and Sunday; $P > 0.10$). Control cows had longer lying bouts on d 19 and shorter lying bouts on d 51 compared with exercise and pasture cows ($P \leq 0.04$), longer lying bouts on d 22 and shorter lying bouts on d 25 compared with pasture cows ($P \leq 0.03$), and shorter lying bouts on d 56 compared with exercise cows ($P < 0.01$). Pasture and exercise cows had similar lying bout durations throughout the dry period ($P > 0.05$).

Reactive Oxygen Species

White blood cell counts differed by treatment × day, with exercise cows having higher values on d 43 compared with pasture cows on d -3 (11.0 vs. 9.8 $10^3/mm^3$, respectively; $P = 0.01$). Percentage of neutrophils generating ROS did not differ by treatment × PMA concentration × day ($P = 0.95$), PMA concentration × day ($P = 0.86$), treatment × PMA concentration ($P = 0.69$), treatment × day ($P = 0.19$), treatment ($P = 0.63$), or day ($P = 0.49$), but did differ by PMA concentration ($P < 0.0001$). More neutrophils generated ROS when activated with 100mM PMA (85.3%) compared with 10mM PMA and HBSS (70.8 and 17.5%, respectively; $P < 0.0001$). Further, more cells generated ROS when activated with 10nM PMA than HBSS ($P < 0.0001$).

Animal Assessments

Body condition score was not affected by the interaction of treatment and day ($P = 0.20$) or treatment ($P = 0.12$); however, BCS differed by day ($P < 0.0001$), with cows being more conditioned 42 d into the dry period and condition gradually decreasing after parturition (Table 4).

DISCUSSION

This was the first study to examine the effect of physical activity on behavior and neutrophil function. Exercise cows were more active than pasture and control cows during exercise days, though pasture cows were more active than control cows on the same days. Treatment did not affect ROS generation, though generation was greater with more cell activation. The ROS ratio was greater for pasture cows on d 42 compared with exercise cows on both days and pasture cows on d 0. No differences occurred among groups for BCS.

It was predicted that exercise cows would have a greater number of steps on exercise days than control groups. However, pasture cows did not experience the same level of physical activity as those exercised, potentially due to environment (i.e. heat, snow) or distance from the barn. Cows were only required to walk a maximum of 330 m to the paddock (660 m roundtrip) from January to April and 15 m to the paddock (30 m roundtrip) from April to December, which is less than the 2 and 3 km implemented to see changes in health in a previous study (Gustafson, 1993). Cows were free to move once in the paddock, but, due to the time of treatment implementation (average: 11:58:40 to 13:40:01 h), cows may have been less willing to walk and explore due to heat load during warmer months and snow cover during colder months. However, even when kept on pasture during the entire dry period, cows only walked between 3,000 and 2,300 steps per day during the far-off and close-up periods, respectively (Black and Krawczel, 2016), which was reached by cows in the current study, indicating cows were hyperactive during turnout compared with cows regularly housed on pasture. Addition of resources that require more travel (i.e. water located further away, feed supplement, heifers/calves in adjacent pen) may encourage cows to participate in more physical activity. Further, turnout during the cooler evening hours may encourage activity, as this is when cows are more likely to graze (Walker et al., 2008) and prefer to be on pasture (Legrand et al., 2009).

Treatment did not affect lying time or lying bout duration and frequency, which is contradictory to previous research where pasture cows spent less time lying during the dry period than confined cows (Black and Krawczel, 2016). This was likely due to a portion of pasture cows' diets coming from grazing, where, while cows could graze in the current study, it is not assumed that a significant proportion of the diet came from grazing; however, this was not measured. In the current study, cows typically spent less than 2 h on pasture and this may not have been enough time to alter their time budget while in the barn.

Neutrophil function, measured in the form of percentage of cells generating ROS, did not change with increased physical activity. This is contradictory to previous research in humans where moderate exercise worked to prime neutrophil killing ability (Smith et al., 1990). Priming of neutrophils allows dormant neutrophils to acquire a state of preactivation, enabling them to provide a more powerful response (Smith, 1994). This priming affect was lost and neutrophil activity depressed 50% when exercise became intense (Smith et al., 1990). This indicates that, while exercise was not intense enough to reduce neutrophil function, it was also not enough to cause an improvement in percentage of neutrophils generating ROS through the priming effect. Time of sampling may also have impacted ROS generation by nuetrophils. Neutrophilia can occur following exercise (Rossdale et al., 1982, Korhonen et al., 2000, Quindry et al., 2003) with immature and less active neutrophils released from bone marrow (Rossdale et al., 1982, Simon, 1991, Iversen et al., 1994). With samples collected the day following exercise, the immune effect may have been lost; however, it was the objective of this research to understand the long term effect of exercise on neutrophil function and not the immediate effect. Employing exercise that would increase workload, either through a more structured exercise method (Anderson et al., 1977) or pasturing cows when they are more willing to graze (Walker et al., 2008, Legrand et al., 2009) may increase the intensity of physical activity and improve neutrophil function. It should be noted that, while ROS generation is important for host defense, over generation of free radicals can cause oxidative stress and tissue damage (Smith, 1994). Therefore, for signs of oxidative stress alongside changes in neutrophil function should occur to ensure that changes in physical activity do not cause tissue damage and negative effects.

Treatments did not affect BCS throughout the study. Previous research reports both exercised cows lost more weight than sedentary controls in previous research (Anderson et al., 1979, Lamb et al., 1981) and weight did not differ (Lamb et al., 1979). Due to the minimal workload required in the current study, no changes in fat metabolism likely occurred to cause exercised cattle a greater reduction in condition during the study. Still, all cows followed the expected changes in BCS during the dry period and early lactation (Roche et al., 2009).

CONCLUSION

Exercise and pasture turnout increased daily activity over control but did not alter lying behaviors. Reactive oxygen species generation was not altered by physical activity, indicating that additional workload need be applied to cows to experience improved neutrophil function. Body condition score did not differ with physical activity and physical activity was not enough to alter fat deposition during the dry period and in early lactation. The current method of exercise was not enough to change neutrophil function and time budget; however, employing physical activity with increased workload, either using a structured exercise machine or by pasturing cows with resources further away, may work to improve neutrophil function.

CHAPTER IV

EFFECT OF PREPARTUM PHYSICAL ACTIVITY DURING THE DRY PERIOD ON HOOF HEALTH IN DAIRY COWS

ABSTRACT

The objective of this study was to determine the effects of exercise, pasture turnout, or total confinement of dry cows on horn growth and wear and sole thickness. Twenty-nine primiparous and 31 multiparous, pregnant, non-lactating Holstein (n = 58) and Jersey-Holstein crossbred (n = 2) dairy cows were assigned to either control (n = 20), exercise (n = 20), or pasture (n = 20) treatments at dry-off using rolling enrollment from January to November 2015. Cows were managed with a 60-d dry period (58.5 ± 5.4 d) divided into far-off (dry-off to 2 weeks before parturition) and close-up periods (two weeks before projected parturition). Cows assigned to control remained in the pen. Exercise cows walked for 1.4 ± 0.1 h at 1.88 ± 0.58 km/h, 5× per week until calving. Pasture cows were turned out 1.7 ± 0.3 h, 5× per week until calving. Hoof growth and wear and sole thickness of the rear hooves were measured on d 2 and 44, relative to dry-off. Data were analyzed using the MIXED procedure of SAS. Cranial and caudal horn wear was greater for exercise cows than control and pasture cows. Exercise cows experienced a more even rate of horn growth and wear both cranially and caudally. Control cows tended to increase sole thickness from d 2 to d 44. Frequent access to exercise on concrete may not impair the hoof health of late gestation dry cows during a brief time period.

INTRODUCTION

Lameness continues to be a major concern within the dairy industry, with clinical lameness prevalence averaging 31% in California and 55% in the Northeastern United States (von Keyserlingk et al., 2012). Lameness is widely considered a welfare concern (von Keyserlingk et al., 2009) as it causes pain (Whay et al., 1998, Shearer et al., 2013), can reduce milk production by more than 1 kg/d (Green et al., 2002, Bicalho et al., 2008), and reduces longevity (Kossaibati and Esslemont, 1997, Booth et al., 2004). The causes of lameness range from infectious disease (i.e. digital dermatitis, foot rot), claw horn disruptions (i.e. white line separation, ulcers, hemorrhage), or management factors (i.e. concrete flooring zero grazing, uncomfortable stalls) and all increase the risk of lameness (Cook and Nordlund, 2009).

The majority of US dairy cattle are housed in tie stalls, stanchions, or freestall barns with no access to pasture (58.9%; USDA, 2016). These barns offer little access to exercise outside of traveling to the milking parlor, waterer, feed resources, and social interactions. With the majority of cows housed on concrete flooring (55.6%), few cows have access to softer standing surfaces such as rubber flooring (13.9%), dirt (20%), or pasture (5.1%; USDA, 2010). Concrete flooring was associated with increased incidence of digital dermatitis (48.5%) compared with pasture (28.2%; Wells et al., 1999) and at least one claw disorders (78 to 81%) compared with straw yards (57.5%; Somers et al., 2003), and concrete flooring can often result in unequal hoof horn growth and wear and heel erosion (Hahn et al., 1986, Vanegas et al., 2006, van Amstel et al., 2016). Standing and walking on hard surfaces (Greenough and Vermunt, 1991, Singh et al., 1993) and walking along rough cow tracks (Chesterton et al., 1989) can negatively impact lameness. However, offering cows access to softer surfaces can improve hoof health.

Housing cows on pasture for 3 weeks improved locomotion compared with a total confined control group (Hernandez-Mendo et al., 2007), which may be related to increased activity, as cows housed on pasture are more active than those in confinement (Hernandez-Mendo et al., 2007, Legrand et al., 2009, Black and Krawczel, 2016). Cows given access to exercise 2× or 7× per week tended to have a shorter claw diagonal than non-exercised cows kept in tie-stalls on rubber mats (Loberg et al., 2004). Shorter and steeper claws show less susceptibility to disease (Politiek et al., 1986, Smit et al., 1986) and may be improved with increased physical activity. Therefore, allowing cows access to increased physical activity through pasture turnout may improve hoof health while walking cows excessively on concrete or hard surfaces may lead to negative hoof outcomes.

This may be increasingly important in late-gestation cows when horn quality is weakened, making cows more susceptible to hoof ailments (Kempson and Logue, 1993) and implementing physical activity during late gestation may help to offset reduced horn quality without negative impacts on performance. While studies have determined the impact of different surfaces on hoof health, no studies have examined the impact of activity level on these surfaces on hoof health. Understanding the implications related to regular exercise of cattle, either on concrete or with pasture turnout, may give insight into the impact of physical activity on hoof health during late-gestation. The objective of this study was to determine the effects of exercise, pasture turnout, or total confinement of dry cows on horn growth and wear, sole thickness and lameness.

MATERIALS AND METHODS

Animals, Housing, and Management

Twenty-nine primiparous and 31 multiparous, pregnant, non-lactating Holstein (n = 58) and Jersey-Holstein crossbred (n = 2) dairy cows were assigned to either control (n = 20), exercise (n = 20), or pasture (n = 20) treatments at dry-off using rolling enrollment from January to November 2015. Cows were balanced on parity (1.8 ± 0.9), projected ME FCM (13,831 ± 2,028 kg per lactation), and projected calving date. A 60-d dry period (58.5 ± 5.4 d) was used with cows divided into a far-off group (dry-off to 2 weeks before parturition) and close-up group (two weeks before projected parturition or signs of parturition).

Cows were housed in a naturally ventilated, 4-row head-to-head freestall barn with drive-through feed bunk at the University of Tennessee's Little River Animal and Environmental Unit

(Walland, TN). Sand-bedded freestalls were 2.4 m long and 1.2 m wide with a 1.2 m high neck rail positioned 1.7 m from the curb and a 0.6 m high PVC tube brisket board placed 1.7 m from the curb. Fresh sand was added once per week with manure removed from stalls twice daily before milking (0730 and 1730 h). Fans turned on automatically when temperatures rose above 23 °C. Throughout the study period, study cows were housed in either pen 1, 2, or 6 (Figure 1), with pens measuring 12.1 m wide and 19.4 m long, enclosing 24 freestalls and 26 0.6 m wide headlocks, and containing 2 waterers, one on each end. Study cows were comingled unless the pen was split into far-off and close-up groups, leaving 12 freestalls and 13 headlocks for each group. Cows were maintained below 80% stocking density, assuming one headlock or freestall per cow.

Cows were fed twice daily at 0730 and 1530 h. Far-off cows were fed a TMR from dry-off to two weeks before projected parturition consisting of 4.5 kg ryegrass hay, 3.4 kg orchardgrass hay, 2.3 kg corn silage, and 2.7 kg dry cow grain per cow per day. Close-up cows were fed a TMR up to parturition consisting of 3.6 kg orchardgrass hay, 1.8 kg clover, 11.3 kg corn silage, and 3.0 kg dry cow grain per cow per day. All cows had ad lib access to water except exercise treatment cows during exercise.

Experimental Treatments

Before enrollment, all cows had been housed in the same freestall barn with no previous experience with exercise, aside from pasture access during the dry period before the previous calving. Cows were enrolled into treatments on the day of dry-off. Cows assigned to control remained in the pen at all times, except for general management reasons (i.e. cleaning, rebedding stalls) when cows were moved to an adjacent lane for a maximum of 30 min. Cows were permitted to eat, drink, and move around the pen during exercise times. Cows assigned to exercise were removed from the pen 5× per week, Monday through Friday, and walked for at targeted 1.5 h at 3.25 km/h beginning at 1200 h along the path denoted by a dashed black line in Figure 1, measuring 250 m for each lap. Cows were walked in a group using the cows' flight zones and implements (i.e. rattle paddle) to encourage walking. Exercise pace was calculated by the total exercise time divided by the distance walked. During periods of high heat load, determined subjectively through cow heat stress behavior (i.e. increased respiration rate, panting) and exerciser comfort, cows were offered water at the point where the walking path met the entrance to the milking parlor (Figure 1) from a 19 L bucket. Cows did not have access to feed during the exercise period.

Cows on pasture were moved into a 2.11 hectare pasture (Pasture 1) from January to April 2015 and a 0.42 hectare pasture (Pasture 2) from April to December 2015 5X per week, Monday to Friday. Pasture 1 was 330 m from the barn to the pasture gate while Pasture 2 was 15 m from the barn to the pasture gate. Pasture 1 had rolling hills and a 0.75 hectare wooded area while pasture 2 had a shade structure and trees around one side of the fence line. Both pastures were seeded with orchardgrass and KY-31 fescue and managed by the farm manager for a height of 0.3 to 0.5 m. Cows were put on pasture before and returned to the barn after exercising cows from the exercise treatment group. Cows were put on pasture for a target of 1.5 h beginning at 1200 h. Both pastures had access to water and grass.

Hoof Measures

Hoof growth and wear and sole thickness were measured on d 2 and 44, relative to dry-off. Cows were moved into a mobile, stand-up leg chute between 1000 and 1100 h before daily treatments were imposed. Only the rear hooves were measured, as rear hooves show greater wear and growth patterns and would display more difference over 42 d than front hooves (Hahn et al., 1986). To measure hoof growth and wear, each back claw was grooved horizontally and vertically using a power file, according to van Amstel et al. (2016). Grooves were ground at a 1 mm depth to ensure grooves did not extend past the hoof wall or fade before the lines were measured at d 44. The first vertical line (line B) was ground parallel to the heel, midway between the heel and toe. The second vertical line (line A) was ground parallel to the heel between the first line and the toe. The horizontal line was ground just below the periople of the coronary band. After grooving, the following measures were taken: coronary band to horizontal line using line B (B1), coronary band to horizontal line using line A (A1), horizontal line to edge of the hoof wall using line B (B2), and horizontal line to the edge of the hoof wall using line A (A2). Line segments were using a ruler (accurate to 0.1 mm) on d 2 and 44 and caudal and cranial growth and wear were calculated using the following calculations (van Amstel et al., 2016):

$$\text{Cranial growth} = \text{A1 (d 2)} - \text{A2 (d 44)}$$

$$\text{Caudal growth} = \text{B1 (d 2)} - \text{B2 (d 44)}$$

$$\text{Cranial wear} = (\text{A1 (d 2)} + \text{A2 (d 2)} + \text{cranial growth}) - (\text{A1 (d 44)} + \text{A2 (d 44)})$$

$$\text{Caudal wear} = (\text{B1 (d 2)} + \text{B2 (d 2)}) + \text{caudal growth}) - (\text{B1 (d 44)} + \text{B2 (d 44)})$$

Sole thickness was measured suing a 7.0-MHz curvilinear probe on each day, as described by van Amstel et al. (2004). Hooves were cleaned off using a brush with water and alcohol applied to the sole to improve probe contact. The probe was placed approximately 3.75 cm below the apex of the toe and on the inside of the abaxial line. Sole thickness was measured as the area between the outer margin of the ultrasound image and the inner sole seen as a thin continuous hyperechoic Kofler et al. (1999). All four rear claws were measured.

Statistical Analysis

The experimental and observational units of this study were the cow. Data were analyzed using the MIXED procedure of SAS (SAS 9.4, SAS Inst., Cary, NC). Cow within treatment was considered a random variable. Explanatory variables included treatment (control, exercise, pasture) to analyze hoof growth and wear. Sole thickness was analyzed using treatment, day (d 2 and 44), and their interactions as explanatory variables. Results are reported as least squares means ± SE. A paired TTEST procedure in SAS was used to determine if hoof horn growth and wear were different on the cranial and caudal aspects of the hoof for each treatment. Results are reported as mean ± SE. Means are considered different at $P \leq 0.05$ and a tendency at $P \leq 0.10$.

RESULTS

Treatments

Exercise cows walked for 1.4 ± 0.1 h at 1.88 ± 0.58 km/h. Exercise periods began, on average, at 12:18:50 h, ranging from 10:12 to 14:39 h, and, on average, ended at 13:43:11, ranging from 11:16 to 16:03 h. Pasture cows spent a mean of 2.0 ± 0.3 h on Pasture 1, entering the pasture, on average, at 12:55:58 h, ranging from 11:24 to 14:41 h, and, on average, exiting the pasture at 14:56:17 h, ranging from 13:56 to 17:12 h. Cows spent a mean of 1.7 ± 0.3 h on Pasture 2, entering the pasture, on average, at 11:58:40 h, ranging from 10:04 to 14:32 h, and, on average, exiting the pasture at 13:40:01, ranging from 11:21 to 16:07 h.

Hoof Measurements

Five cows were excluded from hoof growth and wear and sole thickness data (control = 1, exercise = 2, pasture = 2) as cows' hooves were trimmed before d 42 of the study. Treatment did not affect cranial horn growth (control: 0.97 ± 0.08 mm; exercise: 1.11 ± 0.08 mm; pasture: 0.97 ± 0.08 mm; $P = 0.40$) or caudal horn growth (control: 1.02 ± 0.09 mm; exercise: 1.23 ± 0.10

mm; pasture: 1.01 ± 0.10 mm; $P = 0.20$). Cranial horn wear was greater for exercise cows (1.08 ± 0.06 mm) than control (0.69 ± 0.06 mm) and pasture cows (0.76 ± 0.06 mm; $P < 0.0001$). Caudal horn wear was greater for exercise cow (1.05 ± 0.06 mm) than control (0.69 ± 0.05 mm) and pasture cows (0.77 ± 0.05 mm; $P < 0.0001$). Growth and wear did not differ on the cranial aspect of the horn for exercise cows (difference: 0.03 ± 0.08 cm; $P = 0.72$); however, the caudal aspect of the horn tended to grow 0.18 ± 0.10 cm more than the horn wore ($P = 0.08$). Horn growth was greater for the cranial and caudal aspect of the horn in pasture (0.22 ± 0.06 and 0.24 ± 0.06 cm, respectively; $P < 0.001$) and control cows (0.28 ± 0.06 and 0.33 ± 0.06 cm; $P < 0.0001$). Sole thickness tended to be affected by treatment × day (P = 0.07; Figure 11) where control cows tended to increase sole thickness from d 2 to d 44.

DISCUSSION

This is the first study to examine the impact of physical activity on concrete or in pasture on hoof growth and wear and sole thickness. Exercise cows experienced a more even rate of horn growth and wear both cranially and caudally. Exercise cows tended to have thinner soles throughout the course of the dry period, while control cows tended to have thicker soles. Treatment did not affect lameness score.

Horn growth rates have been shown to be seasonal, particularly growing faster in the spring-summer period (Vermunt and Greenough, 1995), greater with higher energy diets (Greenough et al., 1990), and greater in young animals than in older animals (Vermunt and Greenough, 1995). Therefore, it is expected that hoof growth did not differ among treatments as all cows received the same diet and were enrolled into treatments throughout the year evenly. Higher rates of hoof wear were associated with concrete flooring, overstocking, poor cow comfort, claw horn moisture, poor stockmanship, and poor horn quality (Van Amstel et al., 2002). Since exercise cows experienced more time walking on concrete, it is expected that this group would also experience the greatest level of wear.

Exercise cows experienced a more even horn growth and wear rate compared with control and pasture cows. Normal claws are characterized by equal rates of growth and wear (Vermunt and Greenough, 1995) and an imbalance can cause horn lesions (Bazeley and Pinsent, 1984, Greenough and Vermunt, 1991, Cook et al., 2004). Increased horn growth and wear can occur when housed on concrete compared with a softer surface, such as a rubber mat (Vanegas et al., 2006, Telezhenko et al., 2009, van Amstel et al., 2016). Further, walking surface may be even more important around calving when horn quality is weakened from systematic changes with calving and lactogenesis, increasing the likelihood of white line disease and horn lesions (Kempson and Logue, 1993, Webster, 2001a). The current study, however, determined that regular exercise of cows on concrete contributed to an improved growth and wear rate of the horn, potentially improving hoof health. This is in contrast to previous research indicating improved hoof characteristics and health with access to pasture or a straw yard (Hahn et al., 1986, Somers et al., 2003, Chapinal et al., 2010b). This may indicate that, during the dry period, additional locomotor activity on a concrete surface does not impair the hoof health of cows. However, it should be noted that the current study did not record hoof disorders, such as horn lesions, white line disease, or other disorders caused by environmental factors, and additional work looking at hoof disorders is needed to fully understand the interaction of exercise and hoof health.

Walking on concrete has been previously associated with thin soles (van Amstel et al., 2006). Soles provide protection to the claw capsule (Toussaint Raven, 1989) and thin soles are more prone to injury and contusion , particularly in environments with hard or irregular surfaces

(Greenough, 1987, Toussaint Raven, 1989). However, neither exercised nor pasture cow experienced a reduction in sole thickness, illustrating that the exercise and pasture regimens used did not have a negative impact on hoof health. Control cows did experience a tendency to increase sole thickness, but all cows were above the minimum of 7 mm to provide adequate protection to the claw capsule (Toussaint Raven, 1989).

CONCLUSIONS

Exercise cows experienced greater hind hoof horn wear than control and pasture cows but had more equal rates of horn growth and wear. Sole thickness was not altered with exercise or pasture turnout but tended to increase for cows in total confinement and all cows remained above the minimum thickness to provide adequate protection to the claw capsule. Frequent access to exercise on concrete may not impair the hoof health of late gestation dry cows.

CHAPTER V

EFFECT OF PREPARTUM PHYSICAL ACTIVITY DURING THE DRY PERIOD ON CALVING BEHAVIOR AND CORTISOL CONCENTRATION

ABSTRACT

The objective of this study was to assess the effect of exercise and pasture turnout on calving behavior and stress around the time of parturition in dairy cows. Twenty-nine primiparous and 31 multiparous, pregnant, non-lactating Holstein (n = 58) and Jersey-Holstein crossbred (n = 2) dairy cows were assigned to either control (n = 20), exercise (n = 20), or pasture (n = 20) treatments at dry-off using rolling enrollment. Cows assigned to control remained in the pen. Cows assigned to exercise were removed from the pen 5× per week and walked for 1.4 ± 0.1 h at 1.88 ± 0.58 km/h until calving. Cows assigned to pasture were moved to an outdoor paddock 5× per week for 1.8 ± 0.3 h/d. Cows were housed in deep-bedded sand freestalls in a naturally ventilated, 4-row freestall barn at the University of Tennessee's Little River Animal and Environmental Unit (Walland, TN). Cows were moved into maternity pens on the day of projected calving or when cows displayed signs that calving was imminent (i.e. restlessness, holding of tail, ruptured amniotic sac, swollen vulva) and treatments were discontinued. Cameras continuously recorded cows from entry into the pen until farm staff noted a calf and one observer continuously watched video for three visually observable periods throughout the calving process: time from initial observation of amniotic sac to rupture of amniotic sac, time from rupture of amniotic sac to initial observation of calf's feet, and time from initial observation of calf's feet to full expulsion of calf. Assisted calvings were excluded. Accelerometers were attached to the rear fetlock of cows 3 d prior to dry-off and removed 14 d postpartum. Activity was summarized by day for the 7 d before and after calving time recorded from video observation into lying time (h/d), lying bout frequency (bouts/d), lying bout duration (min/bout), and steps (n/d). Plasma total cortisol concentration was determined by a radioimmunoassay procedure using a commercially available kit. Data were analyzed using the MIXED procedure of SAS. Labor was

longer from rupture of the amniotic sac to observation of feet compared with the other two periods, regardless of treatment. Control cows displayed shorter lying bouts and short overall lying time compared with pasture and exercise cows. Cortisol concentrations were higher on the day of calving than 3 d later. Implementing exercise in a more structured manner and offering exercise during cooler periods in paddocks further away may result in improved benefits of physical activity.

INTRODUCTION

Parturition is considered painful (Mainau and Manteca, 2011) and leads to inflammation (Turk et al., 2005, Bionaz et al., 2007). Further, difficulty during calving was rated one of the most painful conditions in cattle by cattle practitioners in the UK (Huxley and Whay, 2006) and can cause subsequent reduction in performance (Dematawena and Berger, 1997). Inadequate expulsive forces (Noakes et al., 2001c, Jackson, 2004), feto-pelvic disproportion (Bellows et al., 1971, Johnson et al., 1988, Noakes et al., 2001b), and malpresnetation (Meijering, 1984, Noakes et al., 2001a) are the primary reasons for difficult calvings. While strategies exist to alter pelvic area (Benyshek and Little, 1982, Morrison et al., 1986) and fetal size (Mee, 2008), no strategies are currently employed in dairy management to improve uterine expulsive forces. However, exercise is often used in human prepartum care to ameliorate this condition, which suggests it may be a useful management strategy to translate to dairy cattle.

Primiparous women participating in strengthening and toning exercise 1 h twice weekly for a minimum of 12 wk had more spontaneous vaginal delivery, less requirement for oxytocin augmentation of delivery, and shorter first and second stages of labor compared with sedentary women (Beckmann and Beckmann, 1990). Exercise during pregnancy in women promoted muscle tone, strength, and endurance, reduced cesarean section incidence, and lowered discomfort at delivery (Wallace et al., 1986, Hall and Kaufmann, 1987, Kulpa et al., 1987). In cattle, prepartum heifers exercised at 5.47 km/h for 1.6 km/d for 4 wk experienced improved ease of calving and faster involution of the uterus by 42 d postpartum (Lamb et al., 1979). Therefore, it was hypothesized that exercise during the prepartum period may improve uterine strength and tone to reduce length of labor.

The addition of exercise may also alter the behavioral response of cattle at parturition. Cow behavior changes as parturition approaches, characterized by reduced lying time, increased lying bout frequency, increased activity, and reduced feed intake (Huzzey et al., 2005, Miedema et al., 2011, Jensen, 2012). These alterations of behavior can be used to monitor the progression and imminence of calving. Access to pasture at calving resulted in increased lying bout frequency compared with confined cows (Black and Krawczel, 2016), so providing cows with access to more physical activity during this period may also exacerbate the behavioral response to calving, improving detection of calving and subsequently improving reproductive efficiency and neonate vitality (Palombi et al., 2013).

Exercise may also improve the calving process by improving endocrine signaling. Increased concentration of corticosteroids before calving signals luteolysis and signals for termination of pregnancy (Adams and Wagner, 1970, Hoffmann et al., 1973). Corticosteroid concentrations return to basal concentrations 3 to 7 d postpartum (Adams and Wagner, 1970). However, difficult calvings can intensify this stress response (Civelek et al., 2008). Exercise may be a means to reduce the level of stress experienced at calving as women who exercised during pregnancy experienced lower cortisol concentrations at birthing compared with sedentary controls (Varrassi et al., 1989).

As the majority of cattle are confined (USDA, 2016), it is important to understand the implication of this sedentary lifestyle on calving behavior. Physical activity during late gestation has the implication to alter the progression of labor, and the behavioral and endocrinological response to calving; however, these changes have not been studied in late gestation dairy cattle. Therefore, the objective of this study was to access the effect of exercise and pasture turnout on calving behavior and cortisol response around the time of parturition in dairy cows.

MATERIALS AND METHODS

Animals, Housing, and Management

Twenty-nine primiparous and 31 multiparous, pregnant, non-lactating Holstein (n = 58) and Jersey-Holstein crossbred (n = 2) dairy cows were assigned to either control (n = 20), exercise (n = 20), or pasture (n = 20) treatments at dry-off using rolling enrollment from January to November 2015. Cows were balanced on parity (1.8 ± 0.9), projected ME FCM (13,831 ± 2,028 kg per lactation), and projected calving date. Cows were managed with a 60-d dry period (58.5 ± 5.4 d) divided into far-off (dry-off to 2 weeks before parturition) and close-up periods (two weeks before projected parturition).

Cows were housed in a naturally ventilated, 4-row head-to-head freestall barn with drive-through feed bunk at the University of Tennessee’s Little River Animal and Environmental Unit (Walland, TN). Sand-bedded freestalls were 2.4 m long and 1.2 m wide with a 1.2 m high neck rail positioned 1.7 m from the curb and a 0.6 m high PVC tube brisket board placed 1.7 m from the curb. Fresh sand was added once per week with manure removed from stalls twice daily before milking the lactating herd (0730 and 1730 h). Fans turned on automatically when temperatures rose above 23 °C. Throughout the study period, study cows were housed in either pen 1, 2, or 6 (Figure 1), with pens measuring 12.1 m wide and 19.4 m long, enclosing 24 freestalls and 26 0.6 m wide headlocks, and containing 2 waterers, one on each end. Study cows were comingled unless the pen was split into far-off and close-up groups, leaving 12 freestalls and 13 headlocks for each group. Cows were maintained below 80% stocking density, based headlock and freestall availability.

Cows were fed twice daily at 0730 and 1530 h. Far-off cows were fed a TMR from dry-off to two weeks before projected parturition consisting of 4.5 kg ryegrass hay, 3.4 kg orchardgrass hay, 2.3 kg corn silage, and 2.7 kg dry cow grain per cow per day. Close-up cows were fed a TMR up to parturition consisting of 3.6 kg orchardgrass hay, 1.8 kg clover, 11.3 kg corn silage, and 3.0 kg dry cow grain per cow per day. All cows had ad lib access to water, except exercise treatment cows during exercise.

Experimental Treatments

Before enrollment, all cows had been housed in the same freestall barn with no previous experience with exercise, aside from pasture access during the dry period before the previous calving. Cows were enrolled into treatments on the day of dry-off. Cows assigned to control remained in the pen at all times, except for general management reasons (i.e. cleaning, rebedding stalls) when cows were moved to an adjacent lane for a maximum of 30 min. Cows were permitted to eat, drink, and move around the pen during exercise times. Cows assigned to exercise were removed from the pen 5× per week, Monday through Friday, and walked for at targeted 1.5 h at 3.25 km/h beginning at 1200 h along the path denoted by a dashed black line in Figure 1, measuring 250 m for each lap. Cows were walked in a group using the cows' flight zones and implements (i.e. rattle paddle) to encourage walking. Exercise pace was calculated by the total exercise time divided by the distance walked. During periods of high heat load, determined subjectively through cow heat stress behavior (i.e. increased respiration rate, panting) and exerciser comfort, cows were offered water at the point where the walking path met the entrance to the milking parlor (Figure 1) from a 19 L bucket. Cows did not have access to feed during the exercise period.

Cows on pasture were moved into a 2.11 hectare pasture (Pasture 1) from January to April 2015 and a 0.42 hectare pasture (Pasture 2) from April to December 2015 5× per week, Monday to Friday. Pasture 1 was 330 m from the barn to the pasture gate while Pasture 2 was 15 m from the barn to the pasture gate. Pasture 1 had rolling hills and a 0.75 hectare wooded area while pasture 2 had a shade structure and trees around one side of the fence line. Both pastures were seeded with orchardgrass and KY-31 fescue and managed by the farm manager for a height of 0.3 to 0.5 m. Cows were put on pasture before and returned to the barn after exercising cows from the exercise treatment group. Cows were put on pasture for a target of 1.5 h, excluding travel time to and from the paddock, beginning at 1200 h. Both pastures had access to water and grass.

Calving Behavior

Cows were monitored for signs of calving by farm staff regularly between 0730 and 2100 h and moved into maternity pens by farm staff on the day of projected calving or when cows displayed signs that calving was imminent (i.e. restlessness, holding of tail, water breaking, swollen vulva) and treatments were no longer continued. Maternity pens were 4.2 × 4.1 m containing a rubber filled mattress covering the entire pen floor (ProMat, Inc., Woodstock, ON) with no bedding. Each pen had access to water and cows were fed using a rubber tub twice daily. Gestation length was calculated from breeding date to calving date.

Video Observations. Video cameras were placed at six points around the maternity pens with one camera in front of each of the four pens and one camera placed at each front corner

(Figure 12). Pens were lit using red lights to observe behavior during night calvings. Cameras continuously recorded cows from entry into the pen until farm staff noted a calf. One unblinded observer viewed all video and noted three visually observable periods throughout the calving process:

Period 1: time from initial observation of the amniotic sac to rupture of the amniotic sac

Period 2: time from amniotic sac rupture to initial observation of one or both of calf's feet

Period 3: time from initial observation of one or both of calf's feet to full expulsion of calf where both back (or front if breech calving) feet are visible

Cows that needed assistance during calving were not included; however, calving time was recorded. Calving ease was assigned by the herd manager using the scoring system (1 = no problem, 2 = slight problem, 3 = needed assistance, 4 = considerable force, 5 = extreme difficulty; Berger, 1994).

Calving Activity. Accelerometers (IceTag, IceRobotics, Edinburgh, Scotland) were attached to the rear fetlock of cows 3 d prior to dry-off and removed 14 d postpartum. Activity was summarized by day for the 7 d before and after calving time recorded from video observation, with d -1 indicating the 24 h period prior to calving and d 1 indicating the 24 h period after calving, into lying time (h/d), lying bout frequency (bouts/d), lying bout duration (min/bout), and steps (n/d). All lying bouts under 2 min were removed (Endres and Barberg, 2007).

Cortisol Measurement

Blood samples were collected from cows on d 0 and 3 postpartum in 6 mL sodium heparin tubes (BD Vacutainer, BD, Franklin Lakes, NJ) from the coccygeal vein while in a palpation chute. Blood was drawn once a recently calved cow was moved into a palpation chute or between 0900 and 1100 h as cows exited the milking parlor on cows that calved after 1700 h the previous day. Blood was drawn between 0900 and 1100 h as cows exited the milking parlor to obtain the d 3 sample. Samples were centrifuged, plasma separated into microcentrifuge tubes, and tubes frozen at -80 °C. Plasma total cortisol concentration was determined by a radioimmunoassay procedure using a commercially available kit (ImmunChem Cortisol 125 | RIA Kit, BP Biomedical, LLC, Orangeburg, NY). Inter- and intra-assay CV for the low control (7 ng/mL) was 24.5% and 28.8%, respectively, and 11.7% and 3.6%, respectively, for the high control (25 ng/mL).

Health Exams

Health exams were performed by farm staff on cows during the first 7 d postpartum. Disorder incidence was recorded for displaced abomasums, mastitis, milk fever, ketosis, and metritis using scoring systems described in (Sterrett et al., 2014). Cows were also assessed for behavioral score, manure score, rumen fill score, respiration rate, heart rate, and uterine score daily during the first 7 d postpartum using scoring systems described in (Sterrett et al., 2014). Health disorders are reported for descriptive purposes.

Statistical Analysis

The experimental and observational units of this study were the cow. Data were analyzed using the MIXED procedure of SAS (SAS 9.4, SAS Inst., Cary, NC). Cow within treatment was considered a random variable. Explanatory variables included treatment, labor period (Period 1, Period 2, Period 3), and their interaction to analyze time for each labor period. Treatment, day (d -7, -6, -5, -4, -3, -2, -1, 1, 2, 3, 4, 5, 6, and 7 relative to calving), and treatment × day were explanatory variables used to analyze lying behavior and activity, with observations repeated by day. Cortisol values were transformed using a forth root transformation to normalize the data

(Miller and Plessow, 2013). Treatment, day (d 0 and 3 relative to calving), and treatment × day were explanatory variables use to analyze cortisol concentrations. Further, a paired ttest was used to determine the difference between the calving ease score of the current and previous calving.

RESULTS

Cow Health

Health disorder incidence is reported in Table 2. Mean (± SD) fresh cow exam scores are reported in Table 3.

Treatments

Exercise cows walked for 1.4 ± 0.1 h at 1.88 ± 0.58 km/h. Exercise periods began, on average, at 12:18:50 h, ranging from 10:12 to 14:39 h, and, on average, ended at 13:43:11, ranging from 11:16 to 16:03 h. Pasture cows spent a mean of 2.0 ± 0.3 h on Pasture 1, entering the pasture, on average, at 12:55:58 h, ranging from 11:24 to 14:41 h, and, on average, exiting the pasture at 14:56:17 h, ranging from 13:56 to 17:12 h. Cows spent a mean of 1.7 ± 0.3 h on Pasture 2, entering the pasture, on average, at 11:58:40 h, ranging from 10:04 to 14:32 h, and, on average, exiting the pasture at 13:40:01, ranging from 11:21 to 16:07 h.

Calving Ease

In the control treatment, 19 cows were given a calving ease score of 1 and one cow received a score of 2. Seventeen exercise cows were scored 1 for calving ease, with two scored a 3 and one scored a 4. Of the pasture cows, 12 cows were scored a 1 for calving ease, seven were scored a 3, and one cow was given a score of 5. No differences occurred between the current and previous calving ease scores ($P = 0.50$).

Calving Behavior

A total of 38 calvings and calving times were recorded (control = 10, exercise = 13, pasture = 15). Nine cows were assisted during calving (exercise = 2, pasture = 7) and not included in analysis of labor periods. Timing of different labor periods were accomplished for 19 cows for Period 1 (control = 7, exercise = 8, pasture = 4), 26 cows for period 2 (control = 9, exercise = 10, pasture = 7), and 29 cows for period 3 (control = 10, exercise = 11, pasture = 8). A total of 42 accelerometers correctly functioned throughout the study period (control = 14, exercise = 14, pasture =14) with 24 cows having a calving time recorded. Therefore, 29 cows (control = 10, exercise = 11, pasture = 8) were used in the labor period analysis and 24 cows (control = 5, exercise = 8, pasture = 11) were used for the activity analysis.

Treatment and treatment × labor period did not affect the time of each labor period ($P \geq 0.31$; Figure 13). However, Period 2 of labor was longer (40.1 ± 5.9 min) than Period 1 (4.4 ± 7.1 min) and Period 3 (24.0 ± 5.6 min; $P < 0.01$). Daily lying time was not affected by treatment × day (P = 0.36) but control cows tended to lie for less time (9.8 ± 0.8 h/d) than exercise (12.2 ± 0.6 h/d) and pasture cows (11.7 ± 0.5 h/d; $P = 0.06$). Cows laid down for the shortest time during the 24 h preceding calving and the longest time during the 24 h following calving (Figure 14; $P < 0.0001$). Lying bout frequency was not affected by treatment × day ($P = 0.32$) or treatment (control: 10.9 ± 1.0 bouts/d, exercise: 9.1 ± 0.8 bouts/d, pasture: 8.8 ± 0.7 bouts/d; $P = 0.22$). Cows changed posture more frequently during the 24 h preceding and following calving (Figure 15; $P < 0.01$).

Lying bout duration was not affected by treatment × day ($P = 0.63$), but control cows had shorter lying bouts (58.8 ± 9.4 min/bout) than exercise (90.7 ± 7.1 min/bout) and pasture cows (87.0 ± 5.7 min/bout; $P = 0.03$). Cows had the shortest lying bouts in the 24 h preceding calving

(Figure 16; $P = 0.01$). Daily steps were not affected by treatment × day ($P = 0.47$) or treatment (control: 2,189.4 ± 192.8 steps/d, exercise: 2,158.9 ± 152.4 steps/d, pasture: 2,030.2 ± 130.0 steps/d; $P = 0.73$). Cows took the most steps during the 24 h preceding calving (Figure 17; $P < 0.001$).

Cortisol Measurement

Cortisol concentrations around calving were not affected by treatment × day (Figure 18; $P = 0.22$) or treatment (control: 3.96 ± 0.52, exercise: 4.01 ± 0.52, pasture: 4.56 ± 0.52; $P = 0.86$). However, cortisol concentrations were higher on the day of calving (6.24 ± 0.47) than 3 d later (2.66 ± 0.50; $P < 0.001$).

DISCUSSION

This was the first study to examine the impact of physical activity on calving behavior and cortisol response of late gestation cows. Exercised and pasture cows displayed less behavioral modification, through increased lying time, and longer lying bouts, compared with control cows during the 7 d preceding and following calving. However, all cows reduced lying time, increased lying bout frequency, reduced lying bout duration, and increased activity preceding calving. Cortisol concentration at and following calving did not differ among treatments but did decrease from calving to 3 d postpartum.

The process of calving is typically separated into three stages: cervical dilation and uterine contractions (stage I), expulsion of the calf (stage II), and expulsion of the fetal membranes (stage III; USDA, 2010). Visual indications of stage I labor (uterine contractions, nest-building behavior, tail raising, olfactory ground checks, grooming, vocalization, restlessness, tail raising, and defecation) can be subjective, vary amongst cow and parity, and may extend across stages (Wehrend et al., 2006). However, visual indicators of stage II labor (appearance of amniotic sac, appearance of the calf, and expulsion of the calf) can be objectively determined, do not extend across stages, and periods within this stage can be objectively assessed (USDA, 2010a, Schuenemann et al., 2011).

Women who exercised during late gestation experienced a shorter length in stage II labor compared with sedentary controls (Beckmann and Beckmann, 1990). The current study did not determine differences in stage II labor time with physical activity. However, this may have been due to a number of factors. First, timing of period 1 and period 2 could not be objectively determined for a subset of cows due to video quality and available lightening in the pens during night calving. This may have been alleviated with more cameras or more light; however, the researchers used red light at night as to not interrupt the photoperiod of cows, which has implications on the immune system and subsequent milk production (Dahl and Petitclerc, 2003). Second, a lack of difference may have also been impacted by the statistical power to determine differences in periods between treatments. Schuenemann et al. (2011) determined differences between unassisted and assisted births in stage II labor (unassisted: 45.1 min; assisted: 84.8 min) with 10 unassisted and 5 assisted calvings. However, the differences in times for this stage only differed by 13 min in the current study with a large amount of variation, which did not allow detection of differences. Lastly, while exercised and pasture cows were more active than the confined controls during the dry period (R. Black, unpublished data), this level of activity may not have been enough to produce an effect in this outcome. Previous work exercising cows used an exercise pace ranging from 3.25 to 4 km/h over 5 to 8 km (Blake et al., 1982, Davidson and Beede, 2009) to attain improved physical fitness, which is greater than that experienced by either exercise or pasture cows.

Behavioral changes occur during all three stages of labor characterized by reduced lying time, increased lying bout frequency, increased activity, and reduced feed intake (Huzzey et al., 2005, Miedema et al., 2011, Jensen, 2012). These changes were observed across all treatments in the current study; however, changes were more pronounced in control cows, particularly through reduced lying bout duration and a tendency to reduce lying time. While increased lying bout frequency is associated with difficult calvings and discomfort, research has not noted a connection between increased standing time and difficult calvings (Wehrend et al., 2006, Proudfoot et al., 2009). Additionally, when kept on pasture during the dry period and calving, cows had similar daily lying times and lying bout durations to confined cows that calved in a maternity pen (Black and Krawczel, 2016). Control cows were not more active at this period either, indicating this difference may not have been related to restless stage I labor behavior (Wehrend et al., 2006). Therefore, this may be related to cow variation independent of treatment and labor stage, as behavior around calving can vary between cows (Wehrend et al., 2006).

A fetal cortisol spike at parturition signals termination of gestation and is coupled with a smaller maternal cortisol spike (Adams and Wagner, 1970, Hoffmann et al., 1973, Hudson et al., 1976). The current study indicates that increased physical activity through exercise or pasture turnout did affect the concentration of maternal cortisol at parturition. While studies in women noted a reduction in cortisol at birthing with prepartum exercise (Varrassi et al., 1989), the exercise performed in the current study may not have been as intense as that performed by cycling. The current study used low stress handling methods to implement exercise to prevent confounding stress levels, which may have inhibited the necessary level of exercise intensity to induce a change. However, implementing exercise in a more stressful manner may have resulted in chronic stress, which could cause hyper-reactivity of the adrenal cortex to other stressors

(Broom, 1988), such as calving, leading to greater levels of postpartum immune dysfunction (Aleri et al., 2016).

Future studies examining the impact of physical activity on calving behavior and cortisol responses can assist in understanding the benefits of physical activity in late gestation as seen in women. However, use of additional cows to increase power may be necessary to detect differences, as variation in calving period times can be quite large. Additional cows may also allow exploration of health benefits at calving, including calving ease, retained placenta, metritis, and hypocalcemia. As cows in the current project may not have been exercised intensely enough, Implementing an exercise program using equipment to apply exercise in a more structured manner (Anderson et al., 1977) may improve performance outcomes. However, it is important that exercise still be carried out in a low stress manner to avoid negative implications on the hypothalamic–pituitary–adrenal axis. Additionally, pasture turnout during the cooler evening hours may encourage activity, as this is when cows are more likely to graze (Walker et al., 2008) and prefer to be on pasture (Legrand et al., 2009).

CONCLUSIONS

Physical activity during late gestation did not affect stage II labor times. However, all cows exhibited decreased lying time and lying bout duration and increased lying bout frequency and activity. This modification in behavior was pronounced in control cows with a further reduced lying bout duration and tendency for reducing daily lying time, though, this difference may be due more to cow variation as this change occurred over the 7 d preceding and following calving. Physical activity did not affect cortisol concentrations, but concentrations were higher the day of calving compared with 3 d later. Future studies should consider using more cows to improve statistical power and examine health benefits of physical activity. Further, studies should use more structured methods to exercise cows and turn cows out to pasture during cooler evenings when cows prefer to be outdoors grazing.

CHAPTER VI

EFFECT OF MATERNAL PHYSICAL ACTIVITY ON CALF PERFORMANCE, BEHAVIOR, AND CORTISOL CONCENTRATIONS

ABSTRACT

The objective of this study was to determine the impact of maternal total confinement, pasture access, or exercise during late-gestation on calf performance, behavior, and stress during disbudding and weaning. Fifty-five Holstein and five Jersey-Holstein crossbred calves were enrolled into the study during gestation. Calves were removed from cows immediately once observed by farm staff, weighed, moved into a straw deep-bedded hutch. Calves were born from pregnant, non-lactating Holstein (n = 58) and Jersey-Holstein crossbred (n = 2) dairy cows assigned to either control (n = 20 cows; 13 female calves, 7 male calves), exercise (n = 20 cows; 8 female claves, 12 male calves), or pasture (n = 20 cows; 11 female calves, 9 male calves) treatments at dry-off using rolling enrollment from January to November 2015. Cows assigned to control remained in the pen. Cows assigned to exercise were removed from the pen 5× per week and walked for 1.4 ± 0.1 h at 1.88 ± 0.58 km/h over 2.66 ± 0.88 km. Cows on pasture were turned out for 2.0 ± 0.3 h or 1.7 ± 0.3 h, depending on date. Data loggers were attached to the rear fetlock of calves in a horizontal orientation using bandaging 3 d prior to and removed 6 d after disbudding and weaning to monitor changes in lying behavior, with data summarized by day to determine daily lying time. Blood was collected on 24 h prior to a 0, 1, and 4 h after dehorning and d -1, 0, 1, and 2 relative to weaning to assess cortisol concentrations. Data were analyzed using the MIXED procedure of SAS. At disbudding, calves gained less weight the day after and tended to have elevated cortisol concentrations 1 h after disbudding, regardless of maternal treatment. At weaning, calves gained less weight the day of and after and had elevated cortisol concentrations the day after weaning, regardless of treatment. Behavior did not differ by treatment at disbudding but calves from pasture cows laid down for less time compared with control and exercise maternal treatments and less frequently than exercise maternal treatments at

weaning. Increased lying bout frequency may be an indication of increased discomfort. However, more research investigating the significance of lying time and restlessness around stressful events is needed to further understand the implications of such behavioral responses.

INTRODUCTION

The maternal environment plays a tremendous role in fetal growth and development during gestation, and manipulations during this time can either improve or impair calf performance. Prenatal heat stress can alter endocrine dynamics, reduce immune function, reduce calf birth and weaning weight, and potentially reduce future milk yield potential of calves (Collier et al., 1982b, Tao et al., 2012, Strong et al., 2015). Prenatal stress during cow transport can reduce cortisol clearance during stressful events, altering the physiological response to stress (Lay et al., 1997). Further, undernutrition of cows during the first trimester resulted in calves with potentially suboptimal fertility, enlarged aortic trunk size, and increased blood pressure (Mossa et al., 2013). Therefore, stress during pregnancy can likely cause impaired performance of calves early in life and potentially into their productive lives.

Exercise increased cortisol during and post exercise in calves and pregnant heifers (Kuhlmann et al., 1985, Arave et al., 1987, Piguet et al., 1994) and may impose similar risks to calves in utero, such as immune dysfunction, altered physiological response to stress, and reduced performance, as seen prenatal with heat stress, transportation stress and malnutrition. However, consumers perceive welfare to be greater on pasture dairies due to freedom to express natural behaviors (Hemsworth et al., 1995) and a number of benefits may be associated with increased access to physical activity, including reduced disease and health disorders (Gustafson, 1993, Popescu et al., 2013) and improved immune function (Raidal et al., 2000, Navarro et al., 2004). Determining the benefits and drawbacks of physical activity may improve recommendations of physical activity levels during gestation for calf welfare.

Therefore, before implementation and recommendation of increased physical activity on farms, an understanding of the risks to calves should be understood. While studies have investigated the impacts of different stressors during gestation of calf performance, none have determined how physical activity may influence calf performance. The objective of this study was to determine the impact of maternal total confinement, pasture access, or exercise during late-gestation on calf performance, behavior, and cortisol concentrations during disbudding and weaning.

MATERIALS AND METHODS

Calves, Housing, and Management

Fifty-five Holstein and five Jersey-Holstein crossbred calves were enrolled into the study during gestation. Calves were removed from cows immediately once observed by farm staff, weighed, moved into a straw deep-bedded hutch, and fed 3.8 L of colostrum during one or two feedings. Calves were fed 1.9 L of milk replacer to 28 d of age and 2.8 L to 60 d of age 2× daily at 0500 and 1500 h. Water and grain starter were available ad libitum inside the hutch with additional water available outside the hutch.

Maternal Treatments

Calves were born from pregnant, non-lactating Holstein (n = 58) and Jersey-Holstein crossbred (n = 2) dairy cows assigned to either control (n = 20 cows; 13 female calves, 7 male calves), exercise (n = 20 cows; 8 female claves, 12 male calves), or pasture (n = 20 cows; 11 female calves, 9 male calves) treatments at dry-off using rolling enrollment from January to November 2015. Cows were balanced on parity (1.8 ± 0.9), projected ME FCM (13,831 ± 2,028 kg per lactation), and projected calving date using a 60-d dry period (58.5 ± 5.4 d). Cows were housed in a naturally ventilated, 4-row head-to head freestall barn with drive-through feed bunk and sand-bedded freestalls at the University of Tennessee's Little River Animal and Environmental Unit (Walland, TN). When parturition was imminent or cows reached their projected parturition date, cows were moved to a maternity pen. After parturition, cows were comingled into the lactating pen within the freestall barn.

Cows were enrolled into treatments on the day of dry-off and treatments were discontinued when cows were moved into the maternity pen. Cows assigned to control remained in the pen at all times except for general management reasons (i.e. cleaning, rebedding stalls). Cows assigned to exercise were removed from the pen 5× per week and walked for 1.4 ± 0.1 h at 1.88 ± 0.58 km/h over 2.66 ± 0.88 km. Cows on pasture were moved into a 21,080 m^2 pasture (Pasture 1) from January to April 2015 and a 4,159 m^2 pasture (Pasture 2) from April to December 2015 5× per week for 2.0 ± 0.3 h on Pasture 1 and 1.7 ± 0.3 h on Pasture 2.

Behavior

Data loggers (HOBO Pendant G Data Logger, Onset Computer Co., Bourne, MA) were attached to the rear fetlock of calves in a horizontal orientation using bandaging tape (Co-Flex, Andover Healthcare, Inc., Salisbury, MA) 3 days prior to and removed 6 d after disbudding and weaning to monitor changes in lying behavior (Bonk et al., 2013). Data were summarized by day to determine daily lying time.

Cortisol Measurement

Plasma total cortisol concentration was determined by a radioimmunoassay procedure using a commercially available kit (ImmunChem Cortisol 125 | RIA Kit, BP Biomedical, LLC, Orangeburg, NY). Inter- and intra-assay CV for the low control (7 ng/mL) was 42.9% and 27.6%, respectively, and 27.5% and 9.7%, respectively, for the high control (25 ng/mL).

Disbudding

Calves were disbudded at 25.6 ± 10.0 d of age. Six mL of 2% (20 mg/mL) lidocaine were administered at each cornual nerve 0 to 10 min prior to disbudding. Calves were disbudded using an electrically heated disbudder (brand) with the iron applied to the horn bud from 10 to 20 s depending on calf age and horn bud size. Six mL of blood was collected via the jugular in sodium heparin tubes (BD Vacutainer, BD, Franklin Lakes, NJ) at -24.0 ± 1.8, 0 ± 0, 1.1 ± 0.1, and 3.8 ± 0.3 h relative to disbudding. Calves were weighed on d -2.9 ± 0.1, -1.0 ± 0.1, 0.1 ± 0.1, 1.0 ± 0.1, 3.0 ± 0.1, 5.0 ± 0.1, and 7.0 ± 0.2 relative to disbudding. Starter grain was weighed daily from 3 d prior to 7 d after weaning to determine daily intake.

Weaning

Calves were abruptly weaned at 62.1 ± 2.4 d of age. Calves were fed a morning bottle at 0500 h and did not receive a bottle at 1500 h. Blood was collected into 6mL sodium heparin tubes (BD Vacutainer, BD, Franklin Lakes, NJ) via the jugular at -26.9 ± 2.3, -2.8 ± 2.3, 21.2 ± 2.2, and 45.5 ± 2.3 h relative to 1500 h on the day of weaning. Calves were weighed on d -3.1, -1.1, -0.1, 0.9, 2.8, 4.9, and 6.8 ± 0.1 relative to 1500 h on the day of weaning. Starter grain was weighed daily from 3 d prior to 7 d after weaning to determine daily intake.

Statistics

The observational unit of this study was the calf and the experimental unit was the calf. Data were analyzed using the MIXED procedure of SAS (SAS 9.3, SAS Inst., Cary, NC). Calf within maternal treatment was considered the random variable in all models. Explanatory variables included maternal treatment (control, exercise, and pasture), day (d -3 to 6), and maternal treatment × day to analyze lying behaviors. Cortisol values were transformed using a forth root transformation to normalize the data (Miller and Plessow, 2013). Cortisol was analyzed with maternal treatment, time (disbudding: -24, 0, 1, and 4 h; weaning: -24, 0, 24, and 48 h), and maternal treatment × time as explanatory variables. Explanatory variables used to analyze birth weight and gestation length included maternal treatment, sex (male or female), and maternal treatment × sex. Feed intake and weight gain at disbudding and weaning were analyzed using maternal treatment, day (feed intake: d -3, -1, 0, 1, 3, 5, and 7; weight gain: d -3, -2, -1, 0, 1, 2, 3, 4, 5, 6 and 7), maternal treatment × day as explanatory variables.

RESULTS

Birth Data

There was no interaction of treatment and sex on calf birth weight (*P* = 0.90; Figure 19) or gestation length (*P* = 0.15) and no effect of sex on gestation length (*P* = 0.45). However, males calves were born heavier than female calves (43.7 ± 1.0 vs. 40.3 ± 1.1 kg; *P* = 0.03). Treatment tended to affect birth weight (control: 39.8 ± 1.4 kg; exercise: 41.8 ± 1.4 kg; pasture: 44.3 ± 1.4 kg; *P* = 0.09) and gestation length (control: 277.3 ± 1.2 d; exercise: 277.6 ± 1.2 d; pasture: 280.7 ± 1.2 d; *P* < 0.10) with the primary difference occurring between calves from pasture and control cows. Six calves were not enrolled after birth and included three that died before or during calving (pasture: 2 female, 1 male), two that were euthanized due to illness (pasture: 1 female; control: 1 female), and one calf that was euthanized due to a broken leg from calving (exercise: 1 female). One calf died from illness (male Holstein from pasture treatment cow) between disbudding and weaning and was not included in weaning data.

Calf Performance

At disbudding, treatment × day (Figure 20; $P = 0.90$) and treatment ($P = 0.63$) did not affect weight gain. Calves gained less weight on d 1 compared with d -3, -1, 0, 3, 5, 7, on d 0 compared with d 3 and 5, and on d -3 compared with 3 ($P \leq 0.04$; Table 5). A treatment × day interaction affects calf feed intake ($P = 0.03$). Calves from pasture cows had a tendency to consume more feed on d 4 compared with calves from control cows (0.72 ± 0.10 vs. 0.47 ± 0.10 kg $P = 0.08$; Figure 22). Day also affected calf feed intake ($P < 0.0001$), where calves ate less on d -3, -2, -1, 0, and 1 compared with d 3, 4, 5, 6, and 7 ($P \leq 0.04$; Table 5). At weaning, treatment × day ($P = 0.25$; Figure 21) and treatment ($P = 0.83$) did not affect weight change. Weight gain did differ by day ($P < 0.0001$) with calves gaining less weight on d 0 and 1 compared with d -3, -1, 3, 5, and 7 (Table 5). Treatment × day ($P = 0.88$; Figure 23) and treatment ($P = 0.14$) did not affect feed intake at weaning. Feed intake did differ by day ($P < 0.0001$), with weights on d -3, -2, -1, and 0 being similar, then increasing gradually from d 1 to 7 (Table 5).

Behavior

At disbudding, there was no significant effect of treatment, day, or their interaction on lying time ($P \geq 0.78$; Figure 24) or lying bout frequency ($P \geq 0.31$; Figure 26). Regardless of treatment at disbudding, calves laid down for longer bouts on d -1 compared with d -3, 0, 1, 2, 3, 4, 5, and 6 (30.9 ± 1.6 vs. 27.0 ± 1.7, 26.1 ± 1.6, 27.7 ± 1.7, 26.9 ± 1.7, 25.4 ± 1.7, 26.3 ± 1.7, 25.4 ± 1.7, and 25.9 ± 1.7 min/bout, respectively; $P \leq 0.04$) and tended to lay down more frequently on d -1 compared with d -2 (30.9 ± 1.6 vs. 28.5 min/bout; $P = 0.09$). There was no treatment or treatment × day effect on lying bout duration at disbudding (Figure 25). At weaning, there was no significant effect of treatment, day, or their interaction on lying bout duration ($P \geq 0.18$; Figure 28) and no effect of treatment × time on lying time (Figure 27; $P =$

0.40) and lying bout frequency (Figure 29; *P* = 0.44). However, both lying time and lying bout frequency were affected by treatment and day. Calves born from pasture cows laid down more frequently than calves born from exercised cow (35.6 ± 2.4 vs. 46.7 ± 2.7 bouts/d; *P* < 0.01; Figure 31) and laid down for less time than calves born from exercise or control treatment cows (15.7 ± 0.3 vs. 16.7 ± 0.3 and 16.5 ± 0.3 h/d, respectively; *P* < 0.04;
Figure 30). Further, regardless of maternal treatment, calves laid down more frequently on d -2, -1, 0, and 1 compared with d 2, 3, 4, 5 and 6 (*P* < 0.01; Table 6) and d -3 compared with d 2 and 5 (*P* ≤ 0.04; Table 6). Further, calves laid down for more time on d 1 compared with d 0, 3, 4, 5, and 6 (*P* < 0.05; Table 6), and d -3, -2, -1, and 1 compared with d 3, 5, and 6 (*P* < 0.05; Table 6).

Cortisol Measurement

Treatment and treatment × time did not affect cortisol concentration at disbudding or weaning (Figure 32 and Figure 33). Cortisol concentration was lowest 4 h after disbudding compared with h -24, 0, and 1 (1.93 ± 0.49 vs. 6.08 ± 0.47, 5.23 ± 0.47, and 6.82 ± 0.49 ng/mL; P < 0.0001) and tended to be higher 1 h after disbudding compared with immediately after (6.89 ± 0.47 vs. 5.20 ± 0.49; *P* = 0.09). Calves also had higher cortisol concentrations the day after weaning compared with the day of and two days after (10.25 ± 0.47 vs. 2.59 ± 0.51 and 1.92 ± 0.47; *P* < 0.0001).

DISCUSSION

The objective of this study was to determine the effect of maternal physical activity on calf performance and cortisol concentrations during stressful life events. At disbudding, calves gained less weight the day after and tended to have elevated cortisol concentrations 1 h after disbudding, regardless of maternal treatment. At weaning, calves gained less weight the day of and after and had elevated cortisol concentrations the day after weaning, regardless of treatment.

However, calved from pasture cows laid down for less time compared with control and exercise maternal treatments and less frequently than exercise maternal treatments.

Birth Data

Calves from pasture cows tended to be heavier than those from exercise and control cows; however, these calves gestated longer. Gestation length can have a positive weak to medium (R = 0.15 to 0.52) correlation with birth weight, depending on breed, season, sex, and sire and dam weight (Andersen and Plum, 1965) and the additional days in utero likely caused increased weight gain in calves.

Disbudding

Maternal treatment did not alter the calf’s ability to cope with the stress of disbudding and all calves displayed similar performance, behavioral, and physiological responses to stress. Disbudding is a painful procedure and calves often show behavioral signs of pain and discomfort from disbudding, including increased head shaking, lying bouts, and hind leg kicks and decreased grooming, rumination, rubbing, and head jerks 4 hours after disbudding (Morisse et al., 1995, Graf and Senn, 1999, Grøndahl-Nielsen et al., 1999). Calves increased lying bout duration and tended to reduce lying bout frequency the day prior to disbudding. However, this was not likely due to the procedure itself and potentially a result of the calves’ initial response to blood collection, as their first exposure was 24 h before disbudding. Handling may impose a stress on calves (Boandl et al., 1989, Wohlt et al., 1994); however, researchers attempted to utilize low stress handling during blood collection (no chute, blood collection within pen while standing with only one or two people) to minimize a confounding cortisol response. Further, though behavior differed, total daily lying time remained the same, indicating calves compensated for the changes in behavior. Morisse et al. (1995) demonstrated similar lying times in calves during the 24 h period prior to and following disbudding. This indicates that, though disbudding is stressful, calves display the majority of behavioral modification during the 2 to 4 h period after disbudding (Morisse et al., 1995, Petrie et al., 1996, Graf and Senn, 1999) and later compensate for important behaviors, such as lying, which is important in young, growing animals that need greater amounts of sleep (Rechtschaffen, 1998, Siegel, 2005).

Though lying behavior did not differ on the day of disbudding, cortisol concentration tended to increase from 0 to 1 h after disbudding. These results are similar to those previously reported (Laden et al., 1985, Morisse et al., 1995, Petrie et al., 1996) indicating a cortisol spike 30 min to 1 h after disbudding with concentrations returning to basal 4 to 24 h later (Morisse et al., 1995). In the current study, cortisol concentrations 4 h after disbudding fell below those 24 h prior. While this may be a depletion of glucocorticosteroids from storage, otherwise termed as "shock" (Selye, 1955, Friend, 1991), it is more likely an adaptation of the calf to handling. The alteration of behavior due to the initial blood collection indicates that calves were likely stressed and the final sample at 4 h may be a more accurate representation of basal cortisol concentrations.

The stress of disbudding is further illustrated by the reduction in daily weight gain and plateauing of feed intake on d 0 and 1. In contrast, Laden et al. (1985) demonstrated that, at 4 wk intervals, disbudded calves grew at similar rates to those that did not experience disbudding while Grøndahl-Nielsen et al. (1999) observed no differences in feed intake or weight gain during the 7 d prior to and after disbudding for disbudded and control calves. Calves in the current study may have experienced more long-term effects than those in previous studies, though the reason is unclear. Calves from pasture cows did tend to consume more feed 4 d after disbudding. However, calves from pasture cows did not gain more weight and ate similar amounts on the following days indicating that the tendency may be more driven by the changes in performance over day than the maternal treatment.

Weaning

While maternal treatment did not affect calves ability to cope with weaning through performance and physiologic measures, calves born from pasture cows exhibited modified behavior at weaning. The period of weaning offers a variety of stressors as calves are typically exposed to new environments, diets, and social relationships. These stresses cause both physiological responses, including reduced feed intake, weight gain, and gastrointestinal function, and behavioral responses, including increased activity and vocalizations (Fraser et al., 1998). Calves born from pasture cows spent less time lying compared with calves from control and exercise treatments but were less restless than calves from exercise cows during the study period. These results are somewhat contradictory as calves spending more time standing may be indicative of hunger (Thomas et al., 2001, De Paula Vieira et al., 2008, Eckert et al., 2015), while increased restlessness is also indicative of weaning stress (Jonasen and Krohn, 1991). However, though cortisol did not statistically differ between maternal treatments, calves from exercise cows demonstrated a higher numerical concentration of cortisol 24 h after weaning. Therefore, while calves from both pasture and exercise cows experienced behavioral modifications around weaning, the maternal stress of exercise may impose a greater risk to the calf's ability to cope with future stress, while maternal stress from pasture access may not negatively impact calves stress capacities later and life. Calves exposed to heat stress during late gestation experienced reduced immune function (Tao et al., 2012) while those exposed to prenatal stress during transport experienced reduced cortisol clearance and impaired stress adaptation (Lay et al., 1997). Therefore, future research may consider the use of pasture access for implementation of physical activity in late-gestation dairy cows to reduce the risk imposed on calves from increased stress. However, more research investigating the significance of lying time and restlessness around stressful events is needed to further understand the implications of such behavioral responses.

Although performance was not affected by maternal treatment, calves did reduce weight gain on d 0 and 1 relative to weaning, while increasing feed intake after weaning, similar to previous research (Sweeney et al., 2010, Eckert et al., 2015). This reduction in performance was likely attributed to a cortisol spike the day following weaning. However, concentrations returned to basal two days after weaning, suggesting that weaning was a short-term stress and calves were able to quickly cope and resume gaining weight and consuming feed.

CONCLUSIONS

Maternal treatment did not affect calves' ability to cope with the stress of dehorning, as calves displayed similar performance, behavioral, and physiological responses. However, calves from pasture cows displayed shorter lying time than calves from control and exercise cows, potentially highlighted increased stress from weaning. In contradiction, calves from exercise cows exhibited more restlessness compared with calves from pasture cows. Future research may consider the use of pasture access for implementation of physical activity in late-gestation dairy cows to reduce the risk imposed on calves from increased stress. However, more research investigating the significance of lying time and restlessness around stressful events is needed to further understand the implications of such behavioral responses.

CHAPTER VII

CONCLUSIONS

Animal welfare research is often defined using three concepts: natural living, affective state, and biological functioning. Used synergistically, these concepts can help determine whether science is inclusive of all aspects of animal welfare. These ideas were used in this research to evaluate the effect of physical activity during the dry period to improve cow welfare. This research held five main objectives, aiming to determine the effect of prepartum exercise, pasture turnout, or confinement on 1.) physical fitness and cortisol concentrations during the dry period, 2.) behavior and neutrophil function during the dry period, 3.) hoof growth and wear and sole thickness during the dry period, 4.) calving behavior and cortisol concentrations at parturition, and 5.) calf performance and cortisol concentrations at disbudding and weaning.

The first objective of this study hypothesized that exercise and pasture turnout during the dry period would improve physical fitness and reduce cortisol concentrations during and after exercise. Cortisol concentrations did decrease from dry-off to 42 d later, but this decrease was similar across all treatments suggesting that the change was related to a habituation to the routine and environment and not the treatments. L-lactate concentrations remained similar from dry-off to 42 d later and did not differ between treatments. Heart rate became less variable for exercise and pasture cows, indicating a maintenance of physical ability compared with confined cows, but heart rates did not improve with physical activity from dry-off to 42 d later. The lack of change from dry-off to 42 d later signifies that cows with exposure to exercise and pasture did not improve physical fitness. Since the biological functioning of cows did not improve with physical activity of cows, welfare remained unchanged with the addition of physical activity.

The second objective of this study hypothesized that exercise and pasture turnout during the dry period would increase activity, reduce lying time, and improve neutrophil function. Exercise cows were most active and confined cows least active during the dry period; however, lying behaviors did not change with increased physical activity, suggesting that cows did not alter daily time budgets with short daily access to physical activity. Neutrophil function did not improve with physical activity. Biological functioning was unaltered; however, increased ability to perform natural behaviors, such as grazing, may suggest pasture cows had improved welfare with increased natural living.

The third objective of this study hypothesized that exercise would increase hoof horn wear and reduce sole thickness, but pasture would not alter hoof characteristics. While exercise did increase horn wear, exercise cows exhibited a more equal rate of horn growth and wear, which is important for maintenance of healthy hooves. Confined cows tended to increase sole thickness from dry-off to 42 d later, but exercise and pasture cows experienced no change. Still, all cows maintained the recommended sole thickness to protect the claw capsule. Therefore, as exercise cows noted improved claw benefits during the short period of increased physical activity, exercise during the dry period may improve welfare through improved biological functioning. Further, natural behavior may also be improved from improved hoof health allowing for maintained freedom of movement, further improving cow welfare.

The forth objective of this study hypothesized that exercise and pasture turnout would reduce labor times, alter calving behavior, and reduce cortisol concentrations at calving. However, stage II labor times did not differ between treatments and, while cortisol concentrations decreased from d 0 to 3 postpartum, treatment did not affect cortisol level. Control cows exhibited altered behavior with less lying time and shorter lying bouts. The importance of this behavioral modification is not fully understood but may elude to discomfort in confined cows at calving and an ability to exercise and pasture cows to recover more quickly after calving. An improvement in comfort at calving would suggest an improvement in affective state, from reduced discomfort, and natural living, from less discomfort behavior, suggesting an overall improvement in welfare at calving with physical activity.

The fifth objective of this study hypothesized that the stress of physical activity would have no negative effect on calves in utero and not impair their response to stressful situations after birth, including disbudding and dehorning. Calf performance, cortisol concentrations, and behavior did not differ between maternal treatments at disbudding. Further, performance and cortisol concentrations were similar between maternal treatments at weaning. However, calves from pasture cows lied down for less time around weaning while calves from exercise cows displayed more frequent lying bouts. As reduced lying time can indicate stress from hunger and increased lying bout frequency can indicate increased stress, it is unclear which behavioral modification is a better indicator of increased stress at weaning. However, calves from pasture cows may have been exhibiting more exploratory behavior, but more research to understand the significance of behavior during stress is needed. If calves from pasture cows were more exploratory, this suggests improved welfare through increased expression of natural behavior. However, if calves from exercise and pasture cows were exhibiting more stressful behaviors, stress during maternal exercise may impair their ability to cope with stress, reducing their welfare.

Overall, physical activity during the dry period had no negative welfare implication on cows. An improvement in welfare was actually noted with improved hoof health in exercise cows and improved behavioral response to calving in exercise and pasture cows. However, a better understanding of the impact of stress from physical activity on the neonatal calf is needed to determine the overall impact of physical activity. Still it can be concluded that physical activity during the dry period did not diminish cow welfare but, instead, improved it.

Physical activity had no negative implications on cows and this research offered a number of interesting future directions for research. Cows in the present study did not improve physical fitness, potentially from too low intensity exercise; however, research studies in women noted positive benefits of low to moderate exercise, suggesting there may be a benefit to just being active instead of improving overall physical fitness. Additionally, understanding if these benefits exaggerated with activity throughout lactation and the dry period and whether there is a consequence of inactivity. Similarly, cows given access to pasture did not improve physical fitness, nor were they as active as cows exercised. Determining methods of pasture access that promote more activity, such as putting cows on pasture when they prefer to be outdoors grazing, may increase overall level of activity. Finally, research better investigating the consequences of behavioral changes during stressful periods could help understand how changes in management alter welfare, such as the importance of lying time at calving or the importance of lying time and lying bouts at weaning.

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APPENDIX

Table 1. Mean, maximum, and minimum heart rates 10 min before, during, and 60 min following exercise challenge on d 0 (n = 12) and d 42 (n = 12) for control (n = 7), exercise (n = 7), and pasture (n = 8) treatments before (pre), during (exercise), and after post) exercise challenge.

Day	Period	Treatment	Mean Heart Rate		Maximum Heart Rate		Minimum Heart Rate	
			Mean (bpm)	Range (bpm)	Mean (bpm)	Range (bpm)	Mean (bpm)	Range (bpm)
0	Pre	Control	78.4 ± 8.1	70.4 – 86.5	116.8 ± 17.6	102.3 – 136.3	53.1 ± 11.8	40.0 – 63.0
		Exercise	86.6 ± 10.7	68.5 – 101.6	120.0 ± 19.0	93.9 – 152.2	72.0 ± 7.9	59.6 – 80.0
		Pasture	105.5 ± 10.8	93.5 – 114.2	184.8 ± 17.3	168.5 – 203.0	79.5 ± 7.2	72.5 – 86.8
	Exercise	Control	81.7 ± 10.2	73.4 – 93.1	103.1 ± 14.8	91.6 – 119.8	71.9 ± 10.5	61.0 – 82.0
		Exercise	92.5 ± 7.6	86.0 – 107.0	110.3 ± 8.7	93.9 – 119.3	78.3 ± 12.9	58.8 – 95.7
		Pasture	105.2 ± 15.7	94.1 – 123.1	175.5 ± 35.2	135.0 – 198.6	71.8 ± 29.5	37.8 – 90.6
	Post	Control	73.8 ± 1.3	72.4 – 74.8	119.4 ± 43.8	89.6 – 169.7	62.8 ± 3.2	59.3 – 65.5
		Exercise	80.5 ± 5.3	73.4 – 87.9	118.0 ± 12.5	108.1 – 138.6	70.1 ± 6.3	62.4 – 78.0
		Pasture	84.5 ± 8.1	77.2 – 93.1	126.2 ± 24.3	102.0 – 150.7	72.6 ± 5.2	66.9 – 77.0
42	Pre	Control	96.3 ± 3.3	91.6 – 99.0	128.8 ± 22.5	110.6 – 159.5	83.0 ± 2.7	78.9 – 84.5

Table 1 continued.

Day	Period	Treatment	Mean Heart Rate		Maximum Heart Rate		Minimum Heart Rate	
			Mean (bpm)	Range (bpm)	Mean (bpm)	Range (bpm)	Mean (bpm)	Range (bpm)
42		Exercise	84.6 ± 7.9	76.1 – 91.7	100.0 ± 10.6	88.0 – 108.0	74.2 ± 7.9	68.5 – 83.3
		Pasture	89.2 ± 6.9	83.2 – 100.5	126.1 ± 41.3	96.9 – 198.0	76.1 ± 2.3	73.5 – 79.2
	Exercise	Control	111.0 ± 4.9	105.0 – 115.3	142.1 ± 23.6	121.9 – 176.2	96.0 ± 4.9	90.0 – 101.9
		Exercise	97.0 ± 4.6	91.8 – 100.0	110.8 ± 9.9	99.4 – 117.1	64.3 ± 33.2	26.0 – 85.4
		Pasture	106.2 ± 14.1	90.3 – 122.6	146.9 ± 46.4	98.7 – 208.0	73.7 ± 11.1	57.8 – 84.3
	Post	Control	90.7 ± 7.1	84.2 – 100.3	176.5 ± 55.8	104.2 – 233.0	74.7 ± 7.4	68.7 – 84.0
		Exercise	85.5 ± 4.5	80.4 – 88.8	117.9 ± 19.9	101.0 – 139.8	74.2 ± 5.2	68.5 – 78.7
		Pasture	85.4 ± 6.2	76.6 – 93.7	157.6 ± 52.1	98.0 – 217.8	70.1 ± 6.0	59.6 – 73.4

Table 2. Number of animals diagnosed with a health disorder by farm staff during the 7 d postpartum for control (n = 20), exercise (n = 20), and pasture treatment cows (n = 20).

Disorder[1]	Control	Exercise	Pasture
Displaced abomasum	1	0	0
Mastitis	1	2	3
Metritis	2	3	5
Ketosis	3	3	4
Milk fever	4	5	2

[1]Disorders were diagnosed using scoring systems described by (Sterrett et al., 2014).

Table 3. Health exam means (± SD) over 7 d for postpartum for control (n = 20), exercise (n = 20), and pasture treatment cows (n = 20).

Parameter[1]	Control	Exercise	Pasture
Behavioral score	1.0 ± 0.1	1.1 ± 0.3	1.1 ± 0.3
Manure score	2.2 ± 0.5	2.4 ± 0.5	2.3 ± 0.5
Rectal temperature (°C)	38.9 ± 0.4	38.9 ± 0.4	38.8 ± 0.5
Rumen fill score	2.2 ± 0.5	2.0 ± 0.4	2.1 ± 0.4
Respiration rate (breaths per min)	38.7 ± 13.5	39.1 ± 15.0	35.8 ± 11.8
Heart rate (beats per min)	82.8 ± 9.2	85.6 ± 13.0	80.4 ± 9.5

[1]Parameters assessed using scoring systems described in (Sterrett et al., 2014).

Table 4. Body condition score of cows on control (n = 20), exercise (n = 20), and pasture (n = 20) treatments.

Parameter	Day[1]							SE
	DO 0	DO 42	PP 0	PP 7	PP 14	PP 28	PP 60	
BCS								
Control	3.40	3.53	3.32	3.28	3.08	3.03	2.88	0.07
Exercise	3.55	3.61	3.30	3.16	3.07	2.87	2.88	0.07
Pasture	3.32	3.42	3.09	2.99	2.92	2.85	2.80	0.07
Mean	3.42[b]	3.52[a]	3.24[c]	3.11[d]	3.02[e]	2.92[f]	2.86[f]	0.04

[1]DO = Day relative to dry-off; PP = day relative to calving.

[abcdef]Different letters within a row indicates a difference ($P < 0.05$).

Table 5. Calf performance at disbudding and weaning.

Parameter	Day[1]											SE
	-3	-2	-1	0	1	2	3	4	5	6	7	
Disbudding												
Weight gain (kg)	0.89^{bc}		1.13^{abc}	0.83^{c}	0.06^{d}		1.46^{a}		1.40^{ab}		1.29^{abc}	0.20
Feed Intake (kg)	0.35^{ab}	0.33^{a}	0.36^{ab}	0.43^{bc}	0.36^{ab}	0.44^{c}	0.50^{d}	0.59^{e}	0.58^{e}	0.66^{f}	0.64^{ef}	0.06
Weaning												
Weight Gain (kg)	2.10^{a}		1.99^{a}	0.86^{b}	0.54^{b}		2.52^{a}		2.45^{a}		2.23^{a}	0.23
Feed Intake (kg)	1.44^{a}	1.61^{a}	1.55^{a}	1.55^{a}	2.08^{b}	2.51^{c}	2.59^{cd}	2.75^{d}	3.05^{e}	3.17^{e}	3.18^{e}	0.10

^{abcdef}Different superscripts within a row indicate significance ($P < 0.05$).

[1]Day relative to disbudding or to weaning

Table 6. Lying Behaviors of calves at disbudding and weaning.

Parameter	Day[1]										SE
	-3	-2	-1	0	1	2	3	4	5	6	
Disbudding											
Lying time (h/d)	17.3	17.5	17.0	17.3	17.4	17.5	17.4	17.2	17.4	17.4	0.3
Lying bouts (n/d)	42.1	41.5	38.9	43.6	42.0	42.8	44.1	42.3	44.2	42.7	2.3
Lying bout duration (min/bout)	27.0^{b}	28.5^{ab}	30.9^{a}	26.1^{b}	27.7^{b}	26.9^{b}	25.4^{b}	26.3^{b}	25.4^{b}	25.9^{b}	1.7
Weaning											
Lying time (h/d)	16.6^{ab}	16.6^{ab}	16.5^{ab}	16.1^{bc}	16.8^{a}	16.3^{abc}	15.9^{c}	16.3b^{c}	15.9^{c}	15.8^{c}	0.2
Lying bouts (n/d)	42.5^{ab}	44.9^{a}	44.8^{a}	44.8^{a}	44.0^{a}	38.3^{c}	39.2^{bc}	38.6^{bc}	36.7^{c}	38.5^{bc}	1.9
Lying bout duration (min/bout)	26.1	26.0	24.3	23.6	24.6	27.1	25.8	26.7	27.1	25.8	1.4

^{abcdef}Different superscripts within a row indicate significance ($P < 0.05$).

[1]Day relative to disbudding or to weaning

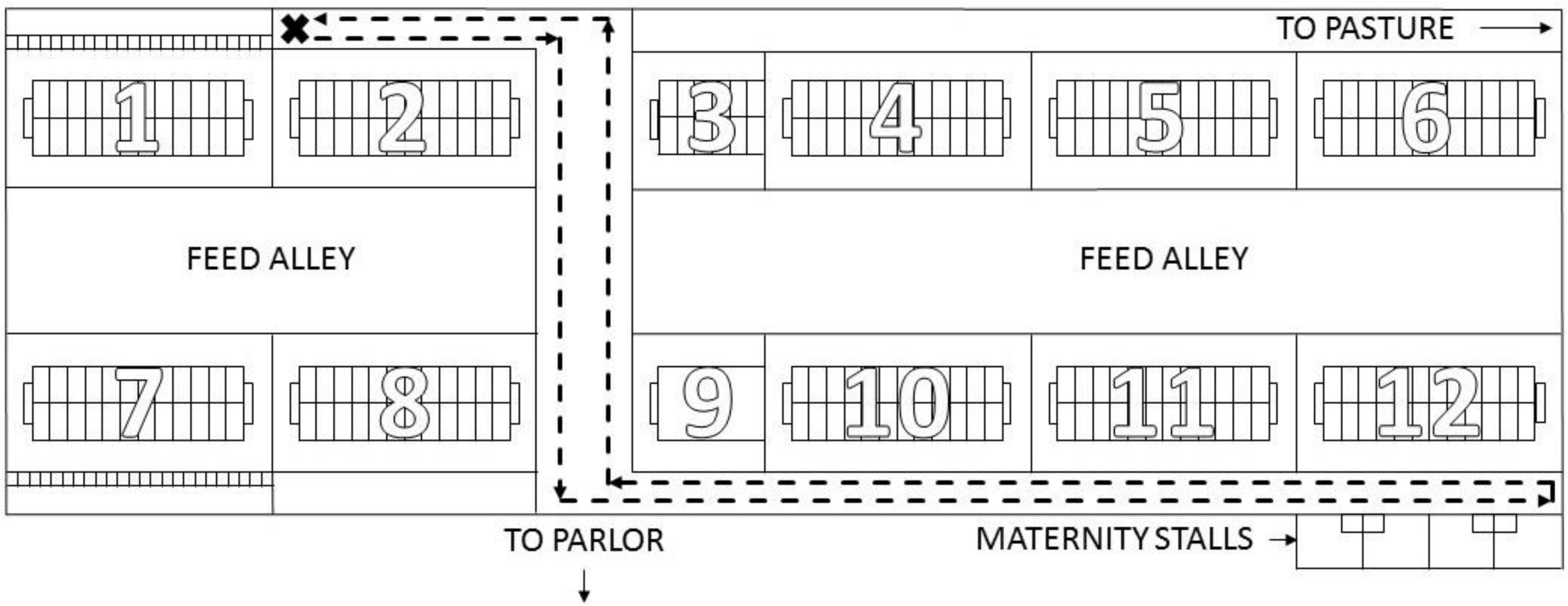

Figure 1. Diagram of University of Tennessee Little River Animal and Environmental Unit freestall barn with the exercise route marked with dashed a line and the start and end location denoted by a black X. The total distance traveled out and back equals 250 m.

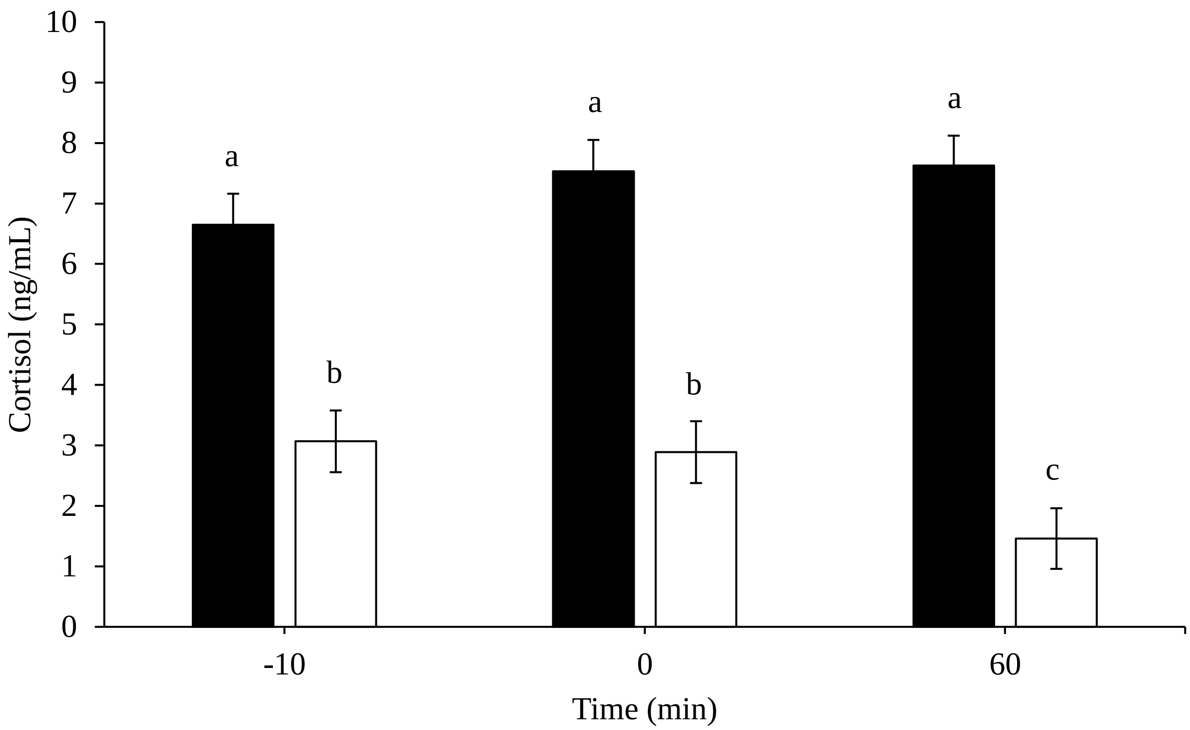

Figure 2. Cortisol concentrations on d 0 (black bars; n = 60) and d 42 (white bars; n = 60) at 10 min prior and 0 and 60 min following exercise challenge (P = 0.07).

[a,b,c]Bars with different letters are significantly different ($P < 0.10$).

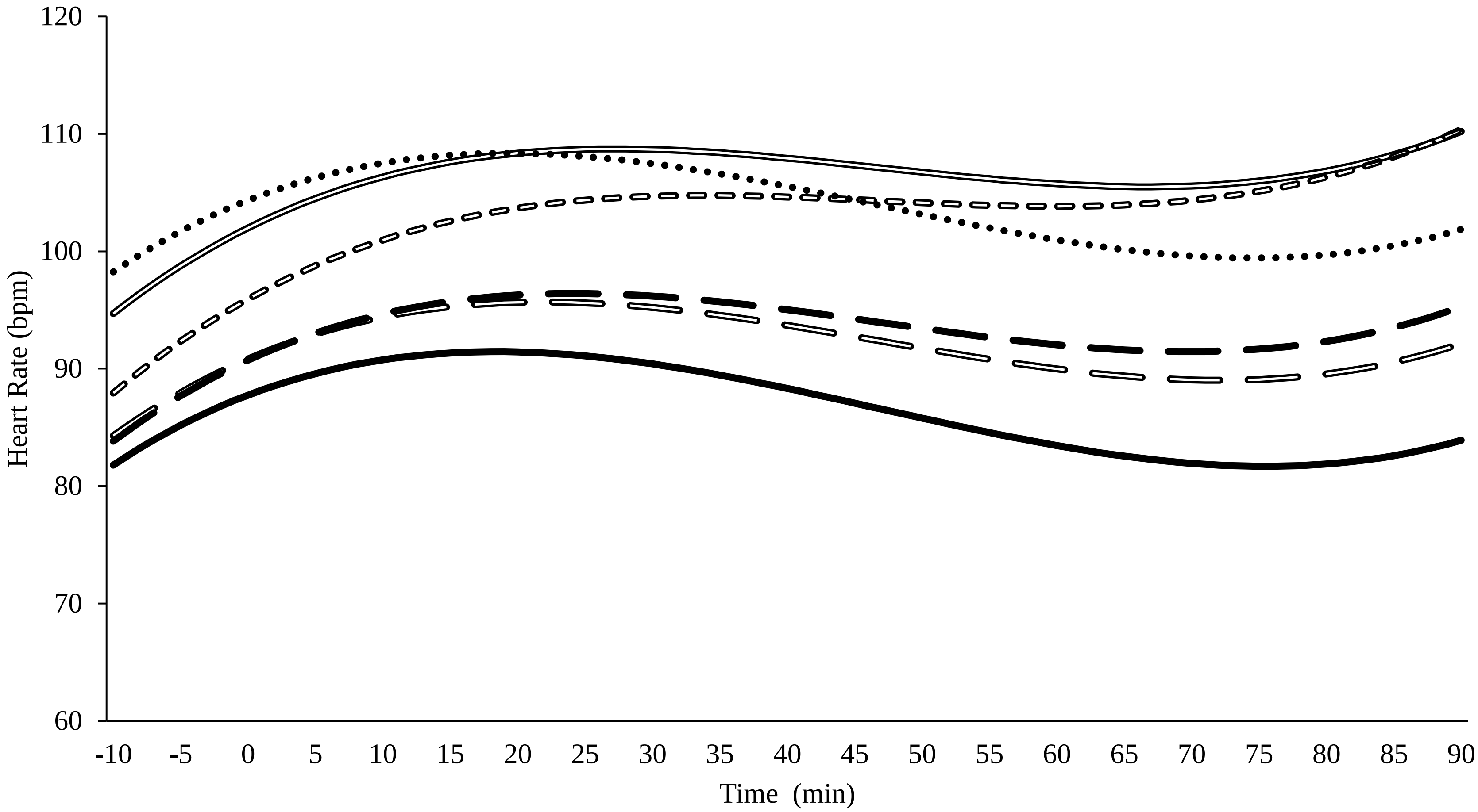

Figure 3. Heart rate prior to and during exercise challenge at dry-off (solid) and 42 days later (open) for control (dashed), exercise (dash-dot), and pasture (solid) treatments.

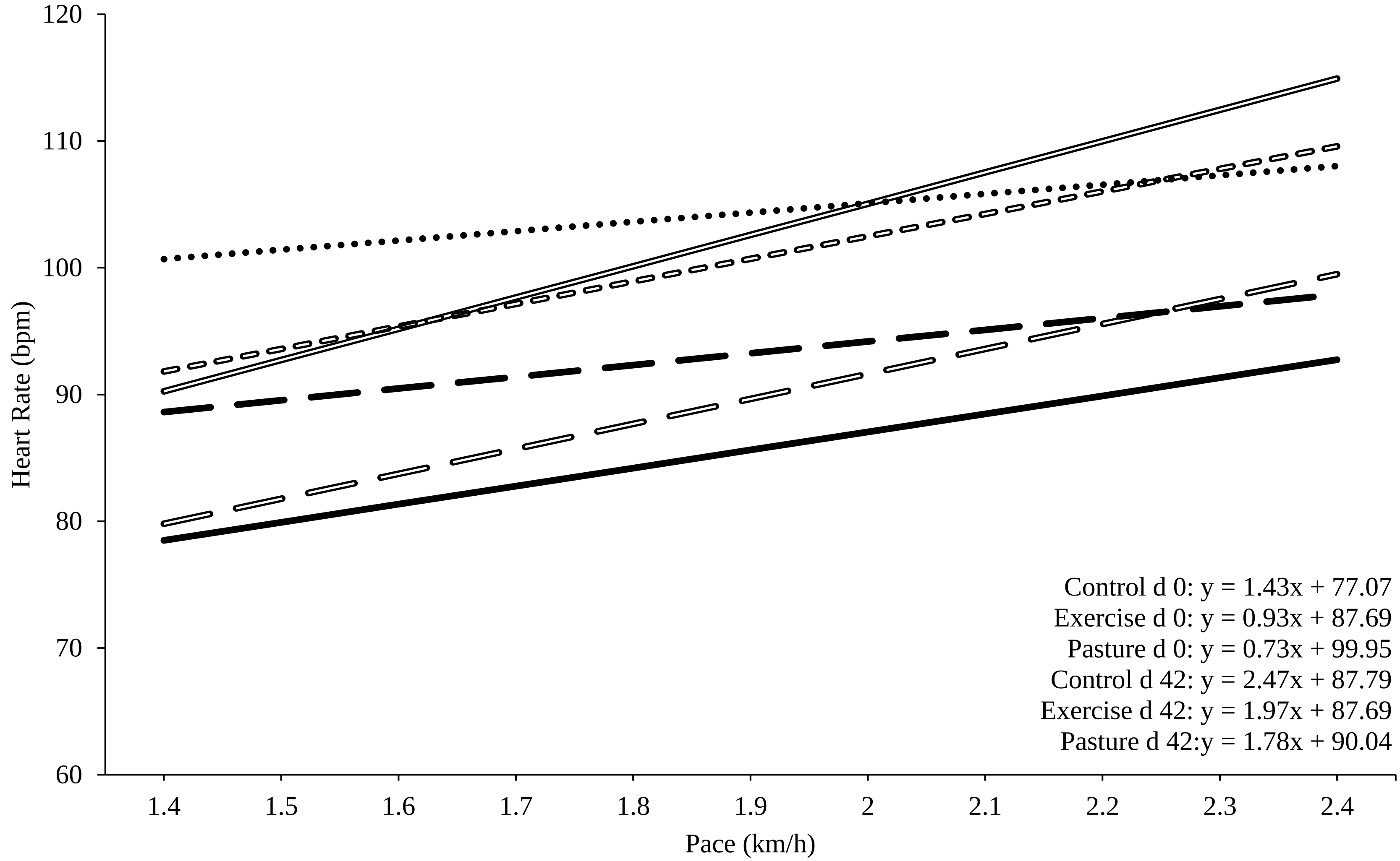

Figure 4. Heart rate with differing pace of exercise challenge at dry-off (solid lines; n = 12) and 42 days later (open lines; n = 12) for control (solid line; n = 7), exercise (dashed line; n = 7), and pasture (dotted line; n = 8) treatments.

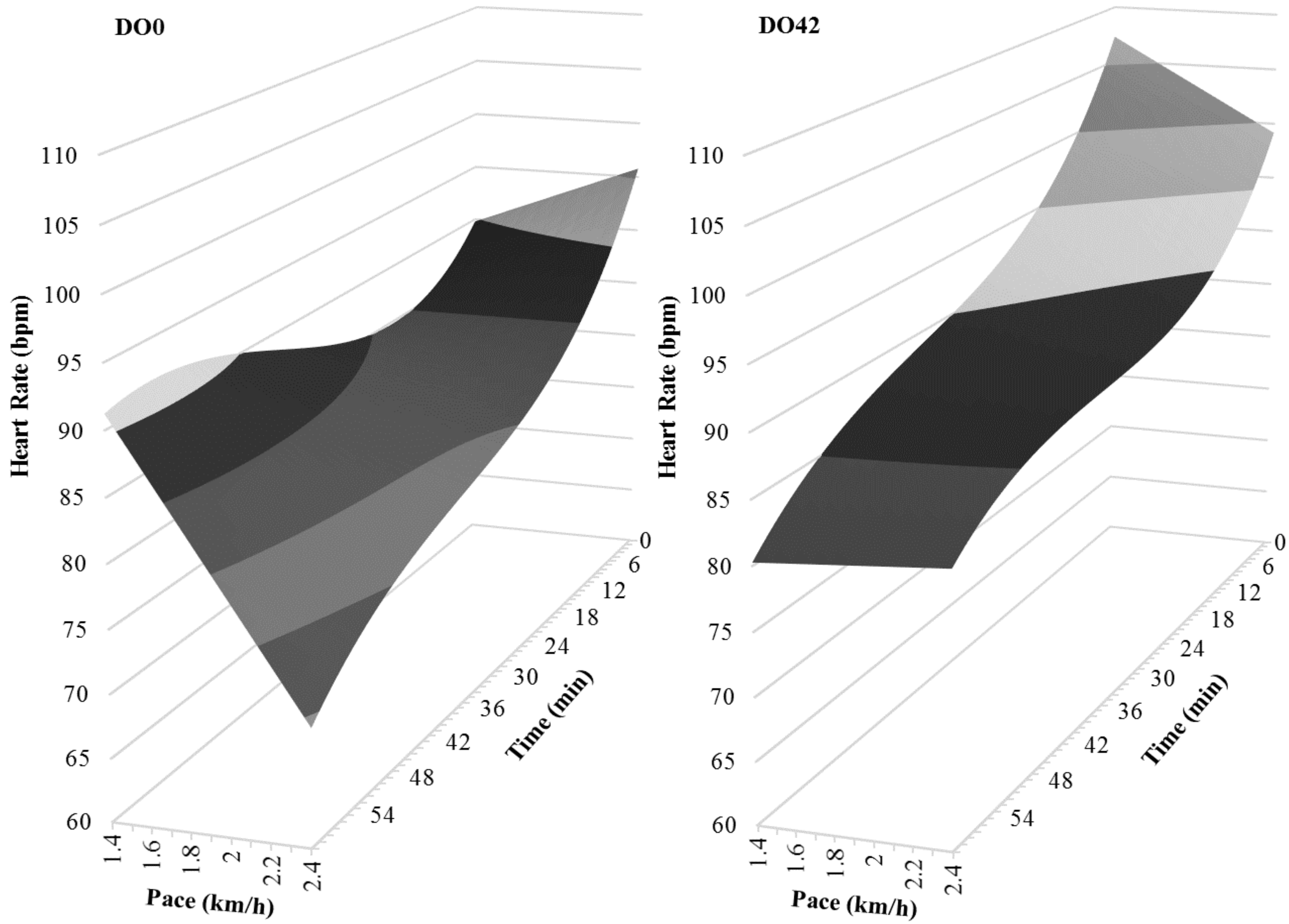

Figure 5. Heart rate with differing pace of exercise challenge at dry-off (solid lines; n = 12) and 42 days later (open lines; n = 12) for control (solid line; n = 7), exercise (dashed line; n = 7), and pasture (dotted line; n = 8) treatments.

Figure 6. Predicted heart rate by time and exercise pace following exercise challenge for control (n = 7), exercise (n = 7), and pasture (n = 8) treatments (P = 0.03).

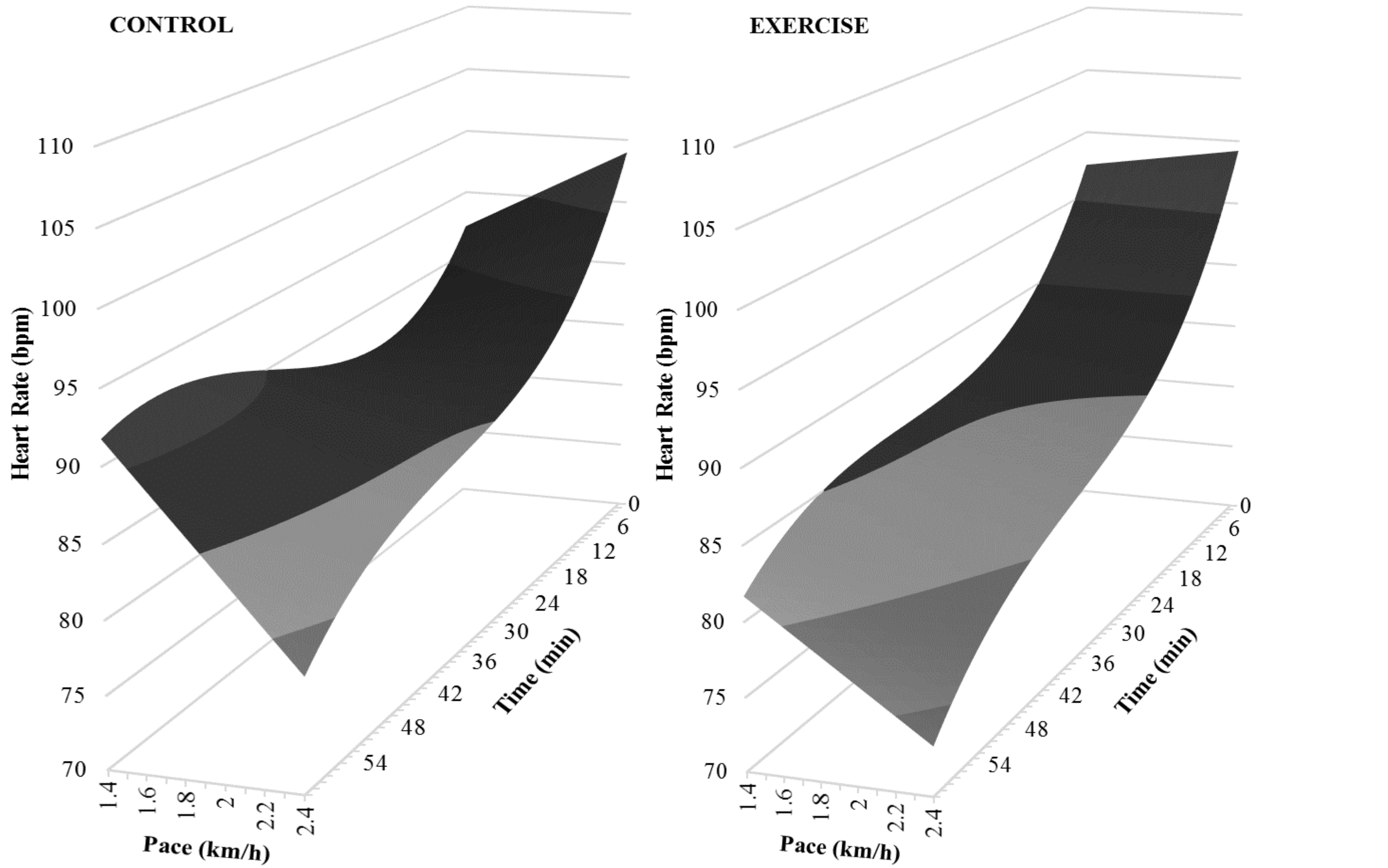

Figure 6 continued.

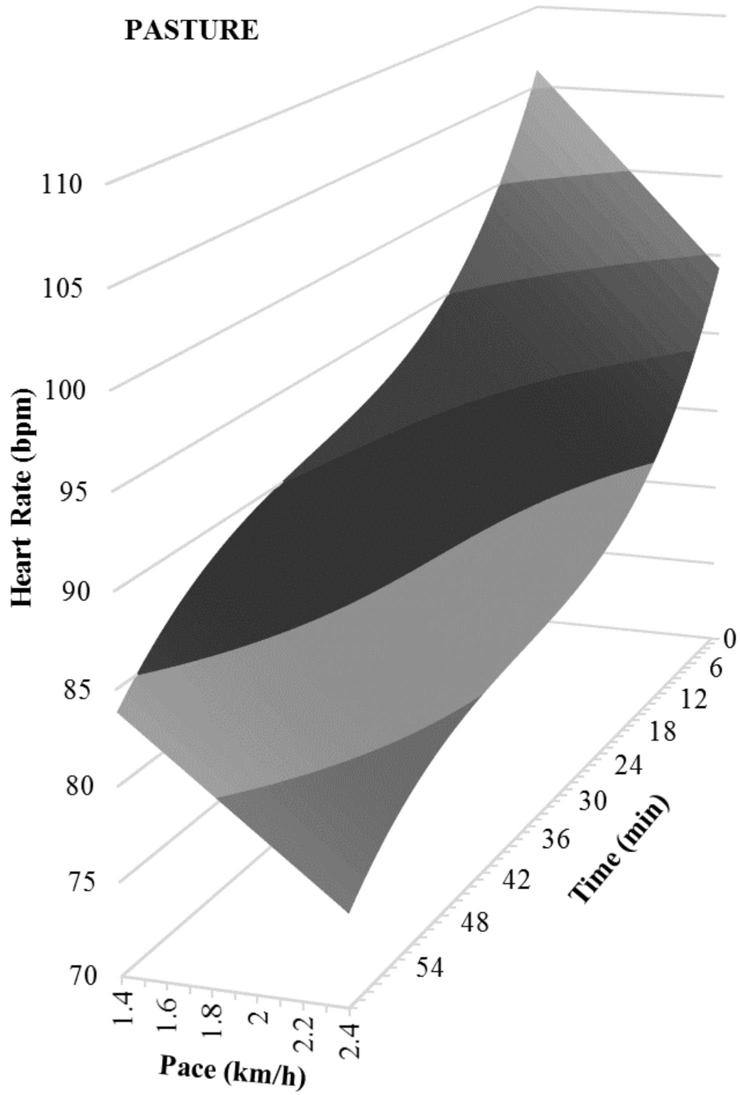

Figure 6 continued.

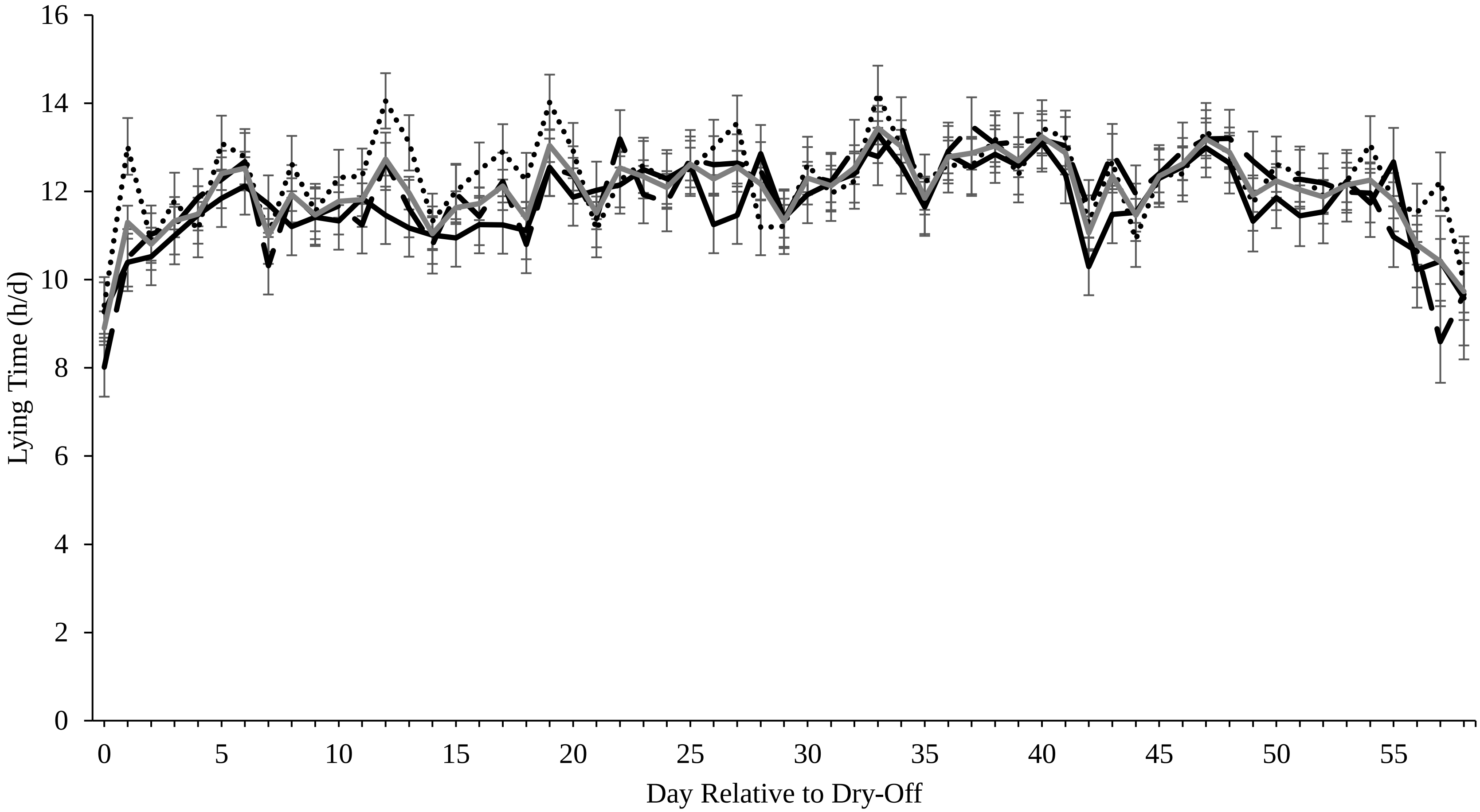

Figure 7. Daily lying time during the dry period for control (solid black line; n = 13), exercise (dashed black line; n = 14), and pasture (dotted black line; n = 14) treatments ($P = 0.16$) and the average across treatments (solid gray line; $P < 0.0001$).

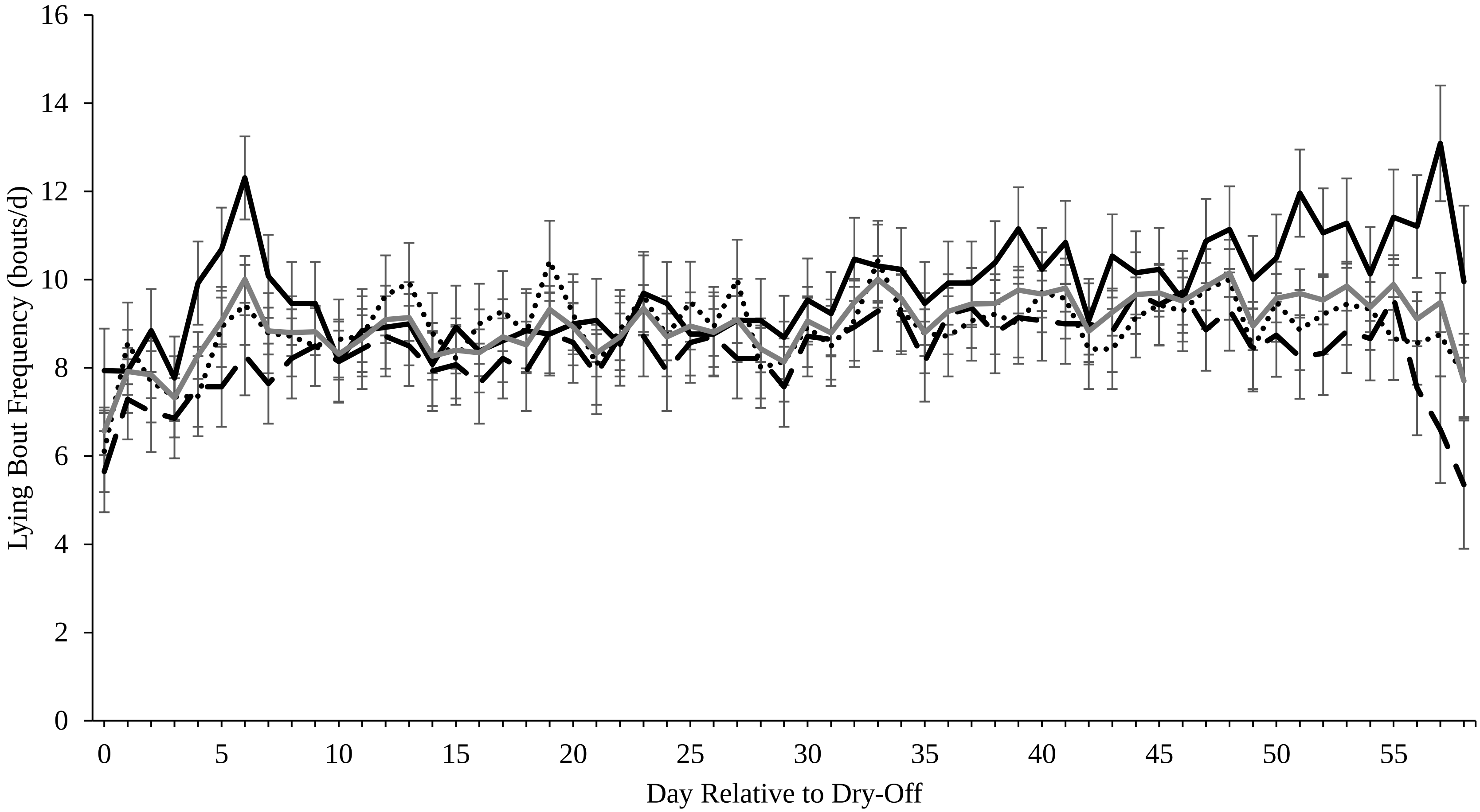

Figure 8. Lying bout frequency during the dry period for control (solid black line; n = 13), exercise (dashed black line; n = 14), and pasture (dotted black line; n = 14) treatments (P = 0.15) and the average across treatments (solid gray line; P < 0.0001).

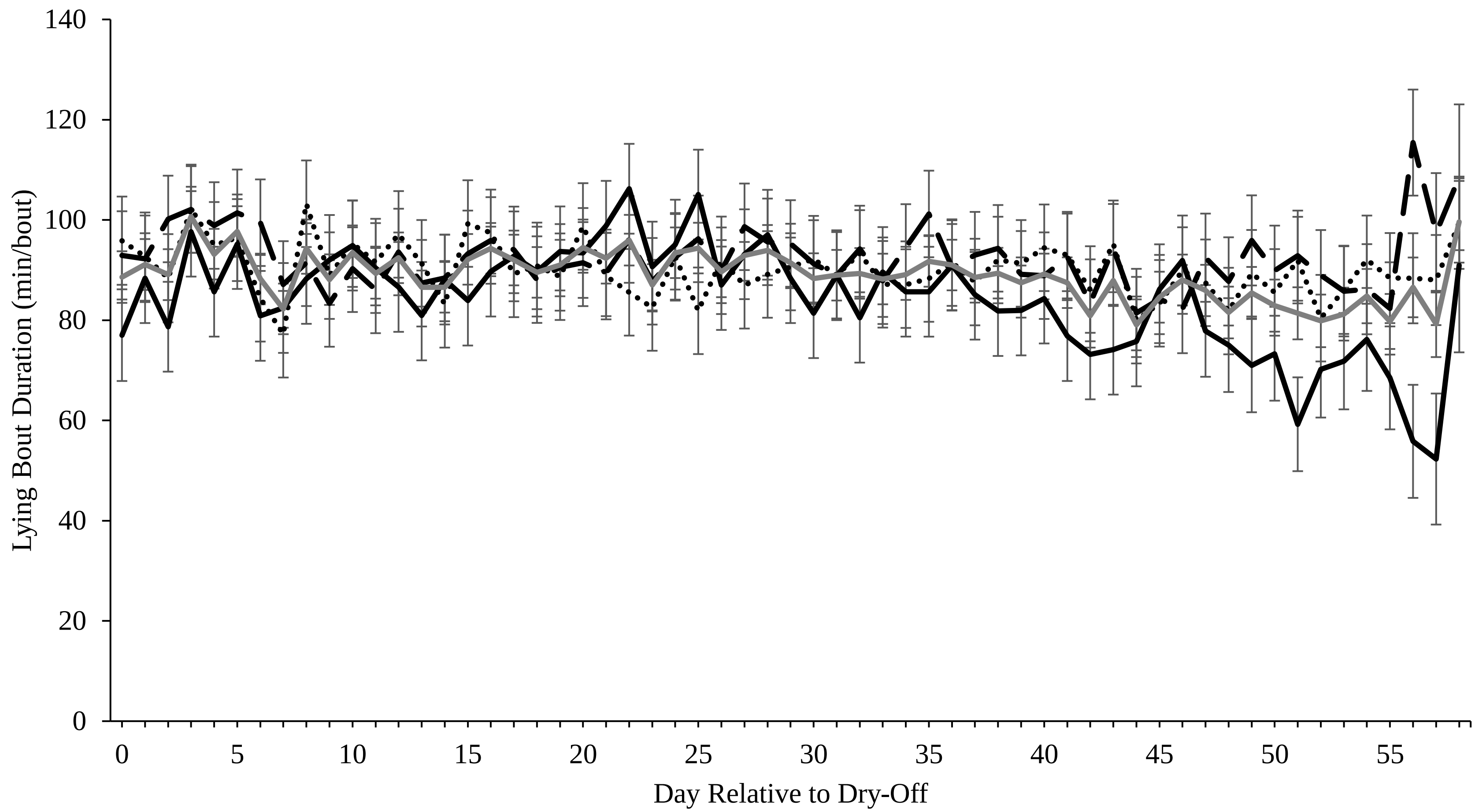

Figure 9. Lying bout duration during the dry period for control (solid black line; n = 13), exercise (dashed black line; n = 14), and pasture (dotted black line; n = 14) treatments (P = 0.22) and the average across treatments (solid gray line; P < 0.01).

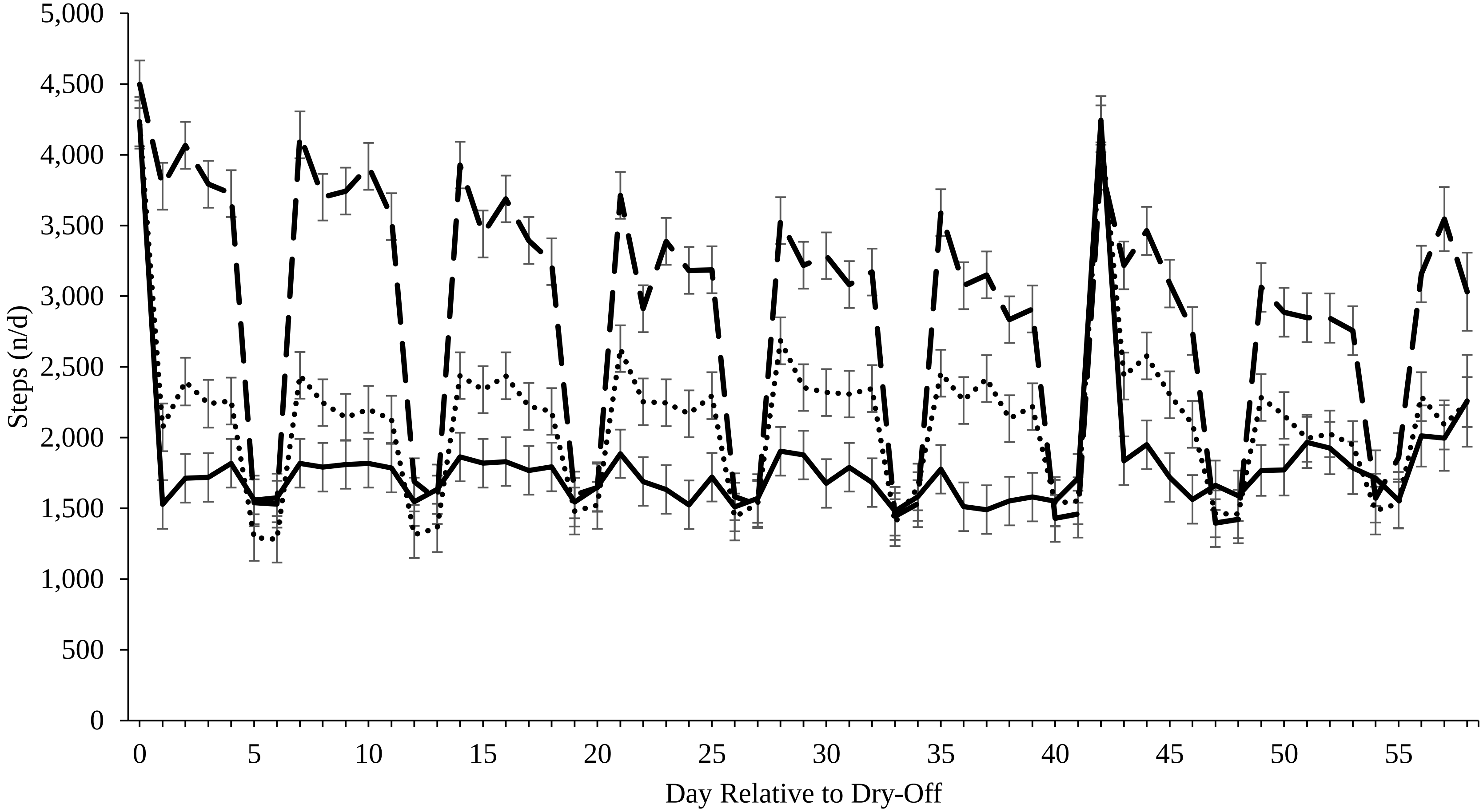

Figure 10. Daily steps during the dry period for control (solid black line; n = 13), exercise (dashed black line; n = 14), and pasture (dotted black line; n = 14) treatments (P < 0.0001).

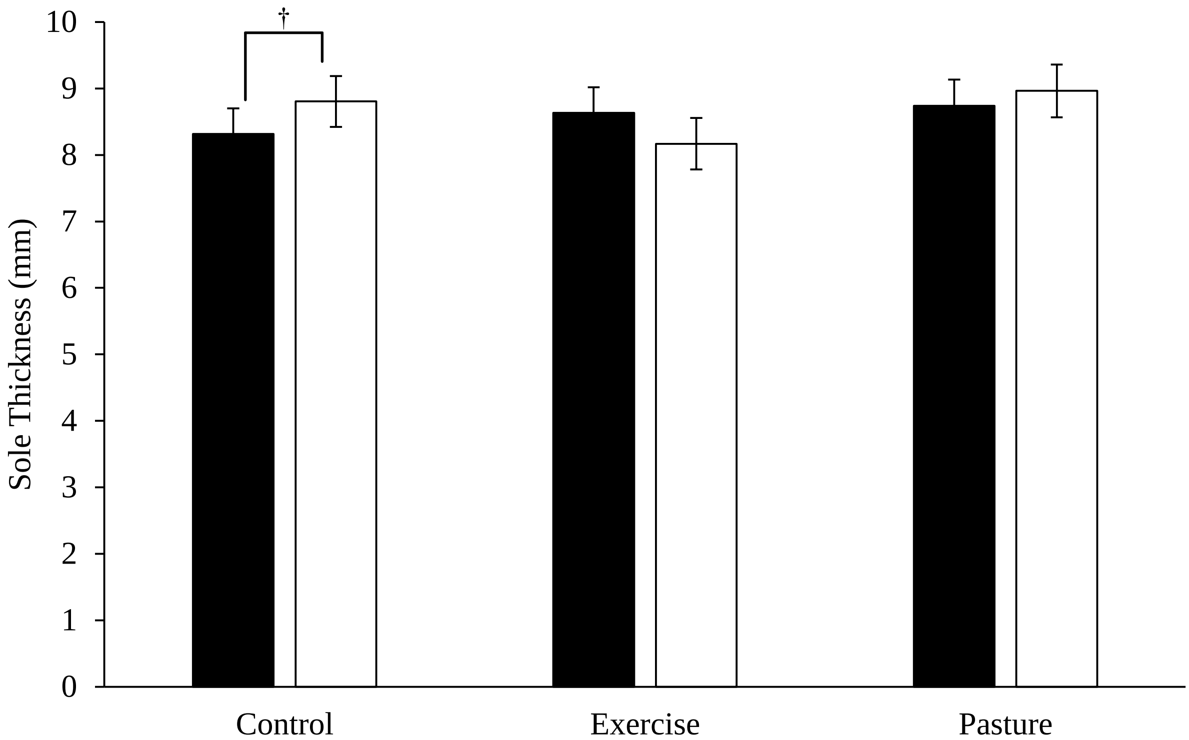

Figure 11. Sole thickness of cows on d 2 (black bars) and d 44 (white bars) relative to dry-off for control (n = 19), exercise (n = 18), and pasture treatment cows (n = 18).

†Differ at $P < 0.10$.

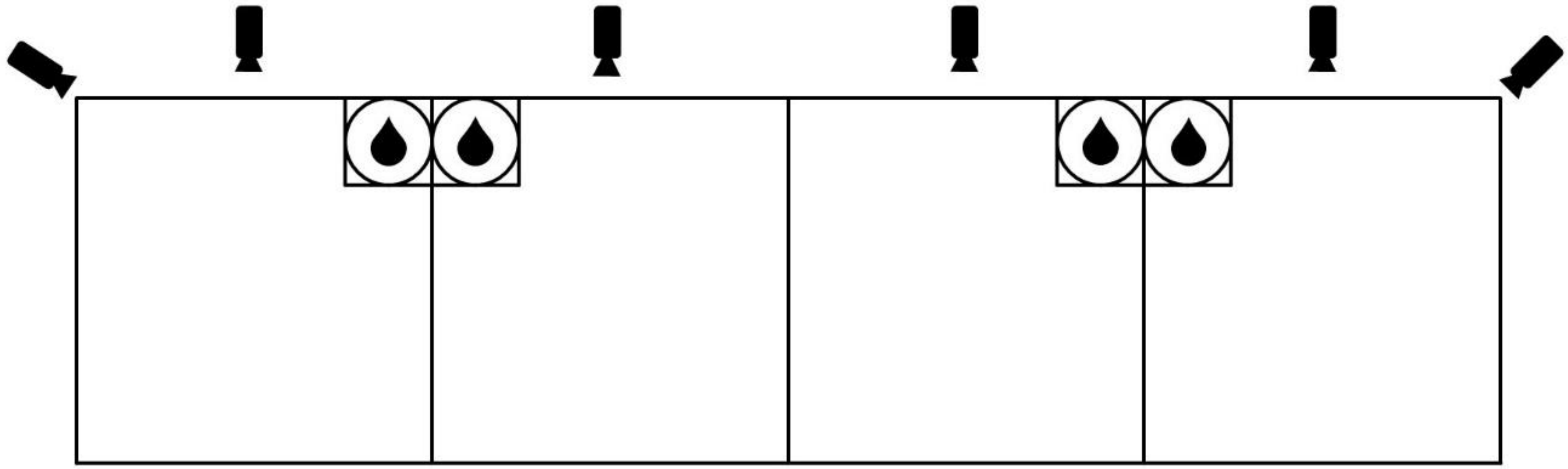

Figure 12. Location of cameras used for behavioral observation above maternity pens.

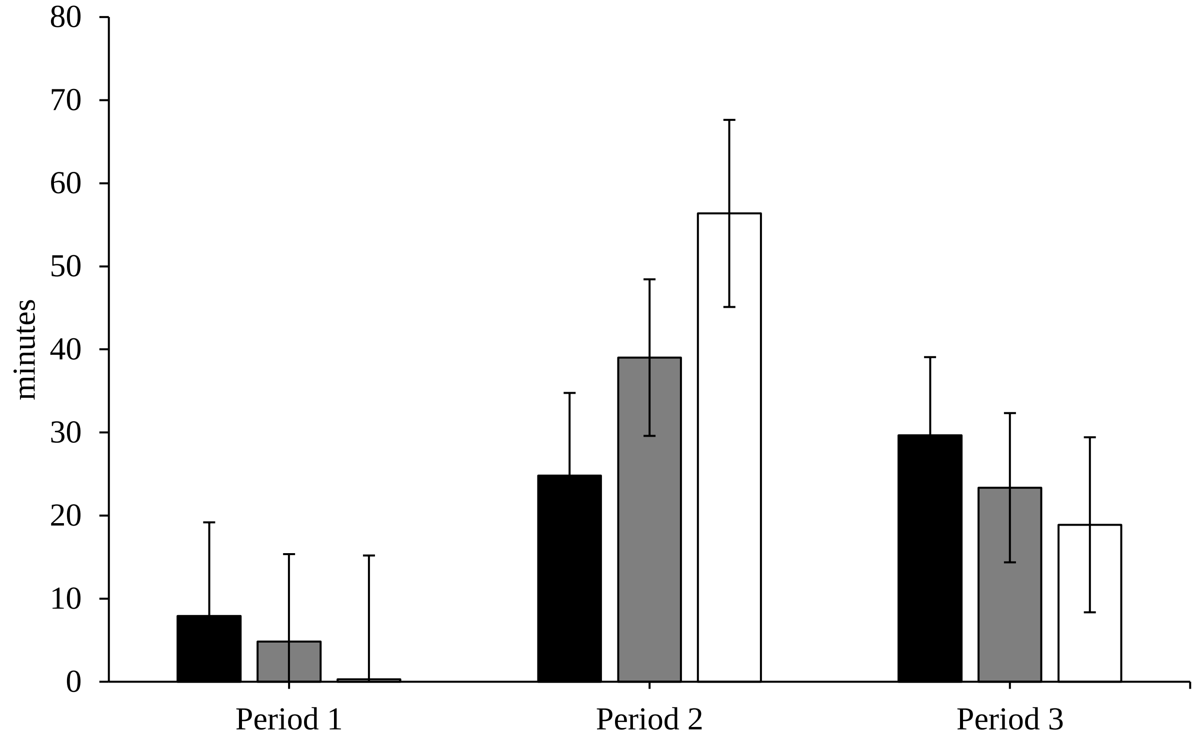

Figure 13. Time of different periods of observable labor1 for control (black bars; n = 10), exercise (gray bars; n = 11), or pasture (white bars; n = 8) treatments (P = 0.31).

[1]Period 1: time from initial observation of fetal membranes from birth canal to water breaking; Period 2: time from water breaking to initial observation of one or both of calf's feet; Period 3: time from initial observation of one or both of calf's feet to full expulsion of calf where both back (or front if breech calving) feet are visible.

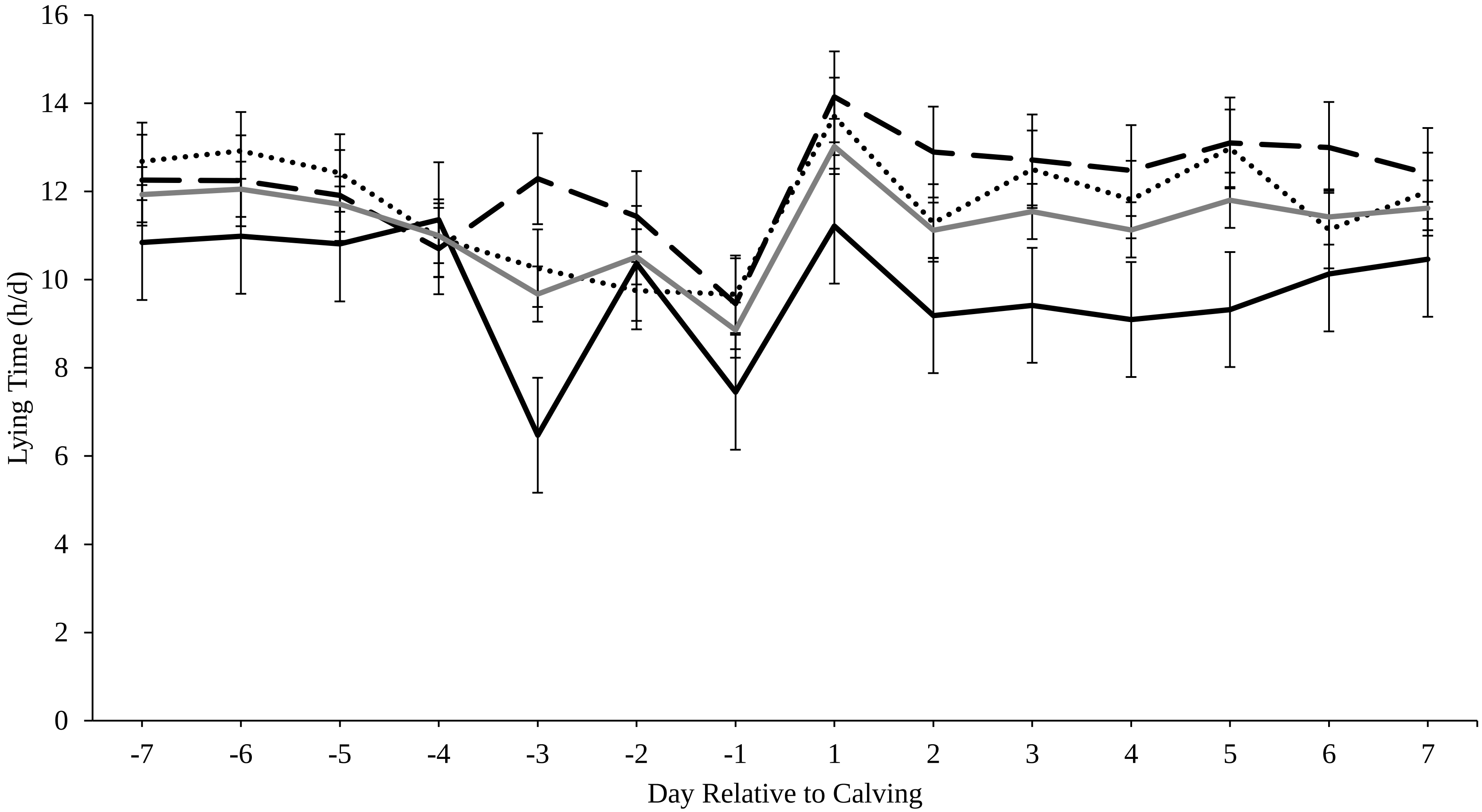

Figure 14. Daily lying time for the 7 d before and after calving for control (solid black line; n = 5), exercise (dashed black line; n = 8), and pasture (dotted black line; n = 11) treatments ($P = 0.36$) and the average across treatments (solid grey line; $P < 0.0001$).

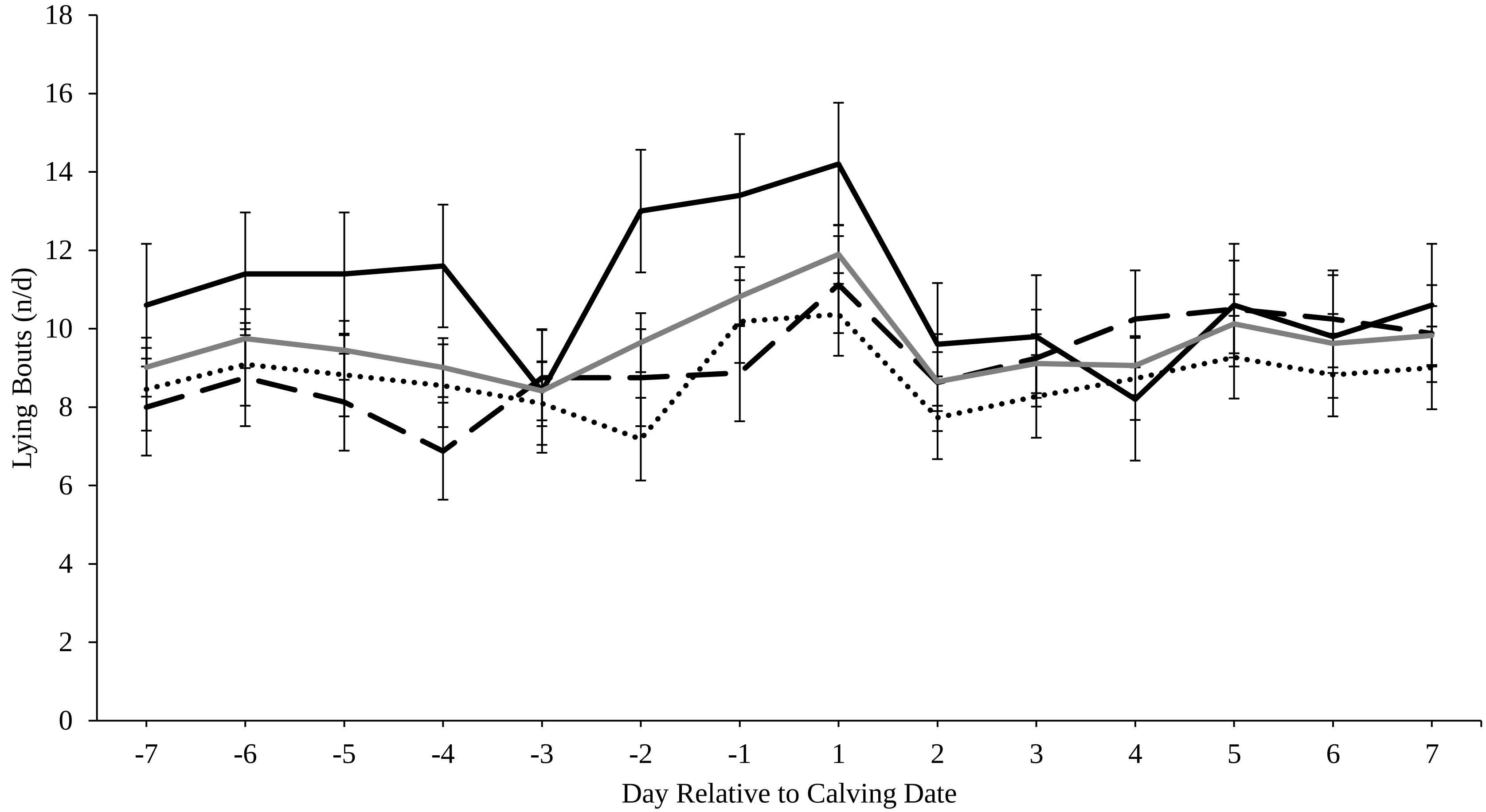

Figure 15. Lying bout frequency for the 7 d before and after calving for control (solid black line; n = 5), exercise (dashed black line; n = 8), and pasture (dotted black line; n = 11) treatments (P = 0.32) and the average across treatments (solid grey line; P < 0.01).

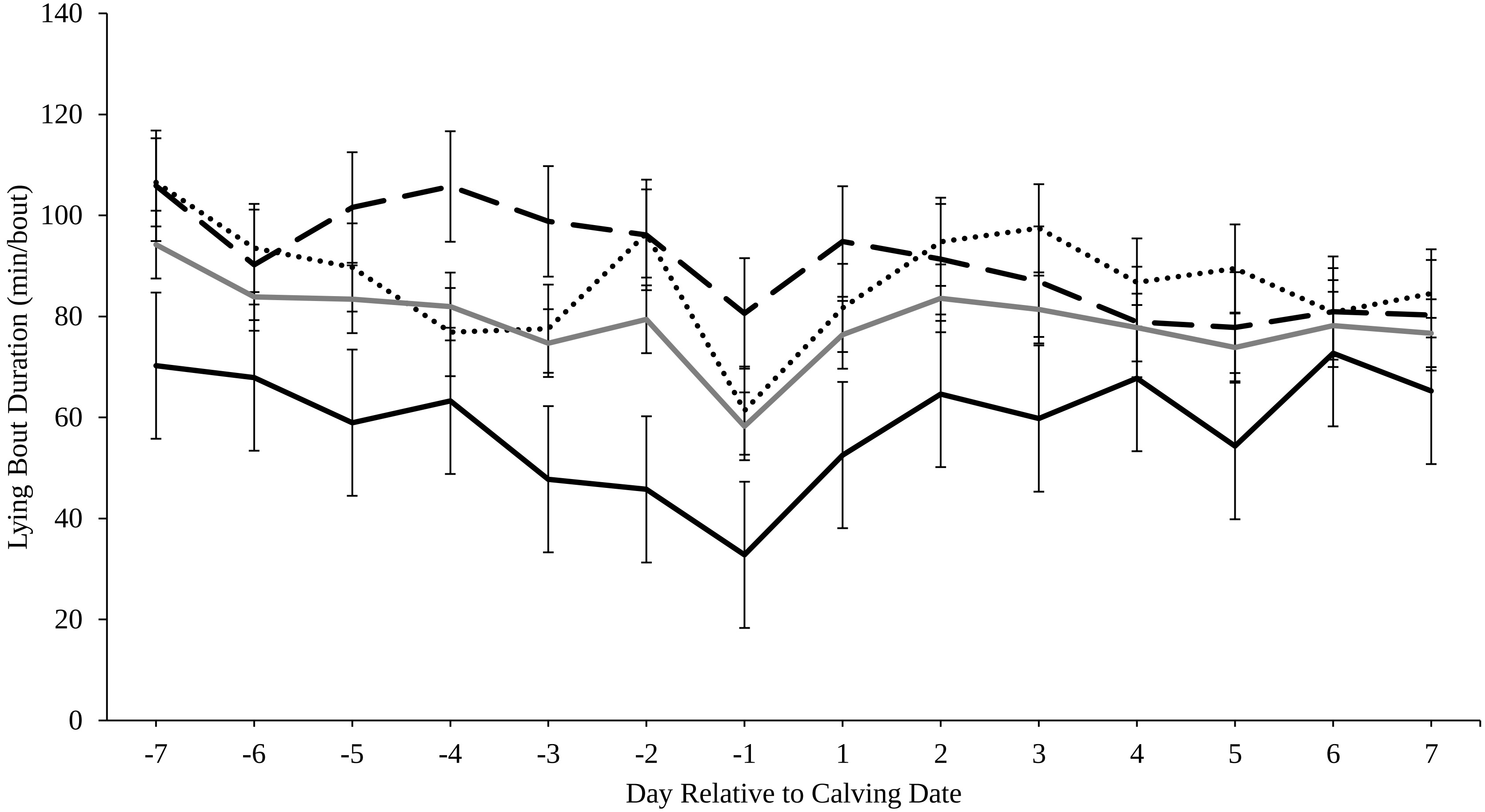

Figure 16. Lying bout frequency for the 7 d before and after calving for control (solid black line n = 5), exercise (dashed black line; n = 8), and pasture (dotted black line; n = 11) treatments (P = 0.63) and the average across treatments (solid grey line; P = 0.01).

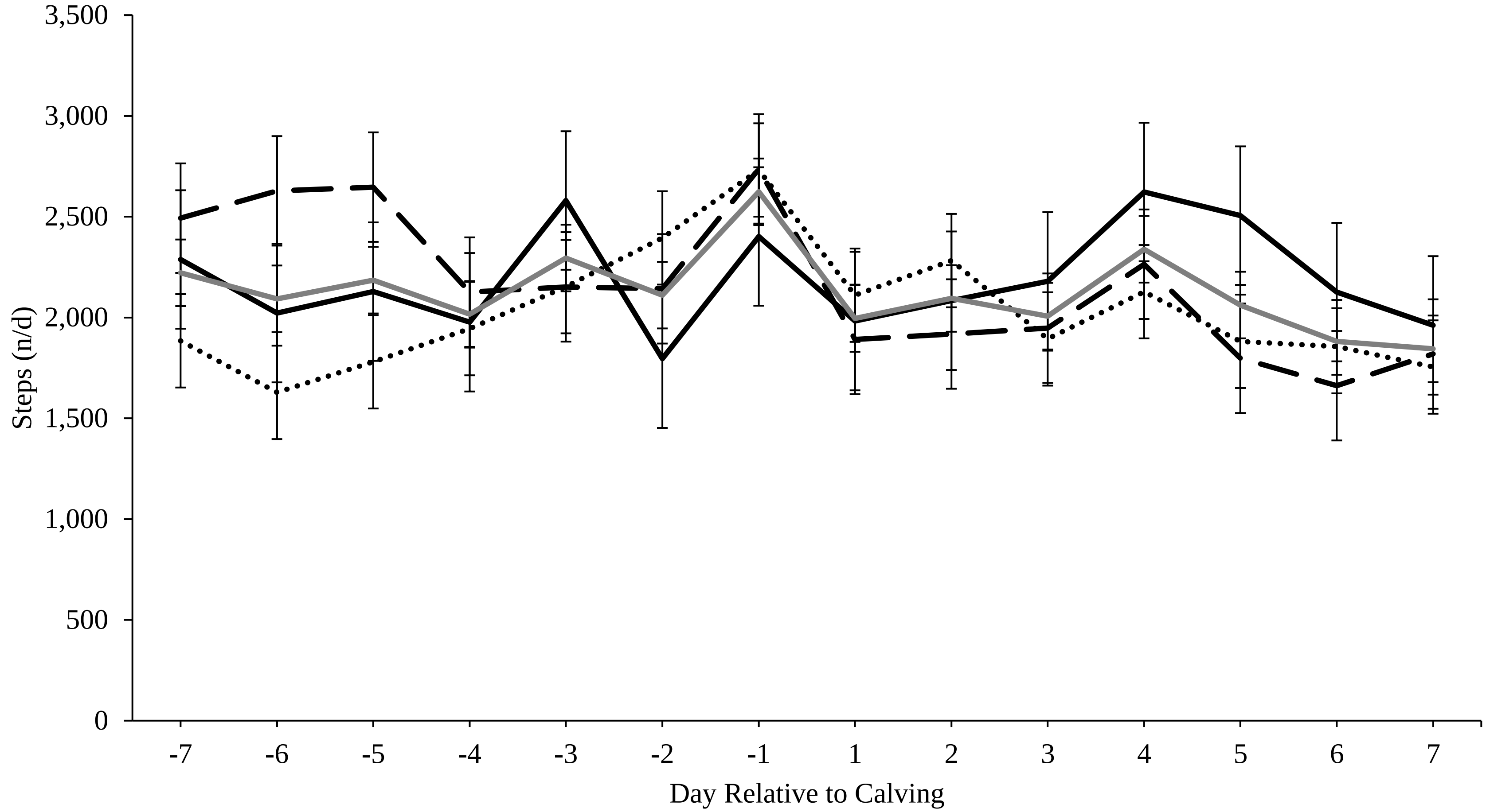

Figure 17. Daily steps for the 7 d before and after calving for control (solid black line; n = 5), exercise (dashed black line; n = 8), and pasture (dotted black line; n = 11) treatments ($P = 0.47$) and the average across treatments (solid grey line; $P < 0.001$).

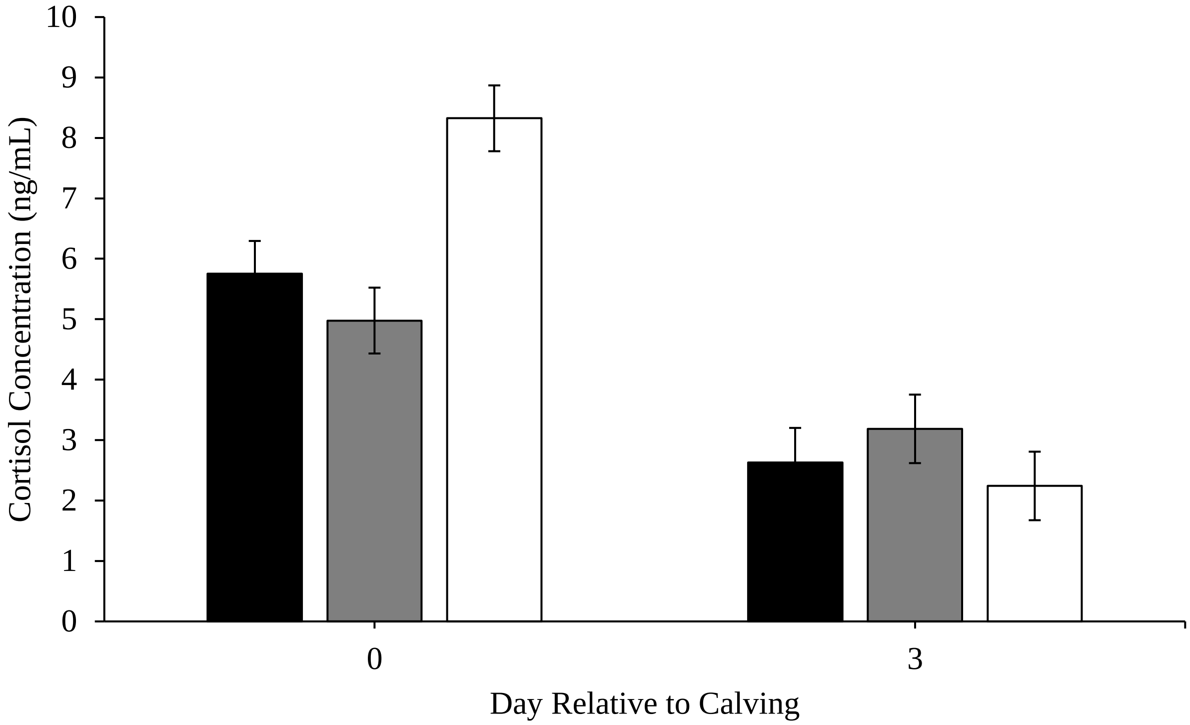

Figure 18. Cortisol concentration for control (black bars; n = 20), exercise (gray bars; n = 20), and pasture (white bars; n = 20) on d 0 and 3 relative to calving (P = 0.21).

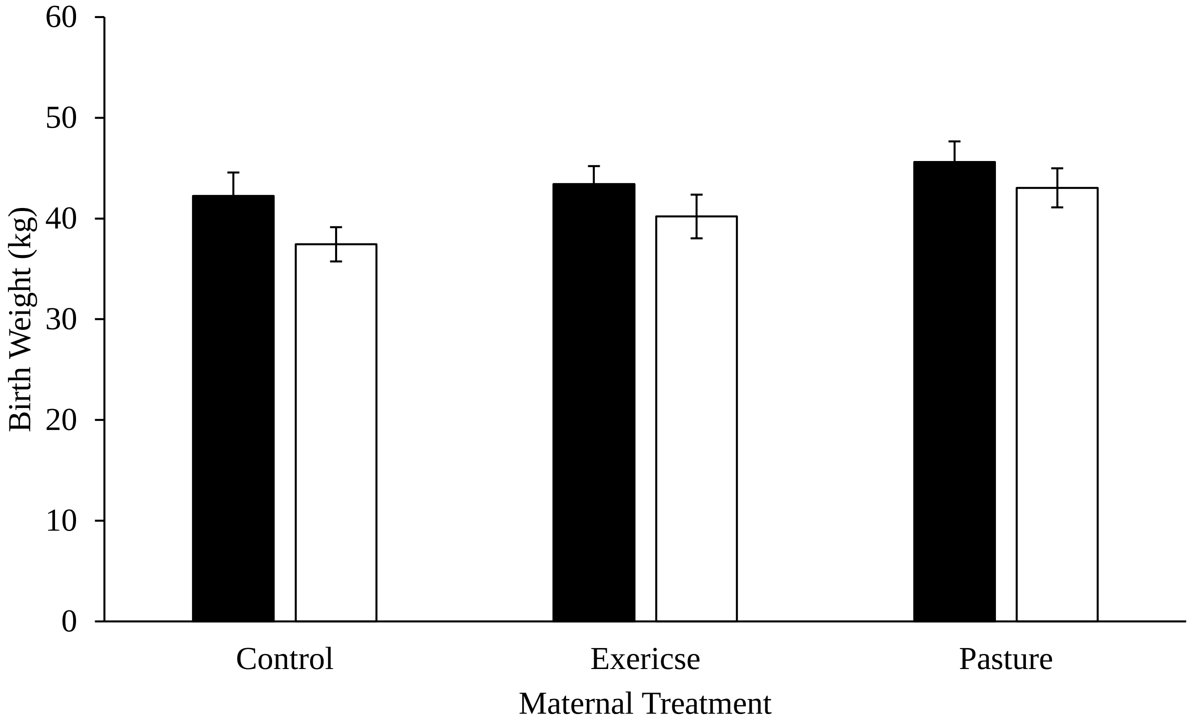

Figure 19. Male (black bars) and female (white bars) calf birth weights from control (n = 20), exercise (n = 20) or pasture (n = 20) cows (P = 90).

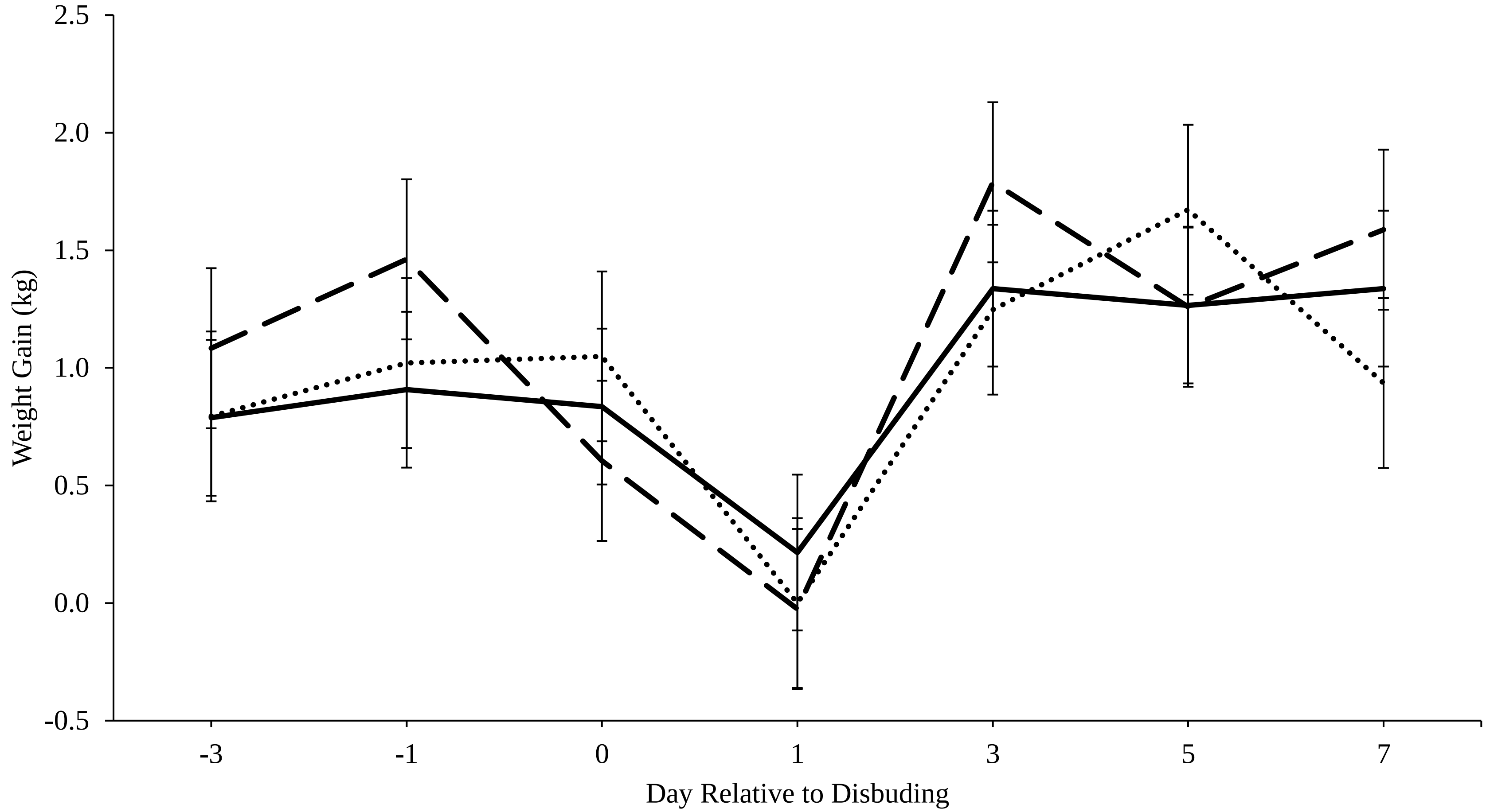

Figure 20. Weight change at disbudding for calves from control (solid line; n = 19), exercise (dashed line; n = 18), and pasture (dotted line; n = 16) (P = 0.83).

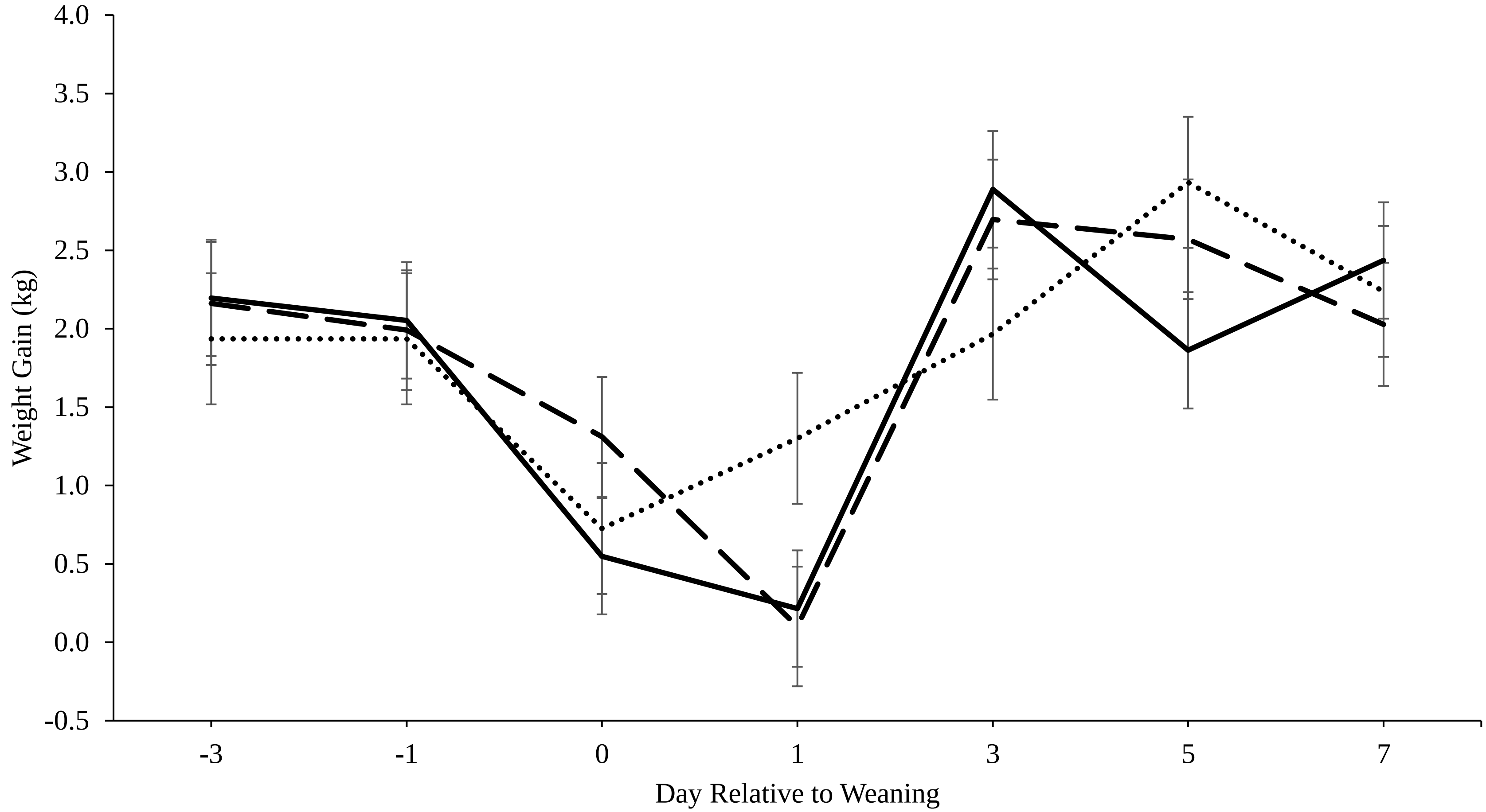

Figure 21. Weight change at weaning for calves from control (solid line; n = 19), exercise (dashed line; n = 18), and pasture (dotted line; n = 15) (P = 0.83).

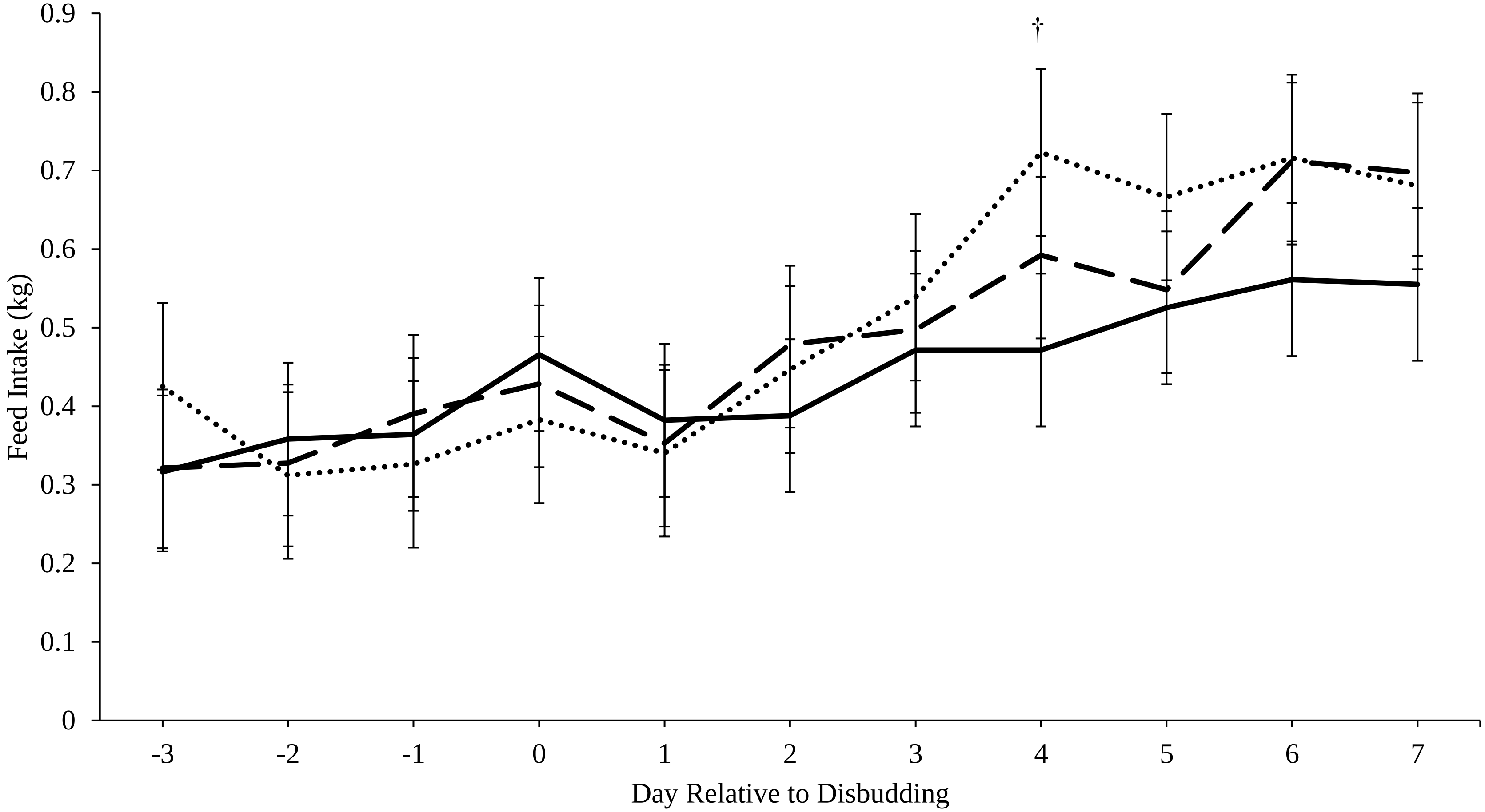

Figure 22. Feed intake at disbudding for calves from control (solid line; n = 19), exercise (dashed line; n = 18), and pasture (dotted line; n = 16) (P = 0.01).

†Pastured calves tended to consume more feed than control calves on d 4 (*P* = 0.08).

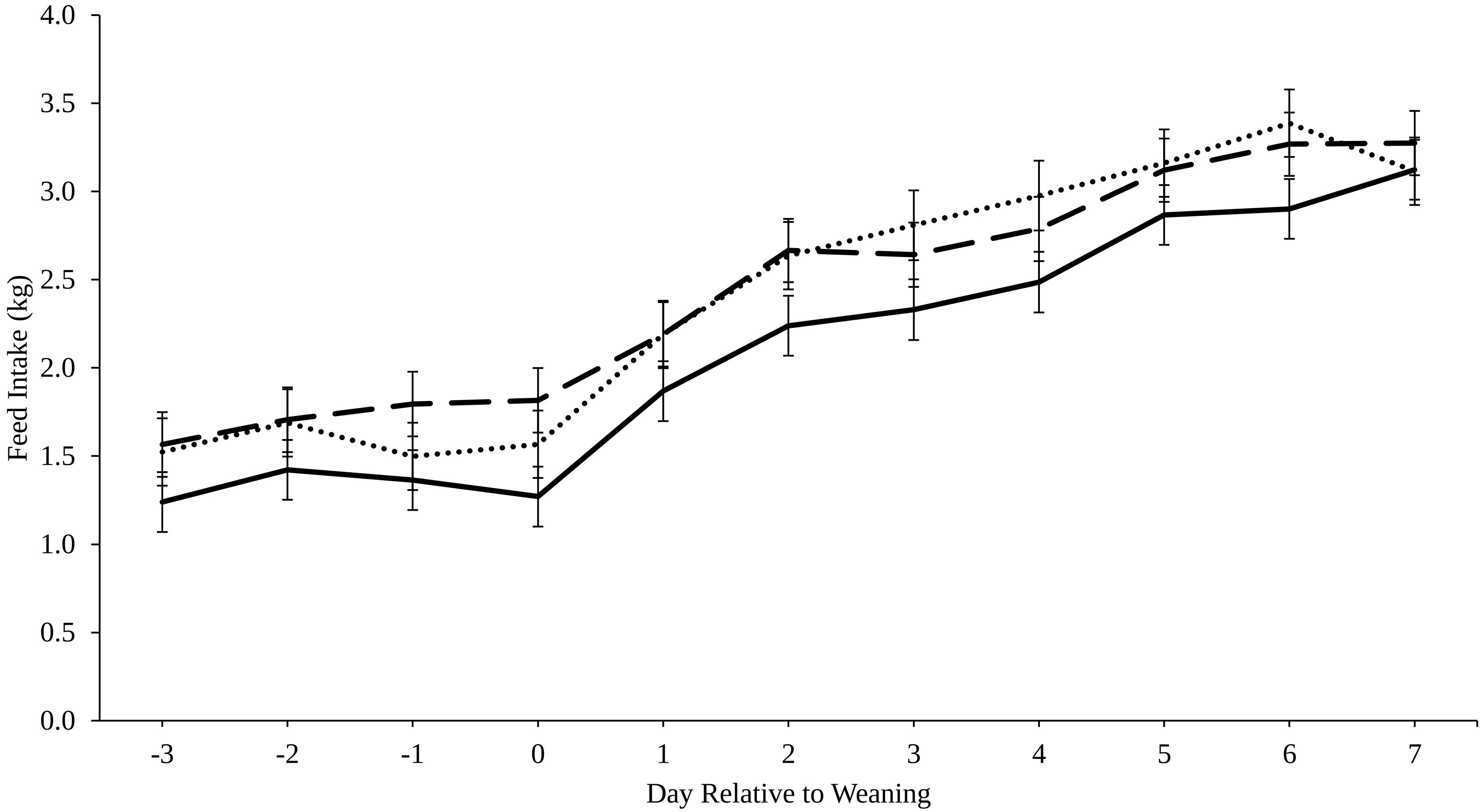

Figure 23. Feed intake at weaning for calves from control (solid line; n = 19), exercise (dashed line; n = 18), and pasture (dotted line; n = 15) (P = 0.86).

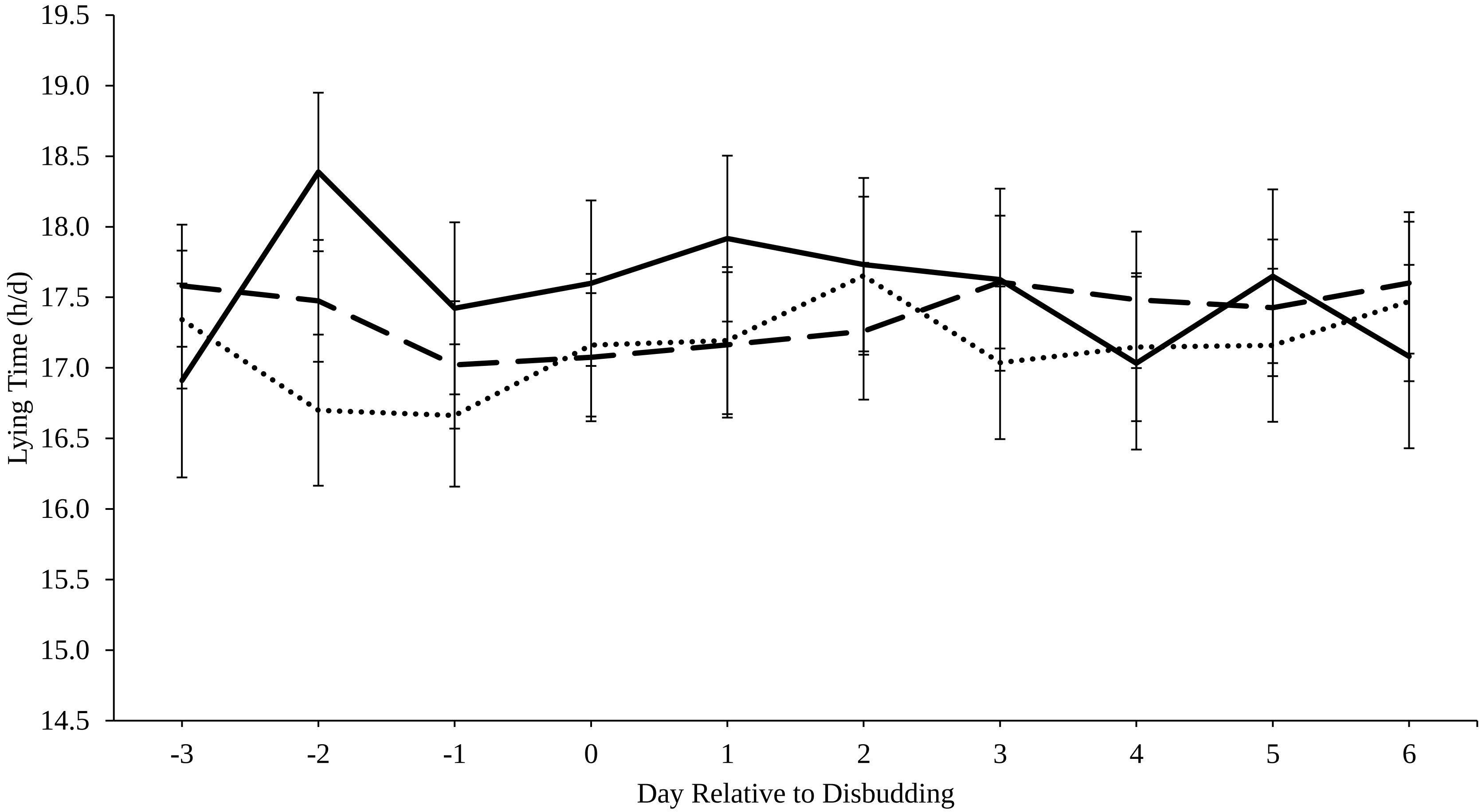

Figure 24. Lying time at disbudding for calves from control (solid line; n = 19), exercise (dashed line; n = 18), and pasture (dotted line; n = 16) (P = 0.79).

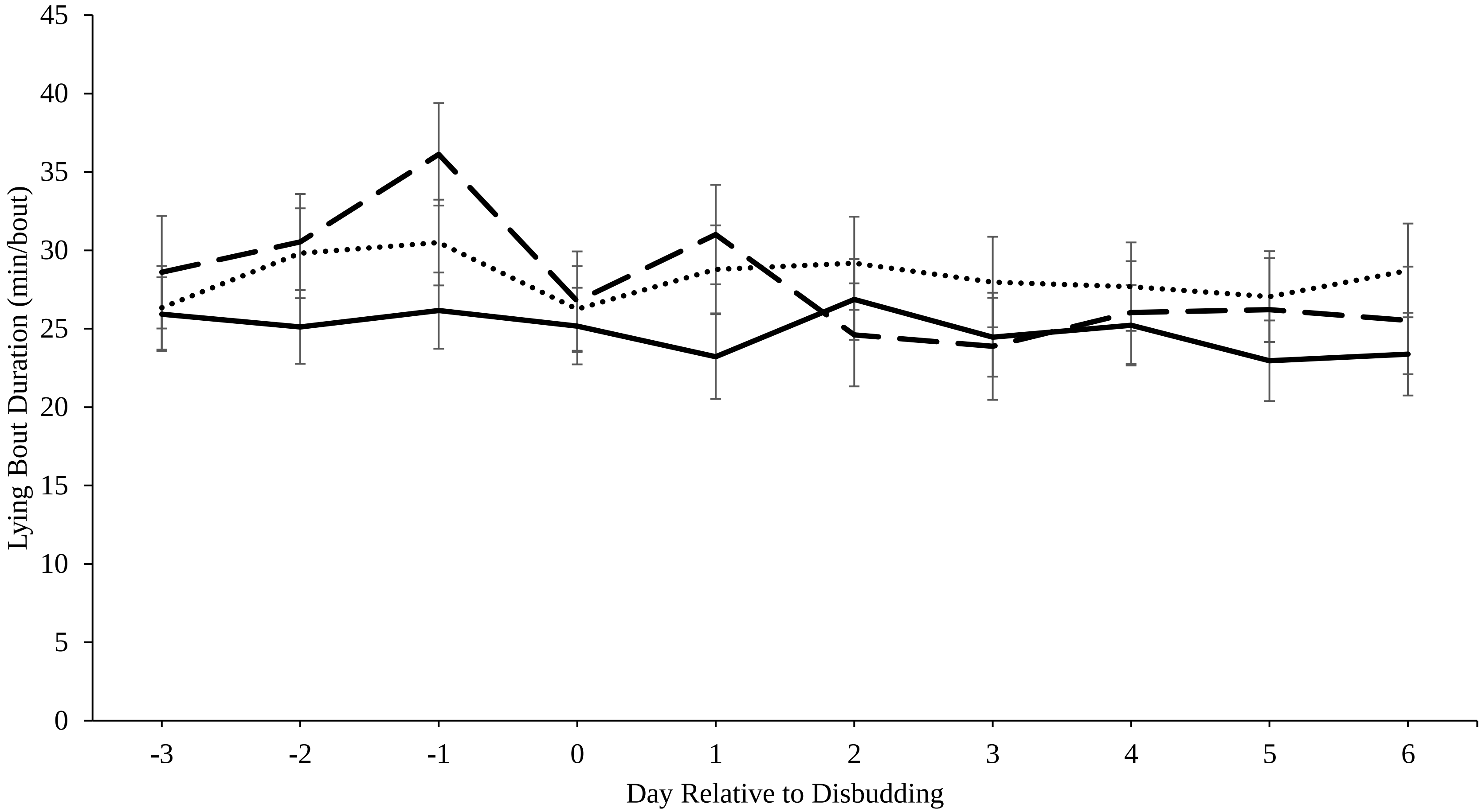

Figure 25. Lying bout duration at disbudding for calves from control (solid line; n = 19), exercise (dashed line; n = 18), and pasture (dotted line; n = 16) (P = 0.35).

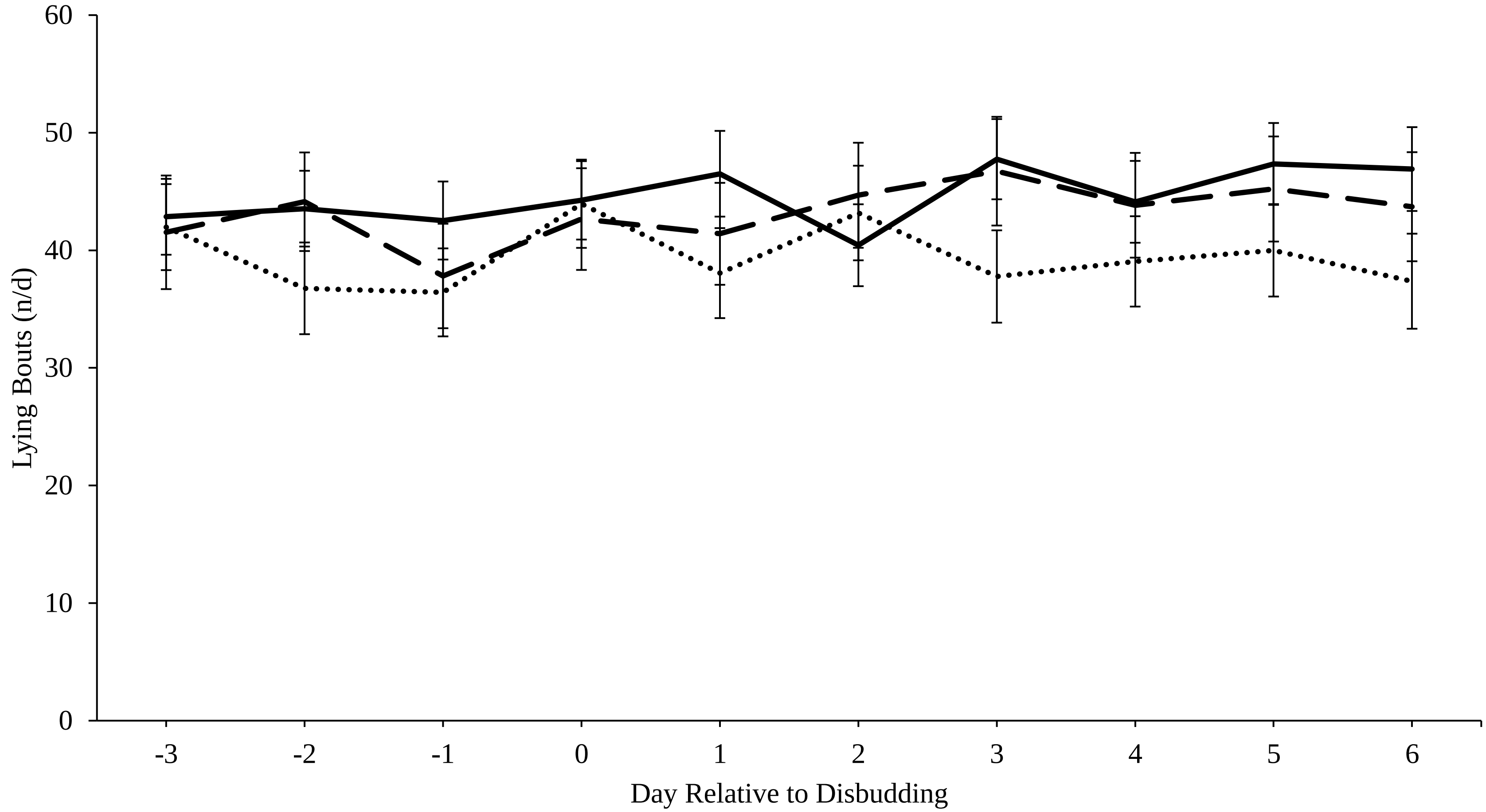

Figure 26. Lying bout frequency at disbudding for calves from control (solid line; n = 19), exercise (dashed line; n = 18), and pasture (dotted line; n = 16) (P = 0.31).

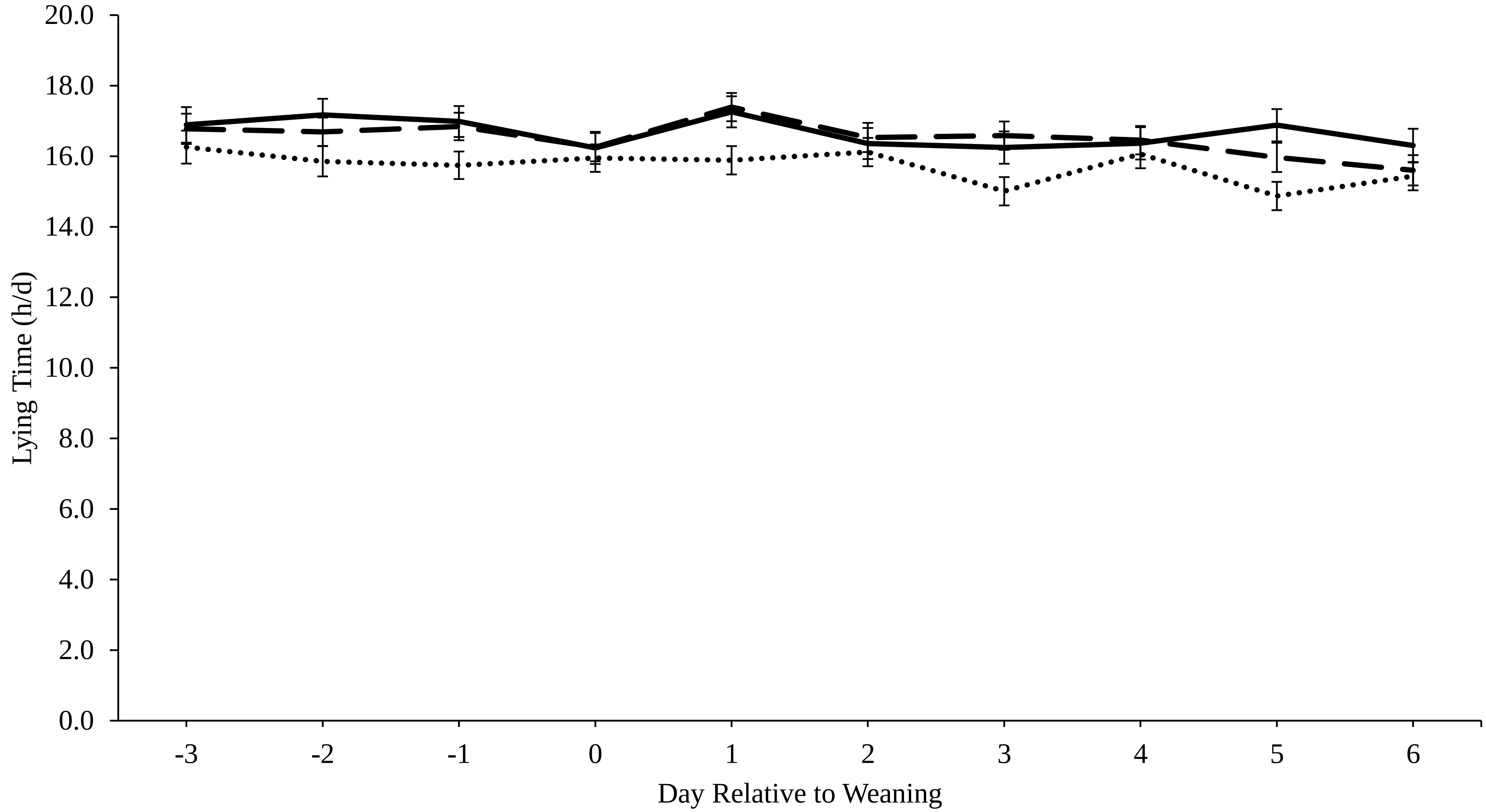

Figure 27. Lying time at weaning for calves from control (solid line; n = 19), exercise (dashed line; n = 18), and pasture (dotted line; n = 15) ($P = 0.40$).

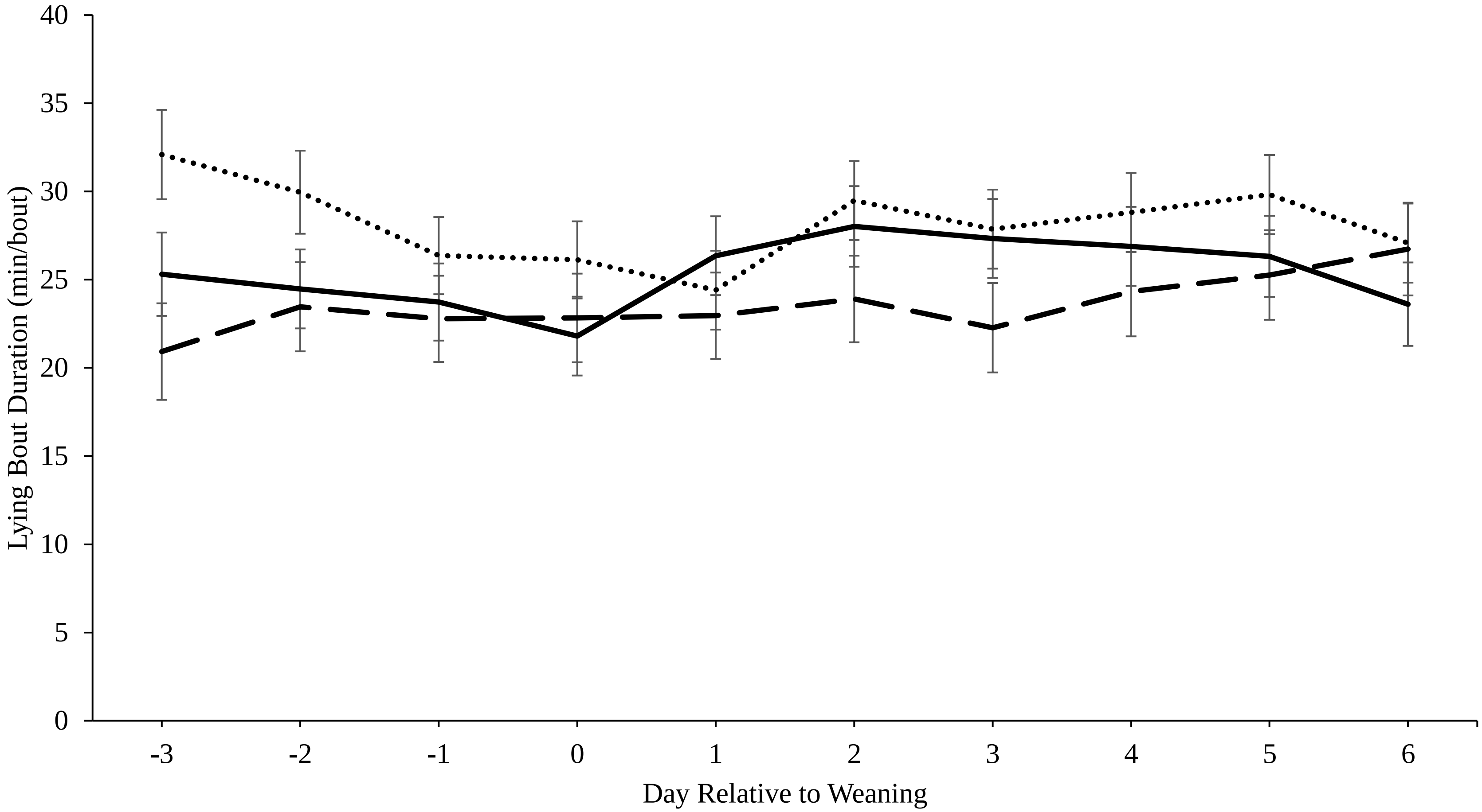

Figure 28. Lying bout duration at weaning for calves from control (solid line; n = 19), exercise (dashed line; n = 18), and pasture (dotted line; n = 15) (P = 0.40).

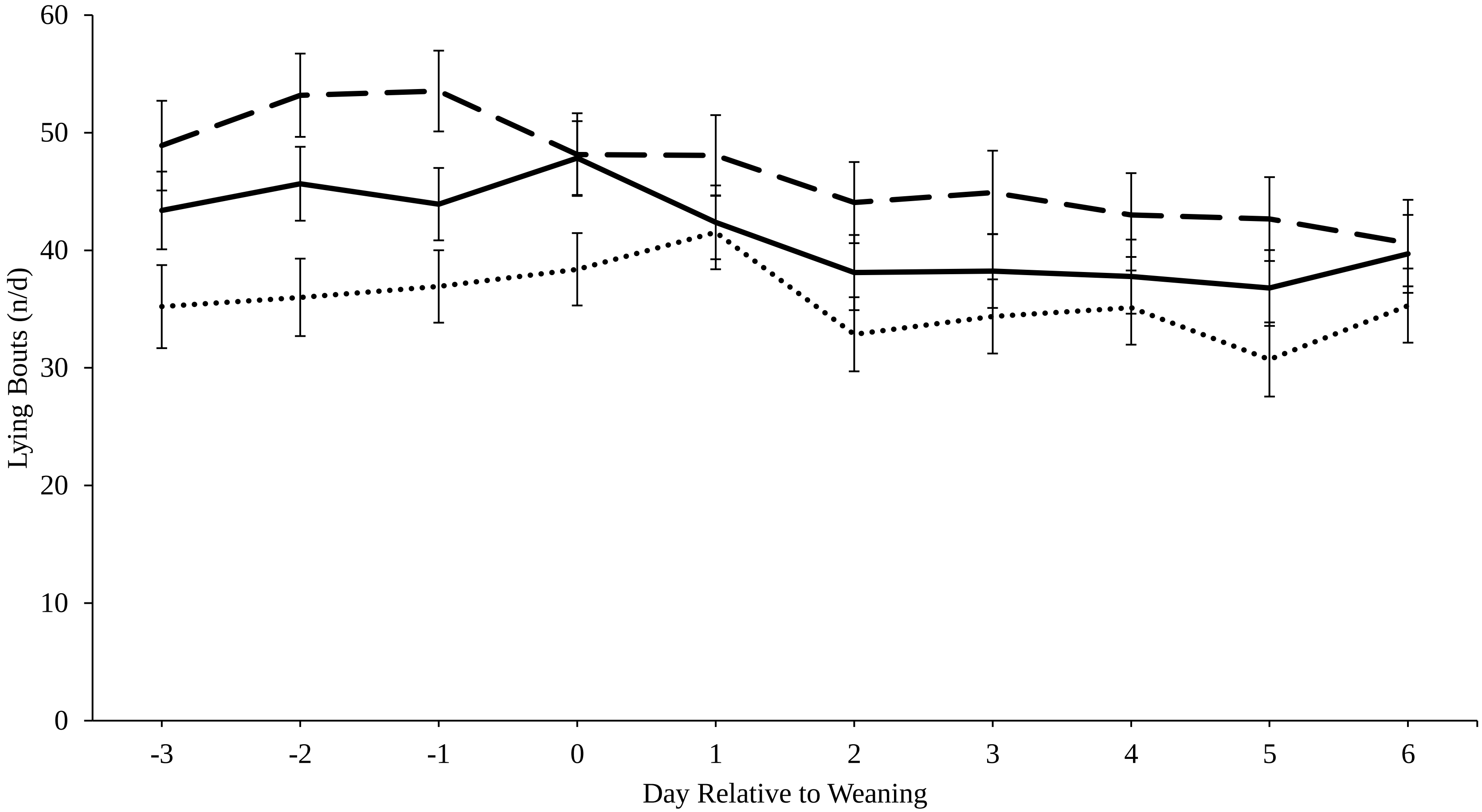

Figure 29. Lying bout frequency at weaning for calves from control (solid line; n = 19), exercise (dashed line; n = 18), and pasture (dotted line; n = 15) (P = 0.44).

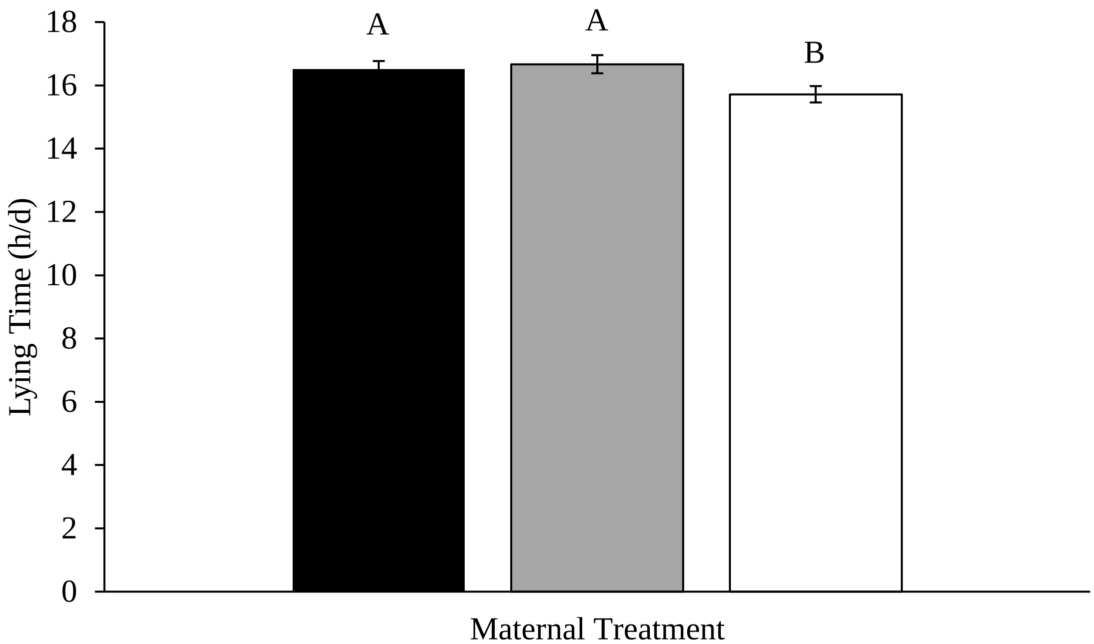

Figure 30. Lying time at weaning for calves from control (black bar; n = 19), exercise (gray bar; n = 18), and pasture (white bar; n = 15) (P = 0.04).

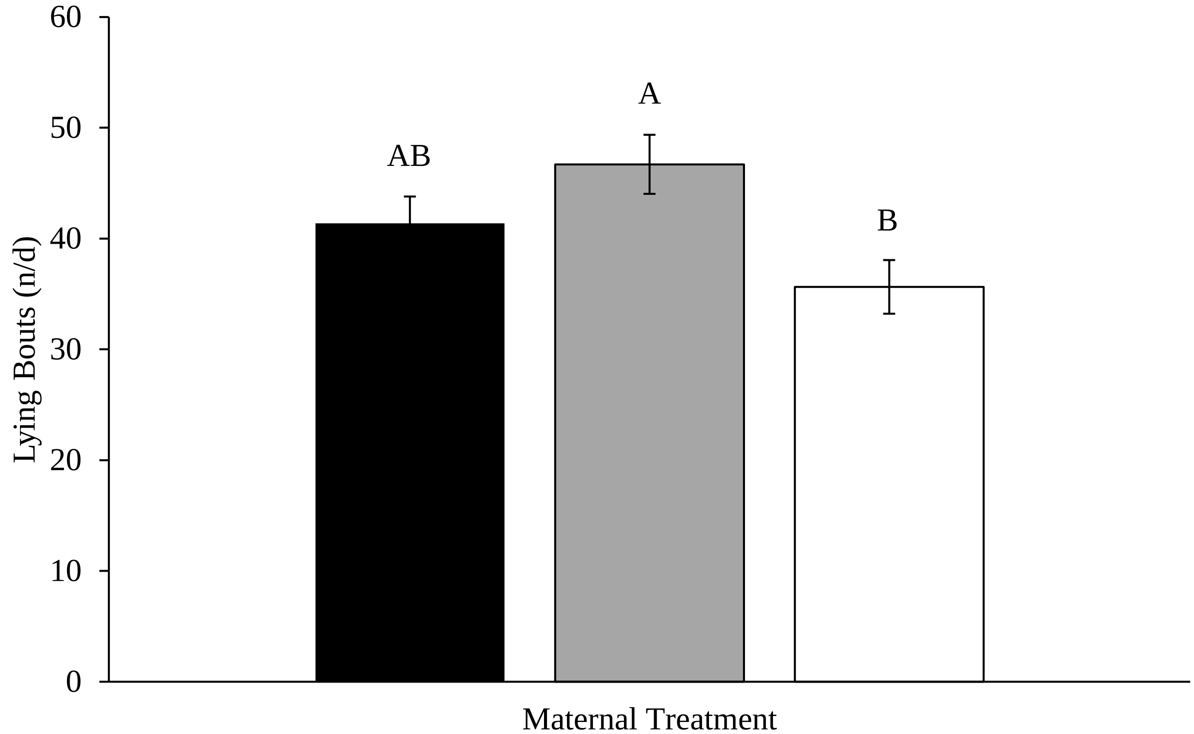

Figure 31. Lying bout frequency at weaning for calves from control (black bar; n = 19), exercise (gray bar; n = 18), and pasture (white bar; n = 15) (P = 0.04).

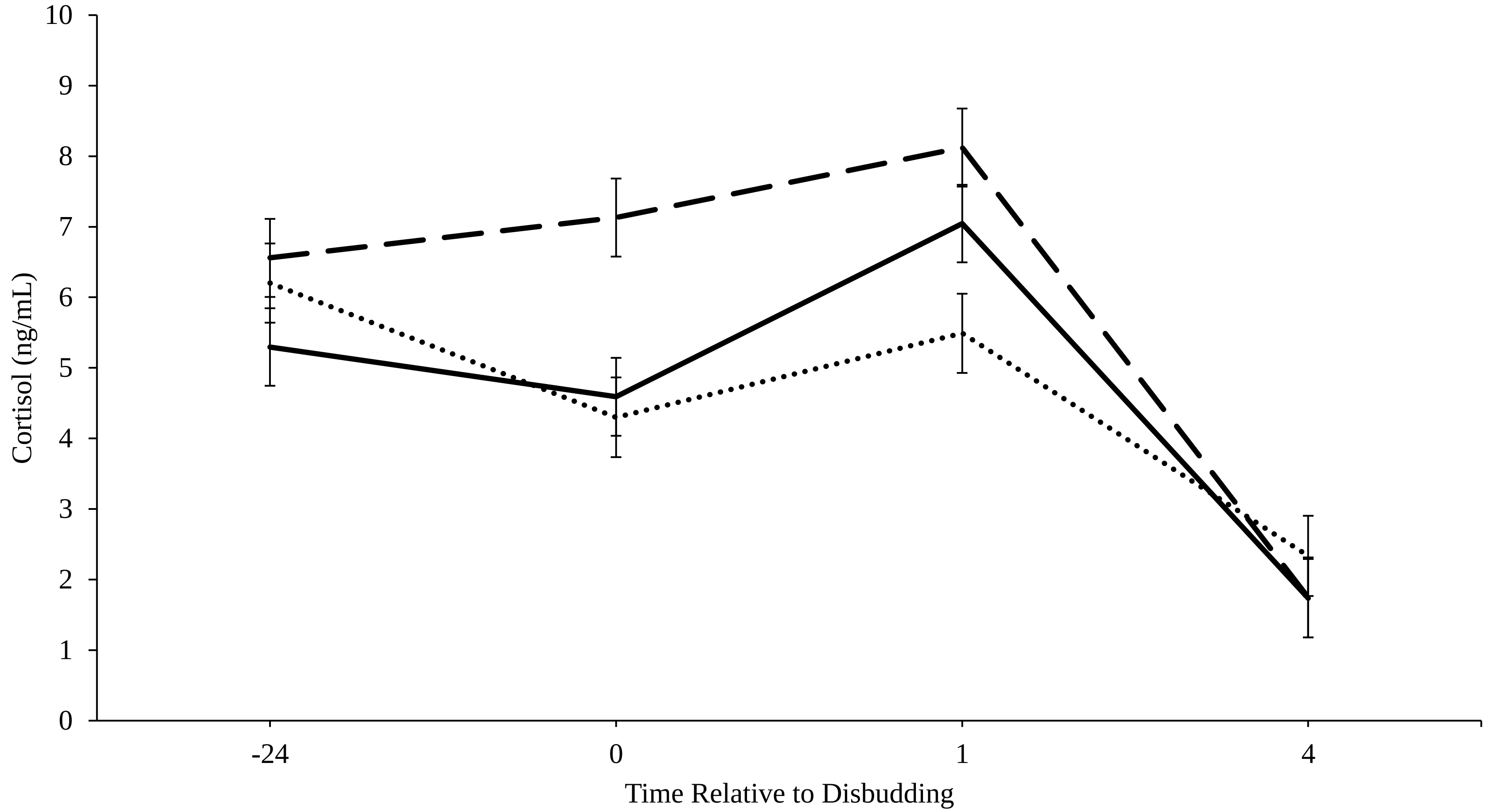

Figure 32. Cortisol concentrations at disbudding for calves from control (solid line; n = 19), exercise (dashed line; n = 18), and pasture (dotted line; n = 16) (P = 0.63).

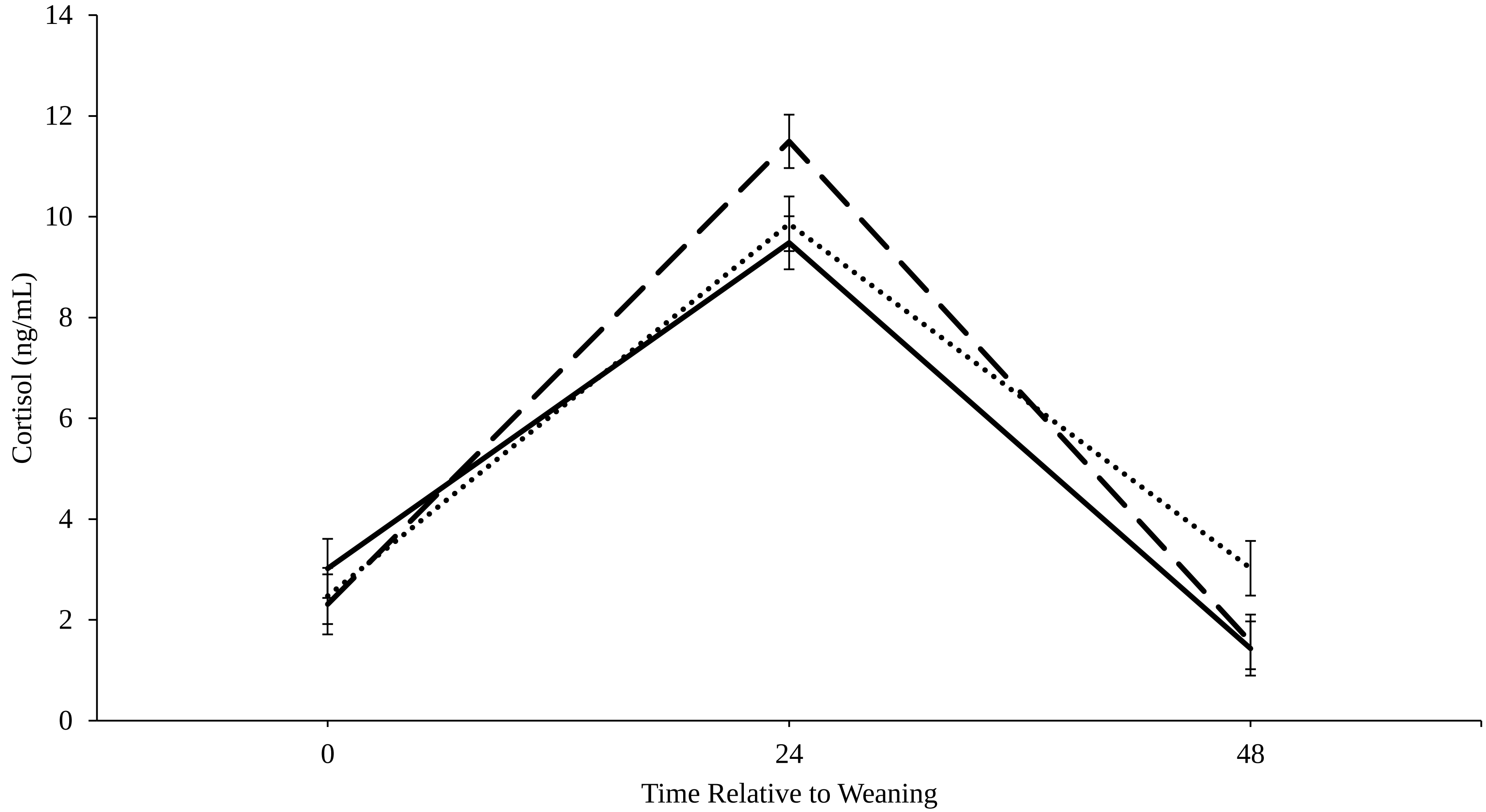

Figure 33. Cortisol concentrations at weaning for calves from control (solid line; n = 19), exercise (dashed line; n = 18), and pasture (dotted line; n = 15) (P = 0.39).

VITA

Randi Black was born on September 11, 1987 in Lexington, KY. Randi graduated from Woodford County High School in 2005 and continued her education at the University of Kentucky, receiving a Bachelor's of Science degree in Animal Science in 2009 and a Master's of Science degree in animal science in 2013. While completing her Master's degree, Randi was active in extension, presenting at multiple statewide extension meetings, professional development, presenting at three national and one international conference, and publication, with two peer-review publications resulting from her Thesis.

In spring of 2013, Randi began studying for her Doctorate of Philosophy under the direction of Dr. Peter Krawczel studying the effect of prepartum exercise during the dry period of dairy cows. Randi has been active in professional development, presenting research at seven national and two international scientific meetings and acting as president of the University of Tennessee Animal Science Graduate Student Association. She has also published two peer-reviewed papers outside of her dissertation research and contributed to industry publications. Randi has mentored four Master's students and eight undergraduate students and participated as a teaching assistant during every semester of her study.