THE EFFECT OF EXERCISE ON THERMO-TOLERANCE IN PREGNANT HOLSTEIN HEIFERS

by

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B.S., College of the Ozarks, 2010
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AN ABSTRACT OF A DISSERTATION

submitted in partial fulfillment of the requirements for the degree

DOCTOR OF PHILOSOPHY

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Abstract

Dairy cows require a low-stress environment in order to efficiently produce milk, and thus stress management is a common focal point for both researchers and producers. A primary source of stress for dairy cattle is associated with the environment, particularly heat, and therefore a considerable amount of research has been done in an attempt to find ways of reducing heat stress. Most of the research, however, has focused on using heat abatement techniques to cool the cow, using evaporative cooling systems to reduce temperature in the environment thus also cooling the cow, and selective breeding to improve thermal tolerance. Whereas cow comfort has been improved, there are still negative responses to heat stress today including decreased milk production and altered milk composition. Cattle remove excess body heat primarily through evaporative and convective cooling in the respiratory system and exercise is likely to improve blood flow and efficiency of heat transfer within the lungs. Furthermore, exercise has been proven to improve performance in humans and horses. This study was designed to determine whether or not exercise improved fitness and heat tolerance, and to observe whether there were any resulting effects on milk production and parturition. Two experiments were carried out during the late summer/early fall of 2014 and summer of 2015. Each experiment utilized a different exercise regimen: experiment 1 used a combination of high-intensity intervals and endurance training, whereas experiment 2 involved an endurance regimen performed during the afternoon in early summer. Pregnant Holstein heifers (Experiment 1, n = 24; Experiment 2, n = 24) were exercised in an 8-panel motorized walker over a period of 8 wk that ended approximately 21 d prior to parturition. In experiment 1, fitness was improved in heifers that were exercised compared with their non-exercised counterparts based on their duration of exercise and speed of exercise at failure ($P < 0.05$). During a cool hour of the day after 6 wk of

exercise, exercised heifers spent more time in body temperature zone 1 (< 39.0°C) compared with their non-exercised counterparts ($P < 0.05$). Exercised heifers also spent less time ($P < 0.05$) than non-exercised heifers in body temperature zone 3 (> 40.0°C) during the hottest hour of a hot day during the 6th week. No treatment effects ($P > 0.10$) were found for weekly milk components or milk production. In experiment 2, exercise resulted in greater milk protein and solids-not-fat (SNF) percentage ($P < 0.05$) compared with contemporaries that did not exercise; however, there was no difference in weekly milk production during the first 150 days ($P > 0.10$). Fat-corrected milk and energy-corrected milk were calculated and no difference was detected between treatments ($P > 0.10$). These results are the first to show that high-intensity intervals and endurance training exercise in pregnant dairy heifers can improve heat tolerance, increase production of milk protein and SNF, and perhaps increase animal comfort and well-being during hot weather.

Keywords: exercise, dairy cattle, heat tolerance

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Chapter 1 - Literature Review

What is stress?

It is well-established that heat stress causes a number of negative responses in dairy cattle including poor reproductive performance, reduced dry matter intake, and decreased milk production. Stress physiology, as defined by Yousef (1985a), is "a study of the animal's physiological, biochemical, and behavioral responses to the various factors of the physical, chemical, and biological environment." Stress will disrupt the body's normal, homeostatic mechanisms to regain homeostasis once the stressor is removed (Yousef, 1985a). Several different types of changes occur as a result of stress, including adaptation, acclimation, acclimatization, and habituation (Yousef, 1985a).

Adaptation can either occur phenotypically or genotypically, in which the body changes in response to some stressor in the environment. Phenotypic changes usually occur within the lifetime of an animal, while genotypic changes occur over generations of genetic selection (Yousef, 1985a). A phenotypic occurrence is a physical expression of a genotype that can be seen based on outward appearances (e.g. hair type), while genotypic changes occur at the molecular level (e.g. DNA). Stress can alter genotypic formation that would result in changes of phenotypic expression. The emerging field of epigenetics focuses on changes at the molecular level in response to external stimuli that affect gene expression, and thus may explain some of the variation in phenotypic changes (e.g. decreased milk production) (Singh et al., 2010).

Acclimation occurs during the lifetime of an animal and occurs when the body changes physiologically in order to accommodate more efficiently an environmental stressor (Bligh and Johnson, 1973; Yousef, 1985a). Changes that occur include blood pressure fluctuations,

respiration rates, and sweat rates, which are all involved in enabling the body to efficiently transfer heat in order to maintain a homeostatic body temperature. Acclimation is usually seen in situations of experimentation, such as a laboratory setting. Once the stressor is removed, physiological changes that had occurred will revert back to functioning as before.

Acclimatization is similar to acclimation except that it is seen in scenarios of natural, stressful changes such as seasonality (Bligh and Johnson, 1973; Yousef, 1985a). As with acclimation, any acclimatizing changes that occur in response to a stressor will revert back to normal functioning.

Habituation is observed in scenarios of repeated stressors resulting in the normal stress responses of the body to become less severe (Yousef, 1985a). Thus, the body becomes attuned to the stressor and begins to respond to it with a normal, homeostatic response.

Heat Stress in Dairy Cattle

Heat stress in ruminants has been defined as a "demand made by the environment for heat dissipation" with dairy cattle being the most susceptible to heat stress under greater ambient temperatures, solar radiation, and humidity compared with other livestock species (Silanikove, 2000). Even in 2003, it was estimated that approximately $900 million were lost annually because of heat stress in the dairy industry alone (St. Pierre et al., 2003) and are more recently predicted to be around $2.2 billion per year by the end of the 21st century (Mauger et al., 2014). In order to improve efficiency of heat loss there are several responses that occur in cattle including increased respiration rates (Yousef, 1985b; Silanikove, 2000), panting, drooling, reduced heart rates, increased sweating (Blazquez et al., 1994; Silanikove, 2000), reduced feed

intake (Silanikove, 1992; Silanikove, 2000), and decreased milk production (Albright and Alliston, 1971; Silanikove, 2000).

Heat Index of Dairy Cattle

Lactating cows prefer a temperature range of 5 to 25°C, which is referred to as the thermoneutral zone (TNZ; Berman et al., 1985; Yousef, 1985b). Above this range, a cow can no longer maintain an unchanged core body temperature and is therefore considered to be in a state of heat stress. The upper critical temperature (UCT), 25 to 26°C, is when a Holstein cow can still maintain a normal body temperature (Berman et al., 1985). Temperatures greater than 26°C, however, result in decreased milk production and changes in the milk composition. Physiological responses of cows in the UCT zone include increases in sweating, body temperature, and respiration rate. Body temperatures are generally greater in lactating cows compared with non-lactating (dry) cows and follow a circadian rhythm with a temperature range of 0.2°C to 0.9°C (Nakamura et al., 1983). An increase in body temperature of lactating cows is partly due to increased demands for energy (greater metabolic heat production) based on increased milk production compared with the non-lactating cow.

The temperature-humidity index (THI) is commonly used to evaluate the environment using a calculation that combines both air temperature and relative humidity and gives a quantifiable indicator of the environmental stress imposed on cattle. This index was first developed by Thom (1958), and then further refined for use in dairy cattle at the University of Missouri (Berry et al., 1964). An index <72 has been considered an environment that is non-stressful to cattle; however, it has recently been argued by Zimbelman et al. (2009) that an index threshold of 68 more appropriately describes the response of high-producing dairy cows above

the TNZ. Because of increased milk production in the modern dairy cow, there is an increase in overall energy utilization to be used for maintenance (including thermoregulation) and lactation and thus an increased susceptibility to heat stress.

Physiological Mechanisms of Heat Transfer

Thermoregulation is largely controlled by neural pathways and thus when environmental changes affect the homeostatic body temperature, the transient receptor potential (TRP) ion channels found on the nerve endings in the dermis and epidermis respond to thermal threshold changes (Collier and Gebremedhin, 2015). The afferent pathway that ensues leads the thermal signal to the preoptic area of the hypothalamus and anterior hypothalamus via the spinothalamic tract; the thermal information also enters the cerebral cortex via the thalamus for cortex sensation (Collier and Gebremedhin, 2015). The efferent autonomic pathways that follow involve signals sent from the hypothalamus to the medulla oblongata, which controls cardiovascular responses, skin blood vessel vasodilation and/or vasoconstriction, and metabolic changes that are all involved in thermoregulatory responses (Collier and Gebremedhin, 2015).

The mechanism of heat transfer follows a concentration gradient that goes from hot to cold. Radiation heat loss occurs by leaving the body in the form of infrared rays; all objects radiate heat above absolute temperature (Kadzere et al., 2002; Hall, 2011; Collier and Gebremedhin, 2015). This can easily be experienced while standing in the sun. If when standing in the sun the environment is warmer than the body temperature of a cow, the heat follows its concentration gradient from the environment to the cow.

Conduction is the transfer of heat from one medium (e.g. liquid, gas, or solid) to another medium both in contact with each other. Convection is heat transferred via moving currents

(Kadzere et al., 2002; Hall, 2011, Collier and Gebremedhin, 2015). An example of conductive heat transfer would be a cow lying on sand bedding. The bedding is the cooler medium thus the heat from the cow will transfer from her body (a place of greater temperature) to the sand (a place of cooler temperature). An example of a moving medium could be a fan or wind traveling over the cow's body, and thus removing some of the heat from the animal. The concentration gradient may lessen during conductive heat transfer as the cooler medium begins to take on more heat, while convective heat transfer is under constant renewal.

Evaporation occurs when water evaporates off the skin surface, and can be referred to as insensible or latent heat loss. Heat can be lost "insensibly" by water evaporating from the skin and lungs (Hall, 2011; Collier and Gebremedhin, 2015). Thus, heat can be lost continuously through evaporation but at varying rates. The most ideal conditions for evaporative heat loss are in hot and dry conditions (Kadzere et al., 2002). Many dairies incorporate sprinkler systems to mimic an evaporative cooling mechanism for the cow since their sweating capacity is very minimal compared with other animals such as horses.

Humans can produce copious sweat through eccrine sweat glands for evaporative cooling from the skin, while animals cannot lose heat as efficiently from the skin's surface. This is largely for two reasons: fur and reduced number of sweat glands in the skin. To compensate, most fur or hair covered animals will use the alternative panting mechanisms for evaporative and convective cooling. The panting response is controlled by the thermoregulatory center in the brain and is carried out by the *panting center* located in the pons by the pneumotaxic respiratory center. Panting allows new air to come into contact with respiratory passages (convection), which cools the blood flowing through the respiratory passage mucosa (conduction and

convection). Water evaporation is also present on the mucosal surfaces in the lungs and on the tongue (Hall, 2011).

Heat Stress and Blood Flow

In any state of heat stress, blood flow is shunted from certain organs (e.g. gastrointestinal tract (GIT)) in order to increase blood flow to the peripheral tissues. Blood flow to the skin increases because of the thermoregulation response overriding that of the sympathetic response. Blood can carry heat from the body core, via conduction through tissues and convection by blood flow, and release it through evaporation or radiation from the skin. As the core body temperature increases, the "active vasodilator system" is activated and is responsible for 80 to 95% of increased skin blood flow (Johnson and Proppe, 1996; Kellogg et al., 1998).

During stress, blood flow is shunted away from the GIT. Reduced blood flow in the intestinal tract creates a state of hypoxia resulting in cellular damage (Hall et al., 2001). Cellular damage in the enterocytes includes: intestinal barrier strength, increased permeability, and the passage of endotoxins (such as lipopolysaccharides (LPS)) into the vascular system resulting in an inflammatory response, including the release of pro-inflammatory cytokines (Lambert, 2009). Because of this, heat stress is also assumed to result in intestinal hyperpermeability, more commonly known as "leaky gut" in ruminants (Sanz-Fernandez et al., 2013). Since the intestinal barrier becomes compromised during heat stress resulting in increased permeability, the luminal contents are released into the portal system and throughout the rest of the vascular system (Sanz-Fernandez et al., 2013). This induces an inflammatory response that may further perpetuate the negative effects of heat stress. Indirectly, the unhealthy rumen environment resulting from heat stress can also lead to other negative side effects, such as laminitis or milk fat depression

(Baumgard et al., 2006). It is important to point out that decreased blood flow to the digestive organs also creates a challenge for dairy cows to acquire and utilize appropriate amounts of nutrients making it difficult to fulfill energy requirements of both maintenance and lactation (West, 2003).

While blood flow is restricted in certain areas, it is maintained in other areas. Interestingly, when comparing rate of mammary blood flow between thermoneutral cows fed ad libitum, thermoneutral cows with restricted intake, and heat-stressed cows fed ad libitum there was no difference between treatments (Lough et al., 1990). Since the mammary gland is a skin gland, we would not expect to see a large reduction in blood flow based on the heat stress response of increased blood flow to skin. This is important because blood carries important hormones, milk precursors, and energy (glucose) that will be discussed later in more detail.

Heat Stress, Nutrition and Digestion

It is well known that heat stress adversely affects rumen health. During heat stress, cows will increase respiration rates, eventually resulting in panting, in an effort to cool the cow. A major disruption to homeostatic function in response to heat stress is a shift in the acid-base chemistry because of increased loss of CO_2 via respiration known as respiratory alkalosis (Dreiling and Carman, 1991; West, 2003). An effective buffering system has a ratio of bicarbonate (HCO_3^-) to CO_2 of 20:1 (Baumgard et al., 2006). Bicarbonate is basic, while CO_2 is acidic and both maintain a homeostatic acid-base balance. Decreased levels of CO_2 reduce the blood concentration of carbonic acid, which aids in stabilizing neutral pH of the blood. Therefore, bicarbonate concentration becomes greater and the animal becomes alkalotic (Benjamin, 1981). In order to compensate for this increase in HCO_3^- concentration, the kidney

will increase urinary excretion, carrying with it excess HCO_3^- (Benjamin, 1981; Baumgard et al., 2006). West (2003) points out, however, that decreasing the HCO_3^- concentration can then result in decreased buffering capacity within the rumen, which is imperative for cows on a high grain diet (such as dairy cattle). Excessive salivation also results in reduced amounts of bicarbonate in saliva available to buffer the rumen (Baumgard et al., 2006). To avoid excessive losses of bicarbonate, producers supplement bicarbonate in feed to heat-stressed cattle to help maintain concentrations. Feed intake in the morning and evening milking periods was also improved in cows that had supplemental sodium bicarbonate (Schneider et al., 1984).

Another nutrient of importance that is lost during heat stress is K^+, which is lost via sweat in the cow. When potassium is supplemented in the diet, however, milk production has increased (Schneider et al., 1984; Mallonée et al., 1985).

While the environment disrupts homeostatic balance, there is also concern regarding the behavioral response of decreased feed intake thus creating a challenge to fulfill energy requirements. Since cattle consume less feed during heat stress, metabolic adjustments must occur in an attempt to restore energy balance. There are two major requirements of a mature dairy cow and they are maintenance and lactation. Because of the great amount of energy needed to sustain lactation, dairy cattle need to be fed a carefully monitored ration in order to meet these increased energy requirements. Unfortunately, since dairy cows reduce their DMI during times of stress fewer nutrients are taken in and therefore less energy is consumed to support lactation (Beede and Collier, 1986; West, 2003).

Usually cattle will end up in a negative energy balance (NEBAL) much like what is seen in transition cows. In early lactation cows, circulating insulin and insulin sensitivity are reduced in response to somatotropin secretion, which results in adipose lipolysis and mobilization of non-

esterified fatty acids (NEFA) to supply the body with stored energy (Baumgard et al., 2006). The end result is reduced glucose uptake by muscle and adipose tissues and the release of stored nutrients (NEFA) to supply more energy to support lactation (Baumgard et al., 2006). Shifts in carbohydrate and lipid metabolism occur and are mediated by endogenous somatotropin, which is naturally increased during periods of NEBAL (Baumgard et al., 2006).

In heat-stressed cows there is no increase in circulating NEFAs because of greater insulin concentrations compared to early lactation cows. Because of greater insulin concentrations there is an increase of glucose entry into cells, implying greater insulin effectiveness than the transition cow in a thermoneutral environment (Baumgard et al., 2006). It is possible that this physiological strategy to shift from fat mobilization to carbohydrate metabolism (glucose use) is to minimize metabolic heat production (Baumgard et al., 2006). Consumed nutrients are utilized to help rid the body of excess heat first before being used for lactation, another reason that milk production is reduced (Sanz-Fernandez et al., 2013).

Heat Stress and Lactation

One of the first papers quantifying the effects of heat stress on lactation in dairy cows found that exposure to heat stress had negative lingering effects on milk production 24-48 hours later (Collier et al., 1981). After exposure to heat stress, cows demonstrated a numerically decreased milk yield compared with their shaded counterparts (Collier et al., 1982; Spiers et al., 2004). Ominski et al. (2000) concluded that short-term, moderate heat stress adversely affects milk production. If a decline in milk production can be avoided in a 48-hour period, this can prevent a decrease in lactation persistency 2 weeks later (Collier et al., 2012). Mammary

epithelial cell numbers are indicative of milk production (Singh et al., 2010), thus a reduction in lactation persistency is indicative of mammary epithelial cells permanently lost.

Milk components are also affected by environmental changes. Reductions in protein and fat percentage in milk can be attributed to decreased dietary energy and protein intake (Ominski et al., 2000). In a more recent study, researchers found that temperatures above the TNZ began to impact milk composition by decreasing solids-not-fat (SNF), protein, lactose and fat percentage of milk (Collier et al., 2012). Solids-not-fat and lactose are both osmotic regulators of milk and are less affected by increased temperatures, thus environmental temperatures >23.9°C have a greater impact on fat and protein percentage compared with SNF and lactose percentage. Milk fat and protein percentage are correlated with decreased ruminal pH (acidosis) and experience decreases of 0.4% and 0.2% respectively (Collier et al., 2012).

An endocrine response to heat stress that impacts lactation is the adrenal gland's release of cortisol. Because lactating dairy cows require a greater amount of energy for lactation, the release of cortisol is especially concerning because a major function of cortisol is the breakdown of glycogen to glucose for use in the stress response (Blum and Eichinger, 1988). Glucose is an important energy source but is also a key component during the milk synthesis process. Glucose is used to make lactose, a milk sugar, by combining with galactose within the secretory cells of the alveoli in the mammary gland. Glucose is taken up by glucose transporters (GLUT) and because of the increase in insulin produced during heat stress, there is a promoted cellular uptake of glucose elsewhere, especially the skeletal muscles (Baumgard et al., 2006). Muscles have an increased concentration of GLUT4 transporters that are insulin-dependent, thus they are dependent upon insulin to regulate glucose homeostasis (Mann et al., 2014). While the mammary gland does not have any GLUT4 transporters present on epithelial cells, there are

other transporters including GLUT1, GLUT8, and GLUT12 (Mann et al., 2014; Zhao et al., 2014). These types of transporters are able to take up glucose passively via facilitative transport, and do not require the presence of insulin. With the increase of insulin in heat-stressed cows and the primary glucose transporter in muscle being GLUT4, this helps explain the increase of glucose partitioned to muscle rather than to the mammary gland.

Milk Letdown and Oxytocin

The primary hormone involved with stimulating milk ejection, or milk letdown, is oxytocin (Gimpl and Fahrenholz, 2001). Milk ejection is an innate reflex that is stimulated in response to tactile stimulation, e.g. teat suckling, or mechanical stimulation by milking machine (Bruckmaier and Blum, 1998; Hall et al., 2001). This neuroendocrine reflex begins with specific pressure-sensitive neural receptors located in the distal ends of the teat, which when stimulated will send signals via the spinal cord to the brain (Bruckmaier and Blum, 1988). The signal then terminates at nerve cell body clusters, the supraoptic and paraventricular nuclei of the hypothalamus, which are the location of oxytocin synthesis (Bruckmaier and Blum, 1998; Hall et al., 2001). An efferent pathway occurs where signals are sent from the cholinergic neurons to the pituitary gland to release oxytocin from the posterior pituitary gland into the blood (Bruckmaier and Blum, 1998). Oxytocin will then bind to oxytocin receptors on the myoepithelial cells surrounding the alveoli and ducts in the mammary gland (Gimpl and Fahrenholz, 2001). Once oxytocin is bound to its receptor, the myoepithelial cells contract on the alveoli and increase the intraluminal pressure. This causes milk ejection into the ducts and eventually pushes the milk towards the gland cistern prior to complete milk removal.

There are 2 different types of inhibition of milk ejection: central inhibition or peripheral inhibition (Bruckmaier and Blum, 1998; Wellnitz and Bruckmaier, 2001). Central inhibition of milk ejection is when oxytocin release is inhibited. This could be from several stressors such as: environmental temperature, estrus, or switching milking methods (Bruckmaier and Blum, 1998; Wellnitz and Bruckmaier, 2001). The autonomic nervous system responds to milk ejection and can largely determine how well, or poorly, a cow can release milk. During milking times, cows undergo a reduced sympathetic response, but this response is heightened between milking times to increase contraction of the teat sphincter muscles in order to prevent milk leaking out of the udder (Bruckmaier and Blum, 1998). There are two types of sympathetic receptors present in mammary smooth muscles and teats: α-adrenergic and β-adrenergic receptors (Bruckmaier and Blum, 1998). Both of these receptors respond to catecholamines, but respond in different ways. The α-adrenergic receptors have been linked to inducing teat contraction and reducing mammary blood flow, while β-adrenergic receptors have been linked to inducing teat relaxation and directly inhibiting milk letdown function (Bruckmaier and Blum, 1998). Hormones that would attach via either α- or β-adrenergic receptors, specifically epinephrine and norepinephrine, are responsible for reducing oxytocin release from the posterior pituitary and blocking its effect during the process of milk ejection.

The other form of inhibition of milk letdown is peripheral inhibition that occurs when oxytocin is released but the mammary gland does not respond to its effects (Wellnitz and Bruckmaier, 2001). Akers (2002) reported that perhaps a reason for reduced milk let down activity is because of increased vasoconstriction of the mammary capillaries in response to the sympathetic response, thus reducing the amount of blood carrying oxytocin to be delivered to the alveoli. Additionally both oxytocin receptors and β-adrenergic receptors are capable of allosteric

modulations and can form heterodimers (Wrzal et al., 2012). This may partially explain the peripheral inhibition of oxytocin's function in the mammary gland in the presence of catecholamines because of receptor changes in response to allosteric conformations.

Physiological Response of Mammary Gland

In order for lactation to be maintained, milk must be removed. The mammary gland is controlled by autocrine-paracrine factors that involve both local and hormonal factors that either stimulate or inhibit milk production. When milk begins to accumulate, a hormone derived from tryptophan called serotonin, also known as feedback inhibitor of lactation (FIL), will increase (Hernandez et al., 2008) locally within the alveoli. Feedback inhibitor of lactation reduces the secretory rate of milk synthesis, key enzymes for synthesis, and prolactin receptor numbers. This initiates a "shut down" of the mammary gland to the point where it can no longer produce milk if FIL is not removed within a certain time period. Hernandez et al. (2008) found that when serotonin receptors (5-HT) were blocked milk synthesis increased indicating that serotonin is indeed a feedback inhibitor of lactation. Serotonin has also been linked with suppressing β-casein gene expression and shrinkage of the alveoli in the mammary gland (Matsuda et al., 2004). This type of feedback ensures that dairy cows will not continually produce milk if they are no longer nursing or mechanically milked.

There are proposed regulatory systems that become activated during heat stress that act as a "safety" mechanism to ensure survival of the animal. This endogenous milk enzymatic system is called the plasminogen activator-plasminogen-plasmin (PA-PG-PL) system (Silanikove et al., 2000; Silanikove et al., 2009). The PA-PG-PL system blocks K^+ channels on the apical side of the secretory cell membranes. Down-regulation of K^+ channels is proposed to play a role in

directing cellular signals that inhibit milk secretion within the epithelia cells (Silanikove et al., 2009).

Heat Stress and Endocrine Function

In heat-stressed conditions cows exhibit a different hormone profile as opposed to non-heat-stressed cows. Collier et al. (1982) compared endocrine profiles of cows in the shade to cows in no shade during the last trimester of pregnancy. Progestin concentration tended to be greater for non-shaded animals until the day of parturition (Collier et al., 1982). Progesterone concentrations were decreased following parturition, most likely because of the termination of the luteal phase at this point in the cycle. There were no statistical differences between estrone and estradiol, however, estrone-sulfate was greater in shaded animals. Decreased estrone-sulfate in heat-stressed animals may cause a negative effect on placental function which may explain in part why there were reduced calf birth weights in non-shaded dams. Pregnant cows had decreased concentrations of T_4 when housed in non-shaded facilities compared with their shaded counterparts, while T_3 concentrations were greater in non-shaded cows. Decreased thyroid function may negatively affect mammary tissue development during pregnancy as well as fetal development and the subsequent lactation (Collier et al., 1982).

Glucocorticoids are increased upon initial exposure to heat stress, but then have been found to subsequently decrease as the heat exposure becomes chronic, suggesting that there was an attempt by the cow to acclimate to the chronic stress (Lee et al., 1976). Heat-stressed cows had elevated levels of norepinephrine compared with pair-fed thermoneutral cows, both antepartum and postpartum (Lamp et al., 2015).

Anti-diuretic hormone is stimulated when there is a decrease in blood volume and therefore stimulates water and electrolyte reabsorption by the kidney (Farooq et al., 2010). Water losses because of increased respiration and sweat result in reduced blood volume (Farooq et al., 2010). Pregnant ewes were exposed to either an acute heat-stressed environment, administered a hypertonic (5%) NaCl infusion, or received intravenous injections of vasopressin or oxytocin. A salt solution was infused to mimic effects of ADH and oxytocin in order to determine the response of uterine blood flow and whether these hormones were involved with regulating blood flow to the uterus during pregnancy. Dreiling and Carman (1991) concluded that both ADH and oxytocin concentrations increased as body temperature increased. In all three treatments there was an inverse relationship between uterine blood flow and concentrations of ADH and oxytocin, such that as ADH and oxytocin increased uterine blood flow decreased (Dreiling and Carman, 1991). While it could not be elucidated whether ADH or oxytocin or both affected uterine blood flow, it was apparent that these hormones play some role in regulating the rate of blood flow to the uterus and gestating fetus during heat stress. Increased ADH concentrations have also been reported in cattle subjected to hot environments (35°C), while aldosterone concentrations were reduced (El-Nouty et al., 1980). Urine output during heat exposure had a small but significant increase. This inverse relationship between ADH and aldosterone may help explain why cattle do not concentrate urine during heat stress (El-Nouty et al., 1980).

The somatotropic axis has become another interest of study in recent years, specifically the "uncoupling" of the axis that occurs during a heat stress response. Growth hormone is a calorigenic hormone, exerting its effects on nearly all body tissues (Farooq et al., 2010). It also has stimulatory effects on thyroid gland activity (Farooq et al., 2010), which means that

increases in GH secretion will result in increased metabolic function (heat production). Hormones involved in the somatotropic axis include growth hormone (GH) and insulin-like growth factor (IGF-1) (Evans and Simpson, 1931; Lucy et al., 2009; Rhodes, 2010). In a minimal stress environment, the GH signals the liver to synthesize and release hepatic IGF-1, a hormone that increases cell proliferation of the mammary gland (Akers, 2002; Rhodes et al., 2010). In chronic states of heat stress, GH has been found at reduced concentrations in the blood (Igono et al., 1988, Rhodes et al., 2010). This is the start of the uncoupling of the somatotropic axis in the heat-stressed dairy cow. It is currently hypothesized by Rhodes et al. (2010) that the reduced IGF-1 concentrations are because of impaired GH responsiveness of the liver, which reduces full milk-producing potential of heat-stressed animals.

Heat stress has been found to affect several different hormones including progestins, estrone-sulfate, thyroxine, glucocorticoids, ADH, GH, and IGF-1. There are some conflicting reports of the endocrine responses to heat stress; however, this may be because longer exposure to heat results in some form of acclimatization.

Heat Stress and Reproduction

Cattle in a heat-stressed environment experience negative reproductive effects including reduced conception rates and poor or delayed expression of estrus. Cows required more insemination services and had a reduced conception risk compared with virgin heifers (Badinga et al., 1985). Conception risk is also affected when the temperature of the day following insemination was under heat-stressed conditions, specifically when temperatures are greater than 30°C (Badinga et al., 1985). Lactating cows had greatly reduced conception risk compared with virgin heifers, 34% versus 50%, respectively (Badinga et al., 1985). Reasons for reduced

conception may be because of poor expression of estrus related to reduced activity (making it more difficult to identify standing estrus). In addition, reduced estradiol secretion from the dominant follicle (Bernabucci et al., 2010) may also contribute to reduced estrous behavior.

Dominant follicles in cows that were shaded were found to be larger than the dominant follicles of their unshaded counterparts (Badinga et al., 1993), a finding that was later re-affirmed by De Rensis et al. (2003). Concentrations of estradiol were also found to be greater in both plasma and follicular fluid in the month of July compared with September (Badinga et al., 1993). Wilson et al. (1998), however, found that cattle in heat stress had decreased concentrations of estradiol from d 11 to 21, and they also found that the average day of luteolysis was delayed 9 days in heat-stressed cows. Because the 2nd follicular wave appears sooner in heat-stressed cows compared with thermoneutral cows, the dominant follicle may in fact be aged thus reducing fertility (Wolfenson et al., 1995).

Research has focused on finding alternative methods to improve reproductive parameters such as cooling the cow and utilizing synchronization protocols including timed artificial insemination (TAI) (Jordan, 2003). Cows that were cooled with ventilation and sprinklers had greater conception rates at first insemination (59% vs. 17%), longer lasting estrous behavior (16 h vs. 11.5 h), and greater pregnancy rate at 90 days postpartum (44% vs. 14%) (Wolfenson et al., 1988). When synchronization protocols were utilized, there were no differences between pregnancy rates over seasons (Burke et al., 1996; Britt and Gaska, 1998). It is also important to note that when utilizing TAI protocols, producers are not dependent on expression of estrus and thus only need to follow the synchronization protocol selected.

Heat Stress, Fetal and Calf Health

Heat stress negatively impacts fetal growth and there is also an increased risk for fetal loss (Bernabucci et al., 2010). De Rensis and Scaramuzzi (2003) stated that glucose is the primary fuel for the ovary and embryo, and therefore is an important nutrient for reproductive function. During heat stress, energy is used for thermoregulation as well as other processes involved in the stress response. Thus, the dairy cow will meet her energy requirements in the following order: maintenance, lactation, and finally reproduction. This means that there may not be much energy left, if any, for reproduction as it is the last priority in the hierarchy for energy use.

Not only are circulating nutrients reduced, but the manner in which nutrients can be brought to certain organs may be compromised by the heat stress response. Research has been done to study the effects of dams experiencing heat stress and how that affects fetal development as well as calf growth. It was argued by Dreiling and Carman (1991) that an explanation for intrauterine growth retardation (IUGR) during bouts of heat stress is because of the reduction in uterine blood flow, thus a shift may occur in the metabolic activity of the fetus from anabolism to catabolism. Reductions in uterine blood flow are most likely linked to adrenergic vasoconstriction, blood pCO_2, and blood pH (Dreiling and Carman, 1991). Because of the acid-base shift due to increased respiration in heat-stressed animals, the vasculature in the uterus is respondent to these changes by shunting blood flow away from the reproductive organs, including the uterus, to the skin to aid in heat dissipation (Dreiling and Carman, 1991).

Calves born to dams that experienced heat stress have reduced birth weights compared with thermoneutral dams (Collier et al., 1982; Tao et al., 2012; Monteiro et al., 2014). Collier et al. (1982) concluded that calves born from dams housed in shade were heavier than their non-

shaded counterparts, thus demonstrating the importance of applying some sort of heat mitigation. Heifer calves also had reduced weaning weight when their dams experienced heat stress (Tao et al., 2012).

When dams were exposed to heat stress, their heifer calves had decreased IgG concentrations, as well as reduced plasma total protein compared with calves whose dams were kept in cooler conditions (Tao et al., 2012). This would indicate that the immune system is compromised and that passive transfer was not complete. Another study evaluated the relationship between passive transfer of calves and the colostrum source from either heat-stressed dams or thermoneutral dams and found that regardless of the source, heat-stressed calves in utero had reduced IgG transfer (Monteiro et al., 2014). Tao et al. (2012) also found that there was no difference overall of serum cortisol concentrations between heat-stressed and thermoneutral dams, though calves born to cooled dams tended to have increased cortisol at birth. In the pre-weaning period, however, there were no differences between calves that came from either cooler dams or thermoneutral dams (Tao et al., 2012). This implies that there is acclimation that occurs under long-term heat stress conditions, and perhaps these adaptations were passed on to their offspring. The authors suggested that perhaps this occurrence may have been in relation to a more sensitive hypothalamic-pituitary-adrenal axis since the calves were subject to cool conditions in utero. This means that the stresses, or lack of stress, experienced by the dam may affect how sensitive or insensitive the calf is to its own stressors.

Heat Stress and Behavior

There are also behavioral differences that occur between heat-stressed and non-heat-stressed cattle, such as increased standing time (Igono et al., 1987; Cook et al., 2007; Allen et al.,

2015). Standing is thought to help cool cows since it exposes most of their surface area to the environment and allows for better cooling, especially when a heat abatement system is implemented. This increased standing time, however, is correlated with increased lameness occurrence (Leonard et al., 1996; Cook et al., 2007). When comparing the hottest period of the day to the coolest, cows were found to increase time spent standing in the alley from 2.6 to 4.5 h/d (Cook et al., 2007). Allen et al. (2015) studied the relationship between standing times and core body temperature in lactating dairy cows and found that core body temperatures greater than 38.93°C would incur a 50% likelihood that the cow will be standing. Grant (2007) proposed that for every additional hour spent resting it resulted in an increase of milk production from 0.91 to 1.59 kg.

Current Heat Abatement Strategies

Through observations and documented research it is very clear that there are detrimental effects of heat stress on dairy cows. Attempts have been made to mitigate the environmental stressors by implementing different management strategies that help cows dissipate heat more efficiently. West (2003) published a review emphasizing the importance of properly managing dairy cows in a heat stressed environment. As temperature and humidity increase, the physiological responses of cows to heat stress are manifested primarily by panting and less frequently by sweating. Bohmanova et al. (2007) found that the limiting factor of heat stress was humidity and dry bulb temperatures in humid and dry climates, respectively.

On-farm Management Strategies

One of the first attempts at abating heat stress in lactating dairy cows was by offering shade to shield the livestock from the sun's radiation, thereby improving lactation performance

and fetal growth (Collier et al., 1981; 1982). Cows given shade also had decreased respiratory rates and rectal temperatures, and increased ruminal contractions and milk production than their non-shaded contemporaries (Roman-Ponce et al., 1977). After shade was established as a useful implementation to reduce heat stress in cattle, other methods were incorporated including water sprinklers and convective cooling (fans). At minimum, one of these methods should be incorporated in dairy management strategies; however, many dairies use a combination of all three. These systems, while helpful in reducing heat stress, have increased costs, energy, or water usage, or a combination of the three. Ortiz et al. (2015) found that dairy producers use anywhere from 0.71 to 1.76 kW/h, depending on the type of cooling system in place. It has also been reported that in order to cool cows approximately 56 to 75 L of water per cow per day is needed during hot months (Harner, 2013). Water waste and run-off, however, must also be managed in these operations and with the increase in electricity and water costs plus reduced water availability these systems are becoming limiting issues in some parts of the United States (Ortiz et al., 2015).

Thus, one avenue of research is to find ways of successfully reducing heat stress in cattle and maintaining a low-cost, eco-friendly system, also referred to as "passive cooling" (Ortiz et al., 2015). Heat-exchanger coils were placed 25 cm below either sand or dried manure bedding in a controlled environment, and cool water (7°C) was passed through the heat exchangers (Ortiz et al., 2015). Sand bedding was cooler over the entire experiment than dried manure bedding and it was suggested that it would be an appropriate method to use in conjunction with sprinklers, fans, and evaporative cooling methods (Ortiz et al., 2015).

Genetics

Researchers have also studied the possibility of selectively breeding for livestock that have an increased thermo-tolerance. Reproductive techniques in the dairy industry that reduce generation interval as well as improved analysis of genetic markers make it possible to breed for increased thermo-tolerance and hopefully maintain a high production status (Collier et al., 2002). Currently, identifying these genetic markers is of great importance. In the past, *Bos indicus* and *Bos taurus* cattle have been cross-bred in an attempt to incorporate the thermo-tolerance of the *Bos indicus* with the European breeds. This, however, reduces milk production compared with the purebred *Bos taurus* cattle. According to Dr. Collier, some avenues that require further research are the genetic improvement of thermo-tolerance in dairy cows as well as exploring the use of passive cooling systems (personal communication, October 2015).

Autonomic Nervous System

Most internal physiological regulation is controlled by two important neural systems that are a part of the autonomic nervous system called the parasympathetic and sympathetic nervous systems. This sympathetic nervous system enables the animal to react in stressful situations, known commonly as the "fight or flight" response, followed by the ability to return to normalcy (increased parasympathetic activity) once the stressful trigger has passed. There are, however, extremes in this scenario where chronic episodes of stress can shift the homeostatic state. The autonomic response is largely controlled by the brainstem and hypothalamus, and controls functions such as arterial pressure, body temperature, rates of salivation, gastrointestinal activity, and bladder emptying (Hall, 2011).

Parasympathetic Nervous System

The primary response of the parasympathetic system is to maintain a balance towards a more relaxed state, commonly referred to as "rest and digest" (Battipaglia and Lanza, 2015). It is largely involved in digestion of feedstuffs and also plays a role in heart rate regulation (Hall, 2011). The vagus nerve controls approximately 75% of the total reaction carried out by the parasympathetic system (Hall, 2011; Battipaglia and Lanza, 2015). Acetylcholine is the primary neurotransmitter (Hall, 2011; Battipaglia and Lanza, 2015). When a mammal is under stress, the parasympathetic control decreases and the sympathetic system becomes dominant.

Sympathetic Nervous System

The sympathetic system initiates the "flight or fight" response. The most basic design to this is that under stress this response enables mammals to either flee or fight the "danger" that is present. This model can, however, be compared to other instances of stress besides a dangerous foe, such as heat stress. During a heightened sympathetic response the following can occur: increased arterial pressure, increased blood flow to active muscles, decreased blood flow to certain organs including the GI tract and kidneys, increased rates of cellular metabolism, increased blood glucose concentration, and increased glycolysis in liver and muscle (Hall, 2011).

There are important hormones in the sympathetic stress response called epinephrine and norepinephrine (catecholamines) and a class of hormones called glucocorticoids (Sapolsky, 2004). Norepinephrine is synthesized in the terminal ends of adrenergic nerve fibers beginning with tyrosine, and then converted to dopamine, and finally completed inside secretory vesicles (Hall, 2011). The release of catecholamines (primarily norepinephrine in cattle) from chromaffin cells in the medulla of the adrenal gland in response to sympathetic stimulation (Hall, 2011) will

result in 1) blocking of insulin release (Blum and Eichinger, 1988) resulting in decreased storage of nutrients, 2) stimulation of release of cortisol from the cortex of the adrenal gland (Hall, 2011), and 3) stimulation of adipocytes to convert triglycerides into free fatty acids (Sapolsky, 2004).

In humans, approximately 80% of norepinephrine is methylated to epinephrine within the adrenal medulla (Hall, 2011). The catecholamines are responsible for rapid responses to stress, while the glucocorticoids lengthen the time of response initiated by the catecholamines (Sapolsky, 2004). There are 2 major types of adrenergic receptors: α and β receptors, including 3 different types of β receptors (β_1, β_2, and β_3). Norepinephrine mainly acts on alpha receptors, while epinephrine has equal effects on both alpha and beta receptors. Since both receptor types can either have excitatory or inhibitory effects on their respective organ locations, they are more appropriately associated with their affinity for the specific catecholamine (Hall, 2011).

While the catecholamines are released by the sympathetic nervous system, glucocorticoid release is controlled primarily by the brain. The brain senses a stressor and triggers the release of corticotropin releasing hormone (CRH). Corticotropin releasing hormone acts on the anterior pituitary to release adrenocorticotropic hormone (ACTH) into the blood and travels to the adrenal gland, where stimulation of glucocorticoid release is initiated from the adrenal cortex (Sapolsky, 2004).

Along with catecholamine and glucocorticoid release during the stress response, the pancreas is also stimulated to release glucagon; the combination of catecholamines, and glucocorticoids and glucagon increase glucose concentrations. Glucose is the major energy substrate used during times of stress. The pituitary also secretes prolactin and vasopressin, hormones involved in suppressing reproduction and contributing to the cardiovascular response,

respectively. Other hormones related to reproduction (estrogen, progesterone, and testosterone) and growth (GH) are inhibited by the stress response as well as insulin (Sapolsky, 2004). There is also a class of neuropeptides that are released from the pituitary in response to a stressor called endorphins and enkephalins. These are endogenous morphine-like substances that have a primary function of blunting pain perception (Sapolsky, 2004).

The gastrointestinal tract is also greatly influenced by stressors and can result in negative responses that can greatly hinder their functions. Three major contributors to stress-related gastrointestinal disorders, such as ulcers in humans, are: acid/base production changes, decreased blood flow, and immune depression. The term "decreased digestion" as a result of stress involves several different mechanistic shifts to help conserve energy for the rest of the body by taking energy away from digestion. One way digestion is decreased is by reducing the production of hydrochloric acid, which results in reduced concentrations of bicarbonate and mucus production. The stomach lining becomes thinner as a result and there is an overall reduction in digestive function. This of course occurs under periods of chronic stress as it continues in a cycle (Sapolsky, 2004).

Physiological Adjustments of Passive Heat Stress

Thermoregulation Adaptation

The core body temperature is carefully monitored by the preoptic and anterior hypothalamic nuclei of the hypothalamus (Hall, 2011). Specific areas in these centers detect temperatures falling out of a specific range (Hall, 2011), which in cattle is closely maintained around 38.6°C (101.5°F). Metabolic rate determines the rate of heat production and includes the following factors: basal rate of metabolism in all cells, greater rate of metabolism caused by

muscle activity and hormones such as thyroxine, and increased metabolic response induced by norepinephrine (Hall, 2011). While core body temperature remains fairly constant under normal conditions, skin temperature varies with the environmental surroundings (Hall, 2011). Unfortunately, as previously discussed, cattle do not maintain homeothermy well under heat-stressed conditions compared with other species (Silanikove, 2000).

An important way that the body modulates core body temperature is by transferring heat from the body core to the skin (Hall, 2011). Increased blood flow (vasodilation) to the skin allows heat to be carried to the outer surface and will help decrease core body temperature, while decreased blood flow (vasoconstriction) to the skin does the opposite by keeping more heated blood in the core of the body. Vasoconstriction is controlled primarily by the sympathetic nervous response. Once heat is brought to the skin surface it can be lost in 4 ways: radiation, convection, conduction, and evaporation (Hall, 2011).

There is less blood flow towards certain organs (e.g. digestive tract, reproductive organs), because of the stress response activated by the body in order to assist in heat removal from the body. For example, blood flow is increased to the skin because it is carrying heat from the body's core through blood flow and body tissues towards the body's surface via convection and conduction. Heat is then released through evaporation (sweat), and radiation. Panting, however, is an important avenue that cows use to release heat more efficiently because of their decreased number of sweat glands.

Skin vasodilation occurs over the entire human body in response to elevated body temperatures, but does not always occur over the whole body in animals (Johnson and Proppe, 1996). An anatomical feature largely used in blood flow to the skin during heat stress is the arteriovenous anastomoses, which are found in only certain areas (Johnson and Proppe, 1996). A

study done with sheep by Hales in 1973 found that blood flow through ovine arteriovenous anastomoses increased from a 1% cardiac output in thermoneutral zones to 11% during mild to severe heat stress (Johnson and Proppe, 1996). Another study from the same lab concluded that blood flow was much greater during bouts of heat stress in the arteriovenous anastomoses and had a similar flow rate compared with capillary blood flow during thermoneutral environments (Johnson and Proppe, 1996).

Cardiovascular Adaptations

Several physiological responses are known to occur in environments of heat stress, otherwise known as passive heat stress (Crandall and González-Alonso, 2010). Because of the thermoregulation response, blood flow will increase to the skin to transport heat from the core to surface and allow for heat dissipation via radiation or other heat transfer mechanisms if available. With the redirection of blood flow, there is increased vascular conductance in this area (Crandall and González-Alonso, 2010). Conductance refers to the amount of blood that passes through a given vessel (Hall, 2011). Arterial blood pressure would decrease in response to this increased conductance; however, the body helps to stabilize arterial pressure by increasing cardiac output and decreasing the vascular conductance of areas that have reduced blood flow because of the increased blood flow to skin (Crandall and González-Alonso, 2010). An increase in heart rate in response to heat stress aids in increasing cardiac output (Crandall and González-Alonso, 2010).

Metabolic Adaptations

A recent study explored how the body mobilized energy during times of heat stress compared with that of an animal stressed because of feed restriction (Lamp et al., 2015). High-

yielding dairy cows were studied in thermo-neutral environments and again in heat-stressed environments using climatic chambers. As previously mentioned, the metabolism in heat-stressed cows is different than that found in NEBAL cows in thermo-neutral conditions. In recent years, several studies have looked more closely at the relationship between mammalian physiology under heat stress and the resulting impeded lactation response. Rhoads et al. (2009) concluded that only 35% of the loss observed in milk production during heat stress can be attributed to reduced feed intake. Part of the remaining 65% is attributed to key hormones involved in the somatotropic axis, in which the growth hormone (GH) responsiveness of the liver is reduced during heat stress and reduces the amount of the IGF-1 released from the liver in response to GH (Rhoads et al., 2009; 2010).

Cows in heat stress during mid-lactation had no elevated non-esterified fatty acids (NEFA), meaning that fat was not being broken down as a first response to low energy (Lamp et al., 2015). Instead, hepatic glucose was elevated in the blood, signifying that there is a preference of carbohydrate oxidation over fat oxidation in the heat-stressed cow (Lamp et al., 2015). On the contrary, cows experiencing a feed restriction in thermoneutral conditions will favor fat catabolism over carbohydrate oxidation, as evidenced by elevated NEFA, β-hydroxybutyric acid (BHBA), and decreased plasma glucose concentrations (Lamp et al., 2015).

Physiological Adjustments to Aerobic Training

During consistent aerobic training, physiological adjustments occur to better handle bouts of eustress experienced during exercise. Aerobic training refers to "the ability of the body to sustain prolonged exercise" and is linked with "improving cardiorespiratory endurance" (Wilmore et al., 2008). These changes can occur within the working muscle, such as improved

transport and utilization of oxygen, and improved circulation in the cardiovascular system (Egan and Zierath, 2013). Different types of exercise such as strength training and aerobic endurance yield different physiological responses. For the purpose of this literature review, however, focus will remain on the physiological adjustments experienced during aerobic training.

Cardiovascular Adaptations

Several changes occur during aerobic endurance training including heart size, stroke volume, heart rate, cardiac output, blood flow, blood pressure, and blood volume (Wilmore et al., 2008). Hypertrophy of the heart muscle can occur in the left ventricle as well as alterations in the thickness of the myocardial wall. Strengthening of the left ventricle would imply a greater contractility of the heart muscle, which would then increase stroke volume, as well as heart rate, cardiac output, blood flow, and blood volume (Blomqvist and Saltin, 1983). Stroke volume is the difference between the end-diastolic volume and the end-systolic volume (Wilmore et al., 2008).

Resting heart rate and submaximal heart rate change when individuals incorporate aerobic training (Wilmore et al., 2008). This is an extremely important adaptation because if heart rate remained elevated as stroke volume increases, the heart would shorten filling time and compromise stroke volume. Reduced heart rate during submaximal exercise also demonstrates the body's way of reducing energy consumption by cardiac tissue since the heart would be contracting less often yet still pumping a greater amount of oxygenated blood to fuel working muscles (Wilmore et al., 2008). With regards to reduced resting heart rate (bradycardia), it appears that aerobic training induces an autonomic response that increases parasympathetic

activity on the heart and a combined decrease in sympathetic activity (Blomqvist and Saltin, 1983).

Cardiac output is made up of 2 components which are heart rate and stroke volume (Wilmore et al., 2008). There are changes that occur in the heart rates and stroke volume of exercising individuals, however, there is little change observed in cardiac output during rest and submaximal exercise (Nielsen, 1998). Generally speaking, cardiac output will match the oxygen consumption demands of the working or sedentary body (Blomqvist and Saltin, 1983). There is, however, a relationship between cardiac output and oxygen consumption (VO_{2max}); as cardiac output increases it positively influences VO_{2max} (Wilmore et al., 2008).

The capacity of the cardiorespiratory system is calculated by determining the greatest rate of oxygen consumption during maximal exercise, commonly called VO_{2max}. This is determined by both cardiac output and the amount of oxygen extracted by the tissues. As the intensity of exercise increases, VO_{2max} will begin to either plateau or slightly decrease, which is the threshold mark for achieving VO_{2max} (Wilmore et al., 2008).

Blood flow and blood volume are both interconnected and also experience changes through aerobic exercise. As one would expect, blood flow increases since there is an increased demand for oxygen and nutrients for working muscles (Wilmore et al., 2008). There are 3 factors that result in increased blood flow: 1) increased capillary formation in trained muscles, 2) increased recruitment of already present capillaries in trained muscles, and 3) increased blood volume. Blood volume increases as a result of aerobic exercise and the effect occurs rapidly. The increase in blood volume is directly linked with increased blood plasma and red blood cells. Since there is an increase in the fluid portion of the blood, the blood's viscosity is reduced and therefore the blood has enhanced oxygen delivery and improved blood movement through blood

vessels, especially capillaries (Wilmore et al., 2008). Plasma volume increase is linked to osmosis or hormonal responses. The first mechanism is a passive response linked to osmosis. Since there are increased levels of plasma proteins, there is increased osmotic pressure that allows for fluid to be reabsorbed from the interstitial fluid into vasculature (Nielsen, 1998). During exercise, there is a shift and the proteins leave the vascular space and enter the interstitial space before returning via the lymph system (Wilmore et al., 2008). The second mechanism is an active response controlled by hormones. Exercise stimulates the release of antidiuretic hormone (ADH) and aldosterone, both of which cause increased reabsorption of water and Na^+ in the kidney and thereby increase blood plasma (Nielsen, 1998).

Respiratory Adaptations

While improvements in cardiovascular function are vital to improved circulation of oxygen and nutrients to working muscle, the body also needs to enhance oxygen uptake. Adaptations occur within the physiological parameters of pulmonary ventilation, pulmonary diffusion, and arterial-venous O_2 difference at different states of aerobic exercise (Wilmore et al., 2008).

Pulmonary ventilation changes occur during submaximal exercise at any given intensity; however, the greatest change is seen at maximal exercise, especially in highly-trained individuals. Increase in tidal volume (defined as "the air inspired or expired during a normal breathing cycle"), and increase in respiration frequency at maximal exercise affect pulmonary ventilation (Wilmore et al., 2008).

Pulmonary diffusion, the gas exchange in alveoli in the lungs, undergoes changes seen only during maximal exercise. Blood flow from the heart to the lungs increases in trained

individuals, which means that lung perfusion is amplified and therefore more blood is made available for gas exchange. With an increase in ventilation and thus more air respired in the lungs, the combination of increased blood flow and increased air allow for enhanced pulmonary diffusion in the alveoli (Wilmore et al., 2008).

An additional change seen in respiration in the trained individual is in the arterial-venous O_2 difference. Total hemoglobin concentration is usually increased as a result of exercise; however, the amount of hemoglobin per unit of blood is usually the same. The changes aren't seen in arterial oxygen content, but are present in venous oxygen content. This means that the venous blood returning to the heart has a reduced oxygen concentration in trained individuals indicative of a greater oxygen extraction at the tissue level and that the blood flow has been more effectively distributed to active tissues (Wilmore et al., 2008). Overall, the biggest changes seen in all of the respiratory adaptations occur during maximal exercise.

Metabolic Adaptations

A common metabolic product formed during exercise is lactate. Since lactate is a metabolic product in working muscles, determining lactate threshold is a key indicator of establishing aerobic endurance performance (Wilmore et al., 2008). As individuals become more fit, lactate concentrations decrease during exercise suggesting that those individuals have improved their aerobic power and a decreased dependence on the glycolytic system for energy, or a combination of the two (Wilmore et al., 2008). Lactate is a by-product of anaerobic glycolysis, which is used a lot more during high-intensity type exercises, such as sprinting (Wilmore et al., 2008). Edge et al. (2005) reported improvements in both high-intensity training

and moderate-intensity training in their respective lactate thresholds (8-10%) with no differences between exercise intensities.

Another metabolic change that occurs as a result of aerobic exercise is in the respiratory exchange ratio, or the ratio between the carbon dioxide that is released and the oxygen that is consumed during metabolism (Wilmore et al., 2008). When the respiratory exchange ratio decreases, it indicates that the body prefers to utilize free fatty acids (FFA) over carbohydrates at different intensities of exercise during submaximal exercise (Wilmore et al., 2008). At lower intensities, circulating FFA are the preferred choice of energy by working muscles, but when the exercise intensity increases the energy source shifts from fat oxidation to carbohydrate oxidation (glucose) (Egan and Zierath, 2013). There is also a change in oxygen consumption, most seen during maximal exercise. In order to determine cardiorespiratory endurance, VO_{2max} is the best indicator to determine in exercising individuals (Wilmore et al., 2008).

While VO_{2max} appears to have a threshold, aerobic training has been shown to continually improve the activity of oxidative enzymes, such as succinate dehydrogenase (SDH) and citrate synthase (Wilmore et al., 2008). Thus, VO_{2max} may be limited by the cardiovascular system's ability to transport oxygen to the muscle and not the muscle's oxidative potential, which break down nutrients to be used for ATP synthesis (Wilmore et al., 2008). Both SDH and citrate synthase are found in greatest activity in type I muscle fibers (Egan and Zierath, 2013), and since type I muscles are largely used in aerobic exercise this increase is intuitive. Continued aerobic exercise improves oxidative function (Egan and Zierath, 2013). A consequence of improved oxidative enzyme capacity within the mitochondria is glycogen sparing (Wilmore et al., 2008). This means that glycogen utilization within the muscle is not picked as the primary fuel source; rather there is a heightened dependence on fat as the primary fuel source (Wilmore et al., 2008).

Muscle Adaptations

Muscles that are used over time, specifically in endurance training, undergo changes that occur in the muscle fibers especially in fiber structure and function (Wilmore et al., 2008). Previous adaptations, such as increased blood flow, play a role in supporting the adaptive changes that take place in muscle fibers as a result of aerobic training. Egan and Zierath (2013) reported that these adaptations occur in response to contractile activity.

Muscle fibers, capillary density and myoglobin content all increase in order to more efficiently facilitate the transfer of oxygen from the blood to the working muscle, specifically the mitochondria (Wilmore et al., 2008). Slow-twitch (type I) fibers are the key muscle fibers used in endurance training and contain greater myoglobin content compared with fast-twitch (type II) fibers (Egan and Zierath, 2013). Type I fibers increase in size under conditions of constant aerobic training and muscle fiber types (types I and II) transform into types more applicable for the work performed (Schiaffino and Reggiani, 2011). For example, type IIx fibers have reduced aerobic capacity and therefore are used less during endurance training, whereas type IIa are more oxidative and are more appropriately used for moderate duration aerobic exercise (Egan and Zierath, 2013). Exercise that continues may cause type IIx muscle fibers to be transformed into type IIa muscle fibers to take on a more oxidative property that produces a more efficient working muscle (Wilmore et al., 2008). Oxidative enzymes also increase with endurance training (Egan and Zierath, 2013). This is important for skeletal muscles, since type I muscle fibers are greatly dependent on oxidative phosphorylation for their energy production (Egan and Zierath, 2013). When comparing 2 different types of exercise, Gibala et al. (2006) reported that short-term sprint intervals and endurance training exhibited similar responses in physiological

acclimations of muscle buffering capacity and glycogen content in the muscle. This indicates that muscular adjustments can occur under different methods of exercise.

Increased capillary density around muscle fibers results from endurance training and allows for increased blood flow, which enables heat, oxygen, and nutrients to be more readily transferred between blood and working muscle fibers. Capillary density is an important factor that aids in increasing VO_{2max}. Once oxygen has been brought to the muscle, it is transported via myoglobin to be either stored for later use or brought immediately to the mitochondria. Importantly, myoglobin concentrations increase with aerobic training, which enable the muscle to more efficiently transport oxygen to the mitochondria. Within the mitochondria is where oxidative energy production takes place, thus the fiber's ability to efficiently produce ATP is improved. The mitochondria size and number also increase in response to aerobic training (Wilmore et al., 2008).

History of Exercise in Cattle

One of the first studies done involving cattle exercise was done by Anderson et al. (1976) at Utah State University. These investigators outlined 2 different exercise models to use for exercising cattle and determined that exercising pregnant dairy cows and heifers could be done comfortably at 3.5 km/h and that most animals refused to continue at anything above 5.5 km/h.

Blum et al. (1979) exercised steers in a high altitude simulation in order to study concentrations of plasma catecholamines, parathyroid hormone (PTH), and heart rate. Steers that began exercising had elevated levels of epinephrine, norepinephrine, and PTH, and were all significantly greater during exercise than pre-exercise. The authors concluded that cattle undergoing exercise induced the stress response, as seen by the elevated catecholamine levels.

Heart rate and PTH both respond quickly to exercise; however, while heart rate remained elevated throughout exercise time, PTH concentrations began to decrease before the end of exercise. Blum et al. (1979) attributed the increase of PTH to sympathetic stimulation not only because of its increased response to exercise but because of its significant correlation with epinephrine as well as simultaneous increases in norepinephrine, lactic acid, and FFA.

A follow up study was done by Anderson et al. (1979) to study post-partum effects during lactation of cows exercised during the dry period. Glucocorticoids and hemoglobin concentrations were both increased post-exercise and there were different glucocorticoid concentrations in cows exercised at different speeds. Those exercised at the same speeds and for different distances, however, did not have any significant differences. Exercise did not affect pre- or post-partum dry matter intake (DMI) and milk production; however, exercised cows had decreased percentages of butterfat. While there were no statistical differences between exercised and non-exercised body weight prior to calving, non-exercised heifers were heavier at parturition (Anderson et al., 1979).

Lamb et al. (1979) studied the effects of prepartum exercise on 2-yr old heifers on parturition, reproduction, mammary health, and milk composition. These researchers found an an improved post-partum response in exercised heifers including improved calving ease, reduced occurrence of udder edema, and improved placental release after parturition. In addition, there was improved milk production and feed intake in exercised heifers. Heifers that were exercised post-partum, however, had both decreased feed intake and milk production.

Lamb et al. (1981) also studied the effects of prepartum exercise in both Holstein heifers and Holstein cows of different ages exercised for either short or long distances and found that there were some differences between milk component percentages and reproductive variables.

Animals exercised for shorter distances had greater protein and SNF percentage during lactation. There were also fewer days open during lactation for exercised cows versus non-exercised cows. Daily feed intake did not increase in any treatment; however, older cows had greater reductions in body weights after longer distance exercise compared with the younger heifers (Lamb et al., 1981).

The previous studies looked only at effects of exercise on production life, including milk production, DMI, and reproductive values. Blake et al. (1982) performed a study that attempted to quantify physical fitness levels in dairy cattle. The objective was to demonstrate whether cows in confinement were in poor physical condition and if exercise could improve their fitness. Both heart rate and respiratory rate were helpful indicators of improved fitness, however, external environmental stressors may have reduced their full reliability. The authors concluded that cattle in a confinement operation are not in the best physical state of fitness.

In the mid to late 1980s, research focused on studying physiological responses to exercise including cardiovascular, ventilatory, and metabolic variables (Kuhlmann et al., 1985; Blum and Eichinger, 1988; Jones et al., 1989; Constantinopol et al., 1989). Oxygen consumption and CO_2 production were increased nearly 10-fold when Hereford calves were exercised at their near maximal load of 2.2 m/s on the treadmill (Kuhlmann et al., 1985). Ventilation patterns demonstrated that tidal volume significantly increased above their resting values, and had an impact on increases in ventilation because respiratory frequency remained consistent at approximately 60 breaths/min even as work load increased (Kuhlmann et al., 1985). Kuhlmann et al. (1985) reported similar responses in cardiovascular, respiratory, and metabolic responses to exercise as in other species. Some different responses, however, included a smaller increase in cardiac output and a greater increase in potassium ion concentrations during exercise (Kuhlmann

et al., 1985). Because of the role of potassium in cardiovascular and respiratory responses, Kuhlmann et al. (1985) suspected that cattle may have a greater limitation on the ability to perform continuous heavy exercise.

Epinephrine and norepinephrine hormones increase in response to exercise in calves, specifically greater concentrations of norepinephrine (Blum and Eichinger, 1988). This increase in catecholamine concentrations inhibits and blocks the secretion and action of insulin as demonstrated by the decreased insulin concentrations. There were also increased concentrations of triiodothyronine (T_3) which when combined with increased epinephrine and norepinephrine concentrations are suspected to act in oxidative processes. Catecholamines also increase both glycogenolysis and lipolysis, resulting in increased circulating concentrations of glucose, NEFAs, lactate, and glycerol. Lactate can indirectly provide energy to the working muscle because it is a substrate in glucose synthesis within the liver (Blum and Eichinger, 1988). This response ensures that glucose is readily available in times of stress.

While heat stress has negative connotations associated with it, exercise stress is a healthier form of stress that has many positive impacts. Arave et al. (1987) found that Holstein heifers undergoing regular exercise had reduced concentrations of glucocorticoids compared with their sedentary counterparts. The authors interpreted this to mean that heifers were either more fit, or that their adrenal response had adapted to the stress of exercise, or a combination of the two (Arave et al., 1987).

Exercise and Thermo-tolerance

Exercise induced heat acclimation, or acclimatization, result in physiological adaptations that can be summarized into 2 major categories: the cardiovascular and fluid regulatory response,

and physiological heat dissipation mechanisms. Although little work has been done in this area with cattle, all of these factors combined have a large influence on exercise performance in hot conditions in both humans and horses.

Exercise in Humans

The degree of acclimation, or acclimatization, depends largely on the amount of time spent exercising, exercise intensity, and the environment (Geor and McCutcheon, 1998; Maughan and Shirreffs, 2004). The major cardiovascular adjustment that occurs during heat acclimation early on is expanded resting plasma volume (Armstrong and Maresh, 1991). Within a week of exercise to improve heat acclimation, there was a 10-25% increase in plasma volume and this expansion was correlated with decreased exercise heart rate (Wenger, 1988). Cardiac output is still maintained even with a decreased heart rate in response to exercise because of the expanded plasma volume. It is hypothesized that this plasma expansion is related to protein inclusion in the vascular space, though its mechanism is not fully understood (Nielsen, 1998). Cardiac output is also redistributed to the peripheral tissues to enhance core body heat removal by conduction through tissues, convection via blood flow, and evaporative loss from skin. Thus, sweating rates are largely impacted by cardiovascular adjustments to the heat.

The sweat response is enhanced by increased sweat sensitivity as well as increases in sweating rate as body temperatures increases (Buono and Sjoholm, 1988). Sweat also becomes more diluted in order to decrease electrolyte loss via sweat (Nielsen, 1998). Sweating rates are linked with cardiovascular capacity because cardiac output influences blood distribution (Geor and McCutcheon, 1998). Blood flow to skin ratio largely increases to aid in heat dissipation via evaporation. The limb areas especially have increased body heat loss potential because of the

high surface area to body mass ratio (Wenger, 1988). As exercise progresses, however, total body sweat production actually has been found to decrease over time (Buono and Sjoholm, 1988). It has been suggested that this decrease may result from body temperatures maintained at reduced temperatures because of improved thermal regulation, as well as improved maintenance of body fluids (Armstrong and Pandolf, 1988). Nielsen et al. (1993) found that in humans following repeated exposure to heat and exercise, resting body temperatures were reduced. While total sweat production has been shown to decrease, the efficiency of sweat gland use appears to be enhanced. Certain areas of the body have been found to reduce their sweat rate, while other areas with previously greater concentrations of inactive sweat glands produce more sweat (Yamacuhi et al., 1997). Therefore there is more evenly distributed sweat activity across the body's total surface providing a greater area for heat dissipation. Sweating rates have also been found to increase when cattle were moved from thermoneutral conditions to a hot environment, though not in response to exercise (Blazquez et al., 1994).

Cheung and McLellan (1998) concluded that exercise-heat tolerance in a heat stressed environment is improved by long-term aerobic fitness and that short-term heat acclimation did not influence exercise-heat tolerance. In highly fit subjects, heat acclimation was enhanced because of improved efficiencies of sweat rate, skin temperature, and rectal temperature. In moderately fit men, however, only sweat rate was improved. Cheung et al. (2000) also reported that long-term physical fitness could provide some aid in "building up" heat tolerance, but that neither heat acclimation nor short-term aerobic training were sufficient in improving heat tolerance and exercise performance. There was another report that found a greater intensity of exercise (75% of VO_{2max}) for 30 min was just as effective in promoting adaptations as lower intensity exercise (50% of VO_{2max}) for 60 min (Houmard et al., 1990). So while a certain level

of fitness enhances heat tolerance, certain combinations of intensity and duration are also linked with improving the ability to acclimate and improve performance in warmer temperatures.

Important factors that determine how well adaptation can occur in hot climates include the rise in body temperature and the sweating response (Maughan and Shirreffs, 2004), and the main limiting factor of performance in the heat is a critically high body temperature (Gonzalez-Alonso et al., 1999; Maughan and Shirreffs, 2004). In humans, adaptation to hot environments occurs in approximately 7-14 days (Montain et al., 1996; Pandolf, 1998) and effects may remain as long as a month (Pandolf, 1998). About 75% of all physiological adjustments occur within the first wk, and it seems that the length of retention of these adjustments are dependent on the individual and the environment making the "level" of physiological adaptations quite variable (Pandolf, 1998). Therefore, when athletes train in the heat it is recommended that no more than 2-3 day elapse without exposure to hot environments in order to gain and keep the best degree of adaptation (Maughan and Shirreffs, 2004).

Exercise in Horses

Physiological adaptations can occur to improve thermoregulatory capabilities if the horse is regularly exercised in cool to moderate temperatures, or if they are repeatedly exposed to hot temperatures. Similar to humans, specific adaptations to the heat include improved cardiovascular function and a greater sweating response.

Improved cardiovascular adjustments involve increased plasma volume and decreased heart rate during exercise (Geor and McCutcheon, 1998). In horses exercised at a low-intensity, there was a 29% increase in resting plasma volume after only 2 wk of walking (McKeever et al., 1987). Another report, however, stated that a 12-15% increase in resting plasma volume was

determined in horses exercised at both low and high intensities for 8 wk (Geor and McCutcheon, 1996). Heart rate was also reduced during the latter part of exercise on d10 of the experiment compared with day 1 (Geor et al., 1996). Average VO_{2max} was not different between 3 wk of training and 10 wk (150 ± 7 ml/kg per min vs. 145 ± 9 ml/kg per min) of training in a hot, humid climate (Geor et al., 1996). Most of the cardiovascular adjustments included increases in plasma volume, cardiac output, and blood flow to skin as well as decreases in heart rate that occurred within 1 wk of exercising in the heat (Geor and McCutcheon, 1998).

Adaptations to exercising in the heat result in increased sweating rates as well as the onset of sweat production at decreased body temperatures (Wenger, 1988; Armstrong and Maresh, 1991). These adaptations usually occur within 2 wk of implementing exercise in the heat (Wenger, 1988; Armstrong and Maresh, 1991; Geor and McCutcheon, 1998).

Rectal temperatures were greater in horses exposed to hot, humid climates compared with cool, dry climates (Geor et al., 1996). By the fifth day of the experiment, rectal temperatures before, during, and after exercise were reduced ($P < 0.05$) when compared with day 1 rectal temperatures (Geor et al., 1996). It was demonstrated in Thoroughbred horses that daily exposure and exercise in hot and humid climates for 3 wk resulted in a reduction in thermal and cardiovascular strain by the decrease in both rectal temperatures and heart rates (Geor et al., 1996). Overall, Geor and McCutcheon (1998) recommended that in order to initiate thermoregulatory adaptation mechanisms by exercise: (1) a 2-3 month period of exercise training will greatly improve thermoregulatory capacity, and (2) a 2-wk period of exercise in warmer temperatures will enhance thermoregulatory function.

Exercise and Pregnancy

Blood flow to the uterus was reduced during exercise in sheep (Lotgering et al., 1983). Physiological changes related to exercise and pregnancy in the maternal cardiovascular system included VO_{2max}, heart rate, and cardiac output (Lotgering et al., 1983). Maternal arterial partial pressure (pO_2) of pregnant sheep exercised at 70% VO_{2max} increased 13%, while arterial partial pressure (pCO_2) decreased by 28%. In the fetus, however, pO_2 and pCO_2 were decreased by 11% and 8%, respectively. Though there was a decrease in fetal pO_2, it was not accompanied with release of catecholamines or with changes in total blood or red blood cell volumes. Because of these changes Lotgering et al. (1983) determined that maternal exercise in sheep did not seem to bring major stress or a hypoxic event to the fetus.

There has been debate whether exercise during human gestation is beneficial or detrimental to the mother and offspring. It is suggested that physical activity during pregnancy in humans is not detrimental and may be beneficial to the mother and offspring (Wang and Apgar, 1998; Olson et al., 2009). Although blood flow is reduced during exercise as a result of the sympathetic nervous response, exercise improves cardiovascular health (Myers, 2003). Because of improved cardiovascular health, exercise may improve blood flow to the uterus during rest, bringing more nutrients to the fetus and affecting growth in-utero and post-parturition. There haven't been any documented studies that show a decrease in fetal growth during exercise and this is thought to be linked with the subsequent glucose delivery after exercise (Kelly, 2005). Though there seem to be no detrimental effects of exercise on women, it should be pointed out that most of the pregnant individuals involved in exercise and pregnancy research have some pre-existing level of fitness.

Justification

It is evident that exploring options to mitigate the deleterious effects of heat stress are needed for dairy cows. From an economic standpoint, not only do cows produce less milk but producers also need to increase input costs in the form of adding sprinklers and fans. These heat mitigation tools significantly reduce the loss of milk. Cow comfort is also important to consider and the physiological changes occurring in the cow during heat stress reduce comfort and can even become life-threatening.

Exercise has been found in other species (e.g. humans and horses) to improve thermo-tolerance and performance during hot, humid conditions. This "performance" can be equated to lactation in dairy cattle because of the great amount of energy required to sustain lactation. Not only is exercise related to improved thermo-tolerance, but the resulting fitness may further enhance cardiovascular and respiratory functions, which are involved in removing heat in cattle.

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Chapter 2 - Effects of exercise on thermo-tolerance in pregnant Holstein heifers

Introduction

Dairy cattle are sensitive to heat stress because of high metabolic heat production and feed intake related to ruminal fermentation and milk yield, already showing signs of heat stress at temperatures as low as 23.3°C and 75% humidity. Environmental temperatures have been found to increase, especially in recent years (Collier and Gebremedhin, 2015). Annually, 2 billion dollars are lost to heat stress in American animal agriculture alone (Baumgard et al., 2006). Energy requirements for maintenance increase during periods of heat stress because of thermoregulatory mechanisms required to dissipate heat. Between 1940 and 1995 in the United States, the average 300-day lactation increased 338% from 2,096 kg of milk to 7,462 kg (Kadzere et al., 2002). From 2005 to 2014, milk production has increased 14% to an average of 10,114 kg/cow per year (US Department of Agriculture, 2015). This increase in milk production makes dairy cattle even more vulnerable to environmental temperatures because more energy is required to support maintenance requirements rather than sustaining milk yield.

Measures have been taken via heat abatement techniques (sprinklers, fans, and shade) to reduce the level of heat stress experienced by cows. Although these heat abatement strategies are extremely useful, financial and environmental costs are necessary to implement these strategies as well as increased water-run off and water usage.

Exercise in hot, humid climates has been found to improve heat tolerance by heat acclimatization in humans and horses (Cheung and McLellan, 1998; Geor and McCutcheon,

1998). In a similar fashion, implementing an exercise regimen for dairy cows could increase heat tolerance, thereby lessening the impact on lactation yields. The objectives of this study were to: 1) develop an exercise regimen and determine heifer fitness, 2) evaluate exercise and heat tolerance after exercise, and 3) determine if a relationship exists between exercise and resulting milk yield or other production traits. We hypothesize a positive relationship between exercise and improved thermo-tolerance exists that may improve animal comfort and alleviate stress during hot environmental conditions.

Materials and Methods

General procedures

All experiments were approved by the Kansas State University Institutional Animal Care and Use Committee (IACUC #3432). Pregnant non-lactating Holstein heifers (n = 24 in experiment 1; n = 24 in experiment 2) from the Kansas State University Dairy Teaching and Research Center were used in this study in a randomized complete block design. Description of treatments and blocking factor are explained later in detail for each experiment. Candidates for these exercise experiments were pregnant heifers from 81 to 101 days before calving (target was 91 d). All heifers were familiarized to the exerciser (Priefert, 8-horse exerciser, Mount Pleasant, TX) 1 wk before the experiment began by forced walking in the exerciser in pairs for approximately 5 min at 3.22 km/h. Eight hanging divider panels connected to a central base housing the motor moved panels either in a clockwise or counter-clockwise fashion (Figure 2.1). The hanging panels were centered between panels that made up 2 concentric rings. The exerciser could be set to specific speeds between 0 and 25.8 km/h.

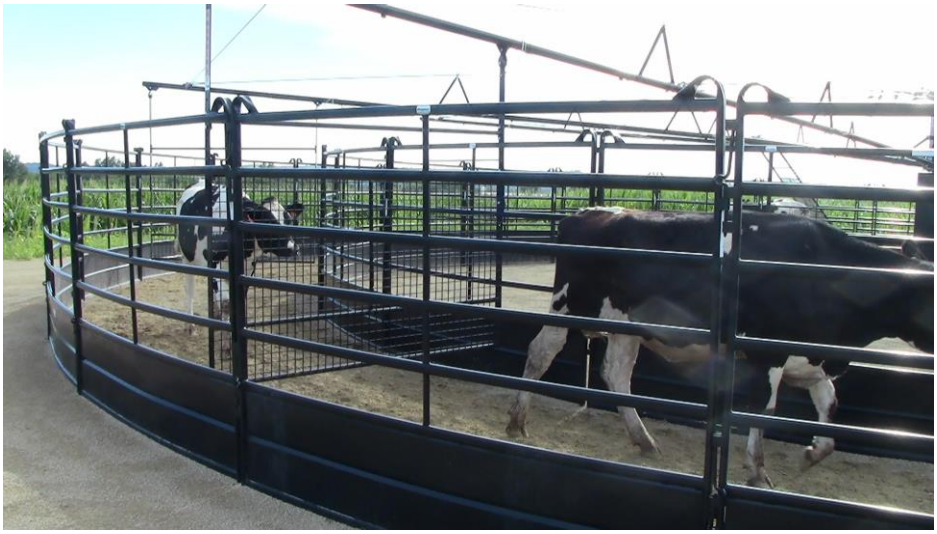

Figure 2.1. Heifers being exercised in 8-panel motorized walker.

Heart rate assessment

Heart rate monitors (Polar, RS800CX, Kempele, Finland) were fitted onto heifers around the withers and sternum and behind the point of the shoulder. Because of the thicker hide and relative dearth of apocrine sweat glands of dairy heifers compared with horses, embrocation (Up & Up, Target©, Minneapolis, MN) was applied to the area behind the point of the shoulder to make the skin more conductive to the electrode sensors of the heart rate monitors. In addition, shower gel (Old Spice High Performance, Proctor & Gamble ©, Cincinnati, OH) was used as an electrode gel because it specifically contained Na^+ and Cl^- ions. Because cattle have fewer sweat glands that are as high-functioning as in horses, this electrolyte gel improved conductivity of the electrode sensors attached to the heart rate monitors.

Environmental temperature loggers

Two environmental logging devices (HOBO Pro V2, Onset Computer Corporation, Pocasset, MA) were used to measure both environmental temperature and relative humidity

(RH). One was placed in the pen where the heifers were housed and the other was placed by the exerciser. Loggers were set to collect temperature and RH every 30 min. Once data were downloaded from the sensors, THI was calculated using the following equation:

= Air temperature(°F) – [(0.55 – (0.55 x RH/100)) x (Air temperature(°F) – 58)]

Blood sampling

Blood samples were collected from each heifer in both experiments before and after each of the 3 fitness tests. Fitness tests were performed differently for experiments 1 and 2 and will be described specifically for each experiment. Blood (6 mL) was collected using sterile evacuated tubes (Monoject Kendall, Na Heparin [Experiment 1], K_2 EDTA [Experiment 2]) from the coccygeal vein. Pre- and post-fitness test blood samples were collected on d 0, 28, and 56 of exercise training and immediately analyzed as a whole blood sample using an iSTAT (Abaxis, VetScan iSTAT 1 Handheld Analyzer, Union City, CA) with cartridge CG8+ (Abaxis, iSTAT CG8+, Union City, CA). The CG8+ cartridge measured: pH, partial pressure oxygen (pO_2), partial pressure carbon dioxide (pCO_2), bicarbonate (HCO_3-), total carbon dioxide (TCO_2), base excess of blood (BE), Na^+, K^+, iCa^{2+}, hematocrit (Hct), hemoglobin (Hb), glucose, and oxygen saturation (sO_2). Once blood samples were collected and initial blood chemistry panel analyzed, samples were placed on ice until transported to the lab for centrifugation (Beckman Coulter, J6-B, Brea, CA; 20 min, 1,200 × *g*, 5°C). Plasma was collected and stored at –20°C until further analyses for lactate and glucose using the YSI analyzer (YSI Inc., YSI 2300 Stat Plus Glucose and Lactate Analyzer, Yellow Springs, OH). Briefly, the YSI analyzer uses an electrode to measure current generated by reactions catalyzed by glucose and lactate oxidase, as it was set up to simultaneously measure both glucose and lactate concentrations. The analyzer aspirated 25 μL

of the plasma sample and performed an autocalibration every 6 samples according to the manufacturer's instructions.

Calving observations

Calving ease was assessed by using a subjective scale of 1 to 5: 1 = no problem, 2 = minor problem, 3 = needed assistance, 4 = considerable force, 5 = very difficult (e.g. C-section) (Weigel, 2010). The difference in calving day was calculated by subtracting the actual calving date from the estimated calving date based on breeding date. Birth weights (for heifer calves only) and gender of calves were recorded. Incidences of any metabolic disorders of cows were observed and recorded by the dairy unit manager during the first week after calving. Fever was recorded when body temperature was > 39.2°C.

Experiment 1-Late Summer/Fall 2014

Three treatments were imposed to examine the impacts of exercise and handling on subsequent heat stress and observational cues to stress during the first postpartum milking in the parlor. Based on performance in the d 0 fitness test, the 3 least fit heifers (as determined by the ratio pace:heart rate) were assigned randomly to each treatment. Stratification continued with the next 3 least fit and so on until all heifers were assigned to treatments. Thus, the block was fitness. The treatments are as follows: 1) sedentary (SC; heifers left in the pen; non-exercised, minimal handling, n = 9); 2) exercise-control (EC; heifers brought to exerciser but not exercised; non-exercised, handled, n = 8); and 3) experimental exercise (EX; exercised heifers; exercised, handled, n = 8). There was 1 heifer with edematous tarsal joints and was therefore assigned non-randomly to the sedentary as a reserve. Animals were acclimated to the exerciser (Priefert, 8-horse exerciser, Mount Pleasant, TX) 1 wk before the start of the experiment. All exercise and

fitness tests were carried out during the morning hours beginning at 0500 and ending before 1100.

Heifers were stratified before assigning to treatments by conducting a preliminary "fitness test" which was modified from that described by Davidson and Beede (2008). Briefly, the fitness test was carried out as follows: On d 0, candidate heifers were fitted with heart rate monitors (Polar, RS800CX) around the withers and sternum, and a temperature sensing device (HOBO U12, Onset Computer Corporation, Pocasset, MA) was fitted to a blank CIDR (Eazi-Breed CIDR Cattle Insert, Pfizer Animal Health, Zoetis, Florham Park, NJ) and placed intravaginally. The fitness protocol was conducted with a single heifer in the exerciser set to perform the following protocol: a warm up (5 min at 3.22 km/h), then an increase every 3 min of 0.64 km/h until failure. Failure was determined using the following physiological indicators: elevated heart rate (HR > 180), lowered heads, excessive salivating, or whenever the heifer refused to continue exercising. Exercise ceased if there were signs of physical distress including but not limited to: lameness, dyspnea, extreme heart rate variations, or vocalization. Heart rate, body temperature and speed of the exerciser at failure were recorded.

Heifers in the exercise treatment were exercised for 8 consecutive weeks. The exercise regimen was performed between 30 and 50 min every morning beginning at 0730-0800 h and varied between tempo paces of walk-slow jog, walk-fast jog, or longer periods of endurance (Appendix A). The exercise regimen used in this experiment was created with the assistance of the CEO of Peaks Coaching Group (Bedford, VA), USA cycling Level 1 coach, and former professional cyclist, Hunter Allen.

Locomotion Score

Heifers were assigned weekly a locomotion score using the Zinpro locomotion scoring outline (Zinpro Corp., Eden Prairie, MN) to ensure that exercise was not negatively affecting gait. The locomotion score was set at a scale of 1 to 5: 1 = normal; 2 = mildly lame; 3 = moderately lame; 4 = lame; 5 = severely lame and adapted from Sprecher et al. (1997). No heifer received a score greater than 2 throughout the entire study.

Temperature data loggers (HOBOs)

Remote temperature sensors were fitted to blank controlled internal drug release (CIDR) inserts placed intravaginally in heifers at the specified times during the exercise period. Because of limited number of temperature sensors (n = 12), heifers from each of the 3 treatments were selected randomly to receive the intravaginal temperature probe as a basis for comparison. During wk 2 and 6, temperature sensors were inserted vaginally and logged temperature data for 3 to 4 d in the same heifers that were previously randomly selected during fitness tests. All temperature data were divided into different temperature zones (zone 1 < 39.0°C, zone 2 = 39.0°C to 40.0°C, or zone 3 > 40°C) and percentage of time spent in each zone was calculated in Microsoft Excel (Microsoft Corporation, Redmond, VA). Research has shown that body temperatures > 39.0°C become hinder performance in lactating dairy cattle (Wolfenson et al., 1988).

Weekly measurements during experiment

Weekly weights were measured (GSE Inc., LBS Scales 350, Livonia, MI) to assure that exercise did not negatively impact the normal increase of body weight during the last trimester of pregnancy. Rectal temperatures (Rectal thermometer M700, GLA Agricultural Electronics, San Luis Obispo, CA) also were recorded at the time of weighing.

Lactation data collection

Residual milk (milk retained in the mammary gland after a normal milking) was recorded at each of the first 3 postpartum milking appointments. Heifers (now cows) from each treatment were milked until the device sensed reduced flow rates and detached from the teats. Cows were then injected with oxytocin (1 mL; 20 USP/mL) and the milking machine was reattached to the teats and allowed to remain on until the device sensed reduced flow rates and detached from the teats for the second time. Milking machines were re-attached 1 min after the oxytocin was administered. Residual milk was then recorded during the post-oxytocin milking. Beginning on d 3 of lactation, weekly milk samples were collected for analyses of milk components: fat, protein, lactose, and somatic cell count (SCC), solids-not-fat (SNF), and milk urea nitrogen (MUN) for 20 wk. Because of the great variability in SCC, somatic cell score (SCS) was used and is defined by the following scale: 0 < 17; 1 = 18 to 34; 2 = 35 to 70; 3 = 71 to 140; 4 = 141 to 282; 5 = 283 to 565; 6 = 566 to 1,130; 7 = 1,131 to 2,262; 8 = 2,263 to 4,525; 9 >4,526.

Observational signs of stress in milking parlor

During the first 3 visits to the parlor after parturition, observational signs of stress were analyzed using an ethogram adapted from Sutherland et al. (2012) to quantify number of actions occurring during milking including: an overall parlor score, number of hoof steps, number of hoof lifts, number of kicks, number of times milking units were kicked off by cows, number of urinations, number of defecations, and number of vocalizations. The scale used for parlor score was a range from 1 to 4 (1 = calm, 2 = slightly nervous, 3 = moderately nervous, 4 = excessively nervous/aggressive). All scores were made by the same individual. Step was defined as the hoof lifted off the ground without going higher than the upper part of the dew-claw. Lift was defined

as the hoof lifted off the ground higher than the upper part of the dew-claw but lower than the middle point between the dew-claw and the point of the hock. Kick was defined as the hoof lifted off the ground higher than the middle point between the dew-claw and the point of the hock. A kick off was recorded when the cows kicked off one or more teat cups of the milking claw during the milking procedure. All cows were assessed for these observational cues beginning at approximately 5 min after entering the parlor and the count was recorded for each heifer before the milking claw was attached (pre-milking). A new count started for each observation as soon as the milking claw was attached and continued until the cows left their respective stall in the parlor.

Statistical analyses

All data were analyzed by the method of ANOVA using both PROC GLIMMIX and PROC MIXED with SAS Enterprise 6.1 (SAS Institute Inc., Cary, NC). Dichotomous variables of ketosis occurrence (1 or 0), fever (1 or 0), and observational variables (1 or 0; defecation, urination, or kick bar use) were analyzed by PROC FREQ and an odds-ratio analysis was carried out using PROC GLIMMIX.

The best-fitting covariance structure was used for repeated measures (first-order autoregressive, heterogeneous autoregressive, or spatial power) based on Bayesian information criterion (BIC) values. Procedure GLIMMIX was used to analyze all data collected on fitness test day. Fixed variables included treatment, time (day, fitness test day), and time by treatment; the random variable was heifer. Because fitness tests were carried out over a series of days the variable day was nested within fitness test day. Procedure MIXED was used to analyze the remaining data including body weight, milk components, milk production, and body temperature

data. Fixed variables included treatment, time (day, week, month), and time by treatment; the random variable was heifer. Orthogonal contrasts between treatments were performed using CONTRAST statements where contrast between EC and SC were first compared and if not different ($P > 0.10$), comparisons of EX vs. EC and SC were made. Differences between each treatment were established using ESTIMATE statements. Confidence intervals are reported at 95% and statistical significance was declared when $P < 0.05$, and trends when $P < 0.10$.

Experiment 2-Summer 2015

Pregnant non-lactating Holstein heifers (n = 24) from the Kansas State University Dairy Teaching and Research Center were enrolled in this study in a randomized complete block design and assigned to 2 treatments: exercise-control (EC; heifers brought to exerciser but not exercised; n = 12), and exercise (EX; n = 12). Following the first fitness test on d 0 (Table 2.1), heifers were randomly stratified into blocks of 2, and each block was assigned randomly to either treatment based on heart rate (HR). Thus, block was HR. The heifers with the 2 greatest HR were assigned randomly to treatments, followed by the next 2 greatest, until all heifers were divided between treatments. For 8 continuous weeks, heifers were exercised 4 d/wk following a pre-programmed exercise regimen that consisted of walking at an average pace of 4.82 km/h (3 mph) for an average time of 30 min (Appendix A). All exercise protocols were carried out between 1300 and 1500 h C.S.T. On d 0, 28, and 56 a fitness test was done for 24 heifers that followed the protocol outlined in Table 1.

Table 2.1. Experiment 2 fitness test outline

Item	Time, min	Speed, km/h
Warm-Up	5	3.22
First Burst	1	4.82
Active Rest	2	4.02
Endurance	10	5.63
Cool Down	5	3.22

On fitness test d 0, 28, and 56, heifers were allocated randomly into 3 exercise groups (Groups A, B, and C), each with equal representation of both treatments. Heifers from each exercise group were exercised at once in the exerciser so that all animals could be exercised in a single day.

Temperature data loggers (HOBOs)

Temperature sensors were fitted and used as outlined in experiment 1. Heifers exercised in group A had the loggers for 1 h after exercise completion. They were then removed and disinfected before inserting into Group C heifers. Both groups B and C had the HOBO devices for 24 h after exercise completion. Loggers recorded body temperature every minute. All temperature data were divided into different temperature zones (Zone 1 < 39.0°C, Zone 2 = 39.0-40.0°C, Zone 3 > 40°C) and percentage of time spent in each zone was calculated in Microsoft Excel (Microsoft Corporation, Redmond, VA) as in experiment 1.

Weekly measurements during experiment

Body weights, skin temperature, rectal temperatures, and respiration rates (RR) were collected weekly on a non-exercise day. Weekly weights were measured (GSE Inc., LBS Scales 350, Livonia, MI) to ensure that exercise was not negatively impacting the normal trend of

increased body weight in the last trimester of pregnancy. Using body weights (kg), surface area was calculated with the following equation (Berman, 2003):

$$\text{Surface Area} = 0.14 \times (\text{Body weight}^{0.57})$$

Skin temperature was also measured in 5 specific locations: cheek, back of ear, withers, thurl and udder using an infrared thermometer (Raytek, RAYMT4U, Mini Temp Portable IR Gun, Quebec City, Canada). Respiration rates were collected while heifers were in their normal housing in order to best assess environmental stress on the heifers without adding handling stress. Flank movements on the right side, which are indicative of respirations, were counted during 15 s time period and then multiplied by 4 to determine breaths/min.

ADH and cortisol data collections

During wk 8, a blood sample was collected from the coccygeal vein using sterile EDTA evacuated tubes (Monoject Kendall, K_2 EDTA, 6ml). Blood samples were then centrifuged and the plasma stored at -20°C for subsequent analysis of cortisol (Enzo Life Sciences Inc., Cortisol ELISA kit, Farmingdale, NY) and anti-diuretic hormone (ADH) (BioAim Scientific Inc., Bovine Vasopressin EasyTest Competitive ELISA Kit, Scarborough, ON, Canada). Cortisol and ADH assays were conducted via ELISA according to their manufacturer's instructions. Concentrations of both cortisol and ADH were measured in duplicates using 2 assays for cortisol, and only 1 for ADH. Both 96-well plates were then read on a plate reader (BioTek Instruments, Eon, Winooski,VT). The intra-assay and inter-assay coefficients of variance (CV) were 12.0% and 11.3% for cortisol, respectively, and the intra-assay CV for the ADH assay was 2.1%. Standard units for cortisol and ADH assays were ng/mL and pg/mL, respectively.

Lactation data collection

Daily milk production were collected from PCDART (Dairy Records Management Systems, PCDart, Raleigh, NC) and lactation persistency calculations were performed to observe percentage increase or decrease in milk production between 2 specific DIM. Each pair of DIM compared were 0 to 50 DIM, 50 to 100 DIM, 50 to 150 DIM, and 100 to 150 DIM. The lactation persistency calculation used was from the Western Canadian Dairy Herd Improvement Services as follows:

= [1-((Milk kg earlier test – Milk kg later test) x 30 d / days between tests)/Milk kg earlier test] x 100

Beginning on d 3 of lactation, weekly milk samples were collected for analysis of milk fat, protein, lactose, SCC, solids, and MUN for 15 wk. Samples were analyzed at the Dairy Herd Improvement Association (DHIA) laboratory in Manhattan, KS until closure, and then the subsequent samples were sent to the MQT Lab Services in Kansas City, MO. Yields of 3.5% FCM and ECM were calculated using weekly milk yield, milk protein, and milk fat in the following equations (Shirley, 2006):

FCM = (milk yield x 0.432) + (milk fat x 16.216)

ECM = (milk yield x 0.327) + (milk fat x 12.95) + (milk protein x 7.65)

Post-calving measurements

Two separate 3-d postpartum periods were selected to measure respiration rate and rectal temperatures in the morning and evening based on greater environmental temperatures (THI > 68). Respiration rates were collected in the normal housing environment to avoid handling stress confounding any environmental stress, and then rectal temperatures were collected and recorded (Rectal Thermometer M700, GLA Agricultural Electronics, San Luis Obispo, CA).

Statistical analyses

All data were analyzed by ANOVA using PROC MIXED with SAS Enterprise 6.1 (SAS Institute©, Cary, NC). Dichotomous variables of ketosis occurrence (1 or 0) and fever (1 or 0) were analyzed by PROC FREQ and an odds-ratio analysis was carried out using Procedure GLIMMIX (SAS Institute©, Cary, NC).

The best-fitting covariance structure was used for repeated measures (first-order autoregressive, heterogeneous autoregressive, or spatial power) based on Bayesian information criterion (BIC) values. Procedure MIXED was used to analyze all repeated measures including data collected on fitness test days, body temperature, body weight, respiration rates, rectal temperatures, skin temperature, milk components, and milk yield. Fixed variables included treatment, time (day, week), and time by treatment; the random variable was heifer. Calculated surface area was included in the model statement when analyzing skin temperature data. Differences between treatments were analyzed using sliced effects. Confidence intervals are reported at 95% and statistical significance was declared when $P < 0.05$, and trends when $P < 0.10$.

Results

Experiment 1-Late Summer/Early Fall 2014

Fitness test

On each fitness test (d 0, 28, and 56), observations were recorded based on each individual heifer's ability to perform a "fitness test" which consisted of a 5 min warm-up at 3.22 km/h, then incremental increases of 0.64 km/h every 3 min until the animal refused to continue (failure). Exercised heifers spent ($P = 0.04$) more time exercising during their fitness test on d 28 than SC (Figure 2.2) illustrating increased endurance for EX heifers. Exercised heifers had greater ($P = 0.01$) speeds of exercise at failure than their SC counterparts on d 28 (Figure 2.3). There was no difference ($P > 0.10$) in duration of fitness tests between EC and EX on any fitness test day (Figure 2.2). The EX treatment had a non-estimable LSM on d 56 because the fitness tests were administered over a series of days rather than on a single day, thus requiring that day (referred to as subday) be nested within fitness test day (Figure 2.2-2.3). One of the subdays during d 56 was without any EX heifers and therefore analysis resulted in a non-estimable value because of unequal balance across subdays.

There were no differences ($P > 0.05$) between starting and ending fitness test vaginal temperatures (Table 2.2). There were also no differences ($P > 0.10$) among treatments (EX, EC, and SC) when differences between beginning and ending vaginal temperature were analyzed (Table 2.2).

Blood parameters pre- and post-exercise

Blood samples were collected from the coccygeal vein approximately 30 minutes before fitness tests and within 10 minutes after fitness test completion. Data were analyzed by subday nested within day and included in the model statement; however, there were no effects ($P > 0.10$) of treatment on any blood parameters. Data are shown by each fitness test day (d 0, 28, 56), and are not broken down by subday (Tables 2.7 and 2.8). No differences ($P > 0.10$) were detected among treatments (EX, EC, or SC) for any of the blood parameters.

Body weight and locomotion

Body weights tended ($P = 0.10$) to be affected by exercise treatment and also were affected ($P < 0.0001$) by the 8-wk exercise regimen. Contrasts between EC and SC were not different ($P > 0.10$), thus EX was compared to both EC and SC and found to have lesser ($P = 0.04$) body weights than both controls. An interaction ($P = 0.01$) of exercise treatment by week of exercise was detected (Figure 2.4). Heifers in all 3 treatments increased in body weight during the experiment. Exercised heifers had reduced ($P = 0.05$) body weights compared with SC during wk 2 (Figure 2.4). There was a difference ($P < 0.0001$) between EX and SC and a difference ($P = 0.02$) between EC and SC, illustrating a continued trend for SC to have greater weights compared with EX during wk 5. During wk 8, EX weighed less ($P = 0.05$) than EC, and there was a tendency for EX to have reduced ($P = 0.07$) weight compared with SC during wk 7 (Figure 2.4).

Locomotion was assessed and scored once weekly for both EX and EC. There were no effects ($P = 0.48$) of exercise treatment on locomotion scores, no effect ($P > 0.10$) of weekly exercise regimens, or an interaction of treatment and time effects ($P > 0.10$) on locomotion.

Locomotion scores were never greater than 2 during the entire experiment for either EX or EC heifers.

Total time spent in each body temperature zone

There were no differences ($P > 0.10$) in time (%) spent between any of the temperature zones between EX, EC, or SC for temperature logged during wk 2 and 6 (Table 2.4). There was a tendency ($P = 0.06$) for an effect of week of experiment on time (%) spent in body temperature zone 1, thus EC and SC spent more time in zone 1 body temperature during wk 2 compared to wk 6, while EX remained similar in time spent in zone 1 during wk 2 and 6. Time spent in body temperature zone 2 was affected ($P < 0.0001$) by exercise treatment. Exercised heifers spent the same percentage of time in zone 2 body temperatures in wk 2 and 6; however, EC and SC spent almost double the amount of time in zone 2 during wk 6 compared with wk 2.

Time spent during hottest hour in each body temperature zone

Exercise-control heifers tended to spend less ($P = 0.04$) time in zone 1 (<39.0 °C) compared with both EX and also less ($P = 0.05$) than SC on d 29 during the hottest hour of wk 6 (Figure 2.5). On d 29, EC spent more time ($P = 0.04$) in zone 2 (39-40°C) than EX and also more ($P = 0.05$) than SC (Figure 2.6).

Sedentary heifers spent ($P = 0.01$) more time than EC in body temperature zone 1 during the hottest hour in d 30 (Figure 2.5). Sedentary heifers also spent ($P = 0.01$) less time in zone 2 than EC heifers on d 30, while EC tended ($P = 0.056$) to spend more time than EX in zone 2 on d 30 during wk 6 (Figure 2.6). Sedentary control heifers spent ($P = 0.01$) more time in body temperature zone 3 (>40°C) than both EX and EC (Table 2.3).

Though there were specific differences on certain weeks, there was no overall effect ($P > 0.10$) of exercise treatment on any of the body temperature zones of EX, EC, or SC or a treatment by day interaction ($P > 0.10$); however, there were effects ($P < 0.01$) of week of the experiment on time spent in temperature zones 1 and 2. During wk 2, both EX and EC experienced sharp increases in the amount of time spent in zone 1 during the hottest hour, while SC spent around the same amount of time. During wk 6, however, heifers from all 3 treatments spent an erratic pattern of time with most time spent in zone 1 on d 42 and 43 of wk 6 and the least amount of time spent on d 44 and 45. During wk 2, time spent in body temperature zone 2 decreased from d 14 to 15 for EX and EC while SC remained the same on both days. During wk 6, however, EC spent between 80 and 95% of the time in zone 2 during the hottest hour of d 42-45. Exercised heifers and SC spent an erratic pattern of time in zone 2 during the hottest hour with the greatest amount of time spent on d 44 and 45 and the least amount of time spent on d 42 and 43.

Time spent during coolest hour in each body temperature zone

There were no effects of exercise treatment, day of exercise treatment, or an interaction of exercise treatment by day on time spent ($P > 0.10$) in body temperature zones 1 and 2 (Table 2.5). There was no time spent in body temperature zones 3 (>40.0°C) by heifers in any treatments.

Time spent from 1300 h to 0000 h in each body temperature zone

There were no exercise treatment effects ($P = 0.45$) and no treatment by day interactions ($P = 0.15$) on time spent in body temperature zone 1, however, there was an effect of exercise day ($P = 0.004$) on time spent in body temperature zone 1 (Table 2.6). Overall, trends in all

treatments were erratic, but SC during wk 6 experienced decreased amounts of time spent in body temperature zone 1.

Time spent in temperature zone 2 was not affected ($P = 0.34$) by exercise treatment; however, there were effects ($P = 0.001$) of exercise day and treatment by day interactions ($P = 0.10$). During wk 2, all treatments experienced a negative linear trend during wk 2 with the most pronounced decrease in SC. During wk 6, however, EX and SC experienced a positive linear trend in time spent in zone 2 with a sharper increase in SC, while EC experienced a quadratic trend (Table 2.6).

There were no effects ($P = 0.42$) of exercise treatment, treatment by day interactions ($P = 0.89$), or an effect ($P = 0.20$) of exercise during wk 2 or 6 for time spent in body temperature zone 3 (>40.0°C) (Table 2.6).

Health variables measured at parturition and first week postpartum

Least squares means were compared for calf birth weights, difference in day between predicted calving date and actual calving date, and calving ease (Table 2.10). There was no effect ($P = 0.99$) of exercise treatment on calf birth weights, calving dates ($P = 0.22$), or calving ease ($P = 0.45$). Calving ease scores were given based on a 1-5 scale: 1 = no problem, 2 = minor problem, 3 = needed assistance, 4 = considerable force, 5 = very difficult (e.g. C-section) (Weigel, 2010). There were no calving ease scores of "4" or "5" births from any of the treatments (n = 0). Out of all treatments, there was 1 heifer that aborted from the SC heifers due to reasons not related to the experiment.

Mild-moderate ketosis occurrence was 25% for EX (n = 2), 38% for EC (n = 3), and 44% for SC (n = 4). There were 3 cases of fever after parturition, all occurring in EX heifers.

Ketones and fever occurrence after parturition were not affected ($P > 0.10$) by exercise treatment.

Observational signs of stress during first 3 milking times

Observational signs of stress were observed in response to parlor entry for milking and analyzed to see whether or not there were carry over effects from exercise on stress responses of heifers exposed to a new experience. There were no differences ($P > 0.10$) in parlor scores, kicks, kicking off milking apparatus, urinations, and defecations among treatments (Table 2.9). There were no observed vocalizations from either EX or EC during any of the first 3 milking times (n = 0). During the 1st and 2nd milking periods, there were no differences ($P > 0.05$) between lifts or steps. During the 3rd milking, EX tended ($P = 0.08$) to exhibit a greater number of lifts than SC (Table 2.9). The number of defecations, urinations, or whether or not a kick bar was used was not affected ($P > 0.10$) by exercise treatment.

Milk components

Milk components were measured for the first 6 wk of lactation. Milk fat percentage was not affected ($P = 0.60$) by exercise treatment or an interaction ($P = 0.98$) of lactation week and exercise treatment, but there was an effect ($P < 0.0002$) of lactation week (Figure 2.7). Heifers from all treatments experienced a negative linear trend, where milk fat percentage was the greatest at wk 0 and then progressively decreased each week.

For milk protein percentage, there was no effect ($P = 0.70$) of exercise treatment or an interaction ($P = 0.25$) of lactation week and exercise treatment but there was an effect ($P < 0.0001$) of lactation week (Figure 2.8). All treatments experienced a sharp decrease in milk protein percentage during wk 0 to 2 and then plateaued.

Exercised heifers had greater ($P = 0.008$) lactose percentage compared with SC and EC ($P = 0.01$) during wk 3 (Figure 2.9). Exercised heifers tended ($P = 0.06$) to have greater lactose percentage compared with EC and SC ($P = 0.08$) during wk 6 (Figure 2.9). Though there were differences among treatments during wk 3 and 6, there was no overall effect ($P = 0.83$) of exercise treatment or an interaction effect ($P = 0.31$) of lactation week and exercise treatment, but there was an effect ($P < 0.0001$) of lactation week on lactose percentage with a steady increase from wk 0 to wk 6 (Figure 2.9).

Sedentary heifers tended to have reduced ($P < 0.10$) SNF percentage compared with EX during wk 3 of lactation (Figure 2.10). Exercised heifers had greater ($P < 0.05$) SNF percentage compared with EC during wk 6 of lactation (Figure 2.10). Though there were specific differences between treatments during wk 3 and 6, there was no overall effect ($P = 0.74$) of exercise treatment and no interaction effect ($P = 0.14$) of lactation week and treatment, but there was an effect ($P = 0.005$) of lactation week on SNF percentage with a sharp decrease from wk 0 to wk 1, and a plateau afterwards.

Milk urea nitrogen was not affected ($P = 0.21$) by either exercise treatment or exercise treatment by lactation week interaction ($P = 0.85$), but there was a time effect ($P = 0.01$) of lactation week on MUN (Figure 2.11). Week 0 was very erratic between treatments; however, MUN plateaued after wk 0 in the range of approximately 11-13 mg/dL.

Because of the non-normal distribution of SCC, a log function was used [Log2 x (SCC/100) + 3] to produce a somatic cell score (SCS). There were no differences between EX, EC, and SC during any of the lactation weeks on SCS after exercise treatment and parturition ($P > 0.10$) (Figure 2.12). There was an interaction ($P < 0.0001$) of lactation week on SCS; however, there were no effects ($P = 0.76$) of exercise treatment or an interaction ($P = 0.70$) of

lactation week and exercise treatment. Somatic cell count sharply decreased from wk 0 to 2 and then plateaued at values between 0.5 and 1.5.

Lactation

The impacts of exercise were analyzed on milk production and residual milk percentage (remaining milk released after an oxytocin injection) of the first 3 milking periods as well as overall milk production of the first 6 months of lactation. Sedentary heifers released ($P = 0.01$) more milk than EC during the first milking prior to an oxytocin injection (Table 2.11). Exercised heifers tended ($P = 0.07$) to give more than their EC counterparts during the first milking (Table 2.11). Sedentary heifers tended ($P = 0.08$) to give more milk compared with EX during the 2nd milking (Table 2.11). The amount of milk produced prior to an oxytocin injection was affected by exercise treatment ($P = 0.0308$) during the first 3 milking times. Exercised heifers experienced a quadratic trend, where milk production was greater for the first and 3rd milking but reduced during the 2nd milking, while both EC and SC experienced negative linear trends as each milking period produced less milk. There was also an effect ($P < 0.0001$) of milking time on the first 3 milking periods, thus all treatments produced the most milk during the first milking. There were no differences ($P > 0.10$) among treatments for residual milk percentage (Table 2.12).

There were no differences ($P > 0.10$) between treatments on milk production as well as no effect ($P > 0.10$) of exercise treatment on overall milk production around d 7, 30, and 60 of lactation (Figure 2.13). Days within the first 2 months of lactation were affected ($P < 0.0001$) by time, as all treatments experienced an increase in milk production during the first month (d 7 and

30) (Figure 2.13). Exercise treatment did not affect (P = 0.98) milk production on d 7, 30, and 60 of lactation.

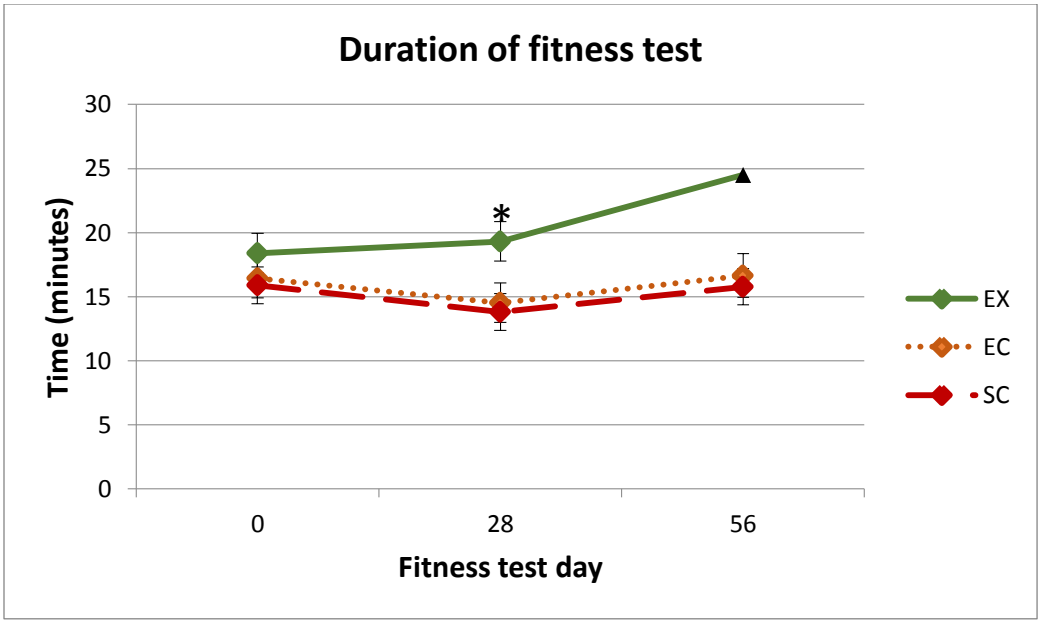

Figure 2.2. Least squares means of total time (min) spent during fitness tests on d 0, 28, and 56 during experiment 1.

Treatments: EX = exercised heifers (n = 8); EC = exercise-control (n = 8) brought to exerciser but not exercised; SC = sedentary control (n = 9) remained in pen. Day 56 LSM for minutes spent exercising was non-estimable, thus a raw mean (▲) is included to illustrate the trend.

*$P < 0.05$; EX vs. SC on d 28

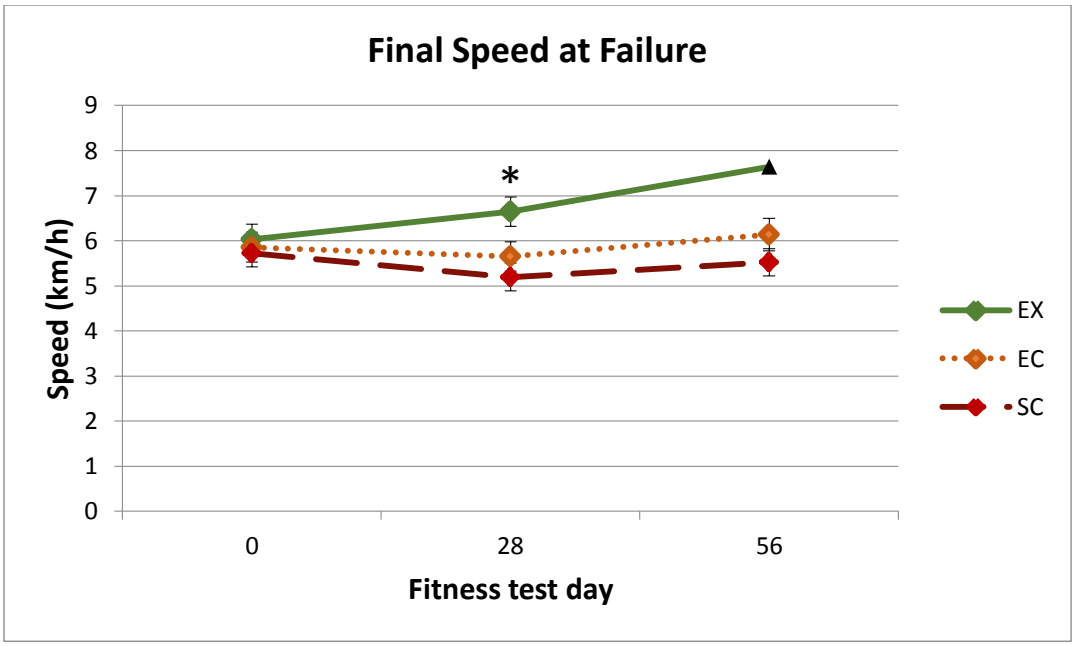

Figure 2.3. Least squares means of final speed (km/h) at failure to continue exercise for fitness tests on d 0, 28, and 56 during experiment 1.

Treatments: EX = exercised heifers (n = 8); EC = exercise-control (n = 8) brought to exerciser but not exercised; SC = sedentary control (n = 9) remained in pen. Day 56 LSM for minutes spent exercising was non-estimable, thus a raw mean (▲) is included to illustrate the trend.

*$P < 0.05$; EX vs. SC on d 28

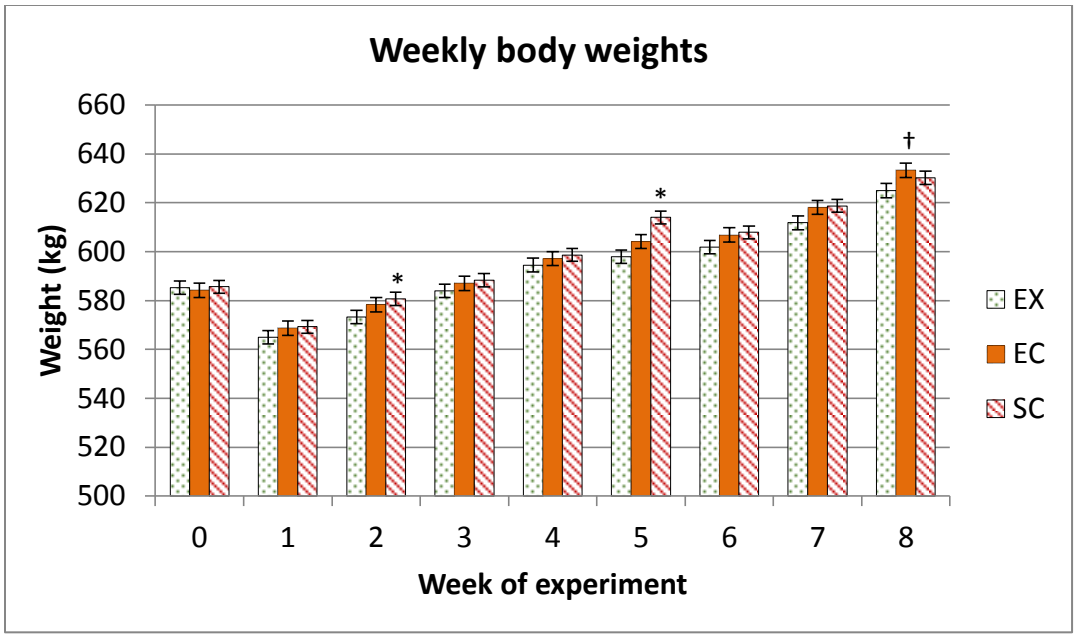

Figure 2.4. Least squares means of weekly body weights (kg) during wk 0-8 of experiment 1. Treatments: EX = exercised heifers (n = 8); EC = exercise-control (n = 8) brought to exerciser but not exercised; SC = sedentary control (n = 9) remained in pen. W 0 was included as a covariate because of significant differences.

*$P < 0.05$ (SC vs. EX, wk 2) (SC vs. EX and SC vs. EC, wk 5)

†$P < 0.10$ (EC vs. EX, wk 8)

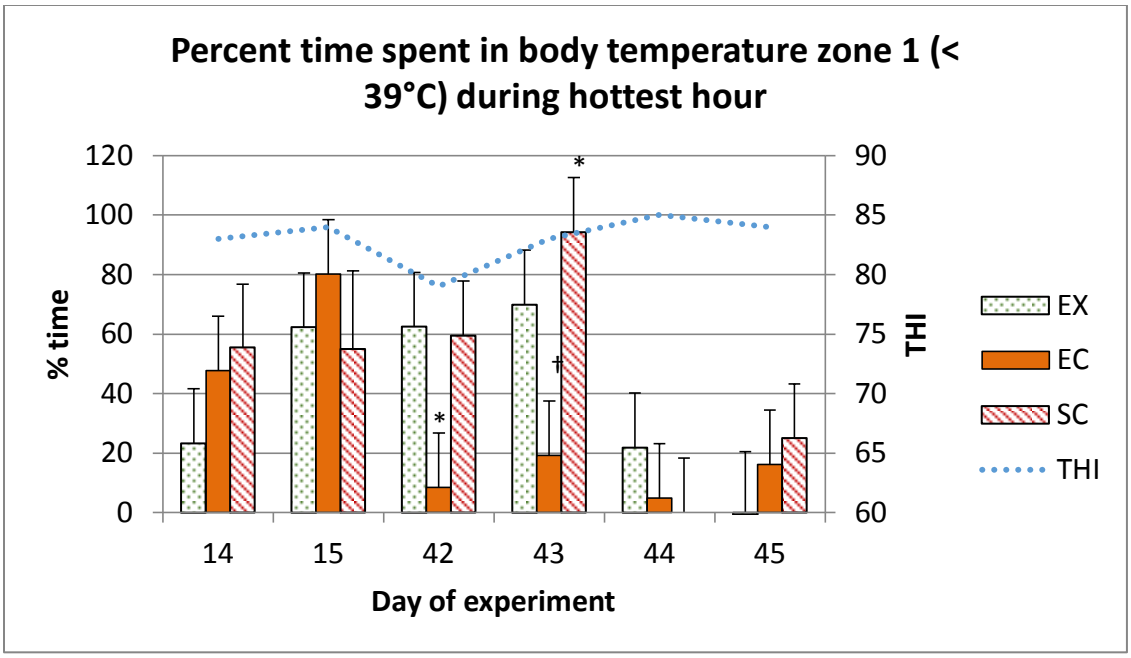

Figure 2.5. Least squares means of percentage time spent in zone 1 body temperature (<39°C) of the hottest hour in each day (THI > 79) during wk 2 and 6 of experiment 1.

Treatments: EX = exercised heifers (n = 4); EC = exercise-control (n = 4) brought to exerciser but not exercised; SC = sedentary control (n = 4) remained in pen. Days 14-15 occurred during wk 2, and d 42-45 occurred during wk 6.

*$P < 0.05$ (EC vs. SC and EC vs. EX on d 42; SC vs. EC on d 43)

†$P < 0.10$ (EX vs. EC on d 43)

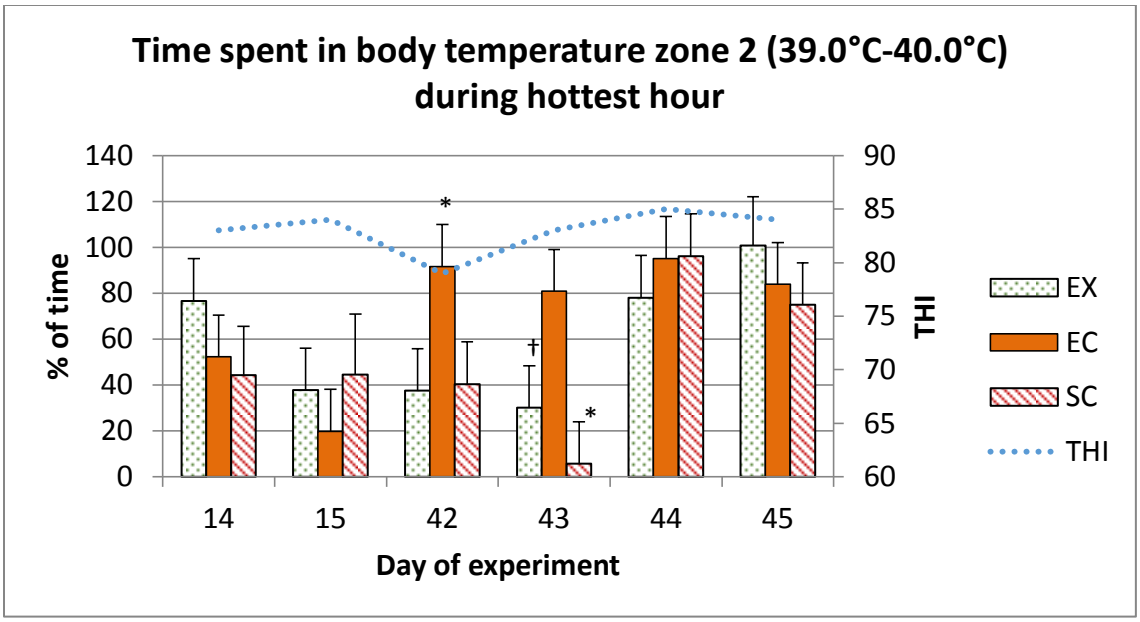

Figure 2.6. Least squares means of time spent (%) in body temperature zone 2 (39.0°C-40.0°C) during the hottest hour (THI > 79) during wk 2 and 6 of experiment 1.

Treatments: EX = exercised heifers (n = 4); EC = exercise-control (n = 4) brought to exerciser but not exercised; SC = sedentary control (n = 4) remained in pen. Days 14-15 occurred during wk 2, and d 42-45 occurred during wk 6.

*$P < 0.05$ (EC vs. EX and EC vs. SC on d 42; SC vs. EC on d 43)

†$P < 0.10$ (EC vs. EX on d 43)

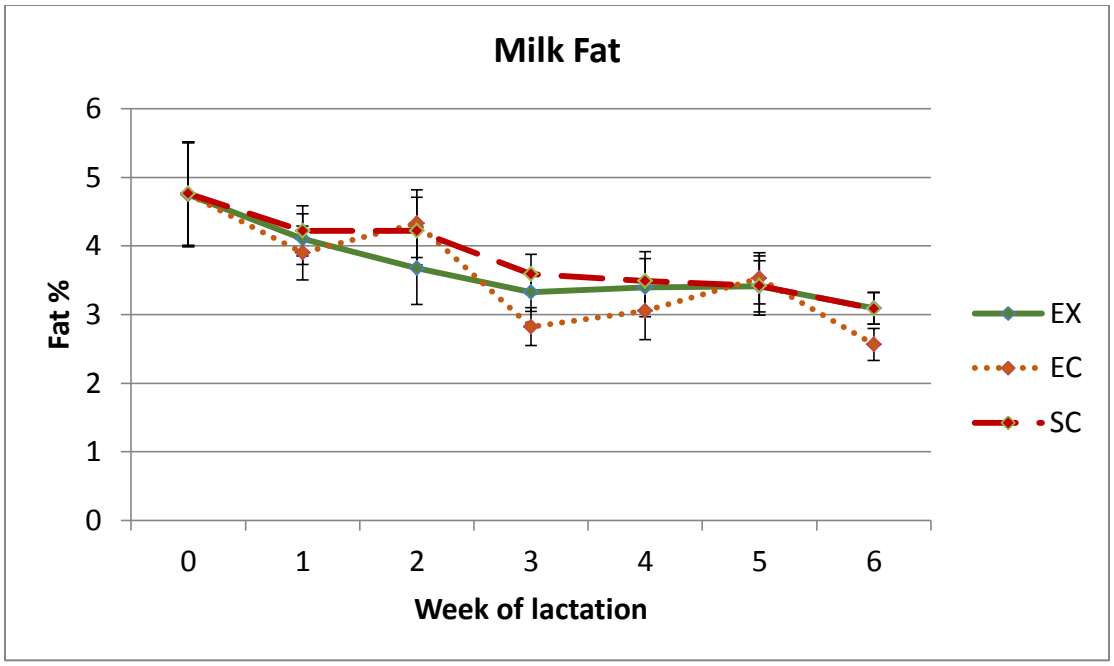

Figure 2.7. Least squares means for milk fat percentage during the first 6 wk of lactation after experiment 1.

Treatments: EX = exercised heifers (n = 8); EC = exercise-control (n = 8) brought to exerciser but not exercised; SC = sedentary control (n = 8) remained in pen.

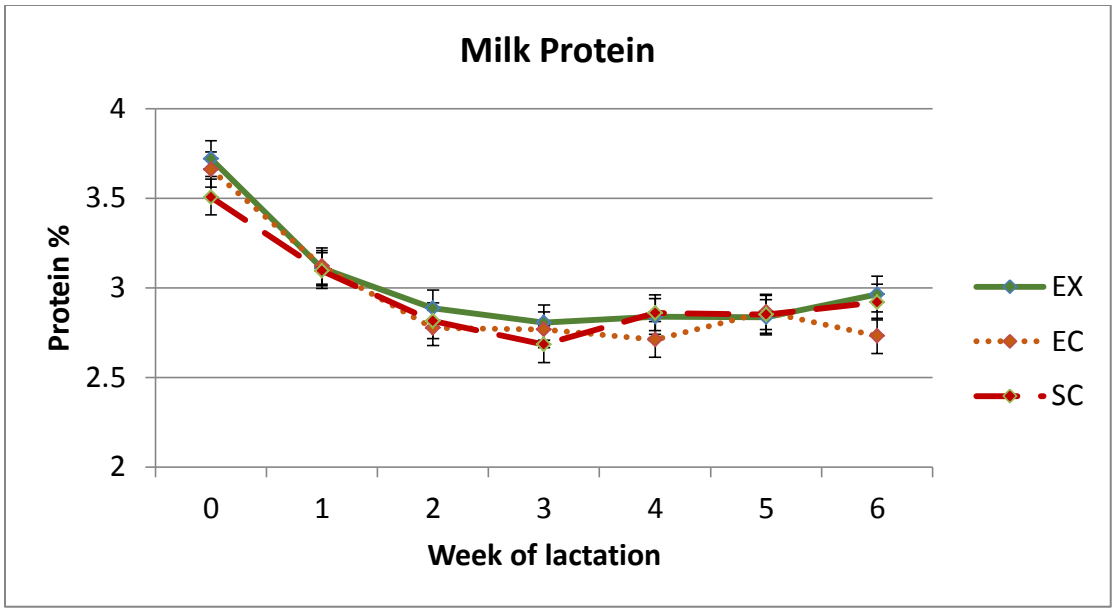

Figure 2.8 Least squares means for milk protein percentage during the first 6 wk of lactation after experiment 1.

Treatments: EX = exercised heifers (n = 8); EC = exercise-control (n = 8) brought to exerciser but not exercised; SC = sedentary control (n = 8) remained in pen.

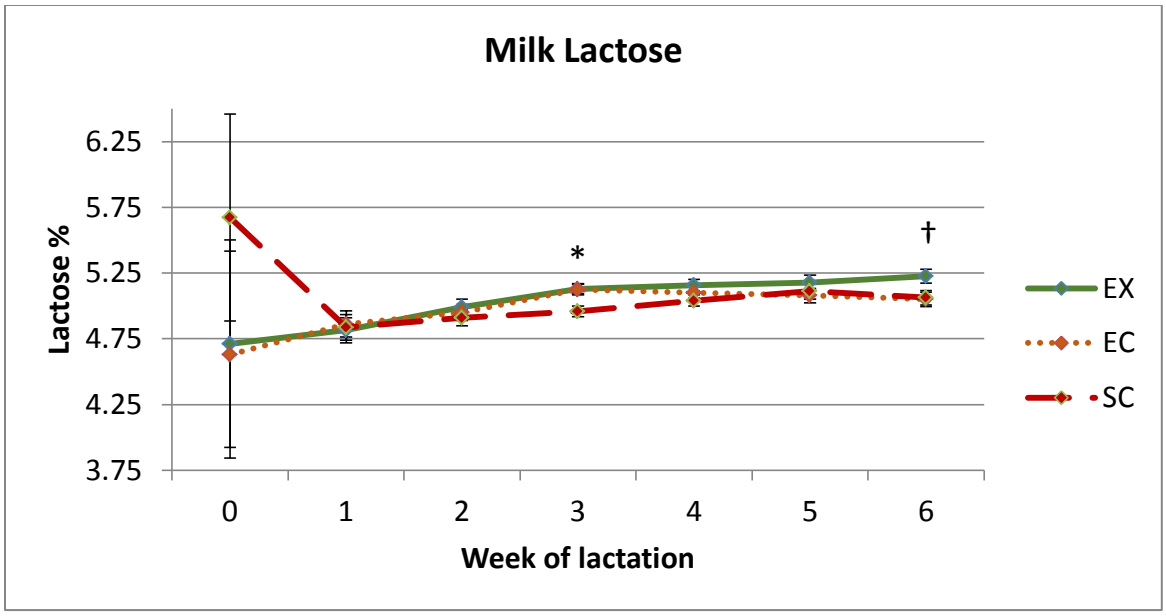

Figure 2.9. Least squares means for milk lactose percentage during the first 6 wk of lactation after experiment 1.

Treatments: EX = exercised heifers (n = 8); EC = exercise-control (n = 8) brought to exerciser but not exercised; SC = sedentary control (n = 8) remained in pen.

*$P < 0.05$ (SC vs. EX and SC vs. EC)

†$P < 0.10$ (EX vs. EC and EX vs. SC)

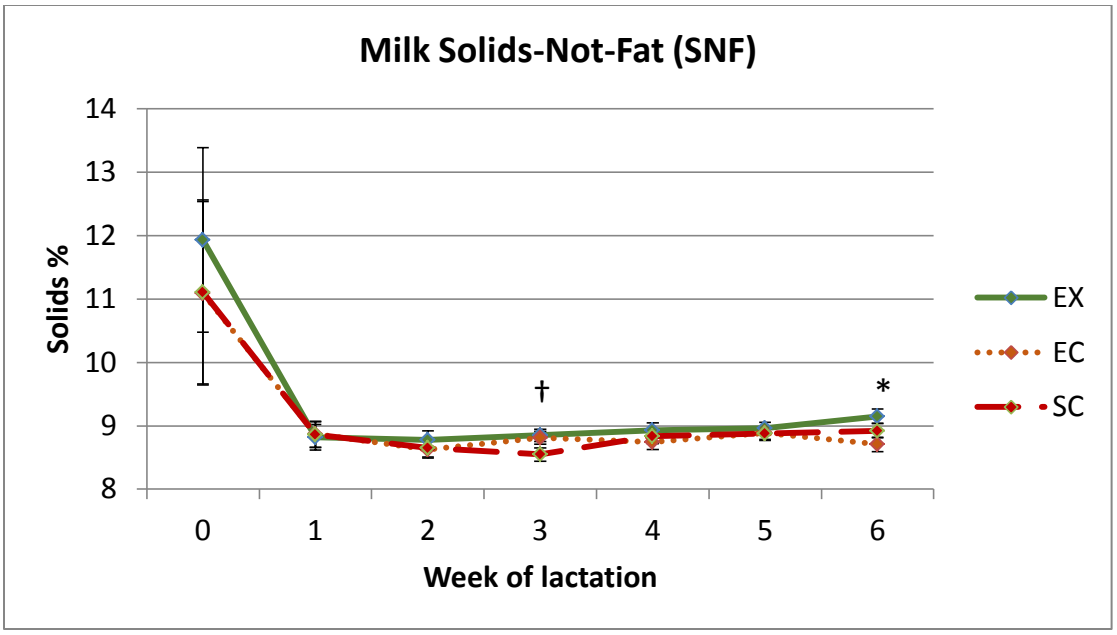

Figure 2.10. Least squares means for milk solids-not-fat (SNF) percentage during the first 6 wk after experiment 1.

Treatments: EX = exercised heifers (n = 8); EC = exercise-control (n = 8) brought to exerciser but not exercised; SC = sedentary control (n = 8) remained in pen.

*$P < 0.05$ (EX vs. EC)

†$P < 0.10$ (SC vs. EX)

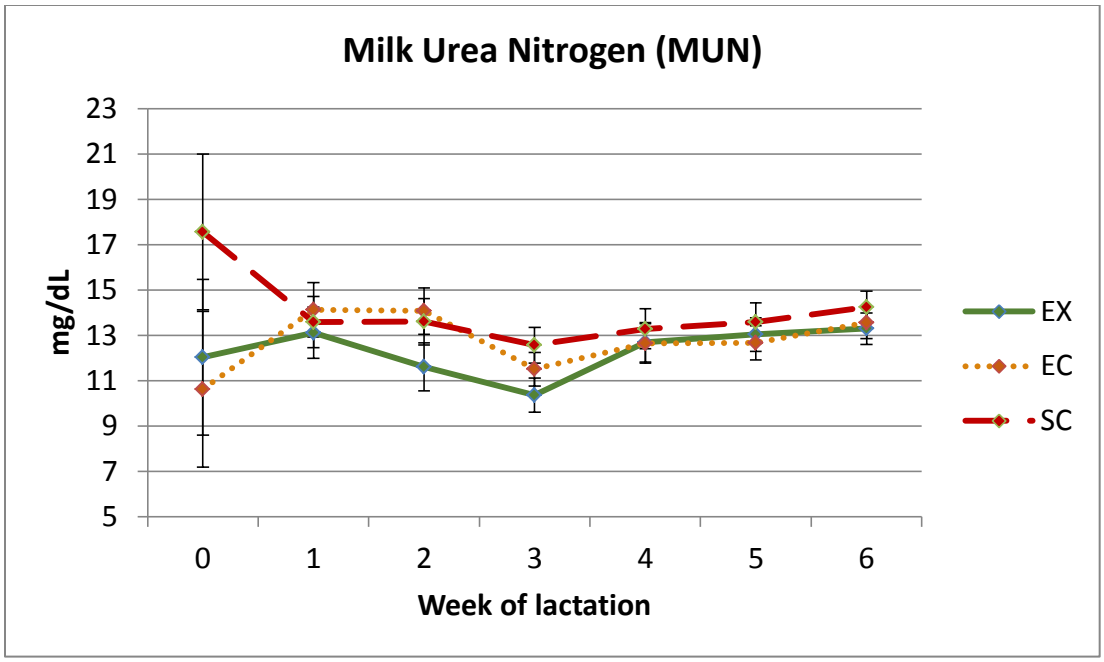

Figure 2.11. Least squares means for milk urea nitrogen (MUN) during the first 6 wk of lactation after experiment 1.

Treatments: EX = exercised heifers (n = 8); EC = exercise-control (n = 8) brought to exerciser but not exercised; SC = sedentary control (n = 8) remained in pen.

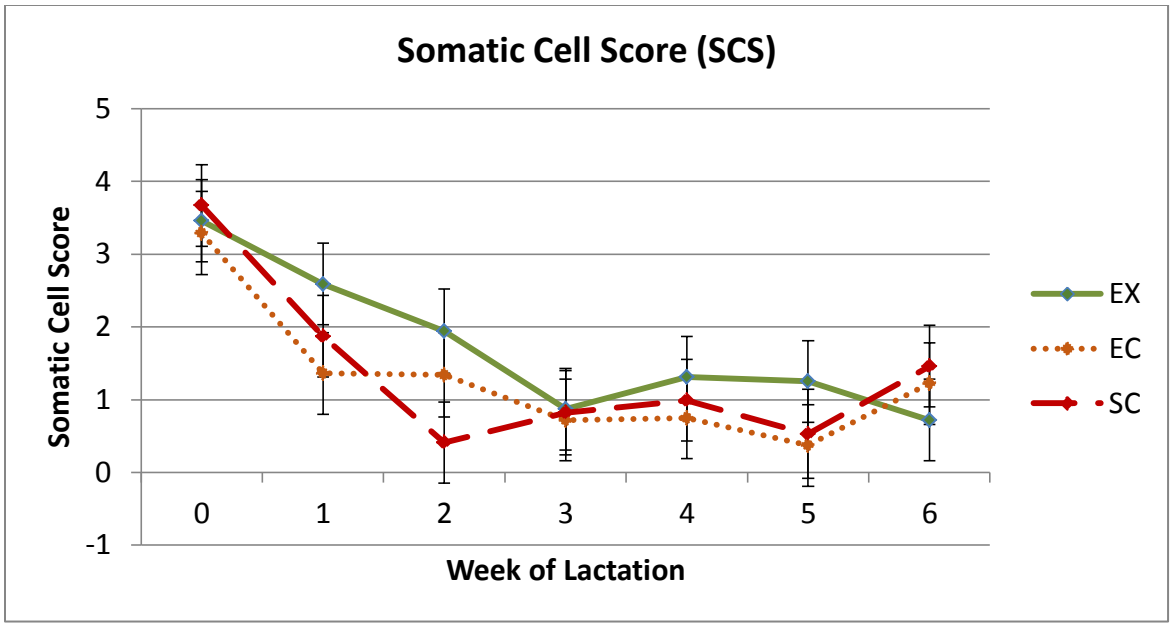

Figure 2.12. Least squares means for somatic cell score (SCS) during the first 6 wk of lactation after experiment 1.

Treatments: EX = exercised heifers (n = 8); EC = exercise-control (n = 8) brought to exerciser but not exercised; SC = sedentary control (n = 8) remained in pen.

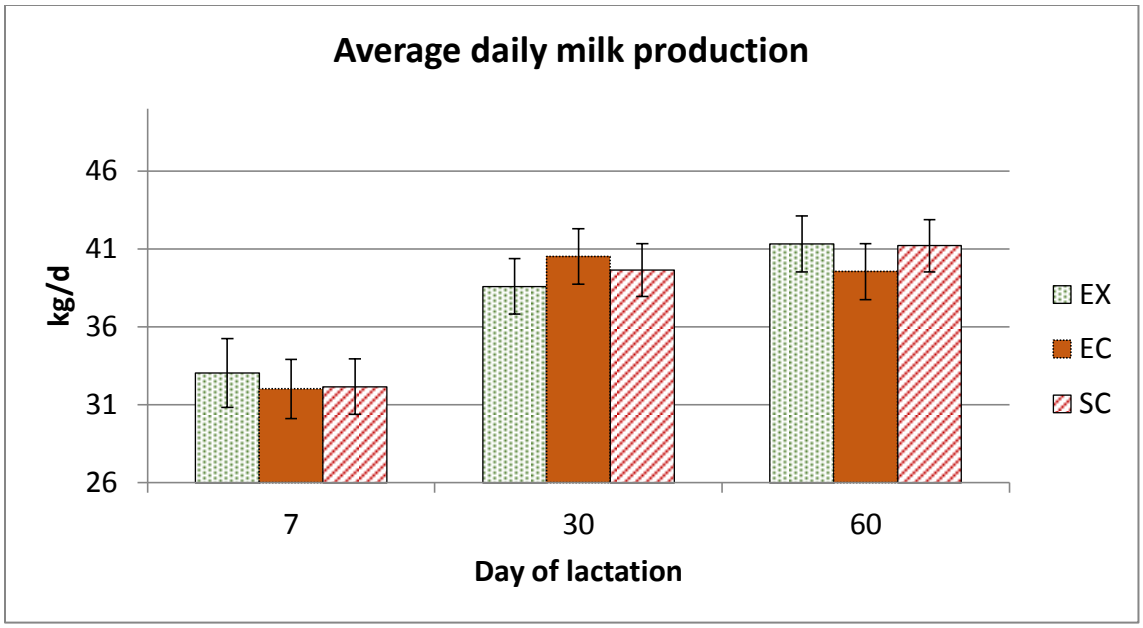

Figure 2.13. Least squares means of average daily milk production on milk test days during the first 2 months after experiment 1.

Treatments: EX = exercised heifers (n = 8); EC = exercise-control (n = 8) brought to exerciser but not exercised; SC = sedentary control (n = 8) remained in pen.

Table 2.2. Least squares means of vaginal temperatures at the beginning and end of fitness tests during experiment 1.

Item	Fitness test day[2]	Treatment[1]		
		EX	EC	SC
			°C	
Pre-exercise	0	38.4 ± 0.14	38.5 ± 0.14	38.7 ± 0.13
	28	39.0 ± 0.09†	38.7 ± 0.08	38.9 ± 0.08
	56[3]	38.4	38.7 ± 0.19	38.9 ± 0.18
Post-exercise	0	39.0 ± 0.08	39.2 ± 0.08	39.1 ± 0.07
	28	39.2 ± 0.08†	39.0 ± 0.08	39.1 ± 0.07
	56[3]	39.0	38.9 ± 0.09	39.1 ± 0.08

[1]Treatments: EX = exercised heifers (n = 8); EC = exercise-control (n = 8) brought to exerciser but not exercised; SC = sedentary control (n = 9) remained in pen.

[2]Fitness test days were done over a series of days and therefore subday was nested in the variable day for analysis. There was unequal treatment representation on each of the subday and in some cases no representation, resulting in non-estimable data on d 56. Any differences could be considered confounded by unequal treatment representation per subday, therefore only simple comparisons of LSM of treatments by day are shown. There were no significant differences.

[3]Exercised heifers had non-estimable data on d 56; therefore raw means are shown for all variables on d 56.

†$P < 0.10$ (Pre-exercise: EX vs. EC; post-exercise: EX vs. EC)

Table 2.3. Least squares means of percentage time spent in zone 3 (>40.0°C) during the hottest hour of several day (THI > 79) during wk 2 and 6 of experiment 1.

Day	Treatment[1]		
	EX	EC	SC
		% time	
14	0.0 ± 0.93	0.0 ± 0.93	0.0 ± 1.07
15	0.0 ± 0.93	0.0 ± 0.93	0.0 ± 1.33
42	0.0 ± 0.93	0.0 ± 0.93	0.0 ± 0.93
43	0.0 ± 0.93	0.0 ± 0.93	0.0 ± 0.93
44	0.0 ± 0.93	0.0 ± 0.93	3.8 ± 0.93*
45	0.0 ± 1.08	0.0 ± 0.93	0.0 ± 0.93

[1]Treatments: EX = exercised heifers (n = 4); EC = exercise-control (n = 4) brought to exerciser but not exercised; SC = sedentary control (n = 4) remained in pen. Day 14-15 were during wk 2 of the experiment and d 42-45 were during wk 6 of the experiment.

*$P < 0.05$ (EX vs. SC and EC vs. TB on d 44)

Table 2.4. Least squares means of total time spent (%) in body temperatures zones during wk 2 and 6.

Item	Body Temperature Zones[2]	Treatment[1]		
		EX	EC	SC
		% time		
Week 2	1	70.6 ± 6.32	84.4 ± 6.32	84.6 ± 7.30
	2	25.2 ± 5.89	15.6 ± 5.89	15.5 ± 7.06
	3	4.3 ± 2.03	0.0 ± 2.03	0.0 ± 2.35
Week 6	1	74.6 ± 6.32	62.5 ± 6.32	65.8 ± 6.32
	2	22.8 ± 5.89	36.9 ± 5.89	32.3 ± 5.89
	3	2.6 ± 2.03	0.7 ± 2.03	2.0 ± 2.03

[1]Treatments: EX = exercised heifers (n = 4); EC = exercise-control (n = 4), brought to exerciser but not exercised; SC = sedentary control (n = 4), remained in pen.

[2]Body temperature zones: zone 1 = <39.0°C; zone 2 = 39.0-40.0°C; zone 3 = >40.0°C

Table 2.5. Least squares means of percentage time spent in body temperature zones 1 and 2 during cool hours of wk 2 and 6 in experiment 1.

Item	Day[1]	Treatment[2]		
		EX	EC	SC
		% time		
Zone 1	14	100.0 ± 10.15	100.0 ± 10.15	100.0 ± 11.72
	15	100.0 ± 10.15	100.0 ± 10.15	100.0 ±11.72
	16	79.3 ± 10.15	100.0 ± 10.15	100.0 ± 14.28
	42	100.0 ± 10.15	100.0 ± 10.15	100.0 ± 10.15
	43	100.0 ± 11.6	90.9 ± 10.15	100.0 ± 10.15
	44	100.0 ± 10.15	100.0 ± 10.15	50.0 ± 10.15*
	45	100.0 ± 11.67	100.0 ± 10.15	75.0 ± 10.15†
Zone 2	14	0.0 ± 10.15	0.0 ± 10.15	0.0 ± 11.72
	15	0.0 ± 10.15	0.0 ± 10.15	0.0 ± 11.72
	16	20.7 ± 10.15	0.0 ± 10.15	0.0 ± 14.28
	42	0.0 ± 10.15	0.0 ± 10.15	0.0 ±10.15
	43	0.0 ± 11.6	9.2 ± 10.15	0.0 ±10.15
	44	0.0 ± 10.15	0.0 ± 10.15	50.0 ±10.15*
	45	0.0 ± 11.67	0.0 ± 10.15	25.0 ±10.15†

[1]Day: d 14-16 are within wk 2 of experiment, and d 42-45 are within wk 6 of experiment.

[2]Treatments: EX = exercised heifers (n = 4); EC = exercise-control (n = 4), brought to exerciser but not exercised; SC = sedentary control (n = 4), remained in pen.

Body temperature zones: Zone 1 <39°C, Zone 2 = 39-40°C, no time was spent in Zone 3 >40°C

*$P < 0.05$ (SC vs. EX and SC vs. EC on d 44 in zones 1 and 2)

†$P < 0.10$ (SC vs. EC on d 45 in zones 1 and 2)

Table 2.6. Least squares means of percentage time spent in body temperature zones from 1300 h to 0000 h on certain days during wk 2 and 6.

Item	Day[1]	Treatment[2]		
		EX	EC	SC
		% time		
Zone 1	14	59.2 ± 14.09	55.8 ± 14.09	61.0 ± 16.12
	15	51.6 ± 14.09†	79.6 ±14.09	98.8 ± 20.16
	42	79.6 ± 14.09	45.1 ±14.09†	79.7 ±14.09
	43	45.1 ± 14.09	19.5 ± 14.09	45.8 ± 14.09
	44	61.1 ±14.09	42.5 ±14.09	31.4 ±14.09
Zone 2	14	40.8 ± 13.00	44.3 ± 13.00	40.3 ± 14.53
	15	30.0 ± 13.00	20.4 ± 13.00	3.8 ±17.77
	42	20.4 ± 13.00	55.0 ±13.00†	20.4 ±13.00
	43	36.7 ±13.00*	75.3 ±13.00	41.1 ± 13.00†
	44	38.9 ± 13.00	57.6 ± 13.00	66.6 ± 13.00
Zone 3	14	0.0 ± 7.40	0.0 ± 7.40	0.0 ± 8.54
	15	18.4 ± 7.40	0.0 ± 7.40†	0.0 ± 10.54
	42	0.0 ± 7.40	0.0 ± 7.40	0.0 ± 7.40
	43	18.3 ± 7.40	5.2 ± 7.40	13.1 ± 7.40
	44	0.0 ± 7.40	0.0 ± 7.40	2.0 ± 7.40

[1]Day: d 14-15 are in wk 2 and d 42-44 are in wk 6.

[2]Treatments: EX = exercised heifers (n = 8); EC = exercise-control (n = 8), brought to exerciser but not exercised; SC = sedentary control (n = 8), remained in pen.

Body temperature zones: zone 1 <39.0°C, zone 2 = 39-40°C, zone 3 >40.0°C

*$P < 0.05$ (zone 2: EX vs. EC on d 43)

†$P < 0.10$ (zone 1: EX vs SC on d 15, EC vs. EX and EC vs. SC on d 42; zone 2: EC vs. EX and EC vs. SC on d 42, EC vs. TB on d 43; zone 3: EX vs. EC on d 15)

Table 2.7. Least squares means of acid-base blood variables pre- and post-fitness tests between treatments from experiment 1.

Item	Fitness test day[2]	Treatment[1]					
		Pre-fitness test			Post-fitness test		
		EX[3]	EC	SC	EX	EC	SC
pH	0	7.44 ± 0.02	7.48 ± 0.02	7.45 ± 0.02	7.49 ± 0.02	7.50 ± 0.02	7.48 ± 0.01
	28	7.47 ± 0.02	7.44 ± 0.02	7.46 ± 0.02	7.53 ± 0.02	7.48 ± 0.02	7.48 ± 0.01
	56	7.50	7.52 ± 0.02	7.49 ± 0.02	7.48	7.48 ± 0.02	7.48 ± 0.01
pCO_2, mmHg	0	40.8 ± 1.87	39.0 ± 1.87	38.6 ± 2.15	36.9 ± 1.27	37.0 ± 1.32	38.1 ± 1.19
	28	38.7 ± 1.88	40.4 ± 1.88	38.4 ± 2.17	33.2 ± 1.33	36.4 ± 1.33	36.5 ± 1.24
	56	37.9	34.8 ± 2.1	37.1 ± 1.93	38.2	38.0 ± 1.38	39.5 ± 1.17
pO_2, mmHg	0	47.5 ± 11.22	62.5 ± 11.22	39.7 ± 10.45	69.0 ± 15.63	44.0 ± 15.63	46.9 ± 14.55
	28	87.2 ± 22.46	45.4 ± 22.45	59.6 ± 20.89	112.0 ± 15.67	88.2 ± 15.67	60.2 ± 14.58
	56	42.5	142.5 ± 25.65	102.0 ± 20.85	90.1	79.1 ± 17.49	85.1 ± 14.87
TCO_2, mmol/L	0	29.2 ± 0.69	29.7 ± 0.69	30.4 ± 0.64	29.2 ± 0.77	29.6 ± 0.77	29.9 ± 0.72
	28	29.2 ± 0.68	28.9 ± 0.68	28.7 ± 0.64	28.5 ± 0.77	28.7 ± 0.77	28.2 ± 0.72
	56	29.1	29.3 ± 0.74	30.0 ±0.62	28.9	29.4 ± 0.85	30.9 ± 0.73
HCO_3^-, mmol/L	0	27.9 ± 0.67	28.8 ± 0.67	29.1 ± 0.63	28.1 ± 1.03	30.3 ± 1.03	28.7 ± 0.96
	28	28.2 ± 0.66	27.8 ± 0.67	27.6 ± 0.62	27.4 ± 0.54	27.4 ± 0.55	27.1 ± 0.51
	56	27.8	28.4 ± 0.73	28.9 ± 0.61	27.8	28.3 ± 0.86	29.7 ± 0.73
sO_2, %	0	71.2 ± 5.99	84.3 ± 5.99	70.7 ± 5.58	82.4 ± 4.58	76.6 ± 4.58	75.1 ± 4.26
	28	83.4 ± 6.02	71.8 ± 6.01	77.1 ± 5.6	91.1 ± 4.57	90.1 ± 4.58	82.7 ± 4.26
	56	71.5	96.8 ± 6.73	89.3 ± 5.45	87.6	80.0 ± 5.09	87.5 ± 4.34
Base Excess, mmol/L	0	3.7 ± 0.9	5.2 ± 0.91	5.2 ± 0.84	4.6 ± 0.86	5.4 ± 0.86	5.2 ± 0.80
	28	4.5 ± 0.9	4.0 ± 0.90	4.0 ± 0.84	4.5 ± 0.86	3.9 ± 0.86	3.7 ± 0.80
	56	3.38	5.5 ± 0.99	5.64 ± 0.82	4.6	4.5 ± 0.96	6.2 ± 0.81

[1]Treatments: EX = exercised heifers (n = 8); EC = exercise-control (n = 8), brought to exerciser but not exercised; SC = sedentary control (n = 9), remained in pen.

[2]Fitness test day had to be done over a series of day and therefore subday was nested in the variable day for analysis. There was unequal treatment representation on each of the subdays and in some cases no representation, resulting in non-estimable data on d 56. Any differences could be considered confounded by unequal treatment representation per subday, therefore only simple comparisons of LSM of treatments by day are shown for clearer output.

[3]Exercised heifers had non-estimable data on d 56, therefore raw means are shown for all variables on d 56.

Table 2.8. Least squares means of blood variables pre- and post-fitness tests during experiment 1.

Item	Fitness test day[2]	Treatment[1]					
		Pre-fitness test			Post-fitness test		
		EX[3]	EC	SC	EX	EC	SC
Na, mmol/L	0	139.7 ± 0.51	139.9 ± 0.51	139.9 ± 0.48	140.6 ± 0.52	140.1 ± 0.52	140.8 ± 0.49
	28	139.4 ± 0.50	138.6 ± 0.51	139.0 ± 0.48	139.8 ± 0.52	139.0 ± 0.52	140.3 ± 0.48
	56	139.6	138.1 ± 0.54	138.9 ± 0.47	140.4	139.1 ± 0.56	139.6 ± 0.49
K, mmol/L	0	3.9 ± 0.09	3.7 ± 0.09	3.9 ± 0.09	3.9 ± 0.09	4.0 ± 0.09	4.1 ± 0.09
	28	4.2 ± 0.09	4.1 ± 0.09	4.2 ± 0.09	4.2 ± 0.09	4.1 ± 0.09	4.2 ± 0.09
	56	4.0	3.9 ± 0.10	3.9 ± 0.08	3.9	4.3 ± 0.10	4.2 ± 0.09
iCa[2], mmol/L	0	1.20 ± 0.02	1.19 ± 0.02	1.21 ± 0.01	1.18 ± 0.01	1.18 ± 0.01	1.19 ± 0.01
	28	1.21 ±0.01	1.22 ± 0.01	1.22 ± 0.01	1.18 ± 0.01	1.20 ± 0.01	1.19 ± 0.01
	56	1.21	1.18 ± 0.01	1.18 ± 0.01	1.16	1.18 ± 0.01	1.18 ± 0.01
Glucose, mg/dL	0	75.8 ± 1.86	77.8 ± 1.86	76.0 ± 1.73	76.8 ± 2.49	80.3 ± 2.49	77.1 ± 2.32
	28	72.2 ± 1.84	70.1 ± 1.84	71.5 ± 1.72	74.3 ± 2.48	74.4 ± 2.48	72.6 ± 2.31
	56	74.8	75.2 ± 2.01	73.5 ± 1.69	80.0	75.1 ± 2.75	73.6 ± 2.35
Hematocrit, %PCV	0	25.8 ± 0.74	24.7 ± 0.74	25.6 ± 0.69	25.5 ± 0.69	25.0 ± 0.69	25.6 ± 0.64
	28	27.2 ± 0.73	25.7 ± 0.74	27.4 ± 0.69	25.8 ± 0.68	24.9 ± 0.68	26.0 ± 0.63
	56	26.6	26.3 ± 0.79	26.3 ± 0.79	25.8	25.6 ± 0.74	26.8 ± 0.64
Hemoglobin, g/dL	0	8.8 ± 0.25	8.4 ± 0.25	8.7 ± 0.23	8.7 ± 0.23	8.5 ± 0.23	8.7 ± 0.22
	28	9.3 ± 0.25	8.7 ± 0.25	9.3 ± 0.23	8.8 ± 0.23	8.5 ± 0.23	8.8 ± 0.22
	56	9.1	8.9 ± 0.27	9.3 ± 0.23	8.8	8.7 ± 0.25	9.1 ± 0.22
Lactate, mmol/L	0	1.46 ± 0.24	1.35 ± 0.24	1.23 ± 0.23	1.06 ± 0.29	0.83 ± 0.30	0.87 ± 0.28
	28	1.10 ± 0.24	1.31 ± 0.24	1.24 ± 0.22	0.68 ± 0.30	1.16 ± 0.30	1.17 ± 0.28
	56	1.47	0.92 ±	1.09 ±	1.12	1.13 ±	1.05 ±

		0.26	0.22		0.33	0.30

[1]Treatments: EX = exercised heifers (n = 8); EC = exercise-control (n = 8) brought to exerciser but not exercised; SC = sedentary control (n = 9) remained in pen.

[2]Fitness test day had to be done over a series of day and therefore subday was nested in the variable day for analysis. There was unequal treatment representation on each of the subdays and in some cases no representation, resulting in non-estimable data on d 56. Any differences could be considered confounded by unequal treatment representation per subday, therefore only simple comparisons of LSM of treatments by day are shown for clearer output.

[3]Exercised heifers had non-estimable data on d 56; therefore raw means are shown for all variables on d 56.

Table 2.9. Least squares means of observational signs of stress in the parlor between treatments after experiment 1.

		Treatment[1]					
		Before and during milking			After mechanical milker removal		
Item	Milk Time	EX	EC	SC	EX	EC	SC
Parlor Score[2]	1	2.1 ± 0.36	1.7 ± 0.31	1.6 ± 0.36	-	-	-
	2	2.0 ± 0.36	2.5 ± 0.31	2.0 ± 0.36	-	-	-
	3	2.1 ± 0.36	2.3 ± 0.31	1.6 ± 0.36	-	-	-
Step[3]	1	1 ± 0.64	1 ± 0.55	2 ± 0.64	2 ± 0.68	1 ± 0.58	1 ± 0.68
	2	2 ± 1.37	3 ± 1.19	0 ± 1.37	1 ± 0.68	1 ± 0.58†	3 ± 0.68
	3	1 ± 0.79	2 ± 0.69	1 ± 0.79	0 ± 0.68	1 ± 0.58	1 ± 0.68
Lift[4]	1	2 ± 1.82	3 ± 1.58	4 ± 1.82	2 ± 1.00	0 ± 0.87	1 ± 1.00
	2	3 ± 1.05	3 ± 0.91	2 ± 1.05	1 ± 1.00	4 ± 0.87†	1 ± 1.00
	3	4 ± 1.09	3 ± 0.95	2 ± 1.09	4 ± 1.00†	2 ± 0.87	1 ± 1.00
Kick[5]	1	0 ± 0.43	1 ± 0.37	1 ± 0.43	0 ± 0.52	1 ± 0.45	0 ± 0.52
	2	1 ± 0.69	1 ± 0.6	1 ± 0.69	0 ± 0.52	1 ± 0.45	1 ± 0.52
	3	2 ± 1.25	1 ± 1.09	1 ± 1.25	0 ± 0.52	1 ± 0.45	1 ± 0.52
Kick Off[6]	1	1 ± 0.51	0 ± 0.44	0 ± 0.51	-	-	-
	2	0 ± 0.51	1 ± 0.44	1 ± 0.51	-	-	-
	3	1 ± 0.51	1 ± 0.44	0 ± 0.51	-	-	-

[1]Treatments: EX = exercised heifers (n = 8); EC = exercise-control (n = 8) brought to exerciser but not exercised; SC = sedentary control (n = 8) remained in pen.

[2]Parlor Score: an overall subjective score on a scale of 1-4; 1 = calm, 2 = moderately calm, 3 = moderately crazy, 4 = crazy

[3]Step: the hoof lifted off the ground without going higher than the upper part of the dew-claw.

[4]Lift: the hoof lifted off the ground higher than the upper part of the dew-claw but lower than the middle point between the dew-claw and the point of the hock.

[5]Kick: the hoof lifted off the ground higher than the middle point between the dew-claw and the point of the hock.

[6]Kick Off: when a heifer kicked off one or more arms of the milking unit during milking period.

†$P < 0.10$ (Step: EC vs. SC during 2nd milking; Lift: EC vs. EX and SC during 2nd milking, EX vs. SC during 3rd milking)

Table 2.10. Least squares means of parturition and post-calving data after experiment.

	Treatment[1]		
Item	EX	EC	SC
Calving ease	1.6 ± 0.28	1.1 ± 0.26	1.5 ± 0.26
Calf birth weights[2], kg	36.9 ± 2.29	36.5 ± 2.05	36.6 ± 3.24
Calving date[3]	5 ± 2.39	1 ± 2.39	7 ± 2.25

[1]Treatments: EX = exercised heifers (n = 8); EC = exercise-control (n = 8) brought to exerciser but not exercised; SC = sedentary control (n = 8) remained in pen.

[2]Calf birth weights: data presented are weights of heifer calves only; EX (n = 4); EC (n = 5); SC (n = 2).

[3]Calving date: difference of predicted calving date and actual calving date; a positive number means that actual calving date was earlier than predicted calving date.

Table 2.11. Least squares means of milk production (kg) during the first 3 milking periods.

	Treatment[1]		
Milking time[2]	EX	EC	SC
1	5.3 ± 0.90†	3.0 ± 0.78	6.3 ± 0.90*
2	2.0 ± 0.49†	2.3 ± 0.39	3.2 ± 0.45
3	2.8 ± 0.59	2.1 ± 0.51	3.5 ± 0.59†

[1]Treatments: EX = exercised heifers (n = 8); EC = exercise-control (n = 8) brought to exerciser but not exercised; SC = sedentary control (n = 8) remained in pen.

[2]Milking time refers to the first 3 milking periods post-parturition.

*$P < 0.05$ (SC vs. EC)

†$P < 0.10$ (Milk 1: EX vs. EC; Milk 2: EX vs. SC; Milk 3: EC vs. SC)

Table 2.12. Least squares means of residual milk (%) during the first 3 milking periods.

	Treatment[1]		
Milking time[2]	EX	EC	SC
1	54.2 ± 24.7	30.9 ± 21.4	11.9 ± 24.7
2	90.4 ± 30.5	61.2 ± 23.8	29.6 ± 27.5
3	64.8 ± 82.7	159.1 ± 71.6	41.8 ± 82.7

[1]Treatments: EX = exercised heifers (n = 8); EC = exercise-control (n = 8) brought to exerciser but not exercised; SC = sedentary control (n = 8) remained in pen.

[2]Milking time refers to the first 3 milking periods post-parturition.

Experiment 2-Summer 2015

Fitness test

When heart rates were recorded after 10 min of walking at 5.63 km/h, there were no differences between EX and EC on any of the fitness test days. Fitness test day affected (P = 0.0002) heart rate before fitness tests began, demonstrated by a sharp increase of HR in both treatments on fitness test d 28, but it did not affect ($P > 0.10$) HR after the 10 min brisk walking pace or at the end of fitness tests (Table 2.13). There was an influence ($P < 0.0001$) of fitness test day on RR at the beginning of the fitness test and at the end of the 10 min endurance portion where a sharp increase is observed on d 28. Respiration rates and heart rates were not affected ($P > 0.10$) by treatment or the interaction of treatment and fitness test day before, during, or after fitness tests. The RR collected at the end of the 10 min endurance portion of the fitness test was different ($P < 0.05$) between exercise and exercise-control treatments on d 0, and therefore d 0 values were included in the model as a covariate (Table 2.13).

There were no differences ($P > 0.10$) between EX or EC on skin temperatures of the cheek, withers, ear, and udder before or after fitness tests (Table 2.16). Fitness test day affected ($P < 0.0001$) skin temperatures between treatments at the cheek, withers, thurl, ear, and udder pre- and post-fitness tests, and is seen on d 28 where both EX and EC experienced sharp increases in skin temperature. There was a tendency for EX heifers to have reduced ($P = 0.07$) thurl skin temperatures compared with EC after exercise on fitness test d 28. Calculated surface area of heifers was included in the model when analyzing skin temperatures.

Blood parameters pre- and post-exercise

Similar to experiment 1, blood samples were collected from the caudal vein to assess exercise effects on blood parameters. There was no effect ($P = 0.35$) of exercise treatment and no interaction ($P = 0.52$) of fitness test day and exercise treatment on hemoglobin (Hb) concentration after fitness tests on d 0, 28, and 56. There was an effect ($P < 0.0001$) of fitness test day on Hb concentrations where concentrations on d 28 were reduced before fitness tests compared with d 0 and 56 (Figure 2.14). Exercise treatment did not affect ($P = 0.38$) Hb concentrations after fitness tests nor was there an interaction ($P = 0.65$) of exercise treatment by fitness test day. Hemoglobin concentrations after fitness tests, however, were affected ($P < 0.0001$) by fitness test day, following a negative linear trend from d 0 to d 56 for both EX and EC. Day 0 pre- and post-fitness test sample were included in the model because there was a difference on d 0 between Hb concentrations of EX and EC (Figure 2.14).

There were no treatment differences ($P > 0.10$) of lactate concentrations pre- and post-fitness tests between EX and EC (Figure 2.15). Lactate concentrations before and after fitness tests were not affected ($P > 0.10$) by exercise treatment, however, post-fitness test samples of lactate were affected ($P = 0.02$) by fitness test day (Figure 2.15). Lactate concentrations were greatest after exercise on d 28 for both EX and EC. There was no fitness test day by exercise treatment interaction ($P > 0.10$) on either pre- or post-fitness test samples of lactate.

Based on fixed effects tests, glucose concentrations before and after fitness tests were not affected ($P > 0.10$) by exercise treatments or by an interaction ($P > 0.10$) of fitness test day and exercise treatment; however, glucose concentrations before and after fitness tests were affected ($P > 0.10$) by fitness test day (Figure 2.16). Before fitness tests, both EX and EC heifers had progressively reduced glucose concentrations as the 56-d experiment was carried out, while post-fitness test glucose concentrations were greatest on d 28. There was a tendency for EC heifers to

have greater ($P = 0.07$) glucose concentrations post-exercise than EX heifers on d 28 (Figure 2.16).

There was a tendency for EX heifers to have greater ($P = 0.06$) hematocrit than EC after the fitness test on d 56 (Figure 2.17). Hematocrit before fitness tests was not affected ($P > 0.10$) by exercise treatment; however, hematocrit was increased ($P = 0.05$) in EX heifers after fitness tests as compared to EC heifers (Figure 2.17). Before and after exercise, hematocrit was affected ($P < 0.01$) by an interaction of fitness test day and exercise treatment. Thus, EX heifers generally maintained similar hematocrit before and after exercise, while EC heifers had reduced after fitness tests.

Base excess concentrations before and after fitness tests were not affected ($P > 0.10$) by exercise treatment, however, they were affected ($P < 0.01$) by fitness test day (Figure 2.18). Base excess concentrations were greatest on d 28 before fitness tests, while concentrations after fitness tests were greatest on d 56 and least on d 0.

Exercise-control heifers had greater ($P = 0.01$) blood pH than EX heifers on d 28 and tended to have greater ($P = 0.08$) pH on d 56 (Figure 2.19). Blood pH before fitness tests was not affected ($P = 0.29$) by exercise treatments, however, pH after fitness tests tended to be affected ($P = 0.06$) by exercise treatment (Figure 2.19). Thus, post-fitness test samples of blood pH were generally greater in EC than EX heifers. There were fitness test day effects ($P < 0.0001$) on both pre- and post-fitness test samples of pH, demonstrated by increased pH before and after fitness tests on d 28. There was also an interaction ($P = 0.04$) of exercise treatment and fitness test day for post-fitness test samples but not ($P = 0.79$) pre-fitness test samples of pH, thus EC generally had greater pH than EX after fitness tests with the greatest concentrations on d 28 (Figure 2.19).

There were no differences ($P > 0.10$) between EX and EC heifers during any of the pre- and post-fitness tests for pCO_2, pO_2, Na, TCO_2, HCO_3, and sO_2 and no effects ($P > 0.10$) of exercise treatment (Table 2.12). Potassium and iCa^{2+} could not be analyzed because values from the assay were either unreadable or the value given was < 0.25 for all treatments on all days.

During the final wk of the exercise experiment (d 51), blood samples were taken in the morning and afternoon to compare ADH and cortisol concentrations in the blood in response to heat stress. There were no differences ($P > 0.10$) between EX and EC heifers for ADH on d 51 (Figure 2.20). Cortisol concentrations were compared from samples taken on d 51 and there were no differences ($P > 0.10$) between EX and EC heifers (Figure 2.21). There was no effect ($P > 0.10$) of fitness test d, exercise treatment, or an interaction of exercise treatment and fitness test day for either ADH or cortisol concentrations.

Weekly body weight, respiratory rates and rectal temperatures during the 8-wk exercise regimen

Body weight, respiration rates and rectal temperatures (RT) were assessed weekly throughout the exercise period. Body weights (Figure 2.22) were not affected ($P = 0.96$) by exercise treatment nor by an interaction ($P = 0.90$) of week of experiment and exercise treatment. There was, however, an effect ($P <0.0001$) of time on weekly body weights of EX and EC heifers, where both EX and EC heifers had an increase in body weight in a positive linear manner (Figure 2.22). Respiration rates and RT were affected ($P < 0.01$) by week, thus the trends of RR and RT were erratic throughout the experiment with greatest RR and RT during wk 4 and 6, however, there were no differences ($P > 0.05$) between EX and EC heifers during any

week (Table 2.20). There was a tendency for EC to have reduced ($P = 0.10$) respiration rates compared with EX (Table 2.20).

Body temperature

There was a tendency for EX to have a greater ($P = 0.09$) body temperature than EC heifers before fitness tests on d 56 (Table 2.13). Beginning and ending body temperatures at the end of fitness tests were affected ($P < 0.0001$) by fitness test day as body temperatures were greatest on d 28 for both EX and EC heifers. Body temperature after fitness tests tended to be affected ($P = 0.06$) by an interaction of exercise treatment and fitness test day, where both EX and EC heifers had greater temperatures on d 28 and EC also had greater temperatures on the final day of the experiment.

There was no difference ($P > 0.10$) between EX and EC heifers on average body temperatures during any fitness tests, however, there was a fitness test day effect ($P < 0.0001$) because of increased body temperatures in both treatments on d 28 compared with d 0 and 56 (Table 2.15). Exercised heifers had reduced ($P = 0.01$) average body temperatures for the hour following exercise than EC heifers on d 28 (Table 2.15). Also, EX heifers had changed less ($P = 0.001$) between average body temperature during exercise and average temperature during the hour after exercise compared with EC heifers on d 28 (Table 2.15). The average body temperatures of the hour after fitness tests were affected ($P < 0.0001$) by fitness test day because of greater temperatures on d 28 (average THI = 83; average temperature: 35.7°C; high temperature: 40.3°C), as well as an interaction ($P = 0.05$) of exercise treatment and fitness test day. Both treatments followed similar trends with greatest body temperatures experienced on d

28; however, EC heifers had numerically reduced body temperatures on both d 0 and 56 compared to EX heifers.

Time spent in zones 1, 2, and 3 were not affected ($P > 0.10$) by exercise treatment or by an interaction ($P > 0.10$) of fitness test day and exercise treatment (Table 2.14). There were fitness test day effects ($P = 0.001$) on time spent in all 3 body temperature zones, such that the least amount of time was spent on d 28 in zones 1 and 2, and the greatest amount of time spent in zone 3 was also on d 28.

Skin temperature

Skin temperature was included in the 2nd experiment to help assess heat dissipation as an indicator of temperature regulation of the body. Calculated surface area was included in the model when analyzing weekly skin temperature data. Exercised heifers had a reduced ($P = 0.01$) cheek skin temperature when compared with EC during wk 7 (Figure 2.24). Exercise-control heifers had greater ($P = 0.004$) thurl skin temperatures during wk 1, and wk 7 ($P = 0.03$), however, during wk 4 EX had greater ($P = 0.02$) thurl skin temperatures than EC (Figure 2.26). Exercise-control had greater ($P = 0.04$) skin temperature of the withers compared to EX during wk 7. Exercise-control heifers had greater ($P = 0.01$) ear skin temperature than EX during wk 6 (Figure 2.27). Exercise-control tended to have greater ($P = 0.07$) udder skin temperatures compared with EX during wk 2 (Figure 2.28).

All areas of skin temperature were affected ($P < 0.01$) by week of exercise. Both EX and EC heifers followed similar trends where skin temperatures were reduced during wk 0-2, however, they began increasing during the remainder of the experiment with only a small decrease during wk 5. None of the other skin temperature regions were affected ($P > 0.10$) by

exercise treatment except for cheek skin temperature ($P = 0.03$). Exercised and EC heifers followed similar trends for the first couple of weeks in which temperatures were reduced, however, both sharply increased from wk 2-4 and EX plateaued for the remainder of the experiment, while EC erratically increased in skin temperature. Thurl skin temperature, however, was affected ($P = 0.01$) by an interaction of week and exercise treatment. During wk 0-2, both EX and EC shared a decreased linear trend in skin temperature of the thurl; however beginning wk 3, EX plateaued in skin temperature for the remainder of the experiment while EC's skin temperature decreased wk 3-4 and then linearly increased for the remainder of the experiment.

Impacts of 8 weeks of exercise on thermal tolerance and production parameters

There were no differences between RR and RT on any day post-exercise (Table 2.17). There were post-experiment day effects ($P < 0.01$) on RR and RT during AM and PM times, but no exercise treatment effects ($P > 0.10$) or interactions ($P > 0.10$) of exercise treatment and post-experiment day. Respiration rates and RT were affected by day because of varying THI on post-experiment day with the greatest RT and RR on the hottest day (THI > 80) and reduced temperatures on cooler day (THI < 79).

Health variables measured at parturition and first week postpartum

Least squares means were compared for calf birth weights, difference of days between predicted calving and actual calving date, and calving ease, similar to experiment 1. There was no ketosis occurrence (n = 0) for EX heifers, however, 25% of EC heifers had ketosis (n = 3). Sixty-seven percent of EX heifers (n = 8) did not have a fever following parturition, while 83% of EC heifers (n = 10) did not have a fever. Ketones and fever occurrence were not affected ($P >$

0.10) by exercise treatment. There was no difference ($P > 0.10$) between calf birth weights, calving ease, or difference of predicted calving date from actual calving (Table 2.18). There were also no effects ($P > 0.10$) of exercise treatment on any of the parturition variables.

Milk components

Milk components during the first 15 wk of lactation were analyzed to determine any residual effects of exercise on milk production. Milk protein percentage was affected ($P = 0.001$) by exercise treatment and lactation week ($P < 0.0001$), but not by an interaction ($P = 0.34$) between week of lactation and exercise treatment (Figure 2.30). Except for wk 5 and 7 of lactation, all other weeks of lactation had consistently greater protein percentage of milk (approximately 0.2%) for EX compared to EC heifers.

Lactose percentage of milk was not affected ($P = 0.24$) by exercise treatment or by an interaction ($P = 0.36$) of lactation week and exercise treatment. There was, however, an effect ($P < 0.0001$) of week of lactation (Figure 2.31).

Milk content of SNF percentage was affected ($P = 0.02$) by exercise treatment and week of lactation ($P < 0.0001$), but there wasn't an interaction effect ($P = 0.52$) of exercise treatment and lactation week (Figure 2.32). Exercised heifers had greater SNF percentage of milk throughout the first 15 wk of lactation compared to EC heifers.

Milk fat percentage was not affected ($P = 0.15$) by treatment and there was no interaction ($P = 0.55$) of week of lactation and treatment. There was, however, an effect ($P = 0.0003$) of week of lactation on milk fat percentage (Figure 2.29).

Milk urea nitrogen in milk was not affected ($P = 0.51$) by exercise treatment or an interaction ($P = 0.34$) of exercise treatment and week of lactation. There was, however, an effect

($P < 0.0001$) of lactation week thus, MUN was numerically greater in EX heifers for a majority of the week, but both treatments maintained similar trends that stayed within the range of 12-14 mg/dL from wk 4-15 of lactation (Figure 2.33). Lactation wk 0-4 had reduced MUN concentrations (<12 mg/dL) in both treatments.

There were no differences ($P > 0.10$) between SCS of EX and EC during any of the weeks of lactation (Figure 2.34). Somatic cell score was affected ($P < 0.0001$) by lactation week; however, there was no effect ($P = 0.71$) of exercise treatment or an interaction ($P = 0.69$) of exercise treatment and week of lactation. During the first few weeks of lactation, SCS was much greater but began plateauing around scores between 0.5-2.5 starting the 3rd week of lactation.

There were no differences ($P > 0.10$) of lactation persistency between EX and EC when comparing 2 different DIM (Table 2.19). There was no affect ($P > 0.10$) of treatment on lactation persistency and no interaction ($P > 0.10$) of week of lactation and treatment.

Daily milk records were recorded and weekly averages were compared. There was no difference ($P > 0.10$) between average weekly milk production between EX and EC heifers during any of the first 15 wk of lactation. Milk production was not affected ($P = 0.73$) by exercise treatment or an interaction ($P = 0.62$) of lactation week and treatment; however, there was an effect ($P < 0.0001$) of week of lactation on average milk production (Figure 2.35). Milk production increased dramatically from wk 0 to 7 and then plateaued for the remaining weeks.

Energy-corrected milk (ECM) and fat-corrected milk (FCM) were calculated and compared. Treatment did not affect ($P = 0.17$) ECM between EX and EC heifers, and there was no interaction ($P = 0.58$) effect of week of lactation by treatment (Figure 2.36). There was also

no effect ($P = 0.20$) of treatment on FCM, or an interaction ($P = 0.57$) of treatment and week of lactation (Figure 2.37). There was, however, a time effect ($P < 0.0001$) of week of lactation on ECM and FCM where both treatments increased during the first 5 weeks and then plateaued between 5 and 15 weeks of lactation.

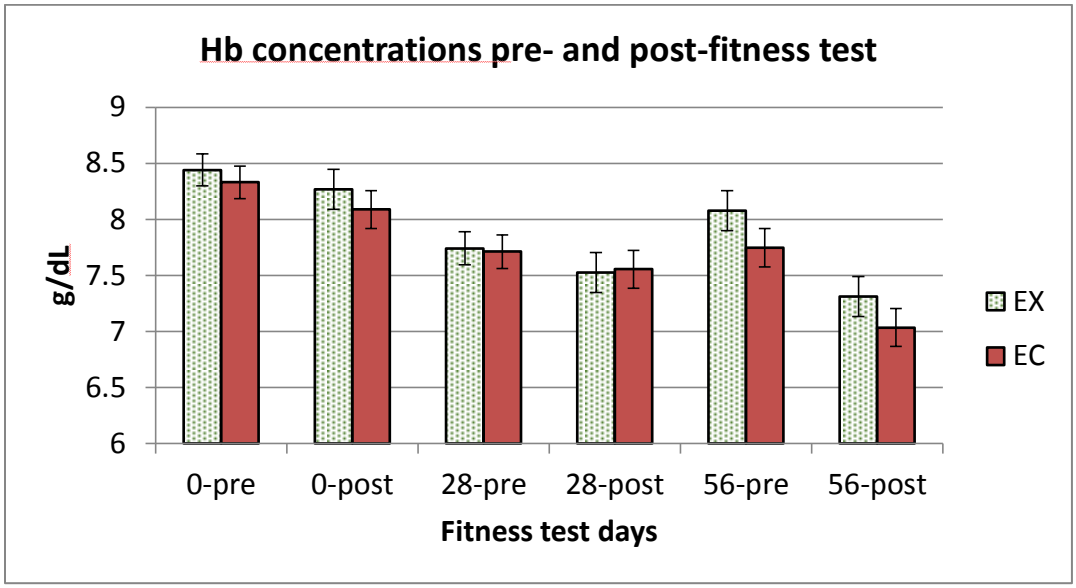

Figure 2.14. Least squares means of hemoglobin (Hb) concentrations of EX and EC on d 0, 28, and 56 during experiment 2.

Treatments: EX = exercised heifers (n = 12); EC = exercise-control (n = 12) brought to exerciser but not exercised.

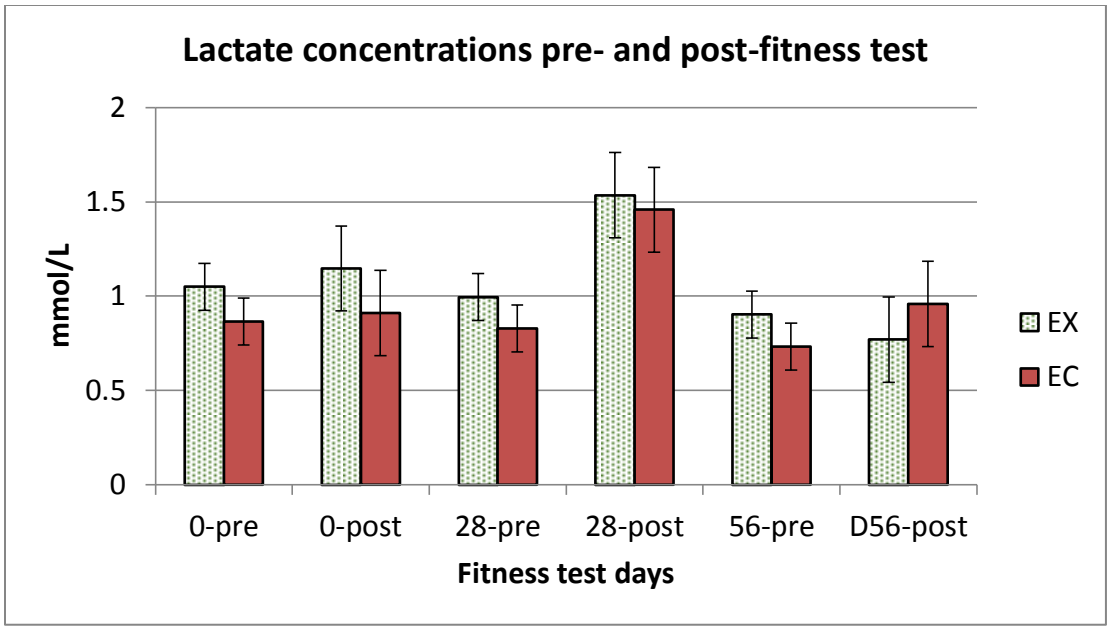

Figure 2.15. Least squares means of lactate concentrations of EX and EC on d 0, 28, and 56 during experiment 2.

Treatments: EX = exercised heifers (n = 12); EC = exercise-control (n = 12) brought to exerciser but not exercised.

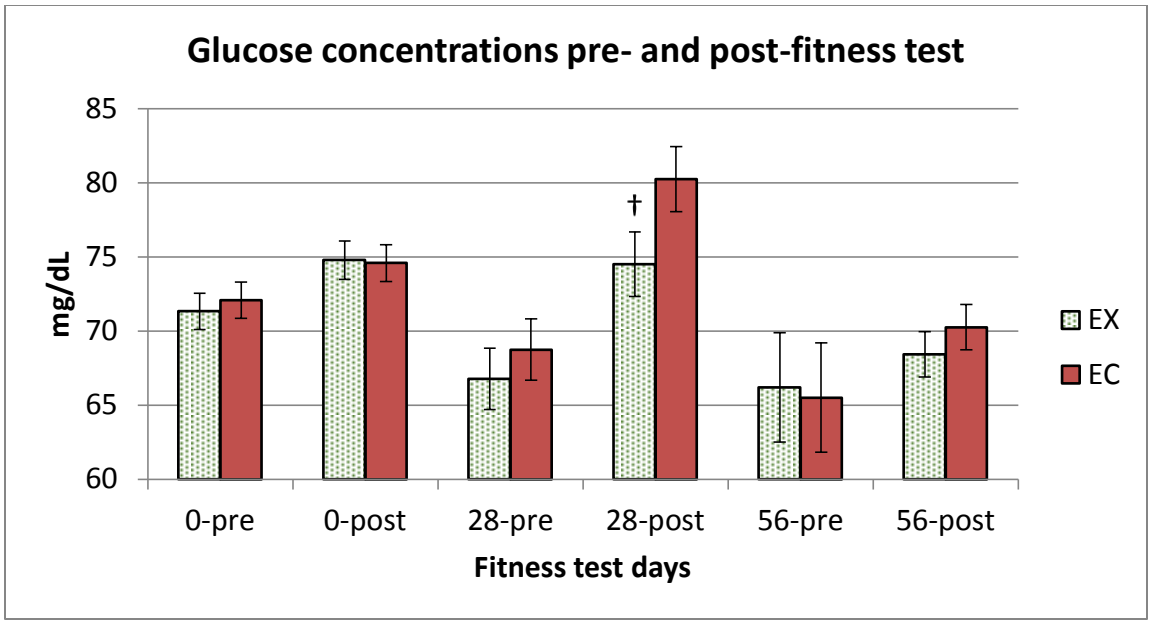

Figure 2.16. Least squares means of glucose concentrations of both EX and EC on d 0, 28, and 56 during experiment 2.

Treatments: EX = exercised heifers (n = 12); EC = exercise-control (n = 12) brought to exerciser but not exercised.

†$P < 0.10$

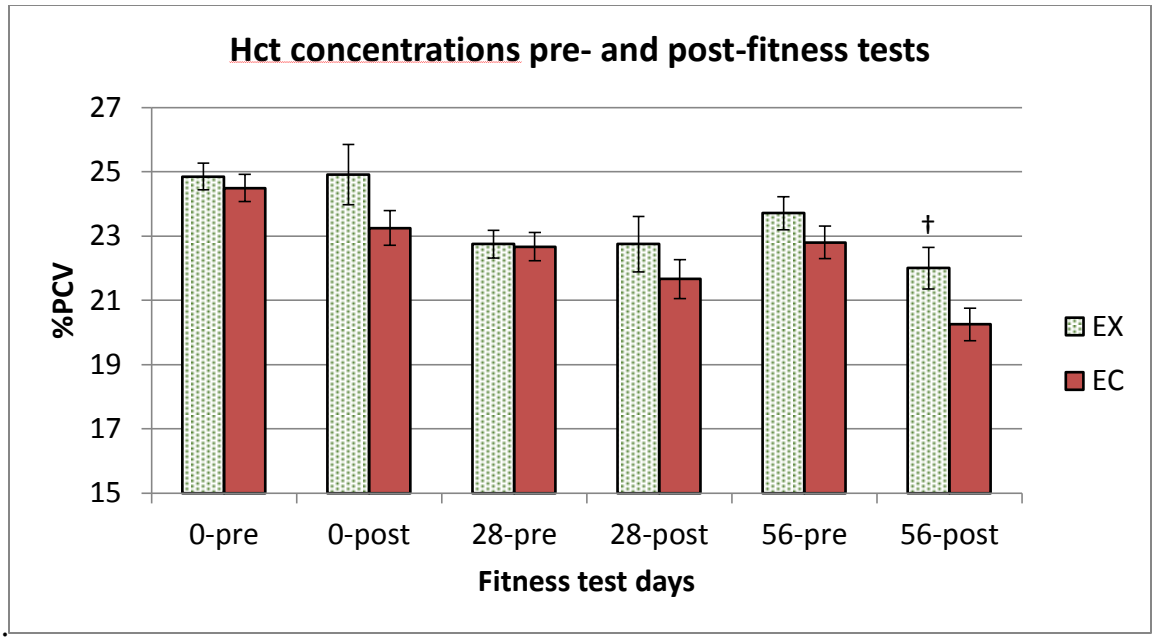

Figure 2.17. Least squares means of hematocrit (Hct) percentage of both EX and EC on d 0, 28, and 56 during experiment 2.

Treatments: EX = exercised heifers (n = 12); EC = exercise-control (n = 12) brought to exerciser but not exercised.

†$P < 0.10$

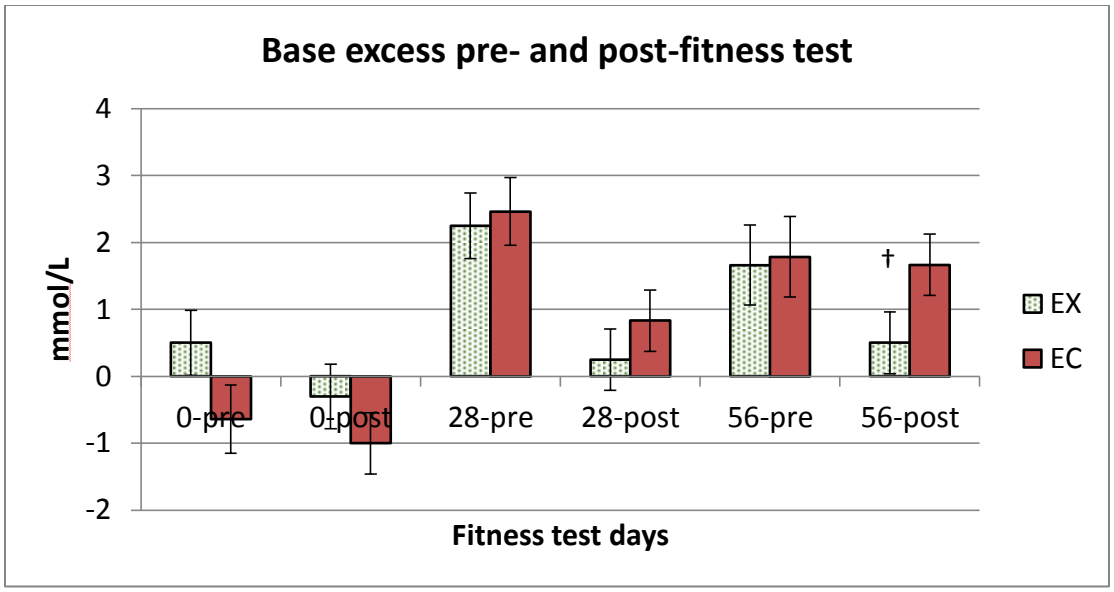

Figure 2.18. Least squares means of base excess (BE) concentrations for both EX and EC on d 0, 28, and 56 during experiment 2.

Treatments: EX = exercised heifers (n = 12); EC = exercise-control (n = 12) brought to exerciser but not exercised.

†$P < 0.10$

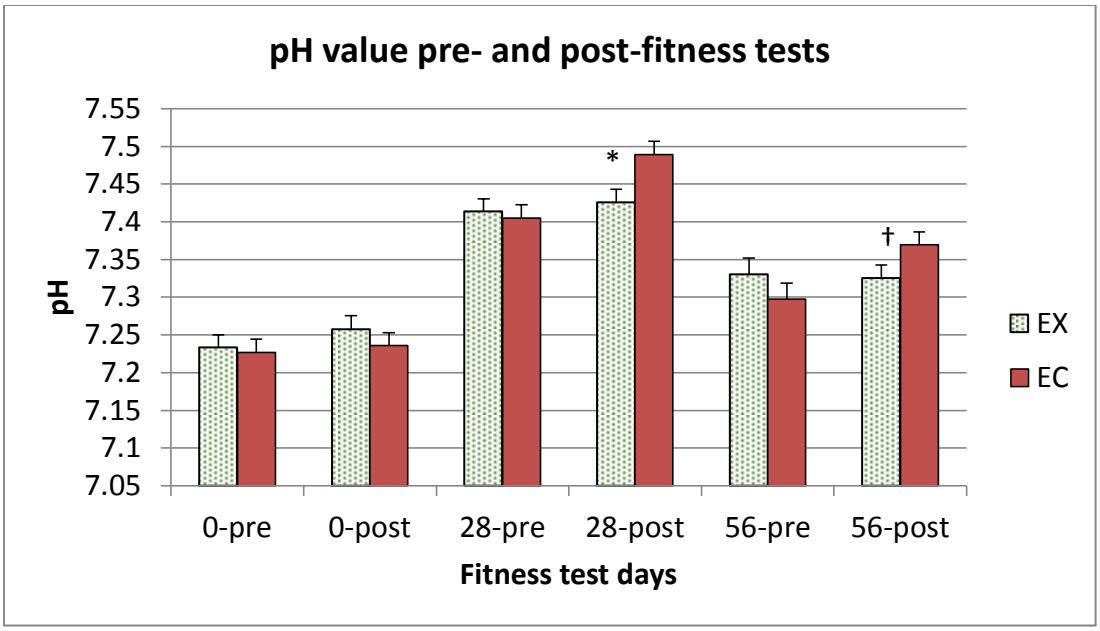

Figure 2.19. Least squares means of pH values of both EX and EC on pre- and post-fitness tests of d 0, 28, and 56 during experiment 2.

Treatments: EX = exercised heifers (n = 12); EC = exercise-control (n = 12) brought to exerciser but not exercised.

*$P < 0.05$

†$P < 0.10$

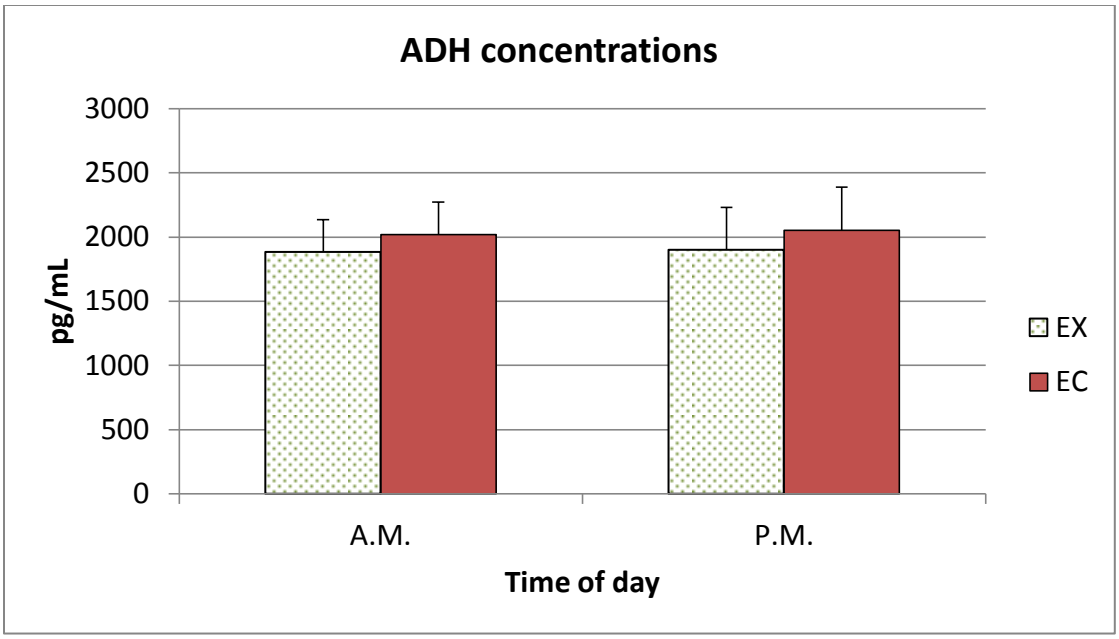

Figure 2.20. Mean plasma ADH concentrations from AM (environmental temperature = 21.1°C) and PM (environmental temperature = 37.8°C) times of both EX and EC during experiment 2 on d 51.

Treatments: EX = exercised heifers (n = 12); EC = exercise-control (n = 12) brought to exerciser but not exercised.

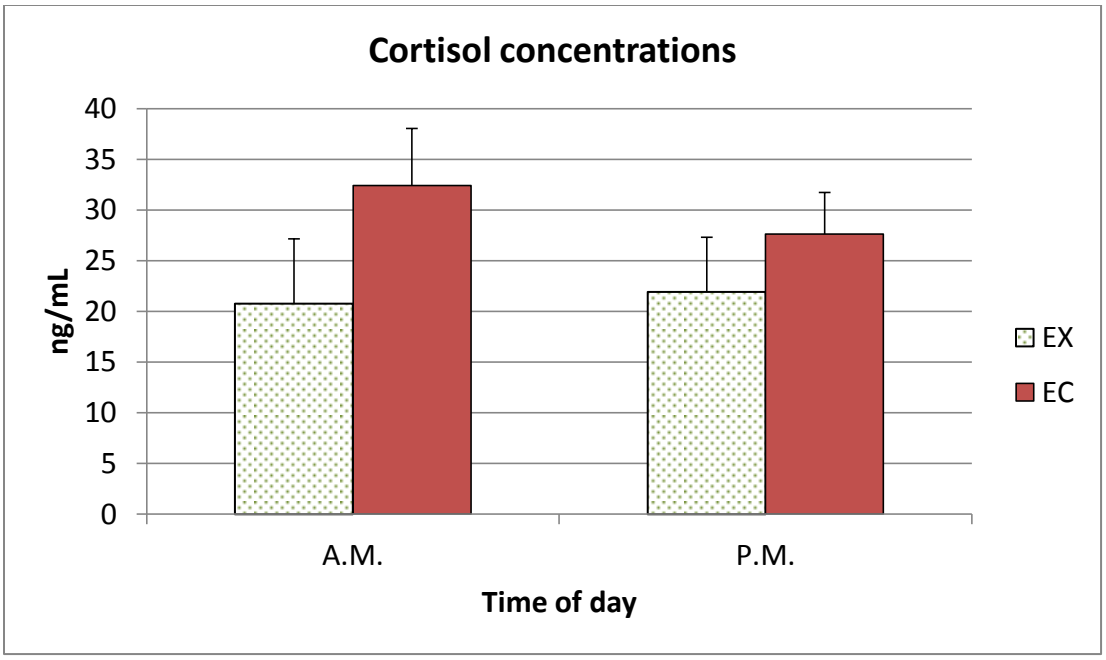

Figure 2.21. Mean cortisol concentrations from AM and PM within 1 day of EX and EC during experiment 2.

Treatments: EX = exercised heifers (n = 12); EC = exercise-control (n = 12) brought to exerciser but not exercised.

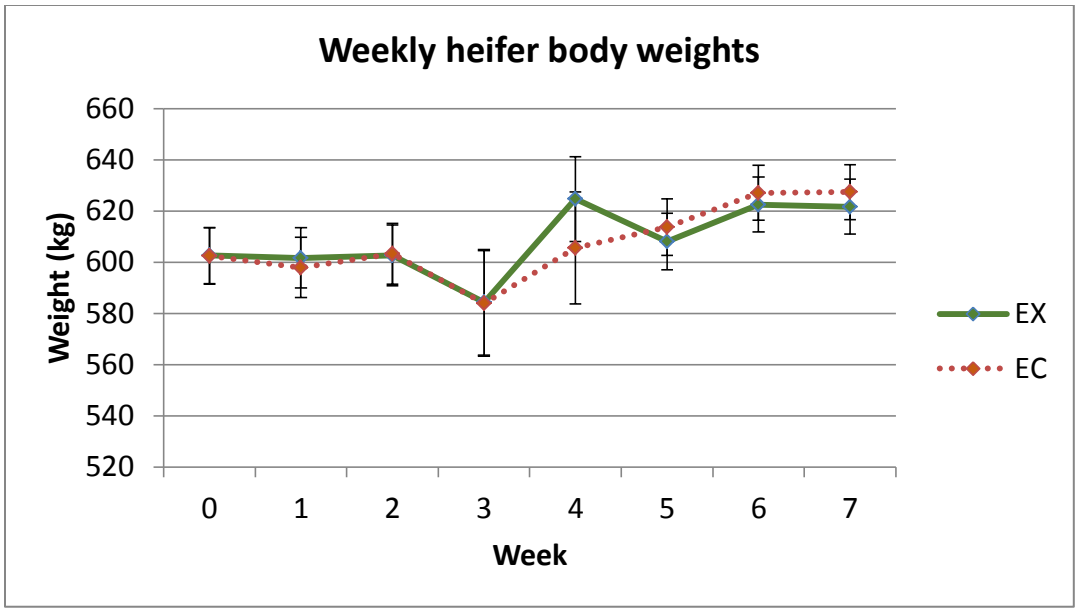

Figure 2.22. Least squares means of weekly body weights (kg) of EX and EC during wk 0-7 in experiment 2.

Treatments: EX = exercised heifers (n = 12); EC = exercise-control (n = 12) brought to exerciser but not exercised.

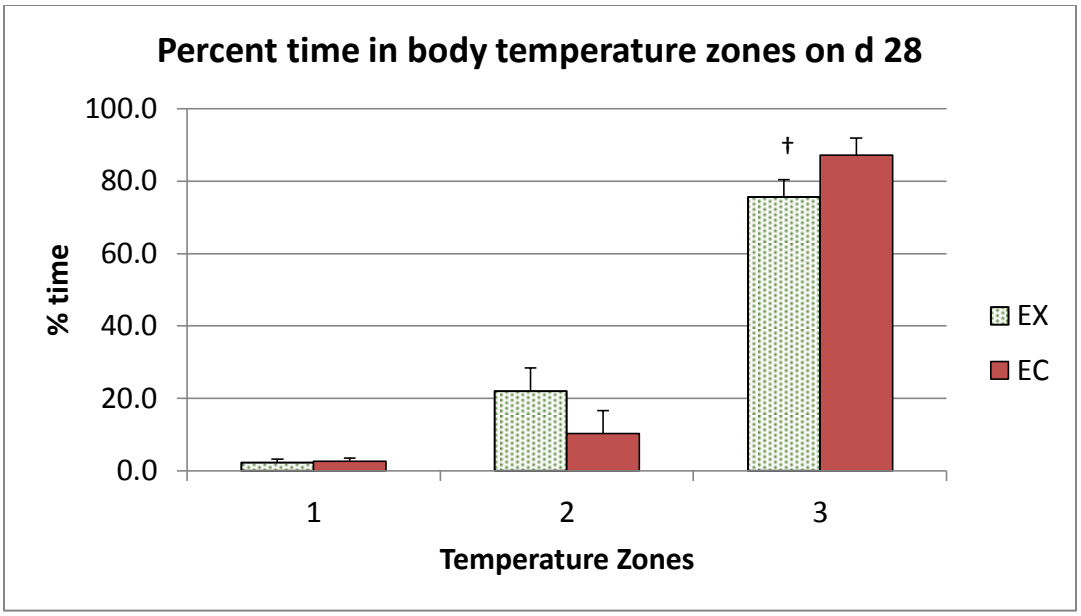

Figure 2.23. Least squares means of the percentage of time spent in each temperature zone (zone 1: <39.0°C; zone 2: 39.0°C-40.0°C; zone 3: >40.0°C) for both EX and EC on d 28 (THI = 81) of experiment 2.

Treatments: EX = exercised heifers (n = 12); EC = exercise-control (n = 12) brought to exerciser but not exercised. Temperature sensors logged body temperature during exercise and continued for approximately 1 h after fitness test completion.

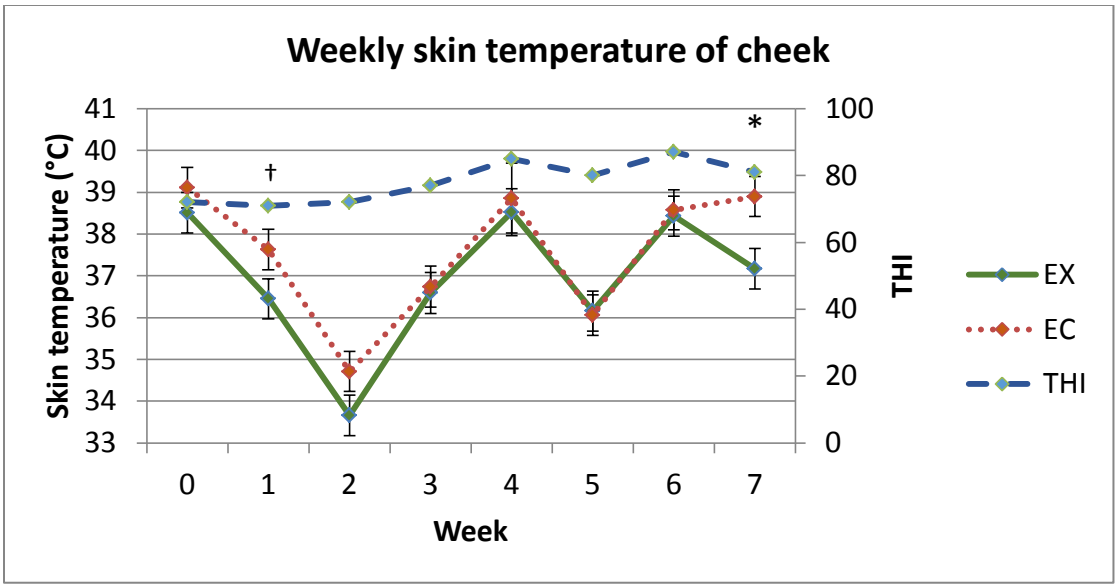

Figure 2.24. Least squares means of weekly skin temperature of the cheek area of EX and EC during experiment 2.

Treatments: EX = exercised heifers (n = 12); EC = exercise-control (n = 12) brought to exerciser but not exercised. Average THI during the time skin temperature was collected is illustrated on the secondary y-axis.

*$P < 0.05$

†$P < 0.10$

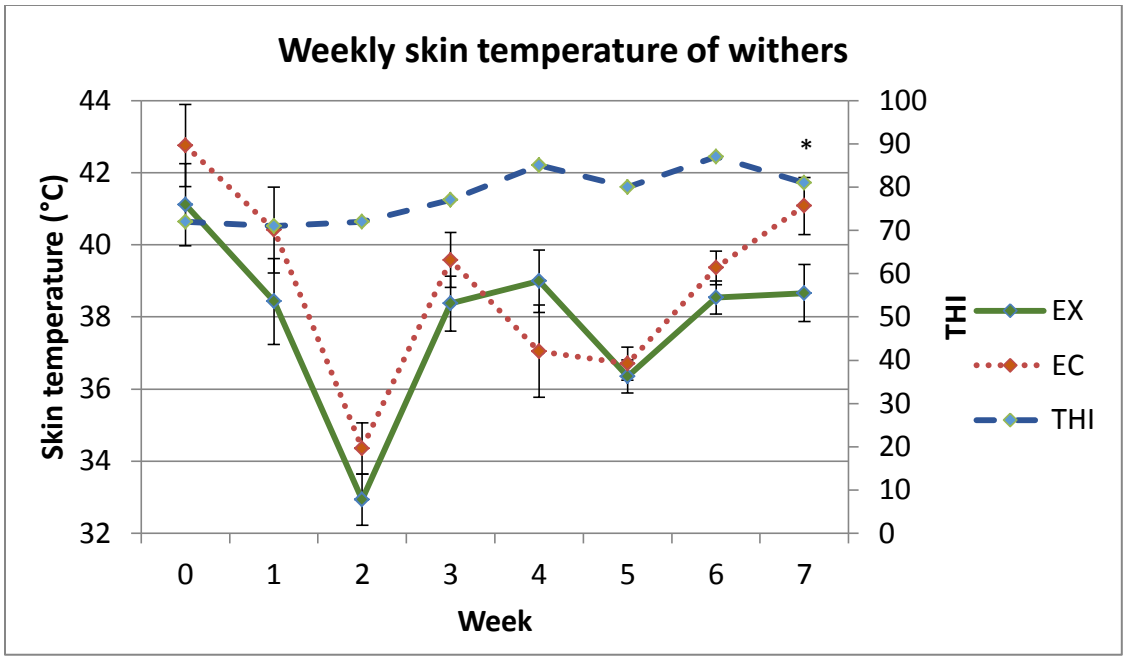

Figure 2.25. Least squares means of weekly skin temperature of the withers area of EX and EC during experiment 2.

Treatments: EX = exercised heifers (n = 12); EC = exercise-control (n = 12) brought to exerciser but not exercised. Average THI during the time skin temperature was collected is illustrated on the secondary y-axis.

*$P < 0.05$

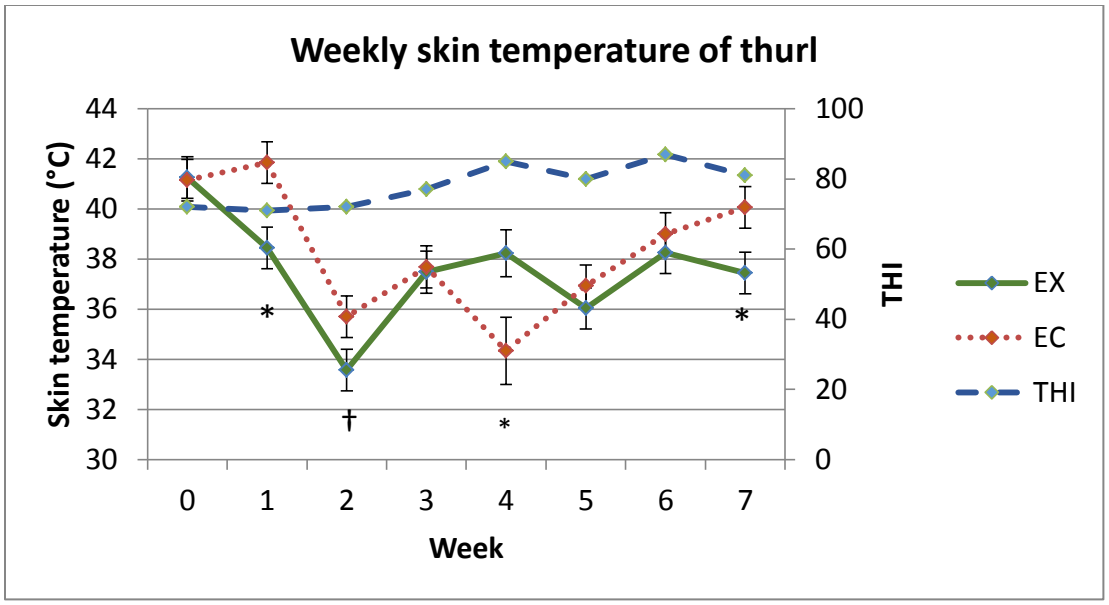

Figure 2.26. Least squares means of weekly skin temperature of the thurl area of EX and EC during experiment 2.

Treatments: EX = exercised heifers (n = 12); EC = exercise-control (n = 12) brought to exerciser but not exercised. Average THI during the time skin temperature was collected is illustrated on the secondary y-axis.

*$P < 0.05$

†$P < 0.10$

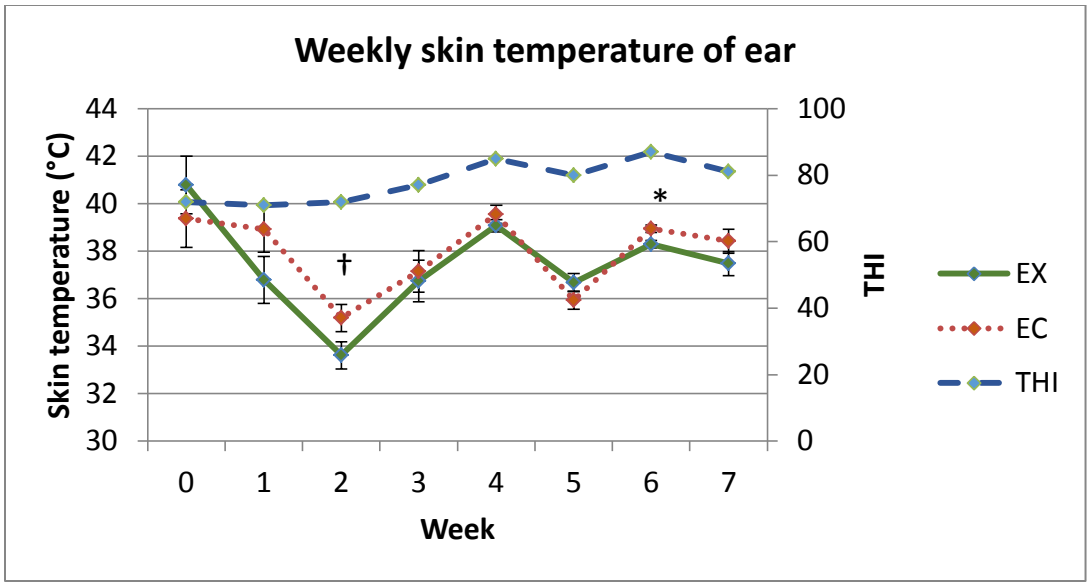

Figure 2.27. Least squares means of weekly skin temperature of the ear area of EX and EC during experiment 2.

Treatments: EX = exercised heifers (n = 12); EC = exercise-control (n = 12) brought to exerciser but not exercised. Average THI during the time skin temperature was collected is illustrated on the secondary y-axis.

†$P < 0.10$

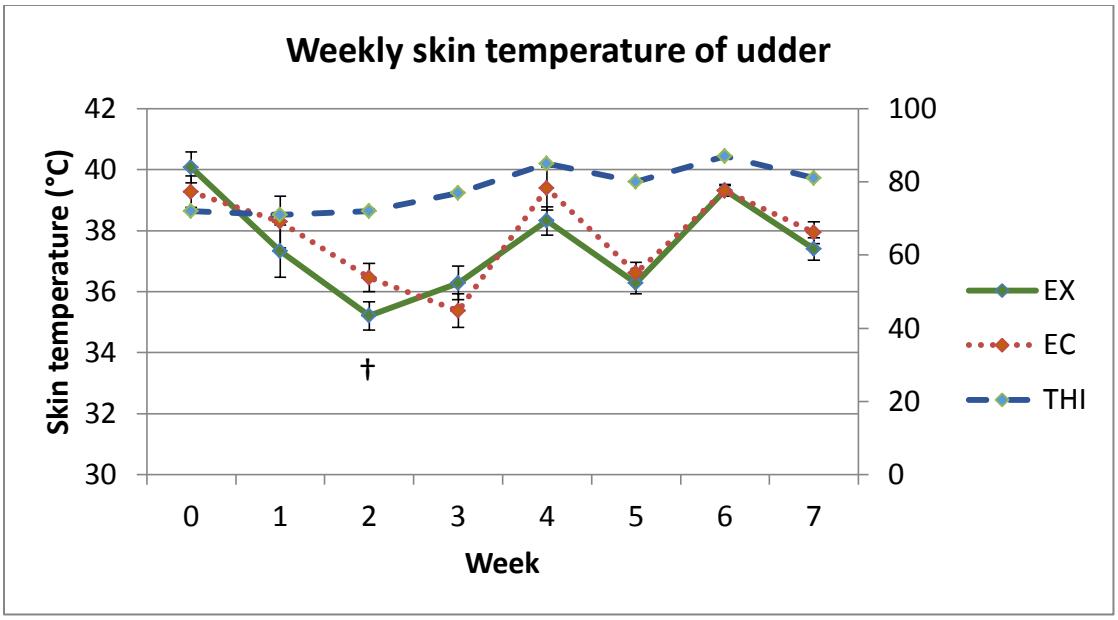

Figure 2.28. Least squares means of weekly skin temperature of the udder area of EX and EC during experiment 2.

Treatments: EX = exercised heifers (n = 12); EC = exercise-control (n = 12) brought to exerciser but not exercised. Average THI during the time skin temperature was collected is illustrated on the secondary y-axis.

†$P < 0.10$

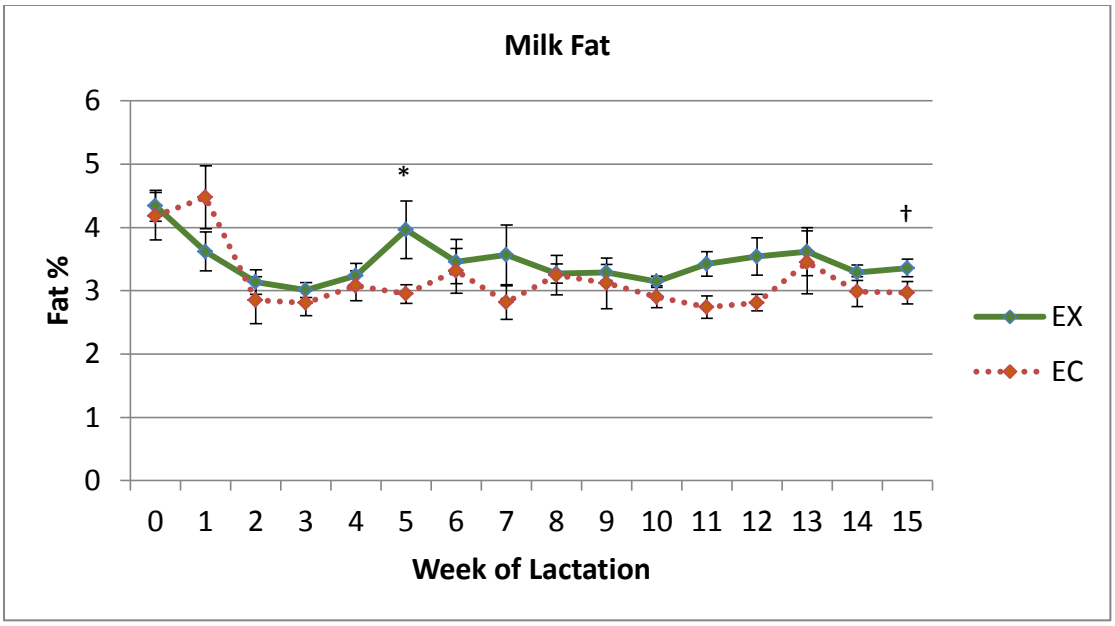

Figure 2.29. Least squares means of milk fat percentage during the first 15 wk of lactation between EX and EC.

Treatments: EX = exercised heifers (n = 12); EC = exercise-control (n = 12) brought to exerciser but not exercised.

*$P < 0.05$

†$P < 0.10$

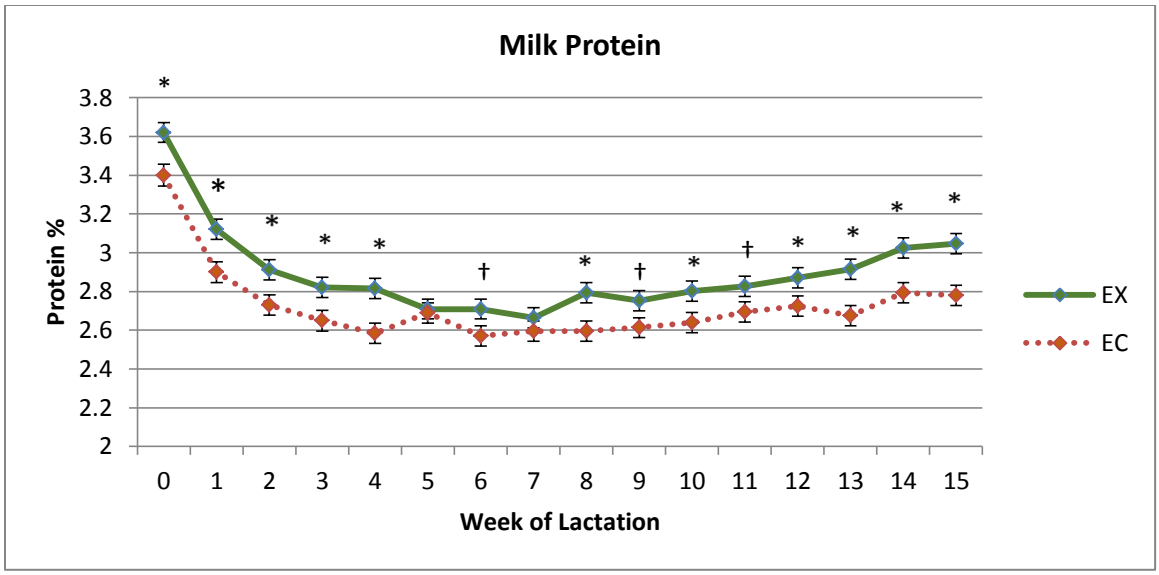

Figure 2.30. Least squares means of milk protein percentage during the first 15 wk of lactation between EX and EC.

Treatments: EX = exercised heifers (n = 12); EC = exercise-control (n = 12) brought to exerciser but not exercised.

*$P < 0.05$

†$P < 0.10$

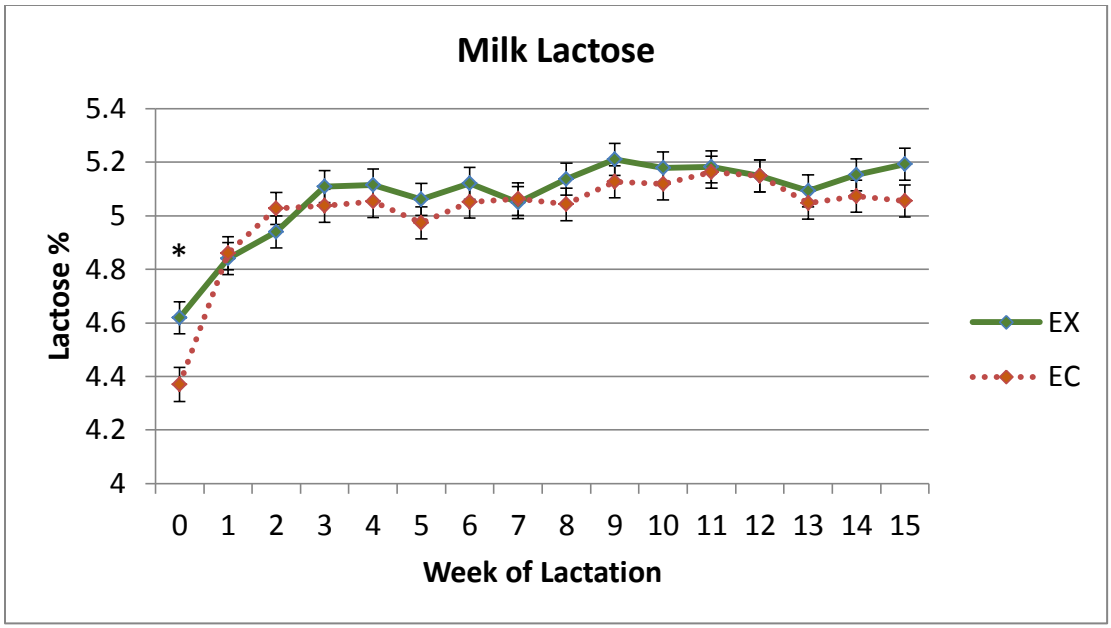

Figure 2.31. Least squares means of milk lactose percentage during the first 15 wk of lactation between EX and EC.

Treatments: EX = exercised heifers (n = 12); EC = exercise-control (n = 12) brought to exerciser but not exercised.

*$P < 0.05$

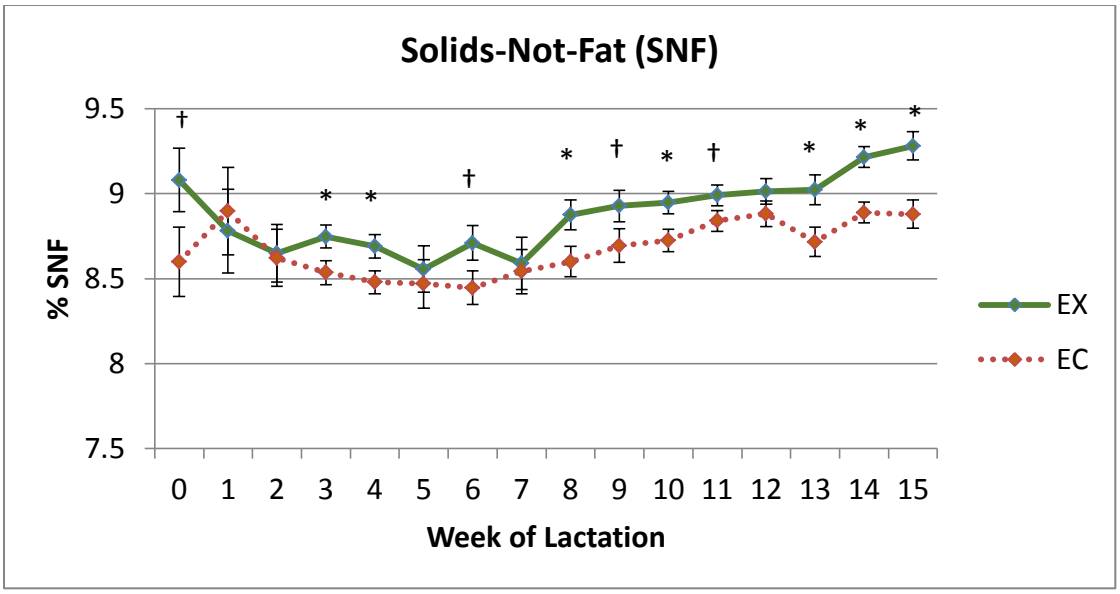

Figure 2.32. Least squares means of milk SNF percentage during the first 15 wk of lactation between EX and EC.

Treatments: EX = exercised heifers (n = 12); EC = exercise-control (n = 12) brought to exerciser but not exercised.

*$P < 0.05$

†$P < 0.10$

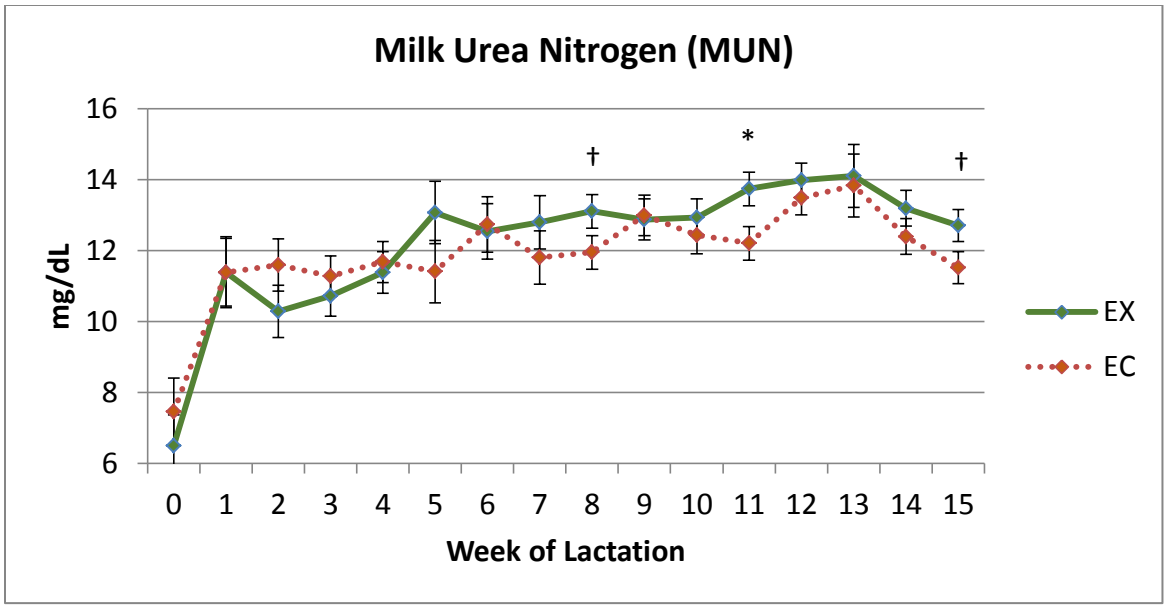

Figure 2.33. Least squares means of MUN during the first 15 wk of lactation between EX and EC.

Treatments: EX = exercised heifers (n = 12); EC = exercise-control (n = 12) brought to exerciser but not exercised.

*$P < 0.05$

†$P < 0.10$

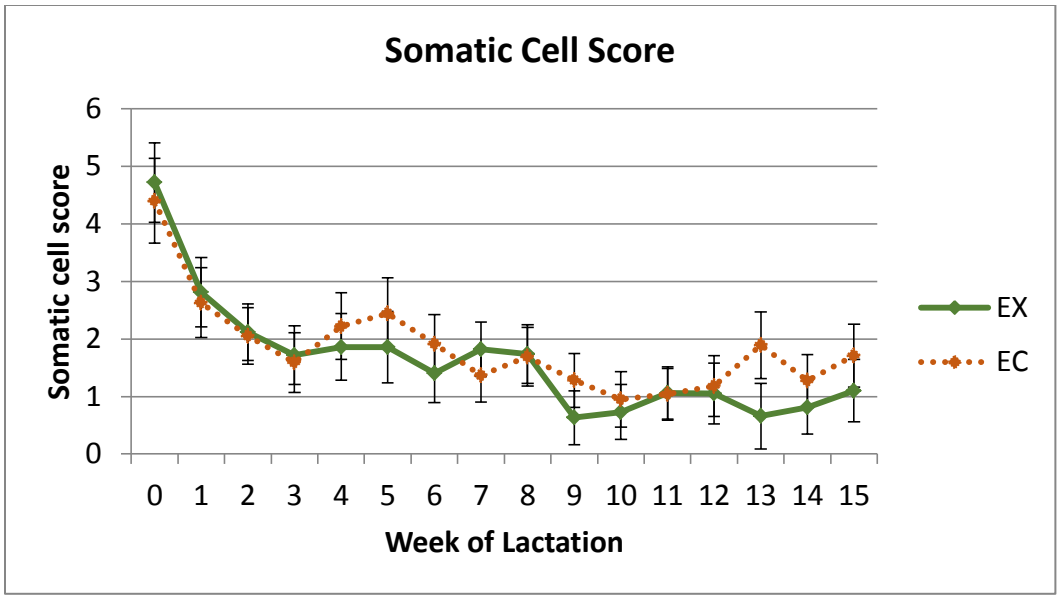

Figure 2.34. Least squares means of somatic cell score (SCS) during the first 15 wk of lactation between EX and EC.

Treatments: EX = exercised heifers (n = 12); EC = exercise-control (n = 12) brought to exerciser but not exercised.

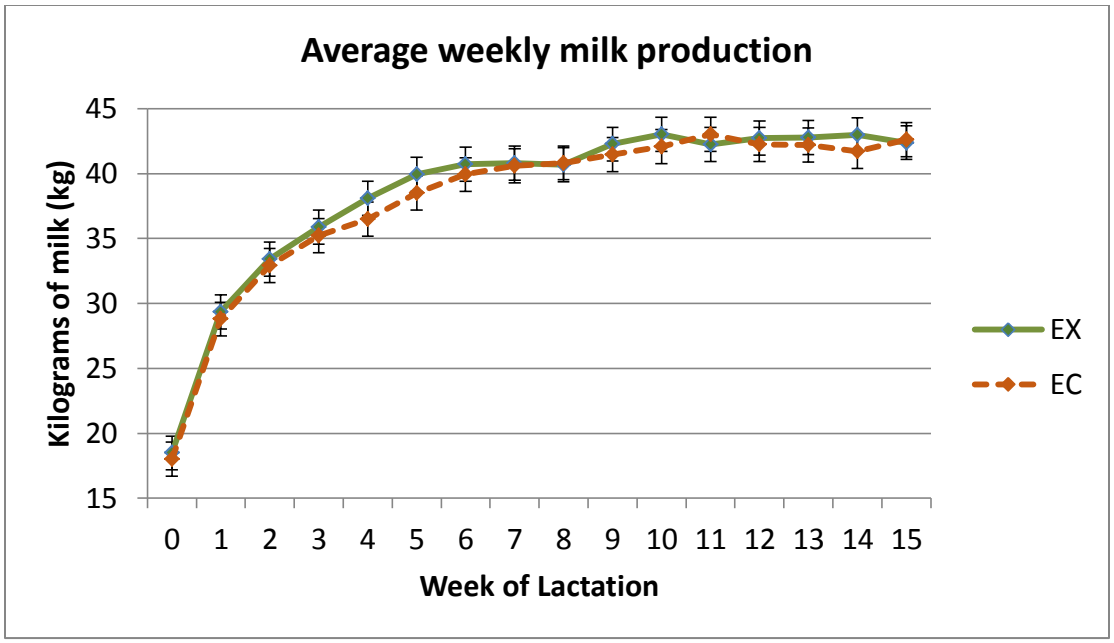

Figure 2.35. Least squares means of the average weekly milk production during the first 15 wk of lactation of EX and EC.

Treatments: EX = exercised heifers (n = 12); EC = exercise-control (n = 12) brought to exerciser but not exercised.

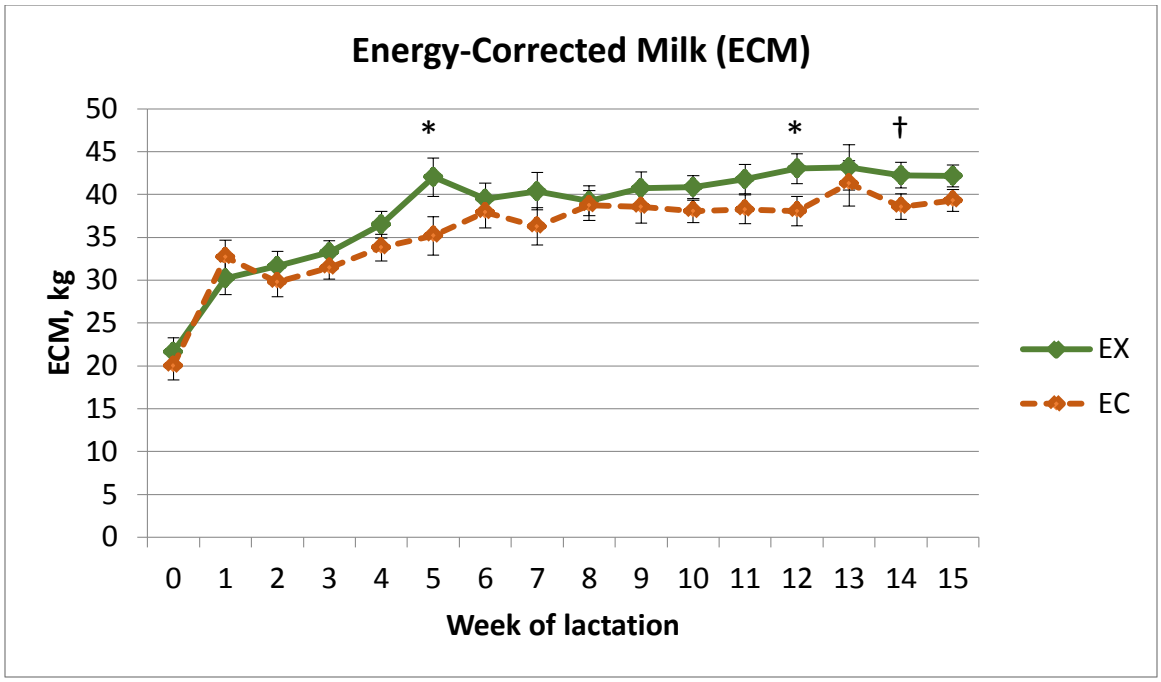

Figure 2.36. Least squares means of energy-corrected milk (ECM) during the first 15 wk of lactation.

Treatments: EX = exercised heifers (n = 12); EC = exercise-control (n = 12) brought to exerciser but not exercised.

*$P < 0.05$

†$P < 0.10$

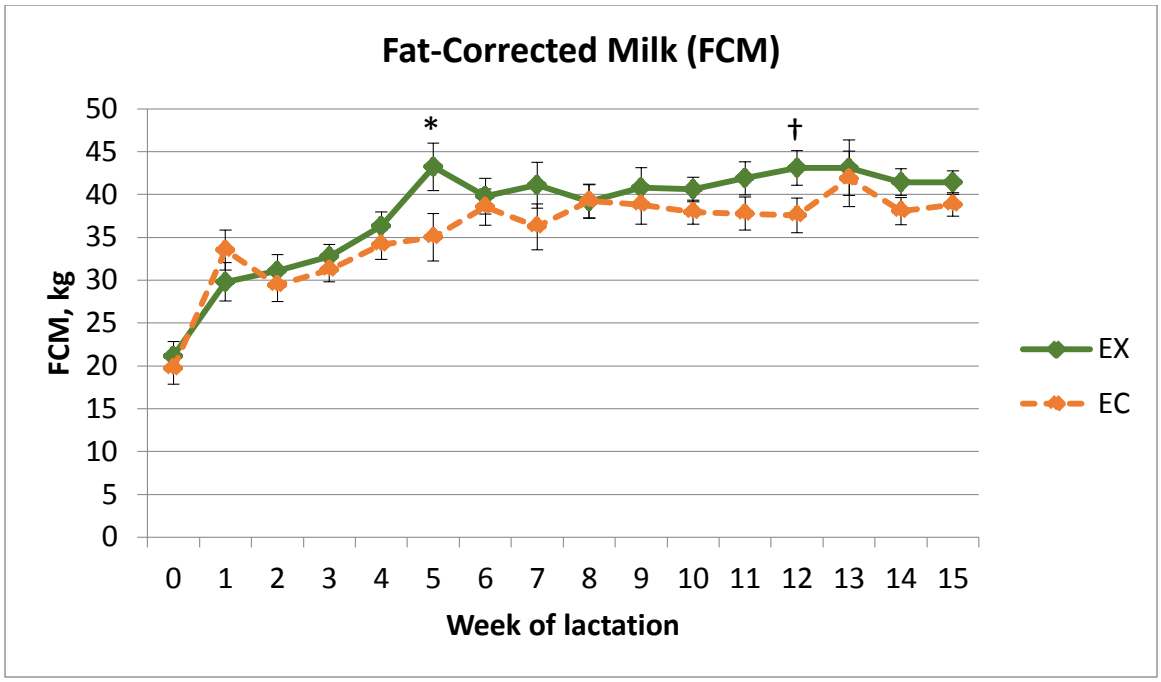

Figure 2.37. Least squares means of fat-corrected milk (FCM) during the first 15 wk of lactation.

Treatments: EX = exercised heifers (n = 12); EC = exercise-control (n = 12) brought to exerciser but not exercised.

*$P < 0.05$

†$P < 0.10$

Table 2.13. Least squares means of additional blood parameter values of both EX and EC on pre- and post-fitness tests of d 0, 28, and 56 during experiment 2.

		Treatment[1]			
		Pre-fitness test		Post-fitness test	
Item	Fitness test day	EX	EC	EX	EC
Na, mmol/L	0	136.3 ± 0.34	136.7 ± 0.34	137.1 ± 0.37	137.3 ± 0.35
	28	137.5 ± 0.34*	136.3 ± 0.36	137.7 ± 0.49	137.2 ± 0.49
	56	135.4 ± 0.43	135.3 ± 0.41	135.3 ± 0.36	135.1 ± 0.36
pCO_2, mmHg	0	66.3 ± 2.4	65.6 ± 2.5	57.6 ± 2.7	62.8 ± 2.5
	28	43.1 ± 2.4	43.1 ± 2.5	38.0 ± 2.5†	31.8 ± 2.5
	56[2]	53.6 ± 3.0	60.2 ± 2.9	52.9 ± 2.5	47.5 ± 2.5
pO_2, mmHg	0	43.4 ± 7.5	61.2 ± 7.5	69.3 ± 9.9	60.3 ± 9.4
	28	32.1 ± 4.3	37.4 ± 4.3	41.3 ± 5.6	41.8 ± 5.4
	56	47.7 ± 12.7	38.0 ± 12.7	49.4 ± 7.2	39.0 ± 7.2
TCO_2, mmol/L	0	30.0 ± 0.53	28.0 ± 0.55	28.5 ± 0.32	28.5 ± 0.31
	28	28.1 ± 0.63	28.6 ± 0.66	25.9 ± 0.75	25.0 ± 0.75
	56	30.2 ± 0.73	30.4 ± 0.74	28.5 ± 0.50	28.6 ± 0.50
HCO_3^-, mmol/L	0	28.0 ± 0.53	27.0 ± 0.55	26.8 ± 0.52	26.6 ± 0.50
	28	26.9 ± 0.53	27.0 ± 0.55	24.7 ± 0.50	24.1 ± 0.50
	56	28.3 ± 0.62	28.8 ± 0.63	26.9 ± 0.50	27.2 ± 0.50
sO_2, %	0	67.0 ± 5.69	70.8 ± 5.96	80.2 ± 6.86	75.7 ± 6.56
	28	57.1 ± 5.96	63.5 ± 5.96	67.4 ± 6.87	70.1 ± 6.56
	56	57.1 ± 7.12	53.0 ± 7.10	65.6 ± 6.56	57.9 ± 6.56

[1]Treatments: EX = exercised heifers (n = 12); EC = exercise-control (n = 12), brought to exerciser but not exercised. Differences are specified between pre-exercise or post-exercise values between treatments.

[2]d56 concentrations of pCO_2 had a greater difference between pre- and post-fitness test values for EC compared with EX.

*$P < 0.05$

†$P < 0.10$

Table 2.14. Least squares means of fitness test physiological parameters pre-exercise, mid-exercise, and post-exercise of EX and EC in experiment 2.

		Treatment[1]					
		Pre-exercise		Mid-exercise		Post-exercise	
Item	Fitness test day	EX	EC	EX	EC	EX	EC
Heart Rate, beats/min	0	90.7 ± 7.72	83.8 ± 7.37	131.2 ± 7.17	118.8 ± 6.86	110.0 ± 7.49	98.6 ± 7.49
	28	120.6 ± 7.37	117.8 ± 7.37	117.1 ± 6.86	117.8 ± 6.86	-	-
	56	93.8 ± 7.37	95.7 ± 7.37	117.6 ± 6.86	120.5 ± 6.86	103.1 ± 7.17	109.9 ± 7.17
RR, bpm[2]	0	49 ± 2.72	43 ± 2.72	71 ± 4.30†	60 ± 4.30	-	-
	28	120 ± 8.04	112 ± 8.04	151 ± 4.53	149 ± 4.30	-	-
	56	47 ± 3.02	44 ± 3.02	59 ± 4.30	62 ± 4.30	-	-
Body temperature, °C	0	38.8 ± 0.07	38.8 ± 0.07	-	-	39.2 ± 0.10	39.2 ± 0.10
	28[3]	39.9 ± 0.13	40.0 ± 0.13	-	-	40.5 ± 0.10*	40.8 ± 0.10
	56	38.9 ± 0.07†	38.7 ± 0.07	-	-	39.2 ± 0.10	39.2 ± 0.10

[1]Treatments: EX = exercised heifers (n = 12); EC = exercise-control (n = 12), brought to exerciser but not exercised. Differences are specified between pre-exercise or post-exercise values between treatments.

[2]Respiration rates (RR), breaths per minute

[3]There was a greater change in temperature from pre- to post-exercise body temperature in EC compared with EX on d 28.

*$P < 0.05$

†$P < 0.10$

Table 2.15. Least squares means of time (%) spent in body temperature zones 1-3 on fitness test d 0, 28, and 56 during experiment 2.

		Treatment[1]	
Item	Fitness test day	EX	EC
		% time	
Zone 1	0	51.3 ± 6.06	55.7 ± 6.06
	28	2.3 ± 1.01	2.6 ± 1.01
	56	49.2 ± 9.49	52.8 ± 9.48
Zone 2	0	48.8 ± 6.06	44.3 ±6.06
	28	22.05 ± 6.34	10.2 ± 6.34
	56	44.2 ± 9.34	47.2 ± 9.34
Zone 3	0	0.0 ± 4.67	0.0 ± 4.67
	28	75.7 ± 4.67	87.2 ± 4.67†
	56	6.6 ± 4.67	0.0 ± 4.67

[1]Treatments: EX = exercised heifers (n = 12); EC = exercise-control (n = 12), brought to exerciser but not exercised. Differences are specified between pre-exercise or post-exercise values.

Body temperature zone 1 = <39.0°C; zone 2 = 39-40°C; zone 3 = >40°C

†$P < 0.10$

Table 2.16. Least squares means of body temperature during and after exercise during experiment 2.

		Treatment[1]	
Item	Fitness test day	EX	EC
		°C	
Avg. temperature during exercise	0	39.1 ± 0.10	39.1 ± 0.10
	28	40.2 ± 0.10	40.4 ± 0.10
	56	39.1 ± 0.10	39.0 ± 0.10
Avg. temperature hour after exercise	0	39.0 ± 0.10	39.0 ± 0.10
	28[2]	40.5 ± 0.10*	40.8 ± 0.10
	56	39.2 ± 0.10	39.1 ± 0.10

[1]Treatments: EX = exercised heifers (n = 12); EC = exercise-control (n = 12), brought to exerciser but not exercised. Differences are specified between pre-exercise or post-exercise values between treatments.

[2]There was a greater change in temperature from average temperature during exercise to average temperature after exercise for EX compared with EC on d 28.

*$P < 0.05$

Table 2.17. Least squares means of skin temperature of specific regions before and after fitness tests during experiment 2.

		Treatment[1]			
		Pre-fitness test		Post-fitness test	
Item	Fitness test day	EX	EC	EX	EC
		°C			
Cheek	0	35.7 ± 1.4	33.4 ± 1.4	36.6 ± 0.8	35.3 ± 0.8
	28	40.8 ± 0.8	40.1 ± 0.8	42.0 ± 0.6	43.2 ± 0.6
	56	37.7 ± 0.5	38.5 ± 0.5	35.2 ± 0.6	35.1 ± 0.6
Withers	0	34.9 ± 2.2	35.4 ± 2.3	36.8 ± 1.4	35.2 ± 1.4
	28	43.9 ±1.4	42.9 ± 1.4	45.5 ± 1.5	45.8 ± 1.4
	56	37.8 ± 1.2	40.1 ± 1.2	35.6 ± 0.7	36.3 ± 0.7
Thurl	0	36.6 ± 1.6	35.2 ± 1.6	35.7 ±1.3	36.4 ± 1.3
	28	44.0 ± 1.7	43.1 ± 1.7	42.8 ±1.4†	46.4 ± 1.4
	56	38.6 ± 1.6	39.6 ± 1.7	36.3 ±1.4	37.1 ± 1.4
Udder	0	37.5 ± 0.8	35.9 ± 0.8	36.6 ± 0.9	36.9 ± 0.9
	28	40.9 ± 0.8	40.1 ± 0.8	41.4 ± 0.9	41.5 ± 0.9
	56	35.5 ± 0.8	36.6 ± 0.8	36.4 ± 0.9	36.3 ± 0.9
Ear	0	37.5 ± 1.2	35.6 ± 1.2	36.4 ± 1.1	35.4 ± 1.1
	28	41.3 ± 0.9	41.2 ± 0.9	42.6 ± 0.6	43.1 ± 0.5
	56	38.7 ± 0.5	39.3 ± 0.6	34.2 ± 0.7	35.0 ± 0.7

[1]Treatments: EX = exercised heifers (n = 12); EC = exercise-control (n = 12), brought to exerciser but not exercised

†$P < 0.10$; tendencies are between EX and EC within pre- or post-fitness tests

Table 2.18. Least squares means of respiration rate (RR) and rectal temperatures on a series of days post-experiment 2.

		Treatment[1]				
		AM[4]		PM[5]		
Item	Day post-exp 2[3]	EX	EC	EX	EC	Avg. THI[6]
RR, bpm[2]	31	55 ± 4.85	61 ± 5.23	96 ± 5.36	101 ± 6.54	77
	32	42 ± 4.28	41 ± 4.85	91 ± 5.36	84 ± 5.69	79
	33	70 ± 4.28	70 ± 4.53	94 ± 5.36	91 ± 5.69	81
	59	66 ± 3.87	60 ± 4.06	85 ± 4.65	84 ± 4.65	78
	60	64 ± 4.29	62 ± 3.71	85 ± 4.65	83 ± 4.65	80
	61	72 ± 3.71	72 ± 3.71	96 ± 4.65	90 ± 4.65	82
Rectal temp, °C	31	38.7 ± 0.24	38.5 ± 0.27	39.3 ± 0.31	39.1 ± 0.36	77
	32	38.6 ± 0.22	38.4 ± 0.25	39.2 ± 0.31	39.2 ± 0.32	79
	33	39.1 ± 0.22	39.0 ± 0.25	39.4 ± 0.31	39.4 ± 0.32	81
	59	38.8 ± 0.23	38.6 ± 0.23	39.0 ± 0.29	39.0 ± 0.26	78
	60†	38.5 ± 0.29	38.8 ± 0.20	39.2 ± 0.28	39.1 ± 0.26	80
	61	39.1 ± 0.20	39.0 ± 0.18	39.5 ± 0.28	39.4 ± 0.26	82

[1]Treatments: EX = exercised heifers (n = 12); EC = exercise-control (n = 12), brought to exerciser but not exercised.

[2]Respiration rates (RR), breaths per minute

[3]Days post-experiment: The first series of d (31-33), not all heifers had calved; EX (n = 7); EC (n = 6). The 2nd series of days (59-61) all heifers had calved; EX (n = 12); EC (n = 12).

[4]AM = morning hour during cooler environmental temperature (0530-0630).

[5]PM = afternoon hour during hotter environmental temperature (1400-1500).

[6]Avg. THI = Average THI of entire day.

†d17, there was a tendency for EX to have a greater difference in temperature between the morning and afternoon hours than EC.

Table 2.19. Least squares means of parturition and post-calving data in experiment 2.

	Treatment[1]	
Item	EX	EC
Calving ease	1.8 ± 0.24	1.3 ± 0.24
Calf birth weights, kg	38.2 ± 1.43	39.1 ± 1.50
Calving date[2], d	6 ± 2.38	6 ± 1.94

[1]Treatments: EX = exercised heifers (n = 12); EC = exercise-control (n = 12), brought to exerciser but not exercised.

[2]Calving date: difference between predicted calving date and actual calving date; a positive number means that actual calving date was earlier than predicted calving date.

Table 2.20. Least squares means of rates (%) of milk production change from two specific time points during lactation.

	Treatment[1]	
Lactation Persistency[2]	EX	EC
	%	
5 DIM vs. 50 DIM[3]	125.8 ± 1.78	129.8 ± 1.93
5 DIM vs. 100 DIM	113.4 ± 0.89	115.5 ± 0.97
5 DIM vs. 150 DIM	112.9 ± 1.33	114.4 ± 1.33
100 DIM vs. 150 DIM	99.3 ± 0.89	98.2 ± 0.89

[1]Treatments: EX = exercised heifers (n = 12); EC = exercise-control (n = 12), brought to exerciser but not exercised.

[2]Lactation Persistency: the LSM percentages shown are calculated based on a lactation persistency calculation that interprets rate of milk production as a percentage when comparing two different milk production values at specific time points (DIM). Percentages > 100 mean that rate of milk production increased, while percentage < 100 signify a decrease in milk production.

[3]DIM: day in milk

Table 2.21. Least squares means of weekly respiration rates and rectal temperatures during experiment 2.

Item	Week	Treatment[1]	
		EX	EC
Rectal temperature, °C	0	39.1 ± 0.32	39.0 ± 0.32
	1	38.7 ± 0.15	38.8 ± 0.15
	2	38.7 ± 0.24	38.7 ± 0.24
	3	38.6 ± 0.20	38.6 ± 0.20
	4	38.9 ± 0.14	39.0 ± 0.14
	5	38.9 ± 0.17	38.7 ± 0.17
	6	38.8 ± 0.18	38.9 ± 0.18
	7	38.7 ± 0.14	38.9 ± 0.14
Respiration rates, bpm[2]	0	47 ± 2.90	48 ± 2.90
	1	46 ± 2.46	42 ± 2.45
	2	43 ± 2.59	36 ± 2.59†
	3	39 ± 2.03	36 ± 2.03
	4	90 ± 4.35	90 ± 4.35
	5	58 ± 3.22	53 ± 3.22
	6	89 ± 4.15	81 ± 4.15
	7	56 ± 4.22	62 ± 4.22

[1]Treatments: EX = exercised heifers (n = 12); EC = exercise-control (n = 12), brought to exerciser but not exercised.

[2]BPM: breaths per minute

†$P < 0.10$

Discussion

The impact of exercise on milk components and lactation

Our results show that exercise had positive effects on the subsequent lactation and milk quality. In experiment 2, there were increases in both protein and SNF percentage in milk from exercised heifers, which is not surprising since milk protein is one of several components measured in SNF. Increased protein and SNF percentage conflicts with some reports that have found no differences between milk protein and SNF percentage in exercised and non-exercised multiparous cows and 2 year old heifers (Anderson et al., 1979; Lamb et al., 1979). Other studies on exercise in dairy cattle, however, have reported increased milk protein (Lamb et al., 1981; Coulon et al., 1998) and SNF percentage (Lamb et al., 1981) for cows that were exercised compared with those that were not exercised. There were 2 different distances that these heifers and cows were exercised and those that were exercised for shorter distances (1.6 km/d at speeds of 4.0 km/h) had greater effects on milk composition compared with those exercised for longer distances (8.0 km/d at speeds of 4.0 km/h) (Lamb et al., 1981). Our exercise regimens ranged from the 1.81 km/d (shortest workout) during the first week to 4.8 km/d (longest workout) during week 7 in experiment 2. Perhaps the reason we saw increased milk protein percentage is in part related to increased muscle size due to exercise. In theory, exercise should increase muscle size, resulting in a greater amino acid pool being released into circulation from catabolism of skeletal muscles. Even though there was no difference between ECM of treatments, muscle growth and composition in relation to exercise could be an area for future research.

Milk fat percentage was not different in our study though this conflicts with some reports that found decreased fat percentage in the milk of exercised cows (Anderson et al., 1979);

however, these were multiparous cows and they were exercised for distances of up to 9.66 km at speeds of 3.54 km/h. Another study found an increased fat percentage in milk from cows that were exercised (Coulon et al., 1998); however, these cows were exercised in mid-lactation. Similar to our study, Lamb et al. (1981) found no change in fat percentage between exercised and non-exercised cows. Milk fat and protein percentage decrease during bouts of heat stress (Kadzere et al., 2002). When 2 pairs of Jersey cows were exposed to either hot conditions (air temperature: 30°C) or cool conditions (air temperature: 15°C) and fed the same diet amount, the cows exposed to increased temperatures had decreased milk fat and protein percentage that was positively correlated to decreased ruminal pH and acetate (Bandaranayaka and Holmes, 1976). Thus, decreases in milk fat and protein percentage are linked to an acidotic shift in the ruminal environment seen during heat stress.

Interestingly, during the 1st week post-partum, heifers exercised in experiment 2 had greater lactose concentrations than their sedentary peers. Throughout the first 15 wk of lactation, exercised heifers had numerically greater lactose concentrations than exercise-control for a majority of the weeks. Theoretically, this could lead to increased milk production in exercised heifers because of an increase in milk lactose content, though this was not seen in our study. Perhaps there were some lingering effects of exercise and its effect on glucose concentrations that allowed for glucose to be more readily available for mammary gland uptake and utilization for lactose. Glucose concentrations before and after fitness tests were reduced in exercised heifers compared with sedentary counterparts. It has been shown that hypoxia states can induce an increase in gene expression of glucose transporters in the mammary gland in dairy cows, specifically GLUT-1 in early lactation (Mattmiller et al., 2011). It would be reasonable to assume that heifers in our experiment underwent episodes of hypoxia to the mammary gland

during bouts of exercise when blood flow is partitioned to other areas, e.g. working muscle. Perhaps there is a relationship with hypoxia-induced mRNA expression of GLUT transporters and exercise in heifers that may have resulted in increased milk lactose early in lactation, even persisting for the at least three week period between the end of the exercise regimen and parturition.

Exercise did not result in changes in milk urea nitrogen (MUN) overall but during 3 of the 15 lactation weeks exercised heifers had greater MUN concentrations than exercise-control. Generally, lactating dairy cows should have a MUN concentration of 10-14 mg/dL (Dinsmore, 2014). Concentrations in our study are within this range.

There were no treatment differences between any of the milk components or milk production in experiment 1. After experiment 1, heifers used in our experiment were put on nutrition trials that may additionally have impacted milk components and lactation results. This may explain the large discrepancy between both experiments 1 and 2, since experiment 2 heifers were not put on any nutrition trials, thus any milk component changes and lactation differences can more directly be associated with our experiment. Different types of exercise were also implemented between experiments 1 and 2, where experiment 1 implemented a combination of high-intensity intervals and endurance and experiment 2 only used an endurance regimen. In experiment 1, exercise was carried out in the morning when environmental temperatures were cooler, while exercise in experiment 2 was carried out in the afternoon when temperatures were much warmer. It is also important to point out that heifers in experiment 1 began lactating in the fall (October-November) when temperatures in Kansas become cooler, while heifers in experiment 2 began lactating in the summer (July-August), when temperatures are still hotter. Because impacts of heat stress are well documented for milk components it is possible that

assessing the impacts of exercise on lactation during the summer revealed more differences in response to our exercise treatments than our earlier assessments in the fall.

Exercise training on fitness parameters

As seen by others (Davidson and Beede, 2003; 2008) we were able to achieve fitness in pregnant heifers. The increase in duration of fitness test and final speed of EX compared with EC and SC imply that heifers were able to withstand longer bouts of exercise, therefore being classified as "more fit". Davidson and Beede (2003; 2008) carried out similar studies where individual cow performance was assessed based on increased length of exercise time. Interestingly, exercised heifers in our experiment 1 were able to reach speeds of 9.01 km/h (5.6 mph) on d 56, which is greater than other reports of 5.5 km/h for dry cows (Anderson et al., 1976), 5.47 km/h for 2-yr old heifers (Lamb et al., 1979), 5.4 km/h for dry cows (Blake et al., 1982), and 5 km/h for non-pregnant, non-lactating cows (Davidson and Beede, 2003).

Blood variables were also collected to help measure physiological status before and after exercise on d 0, 28, and 56. Blood indicators used to quantify the acid-base status are: pH, pCO_2, HCO_3^-, TCO_2, and BE (Davidson and Beede, 2003). Because of tight regulation over acid-base status, it is difficult to use these parameters as reliable indicators of fitness (Davidson and Beede, 2003). Other studies have reported that pH was not different overall during exercise in cows (Blum et al., 1979; Davidson and Beede, 2003). Before and during exercise, pH remained stable throughout, while post-fitness test samples were slightly reduced during the post-exercise phase (0-75 min post-exercise) (Davidson and Beede, 2008). Another study found pH was decreased in bulls undergoing intense exercise (mean pH = 6.81 ± 0.12; Escalera-Valente et al., 2013). The normal range of pH for dairy cattle is 7.37 to 7.5 (Kahn et al., 2005).

In experiment 1, there were no differences between treatments or fitness test day. In experiment 2, pH remained the same for exercised heifers pre- and post-exercise but was greater for exercise-control heifers when comparing post-exercise samples with their respective pre-exercise samples. This may imply that an overcompensation occurred for maintaining acid-base balance during exercise in heifers that did not undergo exercise training. During exercise, respiration rates should increase and therefore more CO_2 would be expired. This would shift the bicarbonate:CO_2 and cause heifers to start becoming more alkalotic. This coincides with the more basic pH seen in exercise-control heifers compared to exercised heifers after exercise, which were able to maintain a numerically similar pH pre- and post-exercise.

Blood gases in our analyses included pCO_2, pO_2, and total CO_2 (TCO_2). Total CO_2 measures all forms of CO_2 including CO_2, HCO_3^-, carbonate anions (CO_3), and carbonic acid (H_2CO_3) (Abbott Point of Care, 2013). Bicarbonate stabilizes the acid-base balance by keeping the blood from becoming too acidic, while CO_2 balances the blood from becoming too basic. Total CO_2 yielded no differences between treatments in experiments 1 and 2; however, in experiment 2, TCO_2 was reduced post-fitness tests. Increased amounts of expelled CO_2 are because of increased respiration rates during exercise, which would explain the decrease of both TCO_2 and pCO_2 recorded in post-fitness tests.

Decreased heart rates are seen in individuals that exercise to the point of improved fitness because of improved cardiovascular function. Heifers in the exercise-control treatment would in theory have increased heart rates during fitness tests, while exercised heifers would have improved cardiovascular and respiratory function, such as improved pulmonary diffusion. We did not see any differences in our experiments; however, we only collected one pre-exercise and one post-exercise HR measurement. In a study conducted by Davidson and Beede (2003), HR

was measured every 3 minutes and found to be reduced after 60 days of exercise compared with their non-exercised counterparts.

Although no differences were detected in pre- and post-fitness test lactate samples in our studies, other studies have found that plasma lactate increases during exercise in cattle (Blum et al., 1979; Lotgering et al., 1983; Kuhlmann et al., 1985; Escalera-Valente et al., 2013; Davidson and Beede, 2003; 2008) and returned to basal levels approximately 15 minutes after exercise in pregnant cows (Davidson and Beede, 2008). In our experiment, however, we only collected 1 sample before exercise and 1 after exercise, while other studies collected serial samples every 3 minutes. In experiment 2, fitness tests were not most likely not intense enough to approach lactate threshold and thus our results are not surprising. Reduced intensity of exercise would likely utilize aerobic metabolism over anaerobic glycolysis, and thus increased blood lactate in post-exercise samples were unlikely to be elevated above that in pre-exercise samples. In experiment 1, however, fitness tests were based on individual maximal effort, thus we expected increases in blood lactate from those samples taken prior to exercise. However, we found that mean lactate concentrations were decreased between pre- and post-fitness tests. We did not take additional blood samples following exercise except for 1 sample immediately after exercise (collected within approximately 10 min of fitness test completion). Other results have found that changes in lactate concentrations were approximately 3.00 mmol/L when comparing pre- and post-exercise values, and lactate concentrations were close to basal concentrations within 10 minutes after exercise (Davidson and Beede, 2003). Because of the rapid clearance of lactate from blood, it is likely that our 1 blood sample collection post-exercise was not immediate enough to illustrate a possible lactate increase from exercise. Though unknown in

many animal species, it is assumed that lactate clears the blood at a similar rate as seen in humans, which is approximately 20 minutes (Tennent-Brown, 2012).

Base excess is defined as the "amount of base needed to return the pH to 7.40" (Abaxis, 2010). Base excess levels were less for exercised heifers compared with exercise-control on the final day (d 56) post-fitness test. Overall, base excess was reduced post-exercise compared with pre-exercise. This coincides with other reports that found decreased levels of base excess because of exercise (Blake et al., 1982; Escalera-Valente et al., 2013). In our 2nd experiment, base excess was reduced on d 0 demonstrating a greater deficit of base (bicarbonate) after exercise. This coincides with the numerically reduced pH seen on d 0 compared with d 28 and 56. After exercise on d 28 and 56, base excess after exercise was numerically reduced for both exercised and exercise-control heifers. When combining the results of pH and base excess, exercise-control heifers were slightly more alkalotic than their exercised counterparts after exercise.

Hematocrit and hemoglobin have been assessed as possible indicators of oxygen-carrying capacity in the blood after exercise (Davidson and Beede, 2003). In our studies, we found that undergoing an 8-wk exercise regimen caused heifers to have increased hematocrit after performing a fitness test, as compared to non-exercised heifers. Exercised heifers had greater hematocrit than exercise-control on d 56 after exercise in experiment 2, while there was no change between treatments on any day for hemoglobin in experiments 1 and 2. There have been other reports of increased hematocrit that increases with exercise intensity (Kuhlmann et al., 1985). In another study, increased hematocrit were present during exercise but returned within approximately 12 minutes to pre-exercise levels (Davidson and Beede, 2003). In another study, increased hemoglobin concentrations were found in response to exercise, but the authors could

offer no link between whether a specific intensity of exercise or duration of exercise affected the magnitude of hemoglobin concentration changes (Arave et al., 1978). Similarly, Blake et al. (1982) reported that hemoglobin increased with exercise, although by the end of the 8-wk trial increases were not as profound. Another study found that pregnancy status affected the concentration of hemoglobin and hematocrit during exercise (Davidson and Beede, 2008). While there was no effect of exercise training on hemoglobin and hematocrit, these investigators found that increases were reduced by about 10% when comparing concentrations of pregnant cows versus non-pregnant cows. These changes were attributed to gestational status on differences in plasma volume.

Glucose concentrations were not different between exercised, exercise-control, and non-exercised heifers on any fitness test day in experiment 1. In experiment 2, exercised heifers had reduced glucose concentrations compared with exercise-control on d 28 after exercise. All post-exercise samples were numerically greater for both exercised and exercise-control heifers in both experiments. Other studies reported minor changes in glucose concentrations throughout exercise but glucose was reduced in exercise treatments in steers (Blum et al., 1979). In another study, glucose was found to be increased in arterial blood in pregnant ewes and increased with exercise intensity (Lotgering et al., 1983). Intuitively, it would be assumed that glucose concentrations would be elevated in response to increased sympathetic tone due to exercise. The sympathetic system releases glucocorticoids (cortisol) and catecholamines (primarily norephinephrine in cattle) which stimulate glycogen break down via glycogenolysis and gluconeogenesis to release glucose into the blood to be taken up by working muscles. The greater glucose concentrations in exercise-control heifers post-exercise may imply a less efficient glucose uptake by muscle, since exercise increases efficiency of energy uptake.

The majority of blood variables in these studies had significant time effects for d 0, 28, and 56 in experiments 1 and 2. Possible reasons for inconsistent blood results may be because of the narrow time frame (10-15 minutes) to analyze the blood immediately after collection and with only 1 machine some samples could not be analyzed within the recommended time frame as well as post-exercise samples collected within 10 minutes.

Blood was also collected to analyze cortisol response one hot day during wk 7 of experiment 2, comparing concentrations of both exercised and exercise-control heifers during AM and PM hours. There were no differences between treatments in response to the hot environment; however, cortisol concentrations were numerically greater in exercise-control heifers even when assessed in only a single snapshot from one sample day. Cortisol response increases to stressors such as exercise (Blum et al., 1979; Kuhlmann et al., 1985) and heat stress (Farooq et al., 2010). The initial response to a stressor is a neural stimulation of the adrenal medulla to release catecholamines. These are the quick-response hormones that act on areas such as the vascular system (vasodilation/vasoconstriction) for a fast response and also act on the brain, specifically the hypothalamus, to stimulate ACTH and subsequent glucocorticoid release. Cortisol is released through a neural pathway where CRH is released from the hypothalamus and stimulates the pituitary to release ACTH, which acts on the adrenal cortex to release cortisol. Cortisol is controlled by a negative feedback loop, where increased concentrations of cortisol decrease hypothalamic and pituitary gland release of CRH and ACTH, respectively. Release of cortisol stimulates glycogenolysis in order to release glucose into the blood for energy use. Though we found no significant effect of exercise on cortisol response in the heat, more sampling would better quantify cortisol response.

Anti-diuretic hormone (vasopressin) increases renal reabsorption of water and Na^+, which increases blood volume and therefore blood pressure as an allostatic mechanism to help improve performance in both distress (fighting or fleeing) and eustress (exercise). Because of increased water losses from both the respiratory tract (increased panting) and skin (sweat), ADH concentrations increase in response to stress to aid in the body's conservation of water (Farooq et al., 2010). Blood samples were compared for ADH concentrations in the cool hours (AM: morning) and hot hours (PM: afternoon) of exercised and exercise-control heifers. There were no differences between exercised and exercise-control heifers in our study, however, other investigators have reported that ADH increases in response to stress (Farooq et al., 2010). As with our assessment of cortisol, taking only a single snapshot of ADH concentrations in the morning versus afternoon of a hot day was likely not adequate to reveal any differences in response to exercise training.

Exercise and thermoregulation based on body temperatures

Although EX and EC heifers had erratic temperatures in experiment 1, the combination of body temperature and skin temperature in experiment 2 suggest a possible relationship with exercise and improved thermal regulation. According to the Merck Veterinary Manual, the normal range of rectal temperatures in dairy cows is 38.0°C -39.3°C (100.4°F-102.8°F) (Robertshaw, 2004). Burfeind et al. (2012) defined fever as body temperatures ranging from 39.4°C - 39.7°C.

As the exercise experiment progressed, sedentary heifers spent less time in cooler body temperature zones during cooler environmental temperatures. Based on this response, exercised heifers seemed to regulate body temperature more efficiently when environmental temperatures

were reduced. Time spent in body temperature zones during the time period between 1300 and 0000 h were also studied because of the circadian rhythms of body temperature during hot months. As the environmental temperature increases, body temperature regulation begins to be less effective during the afternoon and evening hours. Sometimes elevated body temperatures (>39.0°C) are still seen even in the night/early morning hours even after environmental temperature are reduced. This pattern was also observed in our experiments.

Interestingly, the majority of body temperature changes occurred during the 6th week of experiment 1. According to Geor and McCutcheon (1998), horses have improved thermoregulation as a result of exercise in hot, humid environments by 2 wk of training. We did not see similar results in terms of the effectiveness of exercise, although our experiments were not designed specifically to determine the time frame for thermoregulation improvements in response to exercise. Based on experiment 1, we do not see improved body temperature to the environment by wk 2. According to Nielsen et al. (1998), in order to keep physiological acclimations incurred by hot environments, it is important to remain exposed to the heat. In human studies, it is recommended that individuals remain exposed to hot temperatures at least every 2-3 days to maintain adaptive gains in thermo-tolerance (Nielsen et al., 1998). Because ambient temperatures could not be controlled, heifers were exposed to days of cooler temperatures during experiment 1. Inconsistent ambient temperatures may have prevented heifers from building up a level of thermoregulatory acclimation to exercise.

Because of variable vaginal temperatures recorded in experiment 1, skin temperature data were collected in experiment 2 to better assess effects of exercise on thermo-tolerance. Cows are able to dissipate heat using 4 different routes of heat exchange including: convection, conduction, radiation, and evaporation (Collier et al., 2006; Farooq et al., 2010). When skin

temperatures are still below 35°C, cows are able to efficiently lose heat via all 4 routes of heat exchange (Collier et al., 2006). Once above this threshold, however, heat can only be lost by evaporation. Exercised heifers had reduced skin temperatures in the thurl region, which may be an indication that heat is brought to the skin via vasodilation in peripheral tissues more efficiently and is more rapidly dissipated compared with exercise-control heifers. Only a week after starting an exercise program, statistical differences were seen in thurl skin temperatures, whereas the other regions had numerically reduced skin temperatures.

Variables related to animal well-being, parturition, and postpartum health

Importantly, exercise had no negative impacts on locomotion, parturition, metabolic disorders, or observational signs of stress in the parlor. Also, any effects of exercise on thermal regulation were not apparent at 1 month post-experiment. A report found that exercise improved calving ease scores (Lamb et al., 1979). In this experiment, however, animals were exercised until parturition, while animals in our study were only exercised up until 3 wk prepartum. Perhaps in our studies, any potential influence of exercise on calving ease was lost because animals were not exercised until parturition.

In experiment 1, we assessed observational cues indicative of stress between treatments in the first 3 visits to the parlor for milking and no differences were found between exercised, handled but not exercised or sedentary heifers. At a minimum, there was a difference of 3 wk from the end of exercise to the beginning of lactation. Unfortunately, there was a wide range of calving dates of approximately 2 months between all heifers with the average day of parturition being 26 days post-experiment 1. This lag period between exercise and calving may have

reduced the likelihood of seeing any positive impacts of exercise or handling on observational stressors of heifers at calving.

There was a significant time effect for weekly body weights collected throughout the experiment in both experiments 1 and 2. This is to be expected since the heifers were pregnant late in gestation and gained weight as they came closer to parturition. In experiment 1, there was tendency for a treatment effect while in experiment 2 there was no treatment effect. There were 2 different modes of exercise used for each study. Experiment 1 utilized a combination of high-intensity intervals (sprints; speeds $\geq$ 6.44 km/h) and endurance, while experiment 2 only incorporated an endurance regimen. Perhaps the increased intensity of the first experiment may have affected the body weight of exercised heifers more than the endurance regimen implemented in experiment 2. Another reason that sedentary heifers in experiment 1 had greater body weight could be because during the time that exercised and exercise-control heifers were brought to the exerciser in the morning, the pen in which all heifers were housed received feed and the sedentary heifers could begin consuming feed without additional competition from heifers at the exerciser.

In experiment 1, weekly locomotion scores were obtained and found to not have any treatment effects between exercised and exercise-control heifers. Some reports have concluded that some form of physical activity is beneficial, especially for cows housed in confinement (Blake et al., 1982). Our studies demonstrate that increasing activity did not worsen the heifers' locomotive ability. This adds to the literature that providing heifers with some form of exercise, instead of remaining sedentary within confined housing, is not detrimental to their subsequent lactation performance as is believed by many dairy producers.

Conclusion

Heifers became more fit following an interval exercise regimen combining both high-intensity intervals and endurance based on the increased amount of time spent exercising during fitness tests. Less time spent in increased body temperature zones as well as reduced skin temperatures provide evidence for improved thermal regulation in response to exercise. Based on these findings, a positive relationship between attaining fitness and thermoregulation seems to exist in pregnant Holstein heifers. No residual negative effects of exercise were detected on measures of locomotion, parturition, metabolism, or milk production. Milk protein and SNF percentage were increased in exercised heifers compared with the non-exercised controls, though when ECM was analyzed there were no differences between treatments.

Future research

More focused studies to elicit heat acclimation in response to exercise should collect data by increasing the frequency of blood sampling and simultaneous core body temperature. As our experiments had smaller sampling size and no serial blood collections, an increase in experimental units (heifers) as well as increased blood collections, especially during exercise, may add more credibility to our hypothesis that exercise improves thermo-tolerance.

While research in cattle exercise has quantified fitness by observing increased amounts of time spent exercising and decreased heart rates during resting, there is another method that can better measure levels of fitness. Lactate threshold is a common tool used in humans to quantify fitness. While exercise research in cattle has included lactate as a parameter of fitness, it was not collected in such a way that was indicative of a lactate threshold test. If fitness can be quantified

in cattle based on lactate threshold, then levels of exercise and whether or not fitness improved can be properly assessed.

Another area of research that can be looked into based on our results is related to muscle adjustments in response to exercise and the effect of exercise on glucose uptake. As mentioned in the literature review, muscle fiber types change in response to exercise and one benefit to those changes is improved glucose uptake. Muscle biopsies could be collected to assess muscle fiber type changes throughout the experiment. This would establish whether or not glucose uptake in the muscle can be enhanced by exercise, and if so does an increase of glucose uptake by the muscle affect the amount of glucose taken up by the mammary gland during lactation? In our studies, lactose production was greater in exercised animals during the first week of lactation. If this was a mitigated effect of exercise as explained in the discussion, another study could continue exercising heifers right before calving, and subsequent mammary biopsies could be collected to identify the type and number of GLUT transporters present on mammary epithelial cells. Assessing GLUT transporters in the mammary gland and skeletal muscles may aid in quantifying the ability of each tissue to accrue glucose, and whether exercise induced increased expression during late gestation and early lactation.

Because increased milk protein percentage was also observed in our exercised heifers, studies should collect proteolytic enzymes or muscle breakdown byproducts, such as creatinine or 3-methylhistidine, to quantify rates of protein catabolism. In theory, muscle size may increase in response to exercise increasing muscle turnover rate and thus undergoing protein catabolism.

A final study could look at skin temperature and whether differences seen are because of improved cooling or poor thermoregulation due to reduced blood flow to the skin during heat stress. It is generally concluded that decreased skin temperatures are indicative of a well-

functioning thermoregulatory system; however, what if the blood flow to skin is not sufficiently carrying heat to be released via radiation or evaporation? If the latter were the case, this would mean that body temperature would be greater and skin temperature would be less because of a poor functioning "active vasodilator" system. An initial attempt to quantify this would be to collect simultaneous, consecutive vaginal temperatures and skin temperatures to determine if decreased skin temperatures are correlated with decreased core body temperatures.

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Appendix A - Exercise protocols for experiments 1 and 2

Two different exercise regimens were utilized in experiments 1 and 2. Experiment incorporated a combined aerobic endurance and high-intensity interval regimen, while experiment 2 followed an aerobic endurance regimen only.

Exercise regimen in experiment 1

Table 2.22. Exercise regimens during wk 1 of experiment 1.

Day of experiment		Time, min	Speed, km/h
1	Warm-Up	5	4.02
	Endurance	10	5.15
	Pre-intervals	5	4.35
	High-intensity (x4)	1	6.44
	Low-intensity (x3)	1	4.35
	Cool down	2	4.35
	Cool down	3	3.86
4	Warm-Up	5	4.02
	Endurance	10	4.83
	Pre-intervals	5	4.02
	High-intensity (x4)	0.1	7.24
	Low-intensity (x4)	1.9	4.83
	Cool down	5	3.54
5	Warm-Up	5	4.02
	Endurance	10	5.15
	Endurance	5	4.35
	Endurance	10	5.63
	Cool down	5	3.54
6	Low-intensity	15	3.54
7	Warm-Up	5	3.54
	High-Endurance	10	4.83

	Low-Endurance	3	4.02
	High-Endurance	10	5.15
	Low-Endurance	3	4.02
	High-Endurance	10	5.47
	Cool down	5	3.54

Table 2.23. Exercise regimens during wk 2 of experiment 1.

Day of experiment		Time, min	Speed, km/h
10	Warm-Up	5	3.54
	Endurance	10	4.02
	High-intensity (x5)	1	6.44
	Low-intensity (x4)	1	4.02
	Cool down	5	3.54
11	Warm-Up	5	4.02
	High-Endurance (x2)	10	5.63
	Low-Endurance (x2)	3	4.51
	Cool down	5	3.54
12	Warm-Up	5	4.02
	Endurance	10	5.15
	Pre-interval	5	4.35
	High-intensity (x4)	3	6.44
	Low-intensity (x3)	2	4.02
	Cool down	5	3.54
13	Low-intensity	15	3.54
14	Warm-Up	5	4.02
	Pre-interval	5	5.15
	Pre-interval	3	4.02
	High-intensity (x10)	1	7.24
	Low-intensity (x9)	1	4.02
	Cool down	5	3.54

Table 2.24. Exercise regimens during wk 3 of experiment 1.

Day of experiment		Time, min	Speed, km/h
17	Warm-Up	5	4.02
	Endurance	20	4.51
	Low-Endurance	3	3.54
	High-Endurance	10	4.67
	Cool down	5	3.54
18	Warm-Up	5	4.02
	Endurance	10	5.63
	Pre-interval	5	4.02
	Low-intensity (x4)	1.8	5.15
	High-intensity (x4)	0.2	8.37
	Cool down	5	3.54
19	Endurance	30	4.67
	Cool down	5	3.54
20	Warm-Up	5	4.02
	Endurance	25	4.67
	Cool down	5	3.54

Table 2.25. Exercise regimens during wk 4 of experiment 1.

Day of experiment		Time, min	Speed, km/h
24	Warm-Up	5	3.54
	Endurance	30	4.02
	Cool down	5	3.54
25	Warm-up	5	3.54
	Endurance	10	4.83
	Pre-interval	5	4.02
	High-intensity (x4)	0.1	7.24
	Low-intensity (x4)	1.9	4.83
	Cool down	5	3.54
26	Warm-up	5	4.02
	Endurance	15	4.02

27-29	Fitness test day	--	--

Table 2.26. Exercise regimens during wk 5 of experiment 1.

Day of experiment		Time, min	Speed, km/h
31	Warm-Up	5	4.02
	Endurance	10	5.15
	Pre-interval	5	4.51
	Low-intensity (x4)[1]	2	5.15
	High-intensity (x4)	0.5	6.44
	Cool down	5	3.54
32	Warm-Up	5	4.02
	Endurance[2]	10	5.15
33	Warm-Up	5	3.54
	Endurance	10	5.15
	Pre-interval	5	4.35
	High-intensity (x4)	1	6.44
	Low-intensity (x3)	1	4.51
	Cool down	5	3.54
34	Warm-Up	3	2.90
	Endurance	12	3.54

[1]Intensity intervals (both high and low) were interchanged back and forth for number of times indicated

[2]Exercise was stopped that day because of inclement weather (lightning)

Table 2.27. Exercise regimens during wk 6 of experiment 1.

Day of experiment		Time, min	Speed, km/h
38	Warm-Up	5	4.02
	High-Endurance	10	5.15
	Low-Endurance	5	4.35
	High-Endurance	10	5.95
	Cool down	5	3.54
39	Warm-Up	5	4.02

	Endurance	5	5.63
	Pre-interval	3	4.51
	High-intensity (x8)	1	7.24
	Low-intensity (x8)	1	4.02
	Cool down	5	3.22
40	Warm-Up	5	4.02
	Pre-interval	5	5.63
	Low-end. (x2)	3	4.51
	High-end. (x2)	10	6.44
	Cool down	5	3.54
41	Low-intensity	15	4.02
42	Warm-Up	5	4.02
	High-Endurance	10	6.44
	Low-endurance	3	4.51
	High-endurance	10	6.12
	Low-endurance	3	4.51
	Cool down	5	3.54

Table 2.28. Exercise regimens during wk 7 of experiment 1.

Day of experiment		Time, min	Speed, km/h
45	Warm-Up	5	4.02
	Endurance	30	4.51
	Cool down	5	4.02
46	Warm-Up	5	3.54
	Endurance	20	4.02
	Pre-interval	5	3.54
	Low-intensity (x4)	1.8	5.63
	High-intensity (x4)	0.2	8.37
	Cool down	5	3.54
47	Warm-Up	1.5	4.02
	Endurance	30	5.47
	Cool down	5	4.02

48	Low-intensity	15	4.02
49	Warm-Up	5	4.02
	Endurance	30	4.83
	Cool down	5	4.02

Table 2.29. Exercise regimens during wk 8 of experiment 1.

Day of experiment		Time, min	Speed, km/h
50[1]	Fitness test day	--	--
52	Warm-Up	5	4.02
	High-endurance	15	5.31
	Low-endurance	3	4.51
	High-endurance	15	5.31
	Cool down	5	4.02
53	Warm-Up	5	4.02
		25	5.31
		5	4.02
54	Warm-Up	5	4.02
	Endurance	30	4.83
	Cool down	5	4.02
55-57	Fitness test day	--	--

[1]Five heifers needed to be removed from study early because they were 3 wk from predicted calving date at this point and following IACUC protocol were to be removed from study

Exercise regimen in experiment 2

Table 2.30. Exercise regimen during wk 1 of experiment 2.

	Time, min	Speed, km/h
Warm-Up	5	4.02
Endurance	15	4.82
Cool Down	5	3.22

Table 2.31. Exercise regimen during wk 2 of experiment 2.

	Time, min	Speed, km/h
Warm-Up	5	4.02
Endurance	20	4.82
Cool Down	5	3.22

Table 2.32. Exercise regimen during wk 3 of experiment 2.

	Time, min	Speed, km/h
Warm-Up	5	4.02
Endurance	30	4.82
Cool Down	5	3.22

Table 2.33. Exercise regimen during wk 4 and 5 of experiment 2.

	Time, min	Speed, km/h
Warm-Up	5	4.02
Endurance	1	5.63
Cool Down	5	3.22

Table 2.34. Exercise regimen during wk 6 of experiment 2.

	Time, min	Speed, km/h
Warm-Up	5	4.02
Endurance	30	5.63
Cool Down	5	3.22

Table 2.35. Exercise regimen during wk 7 of experiment 2.

	Time, min	Speed, km/h
Warm-Up	5	4.02
Endurance	45	5.63
Cool Down	5	3.22

Table 2.36. Exercise regimen during wk 8 of experiment 2.

	Time, min	Speed, km/h
Warm-Up	5	4.02
Endurance	10	5.63
Cool Down	5	3.22

Appendix B - The impact of "regrades" on student learning in Anatomy and Physiology

The Impact of Corrected Exams (Regrades) on Final Exam Score

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The opportunity to immediately correct mistakes on examinations has the potential to allow students in upper level applied science courses to build a more solid foundation for future exams. The objective of this study was to analyze the impact of percentage back from regrades, and its effect on final exam score. To meet this objective, regrade percentages of exams 1, 2, and 3 and final exam score were analyzed using analysis of variance, regression, correlation, and scatterplots (n=377). Data has been compiled from 5 semesters, beginning in the spring of 2010. Exam 1 regrades had a positive correlation with final exam score. The p-value in the regression analysis was 0.0615, implying that there is a strong tendency for exam 1 regrades to impact final exam score. From our results, it appears that doing regrades on the first exam will have an effect on the final exam score. This may be because the first exam covers many of the foundational concepts of the course, suggesting it is imperative that students obtain a strong understanding in the beginning of the semester to ensure a better comprehension of the more complicated class material that will ensue.