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Heat stress-induced deficits in growth, metabolic efficiency, and cardiovascular function coincided with chronic systemic inflammation and hypercatecholaminemia in ractopamine-supplemented feedlot lambs; Atypical cyclicity at puberty in beef cows is associated with early deficits in muscling, metabolic indicators, and myoblast function in offspring but does not impact feedlot performance.

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by

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Heat stress-induced deficits in growth, metabolic efficiency, and cardiovascular function coincided with chronic systemic inflammation and hypercatecholaminemia in ractopamine-supplemented feedlot lambs; Atypical cyclicity at puberty in beef cows is associated with early deficits in muscling, metabolic indicators, and myoblast function in offspring but does not impact feedlot performance.

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University of Nebraska, 2020

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Our 1st study evaluated the effects of β-adrenergic agonist (ractopamine) supplementation on growth, health, and wellbeing in heat-stressed feedlot wethers. Chronic heat stress impaired growth, metabolism, and wellbeing even when the impact of reduced feed intake was eliminated by pair-feeding. We observed systemic inflammation and hypercatecholaminemia that were likely mediators of these deficits. Moreover, ractopamine did not diminish any wellbeing indicators and improved muscle growth without worsening the effects of heat stress.

Our 2nd study assessed the effects of pubertal cyclicity (weaning to 1st breeding) in cows on myoblast-mediated muscle growth, metabolism, and growth efficiency of their offspring. Maternal fertility and postnatal growth efficiency of offspring are imperative to successful beef cattle production. A maternal condition was previously identified in which cows exhibited irregular cyclicity patterns or were non-cyclic between weaning and 1st breeding. This condition is thought to be associated with high concentrations of androstenedione in their follicular fluid, which causes cows to be sub-fertile but also wean heavier calves when they do become pregnant. We hypothesized that

calves from cows exhibiting irregular pubertal cyclicity would have enhanced growth efficiency compared to calves from cows with normal pubertal cyclicity. To test this hypothesis, we evaluated myoblast function, growth, and metabolism pre-weaning, as well as growth efficiency in the feedlot and carcass characteristics at harvest in calves from cows that were previously identified as having typical, start-stop, or non-cycling pubertal cyclicity patterns. Calves from irregular cycling cows had reductions in insulin sensitivity, plasma proteins and lipids paired with increased myoblast function and reduced myoblast glycolytic capacity. Data independently and combined suggest calves from cows with irregular cyclicity from weaning to 1st breeding have chronic inflammation. While the specific mechanism of inflammation is unknown, further research may allow mediators of abnormal cyclicity and offspring inflammation-induced dysfunction to be elucidated.

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LITERATURE REVIEW

INTRODUCTION

Continued increases in growth efficiency of beef cattle will help to secure the global supply of high-quality protein for the growing population while also increasing environmental sustainability. The use of growth-enhancing supplements including β-adrenergic agonists (βAA) and steroid implants are imperative to increasing beef cattle growth efficiency (Hahn, 1999a; Nienaber and Hahn, 2007; Capper and Hayes, 2012). However, it is critical that the use of growth-enhancing supplements does not increase the risk to animal health and well-being. The effects of βAA on growth are well-documented (Moloney et al., 1990; Beermann, 2002), but they could have detrimental effects on health and well-being, as they stimulate β adrenergic pathways that are part of the canonical stress response. Furthermore, it is well-documented that heat stress has detrimental effects on growth, health, and well-being in livestock (Barnes et al., 2019a). However, it remains unclear if βAA supplementation worsen the effects of heat stress. Understanding the potential interacting effects of βAA and heat stress on growth, health, and well-being is imperative to sustainable beef production and is thus among the primary objectives of our research.

Myoblast function is the rate-limiting step in skeletal muscle growth, and thus proper myoblast function is imperative to growth efficiency in beef cattle and other livestock. Androgenic steroid hormones have been shown to increase growth efficiency (Capper and Hayes, 2012) by increasing myogenesis (Singh et al., 2003), but the androgenic effects of one specific steroid, androstenedione, have not been detailed. The

effects on muscle growth in females is particularly unclear. In general, however, hyperandrogenism in women has been reported to have detrimental health effects, including increasing the risk for obesity, type 2 diabetes, cardiovascular dysfunction, and infertility (Azziz et al., 2009). Like growth efficiency, reproductive soundness is necessary for efficient and profitable beef production, and thus understanding the effects of high concentrations of androstenedione in follicular fluid of beef cows and their offspring is important. Thus, our objective was to identify the effects of high androstenedione in follicular fluid of cows on offspring muscle growth and metabolic efficiency. In this literature review, I begin by discussing the dynamics of skeletal muscle growth (Section II), which is a primary output for beef production and a focal point of the research portions of this thesis. I include a brief overview of the distinct phases of myogenesis as well as the factors responsible for myogenic regulation. In Section III, I review skeletal muscle glucose metabolism and the role it plays in glucose homeostasis, which is the most primary substrate involved in skeletal muscle metabolism and growth. In section IV, I review the physiology of androstenedione and the pathophysiology associated with high concentrations of androstenedione. In section V, I review the use of β-adrenergic agonists as skeletal muscle growth stimulants and their potential effects on animal health and well-being. In section VI, I conclude by reviewing the effects of heat stress on livestock and its impacts on production efficiency.

Stages & Regulation of Muscle Growth

Skeletal muscle growth occurs in several stages, beginning with proliferation of the myogenic precursor cells that give rise to muscle stem cells called myoblasts during

embryonic development (Picard et al., 2002). During muscle growth, myoblasts proliferate to maintain or even increase their population size. Most then exit the cell cycle and differentiate before fusing to form multinucleated myotubes, but a small subset become quiescent and are stored as satellite cells to maintain a source of future myoblasts (Rehfeldt et al., 2000). In the bovine, myoblasts 1st arise in the fetus by 30 days post-conception and differentiate/fuse into myotubes by 180 days post-conception (Chaze et al., 2008). These initial myotubes mature to become the primary skeletal muscle fibers (Rehfeldt et al., 2000). A 2nd myoblast wave will begin proliferating around 90 days post-conception and differentiate/fuse into secondary muscle fibers (Chaze et al., 2008), which are smaller in diameter and form around the primary fibers (Rehfeldt et al., 2000). As fibers form, satellite cells are embedded in the basal lamina surrounding the fibers, where they will remain in quiescence until needed for future skeletal muscle hypertrophy (Rehfeldt et al., 2000). In most non-litter bearing mammals, myofiber number is static by the early 3rd trimester (Chaze et al., 2008). Over the 3rd trimester and early neonatal stages, myosin heavy chain isoforms associated with specific fiber types (i.e. fast-twitch vs. slow-twitch, oxidative vs. glycolytic, red vs. white) become present as myotubes mature into adult fibers (Chaze et al., 2008) by synthesizing proteins specific to muscle cells (Allen et al., 1979). After birth, prenatal myosin isoforms slowly disappear and muscle properties exhibit substantially less developmental plasticity (Chaze et al., 2008).

Skeletal muscle development is associated with and in some cases controlled by expression patterns of transcription factors known as myogenic regulatory factors (MRFs) (Muroya et al., 2002). Thus, these proteins can be used as biomarkers for specific stages of myoblast function. Paired box 7 (Pax7) is unique to the myogenic precursor

cells (i.e. myoblasts and satellite cells) that will ultimately donate their nuclei to myofibers for hypertrophic growth (Kuang et al., 2006). Pax7 is imperative in satellite cell expansion and may also have a role in satellite cell self-renewal and maintenance (Günther et al., 2013). Myogenic factor 5 (Myf5) is expressed in satellite cells, during activation, and proliferation (Muroya et al., 2002). Furthermore, Myf5 is believed to activate skeletal muscle satellite cell self-renewal (Kuang et al., 2007). Myoblast determination protein 1 (MyoD) is lowly expressed compared to Myf5 at the start of proliferation but transitions into greater expression as proliferation proceeds. Functionally, MyoD is the primary driver of differentiation (Muroya et al., 2002). Myogenin expression begins to increase in response to MyoD initiating the differentiation process (Muroya et al., 2002). MyoD expression will decrease as differentiation progresses into the terminal phase, and myogenin expression remains high throughout terminal differentiation and fusion (Muroya et al., 2002). With skeletal muscle fiber numbers determined before birth, postnatal growth is facilitated by muscle fiber hypertrophy (Rehfeldt et al., 2000). This is dependent on the continued activation of myoblasts and their ability to proliferate, differentiate, and effectively donate their nuclei to existing muscle fibers via fusion (Rehfeldt et al., 2000). Increased nuclei accumulation in skeletal muscle fibers increases the capacity for protein synthesis, resulting in greater skeletal muscle hypertrophic growth (Bruusgaard et al., 2010).

In addition to its more canonical role in locomotion, skeletal muscle plays an essential role in whole-body protein homeostasis (Wolfe, 2006). In the post-absorptive state, skeletal muscle releases amino acids into the bloodstream to be utilized by other tissues (Biolo et al., 1995; Wolfe, 2006). Conversely, dietary amino acid intake

replenishes muscle protein to compensate for the loss during the post-absorptive state (Wolfe, 2006). Thus, amino acid influx promotes protein synthesis in order to maintain the balance of protein flux, as protein is constantly broken down (Wolfe, 2006). Proper maintenance of protein cycling is imperative for the essential functions of brain, heart, liver, and other tissues (Wolfe, 2006).

Muscle Glucose Metabolism & Its Role in Glucose Homeostasis

Skeletal muscle is a key facilitator of healthy glucose homeostasis. Skeletal muscle is the most abundant insulin-sensitive mammalian tissue, accounting for 85% of insulin-stimulated glucose clearance (Carnagarin et al., 2015). Glucose homeostasis is maintained in large part through precise regulation of muscle tissues by insulin and of adipose and liver tissues by glucagon (Karlsson and Zierath, 2007). Hyperglycemia following spikes in glucose absorption is the primary stimulus for the secretion of insulin by the β-cells within pancreatic islets (Karlsson and Zierath, 2007). Insulin promotes the clearance of glucose from circulation by stimulating its uptake predominantly into skeletal muscle but also into liver and other soft tissues. It simultaneously suppresses hepatic glucose production (Karlsson and Zierath, 2007). The insulin signaling pathways (Figure 1) contribute to regulation of growth and metabolism in skeletal muscle (Carnagarin et al., 2015). Insulin binds to its tyrosine kinase receptor on the plasma membrane of skeletal muscle, activating a signaling cascade that ultimately results in the translocation of the insulin-sensitive intracellular glucose transporter GLUT 4 to the plasma membrane, thus facilitating glucose diffusion into the cell (Karlsson and Zierath, 2007). Conversely, when hypoglycemia occurs, glucagon is released from the α-cells

within pancreatic islets to stimulate hepatic glucose production, increasing glucose in circulation (Karlsson and Zierath, 2007).

Once within the cell, glucose will either be stored as glycogen or metabolized for energy. Metabolism of glucose begins with glycolysis and then proceeds to either oxidative or non-oxidative metabolism (Karlsson and Zierath, 2007; DeFronzo and Tripathy, 2009). Glycolytic processes produce pyruvic acid under aerobic conditions and produce lactate under anaerobic conditions. Pyruvic acid is further metabolized by the stepwise pathways of the citric acid cycle and electron transport chain of the mitochondria. This oxidative process consumes O_2 but produces high quantities of energy in the form of ATP (Karlsson and Zierath, 2007; DeFronzo and Tripathy, 2009). Conversely, lactate is either transferred from skeletal muscle cells via blood to the liver for glucose synthesis or converted back to pyruvate to yield NAD^+ (Voet et al., 2013).

Physiology & Pathophysiology of Androstenedione

Excessive androstenedione has been identified in women suffering from polycystic ovary syndrome, a leading cause of infertility (Knochenhauer et al., 1998). Androstenedione is an androgenic steroid hormone derived from cholesterol (Auchus et al., 1998). Steroidogenic acute regulatory protein (StAR) delivers cholesterol to the mitochondria of steroid-producing cells and activates P450scc enzyme (Auchus et al., 1998). Upon activation, P450scc sequentially cleaves the side chain of cholesterol to produce pregnenolone, hydroxylates pregnenolone to produce 17α-Hydroxy-pregnenolone, and then cleaves an acetyl group to produce dehydroepiandrosterone (DHEA) (Auchus et al., 1998). Another enzyme called 3β-steroid dehydrogenase 1st

oxidizes and then isomerizes DHEA to synthesize androstenedione (Auchus et al., 1998). Although androstenedione is bioactive, its major downstream fate is aromatization by the p450aro enzyme into estrone, which can subsequently be used in the synthesis of estradiol by 17β-steroid dehydrogenase. Androstenedione can also undergo hydroxylation by 17β-steroid dehydrogenase and synthesized into testosterone (Miller and Auchus, 2011), although this is a less common downstream pathway for androstenedione.

Whether or not androstenedione plays an important role in skeletal muscle growth like testosterone and other androgens is not clear. Some studies suggest that androstenedione does not stimulate protein anabolism, increase lean mass, or elevate serum testosterone but that it does increase serum estradiol (Wallace et al., 1999; Rasmussen et al., 2000). Conversely, another study reported that androstenedione upregulated androgen receptor expression in myoblast, increased their proliferation and differentiation rates by upregulating MyoD, and increased their myosin heavy chain content, albeit to a lesser degree than testosterone (Jasuja et al., 2005). Furthermore, fat-free lean mass and muscle strength were increased and serum cholesterol and high-density lipoprotein cholesterol were reduced after androstenedione supplementation (Jasuja et al., 2005). It is worth noting that differences in skeletal muscle growth attributed to androstenedione could have been due to greater conversion to testosterone rather than due to the action of androstenedione per se. Testosterone, the primary androgenic steroid in many mammalian species, stimulates myoblast-facilitated muscle hypertrophy in part by upregulating expression of the differentiation factor MyoD (Singh et al., 2003). Identifying the specific role of androstenedione on skeletal muscle could result in novel strategies to promote growth in beef cattle to supplement or replace

available hormone therapies that utilize testosterone and estrogen compounds.

Literature on hyperandrogenism and the effects of androstenedione on skeletal muscle in food animals is limited, but these topics have been explored in humans. Hyperandrogenism is a prominent characteristic in women suffering from polycystic ovary syndrome, and is often coupled with ovulatory dysfunction and increased risk for infertility, obesity, type 2 diabetes mellitus, dyslipidemia, and cardiovascular disease (Azziz et al., 2009). Women with polycystic ovary syndrome also exhibit increased visceral and peritoneal (i.e. abdominal) fat in the absence of whole-body obesity (Yildirim et al., 2003), which is associated with greater risk for developing metabolic dysfunction. Moreover, hyperandrogenism is associated with hyperinsulinemia in women, which is a hallmark of insulin resistance and type 2 diabetes mellitus (Burghen et al., 1980). Increased levels of cholesterol, low density lipoprotein cholesterol, high density lipoprotein cholesterol, and triglycerides are associated with polycystic ovary syndrome, independent of obesity (Legro et al., 2001). Hypertriglyceridemia in particular is associated with inflammation and metabolic dysfunction (Feingold et al., 1992; Rosenson et al., 2007). Overall, these pathologies may inhibit normal skeletal muscle growth and overall health and well-being, important factors in beef cattle production. In addition to metabolic pathologies, hyperandrogenism is associated with decreased concentrations of sex hormone binding globulin in circulation (Hogeveen et al., 2002). Sex hormone binding globulin is important for clearance of androgens and serves as a biomarker to estimate free sex steroids in circulation (Hogeveen et al., 2002). Hyperandrogenism and polycystic ovary syndrome in adolescents is usually preceded by premature pubarche, followed by hyperinsulinemia, hyperlipidemia, and reduced sex

hormone binding globulin at puberty (Ibanez et al., 1998) Adolescent females 3-years post menarche still experiencing oligomenorrhea were found to have increased concentrations of testosterone, androstenedione, and luteinizing hormone (van Hooff et al., 1999). Hyperandrogenism is associated with amenorrhea during the onset of puberty.

Beta Adrenergic Agonists (βAA) as Muscle Growth Stimulants

β-adrenergic agonist are commonly used in the feedlot sector of the cattle industry to increase feed efficiency and growth (Hahn, 1999a; Nienaber and Hahn, 2007), which in turn increases profitability and economic sustainability. βAA are nutrient-repartitioning agents that act via β-adrenergic pathways to increase skeletal muscle growth and decrease fat deposition, typically without changing organ or bone mass (Beermann, 2002). Because the direct mode of action for βAA is to stimulate specific signal transduction pathways of the β-adrenergic system, chronic administration eventually causes β receptors to become desensitized and thus they are typically only fed the final 28-45 days prior to slaughter (Byrem et al., 1992; Bittner et al., 2015). Functionally, βAA increase energy production by stimulating greater carbohydrate and lipid metabolism (Yang and McElligott, 1989). Molecularly, βAA biochemical mechanisms are less clear, but β receptors are present on muscle tissues as well as tissues associated with regulation of skeletal muscle growth, including adipose and neuro-endocrine tissues (Yang and McElligott, 1989). β-adrenergic receptors are 7-transmembrane G-coupled glycoproteins that are characterized into three isoform categories: β1, β2, and β3 (Lefkowitz et al., 1983). They can be expressed concurrently in tissues, but the distribution and proportions of subtypes are tissue-specific and species-

dependent (Lefkowitz et al., 1983). In bovine and ovine skeletal muscle and adipocytes, β_2 receptors are the most abundant isoform, with some literature reporting that they make up 75% of total β-receptors (Mersmann, 1998). β_1 receptors have the same binding affinity for epinephrine and norepinephrine, but β_2 receptors have greater binding affinity for epinephrine compared to norepinephrine (Lefkowitz et al., 1983). Norepinephrine is biosynthesized from tyrosine, secreted from the adrenal medulla and the autonomic nervous system, and found in circulation at high concentrations (Figure 2a) (Lefkowitz et al., 1983). Epinephrine is derived from norepinephrine via methylation, secreted from the adrenal medulla only, and found in circulation at lower concentrations unless stimulated by stress (Figure 2b) (Lefkowitz et al., 1983). β_1AA and β_2AA bind primarily to their respective β-receptors on the plasma membrane of cells to actuate the associated guanine nucleotide regulatory protein (G_α). If the associated G-protein is $G_{\alpha s}$, it in turn activates adenylate cyclase to form the second messenger cyclic AMP (cAMP) from ATP (Figure 3) (Lefkowitz et al., 1983). The cAMP binds to protein kinase A, stimulating the catalytic membrane-bound enzyme complex to phosphorylate intracellular proteins in skeletal muscle (Lefkowitz et al., 1983; Mersmann, 1998). These endocrine actions increase blood glucose concentrations through increased glycogen breakdown in liver and hepatic gluconeogenesis (Exton et al., 1972; Norman and Henry, 2014). They also increase ketone bodies from free fatty acids undergoing lipolysis, which serve as additional fuel sources for tissues such as the heart and skeletal muscle (Exton et al., 1972; Norman and Henry, 2014). Epinephrine and norepinephrine regulate pancreatic islet function, as insulin secretion from β cells is decreased and glucagon secretion from α cells is increased, but this occurs via α-receptors rather than β-receptors (Gerich et al., 1974;

Norman and Henry, 2014). β-adrenergic agonists increases RNA transcripts for skeletal muscle proteins such as a-actin and calpastatin, resulting in muscle hypertrophy (Lefkowitz et al., 1983). In addition to stimulating muscle cells, β1 pathways increase contraction rates in the heart, and β2 pathways increase the exchange of O_2 and CO_2 in bronchioles and vasodilation in skeletal muscle arterioles (Jaillard et al., 2001; Norman and Henry, 2014).

Studies have shown that supplementation of βAA rapidly increase skeletal muscle accretion in ruminant livestock and other mammalian species (Moloney et al., 1990) β-adrenergic agonist supplementation increases skeletal muscle growth by 40% but the rate of muscle growth and protein deposition is dependent on the dosage, type of agonist (i.e. β_1AA, β_2AA, or non-selective), age, species, sex, and diet of the animal (Beermann et al., 1987; Moloney et al., 1990; Mersmann, 1998). Skeletal muscle growth resulting from βAA supplementation is due to increased myoblast proliferation by 30%, increased muscle fiber hypertrophy, and altered muscle fiber type proportions in myoblast of mice (Shappell et al., 2000). In addition to stimulating muscle growth, β-adrenergic agonists also stimulate changes in metabolic function and profiles. β-adrenergic agonist infusion caused an increase in circulating insulin, glucose, and non-esterified fatty acid (NEFA) concentrations acutely, but chronic infusion did not change glucose concentrations and in fact decreased insulin concentrations, but NEFA concentrations remained increased (O'Connor et al., 1991). This suggests that βAA initially stimulate metabolism, however, chronic exposure ultimately causes β-receptors to become desensitized and thus the response wanes (O'Connor et al., 1991). Conversely, hyperinsulinemic-euglycemic clamp studies performed in cattle supplemented with the β_2AA clenbuterol for acute and chronic

periods showed no apparent changes in insulin sensitivity for glucose uptake by muscle or liver (Eisemann and Bristol, 1998). In another study, supplementation of the β_2AA cimaterol for 21 d increased hindlimb blood flow and hindlimb extraction of amino acids from circulation by 160% on d 14 but to a lesser degree over the last 7 d of supplementation, and increased protein accretion by 61% and 130% on d 7 and d 14, respectively (Byrem et al., 1996; Beermann, 2002). The β_2AA, clenbuterol, had similar results on protein accretion and muscle growth when administered in feed or via arterial infusion (Aurousseau et al., 1993; Eisemann and Bristol, 1998). When β-adrenergic antagonists were administered concurrently with clenbuterol at 10-fold and 100-fold greater doses fat deposition, heart weight, and energy expenditure were increased but muscle growth was not in rats (Reeds et al., 1988). Increased skeletal muscle growth enables the production of more meat with less cattle. The β_1AA ractopamine HCl in cattle and the β_2AA L-644,969 in pigs and sheep increased α-actin mRNA transcription, stability and synthesis in muscle fibers (Smith et al., 1989; Koohmaraie et al., 1991; Smith et al., 1995). L-644,969 supplement in sheep also decreased protein degradation (Wheeler and Koohmaraie, 1992). More efficient muscle accretion in β-adrenergic agonist-supplemented animals was reflected in their growth characteristics and carcass composition. The β_1AA and β_2AA ractopamine HCl and zilpaterol HCl, respectively, fed to feedlot lambs for 14, 28 or 42 d resulted in better growth performance (López-Carlos et al., 2011). Lambs had increased final body weights, increased average daily gain, and better feed efficiency overall, with no differences observed between the effects of β_1AA and β_2AA (López-Carlos et al., 2011; Barnes et al., 2019a). At harvest, lambs had increased hot carcass weights (HCW), dressing percentages, and loin muscle area and had

decreased fat thickness, with greater results in β_2AA-supplemented lambs compared to β_1AA supplemented lambs, and greater responses as days on supplement increased for both β_1AA and β_2AA (López-Carlos et al., 2011). β_2AA supplementation had its greatest response at 42 d and β_1AA supplementation had its greatest response between 28 d and 42 d (López-Carlos et al., 2011). Overall, lambs supplemented βAA had increased growth performance, increased muscle deposition, and decreased fat deposition (López-Carlos et al., 2011). Fresian steers supplemented the β_2AA, L-644,969, for 12 weeks at 4 doses exhibited no differences on live weight gain, however, supplemented steers had decreased dry matter intake and better feed efficiency (Moloney et al., 1990). At harvest, steers had increased HCW and decreased fat deposition, with the hindlimb being the leanest part of the carcass (Moloney et al., 1990). Overall, dairy steers supplemented βAA had increased muscle deposition and decreased fat deposition (Moloney et al., 1990). The β_2AA terbutaline and metaproterenol were supplemented to culled Moghani ewes on a low-energy diet for eight weeks at four doses, resulting in increased weight gain, decreased dry matter intake, and better feed efficiency (Nourozi et al., 2008). At harvest, these ewes had better carcass characteristics, decreased tail fat deposition, and decreased levels of circulating urea (Nourozi et al., 2008). Blood urea nitrogen is an indicator of protein metabolism and thus, skeletal muscle growth (Kohn et al., 2005). These ewes are a rare breed typically only found in middle eastern countries and were not of a typical finishing age or diet, but the typical responses to βAA were observed including increased muscle deposition and decreased fat deposition (Nourozi et al., 2008).

Growth-enhancing technologies including βAA, steroid implants, ionophores, and

melengestrol acetate (MGA) result in increased economic efficiency and economic and environmental sustainability (Capper and Hayes, 2012). Growth enhancing technology decreases input needs, waste outputs, land and water usage, fossil fuel usage and greenhouse gas emissions by producing more product with less animals and less time (Capper and Hayes, 2012). Without growth enhancing technology, US beef production would decrease by 17.1%, imports would increase by 352%, and the cost of beef production would increase by 9.1% (Capper and Hayes, 2012). US beef industry production value accounts for over $60 billion and US beef exports account for nearly $6 billion annually (USDA, 2019). Although 9% may seem low, it equates to over $5 billion each year. Therefore, the use of growth enhancing technology is imperative for environmental and economic efficiency of beef production.

The Impact of Heat Stress on Livestock

Despite improved management, heat stress is still a major barrier to sustainable livestock production (Nienaber and Hahn, 2007). Heat stress in livestock decreases average daily gain, dry matter intake, and health and wellbeing (Hahn, 1999b; Nienaber and Hahn, 2007). It also causes metabolic dysfunction and altered cardiovascular function, resulting in decreased growth efficiency (O'Brien et al., 2010). For the US livestock industry, heat stress results in $1.5 billion to $2.5 billion in economic losses annually (St-Pierre et al., 2003). In instances when cattle experience *acute* heat stress, they may not present long-term physiological changes or identifiable alterations in growth, although acute heat waves often results in high mortality rates (Eigenberg et al., 2005). Furthermore, animals that survive the onset of sustained heat loads are often able

to adapt and compensate, but not without growth and production losses (Hahn, 1999a). Heat stress causes reduced feed intake that accounts for part of the decreased growth performance, but other physiological changes occur that also impact performance, health, and well-being. These physiological changes include increased body temperature, reduced heart rate, and increased respiration rates (Beatty et al., 2006). Furthermore, heat stress causes reductions in blood carbon dioxide, bicarbonate, pH, sodium, and potassium but increased chloride (Beatty et al., 2006). When exposed to acute heat stress, cattle are typically able to return to homeostasis (Beatty et al., 2006). However, cattle under chronic heat stress are unable to maintain homeostasis of blood gases and metabolites, resulting in alkalosis and metabolic acidosis (Beatty et al., 2006). Intervention strategies including sprinkling, misting, or shade, allowed for partial recovery of growth in feedlot cattle, but cattle given no alleviation from heat stress were unable to recover or compensate for growth losses (Mitlöhner et al., 2001). Furthermore, in climates with substantial overnight cooling, growth reductions were not as severe (Mitlöhner et al., 2001).

β-adrenergic agonists work through pathways typically associated with stress responses, but it is unknown whether βAA further compound the negative effects of heat stress. In our earlier study, ractopamine partially prevented hyperventilation in heat-stressed feedlot wethers, but zilpaterol further increased rectal temperatures (Barnes et al., 2019a). Indicators of lameness and pathologies in liver, lung, heart, and kidney were not increased in heat-stressed feedlot lambs supplemented with βAA (Barnes et al., 2019a). When heat-stressed feedlot lambs were supplemented with βAA, they had improved performance over unsupplemented heat-stressed lambs at the beginning of the

feeding period, but this advantage was transient (Dávila-Ramírez et al., 2014). Feedlot cattle mobility and chute behavior was not affected by βAA supplementation under heat stress conditions (Boyd et al., 2015b).

The objective of the study in Chapter II was to evaluate growth, metabolism, health, and well-being parameters in heat-stressed feedlot wethers supplemented the βAA, ractopamine HCl. The negative effects of heat stress and the positive impact of βAA supplementation on performance and well-being are understood reasonably well, but there is limited information on the potential interacting effects that heat stress and βAA supplementation may have on animals. Elucidating these potential interactions between heat stress and βAA supplementation would allow for better management procedures to be adopted. The objective of the study in Chapter III was to evaluate myoblast function, metabolism, and growth from birth to harvest in the offspring of cows that exhibited abhorrent cyclicity patterns between weaning and their 1st breeding season. These cows are predicted to have high concentrations of androstenedione in their follicular fluid, which we hypothesize is associated with changes in growth and body composition of their offspring as indicated by previously observed increases in weaning weights in calves from these cows.

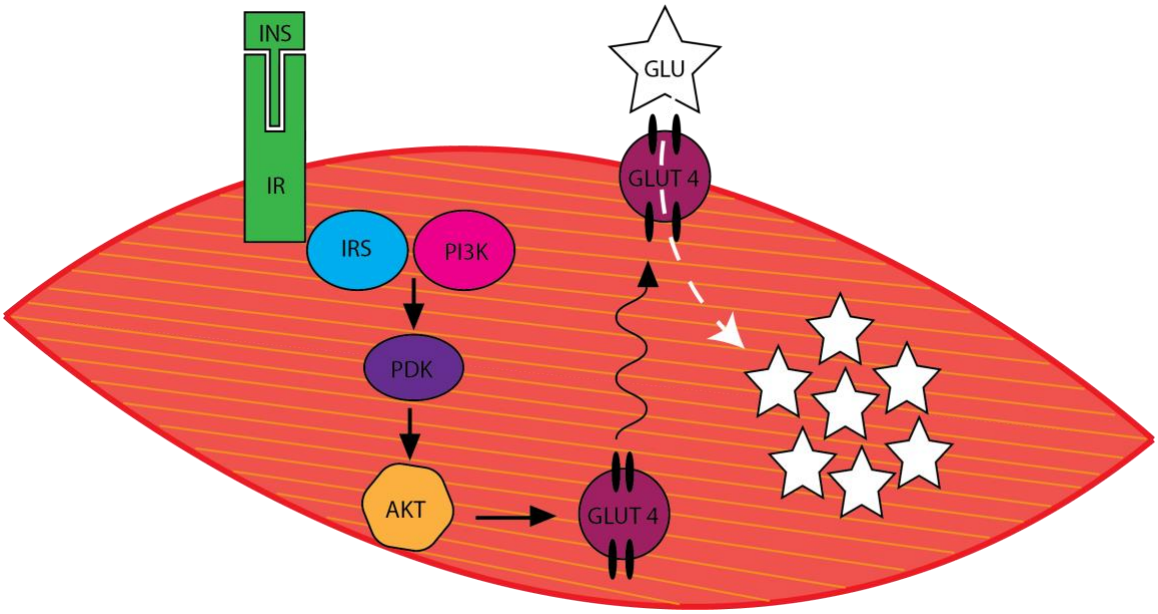

Figure 1. The insulin signaling pathway begins with insulin binding to the insulin receptor, eliciting a signaling cascade that translocates GLUT 4 to the cell membrane and allows for the entrance of glucose.

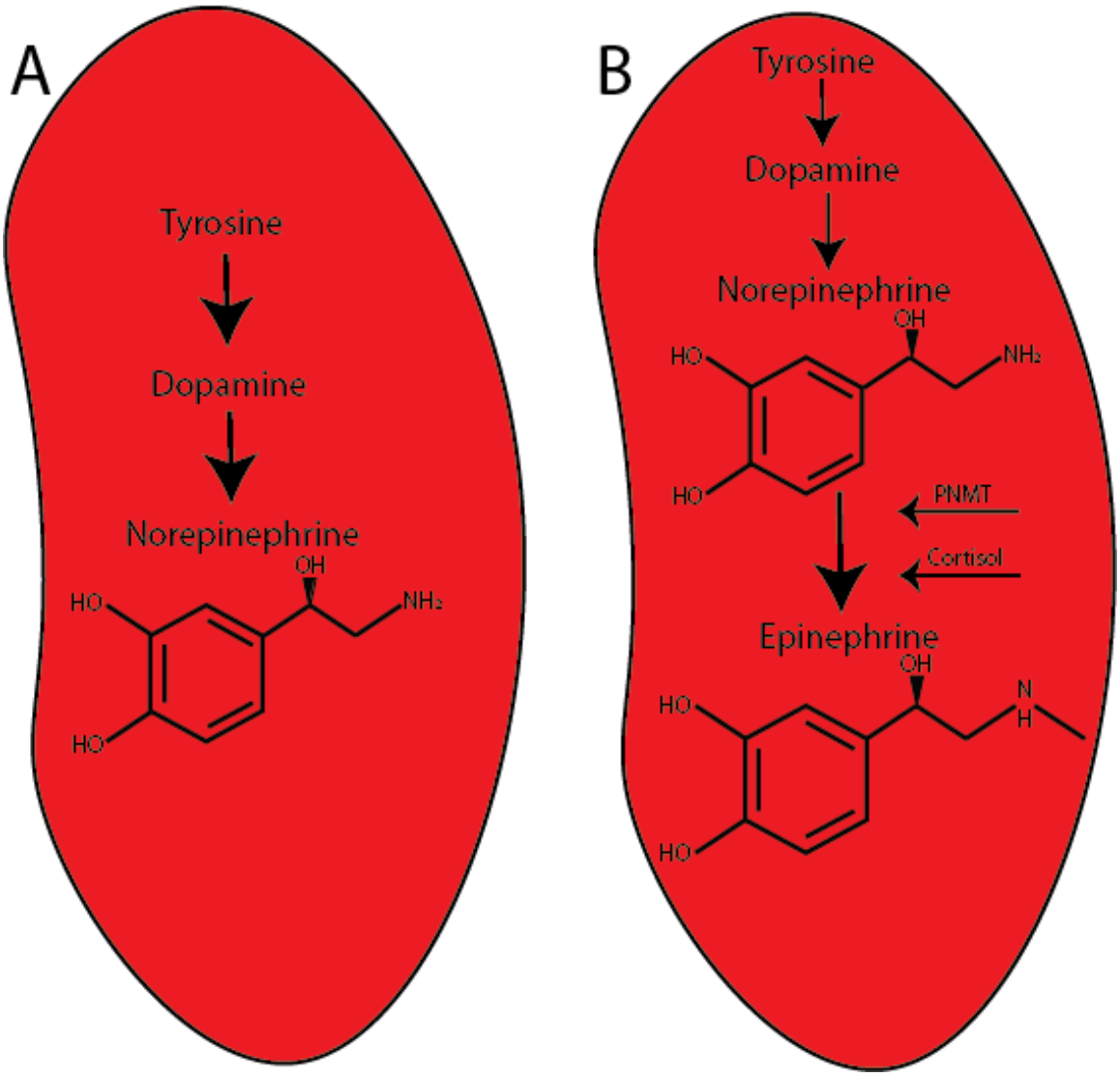

Figure 2. (A) Norepinephrine is derived from tyrosine in the adrenal. (B) Epinephrine is derived from norepinephrine in the adrenal via cortisol or PNMT.

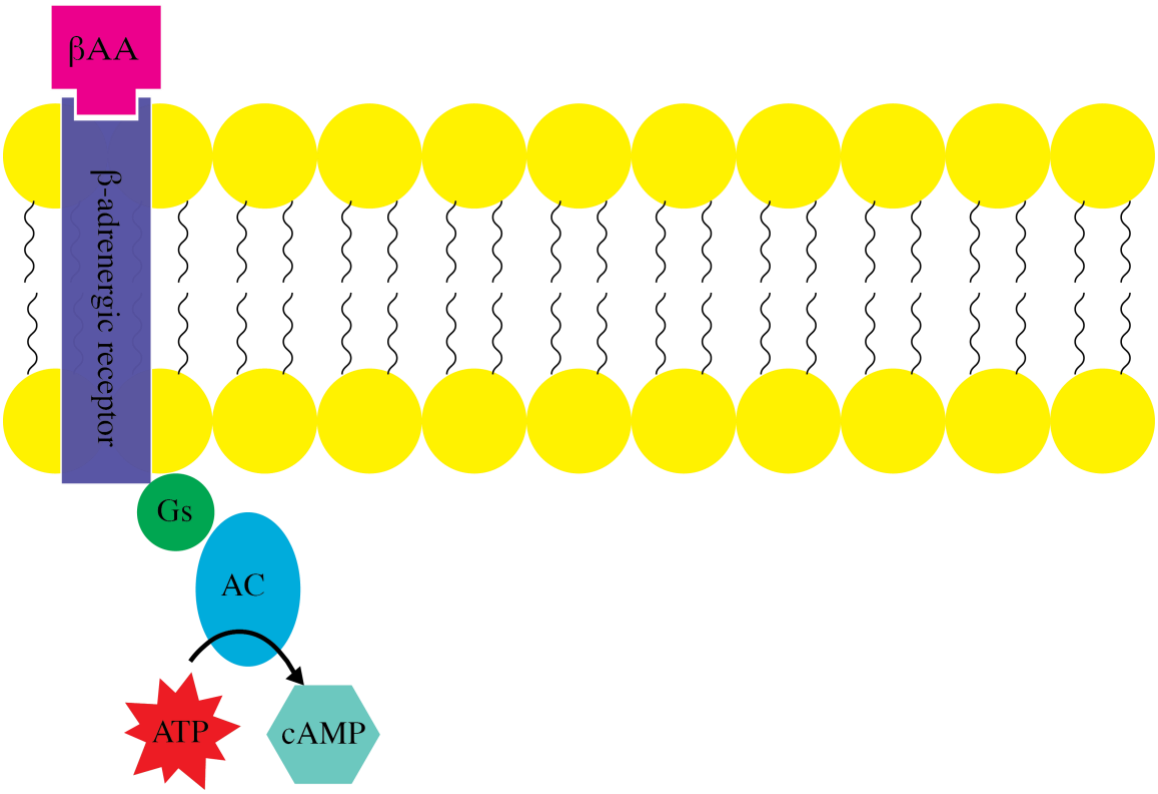

Figure 3: β-adrenergic agonist signaling begins with binding to the β-adrenergic agonist receptor on the plasma membrane, eliciting the Gα signaling cascade in which ATP is converted to cAMP.

CHAPTER 2

Heat stress-induced deficits in growth, metabolic efficiency, and cardiovascular function coincided with chronic systemic inflammation and hypercatecholaminemia in ractopamine-supplemented feedlot lambs

ABSTRACT

Heat stress hinders growth and wellbeing in livestock, an effect that is perhaps exacerbated by the β1 adrenergic agonist ractopamine. Heat stress deficits are mediated in part by reduced feed intake, but other mechanisms involved are less understood. Our objective was to determine the direct impact of heat stress on growth and wellbeing in ractopamine-supplemented feedlot lambs. Commercial wethers were fed under heat stress (40°C) for 30 d, and controls (18°C) were pair-fed. In a 2x2 factorial, lambs were also given a daily gavage of 0 or 60 mg ractopamine. Growth, metabolic, cardiovascular, and stress indicators were assessed throughout the study. At necropsy, 9th-12th rib sections (4-rib), internal organs, and feet were assessed, and *sartorius* muscles were collected for *ex vivo* glucose metabolic studies. Heat stress increased ($P < 0.05$) rectal temperatures and respiration rates throughout the study and reduced ($P < 0.05$) weight gain and feed efficiency over the 1st wk, ultrasonic loin-eye area and loin depth near the end of the study, and 4-rib weight at necropsy. Fat content of the 4-rib and loin were also reduced ($P < 0.05$) by heat stress. Ractopamine increased ($P < 0.05$) loin weight and fat content and partially moderated the impact of heat stress on rectal temperature and 4-rib weight. Heat stress reduced ($P < 0.05$) spleen weight, increased ($P < 0.05$) adrenal and lung weights, and was associated with hoof wall overgrowth but not organ lesions. Ractopamine did not

diminish any measured indicators of wellbeing. Heat stress reduced ($P < 0.05$) blood urea nitrogen and increased ($P < 0.05$) circulating monocytes, granulocytes, and total white blood cells as well as epinephrine, TNFα, cholesterol, and triglycerides. Cortisol and insulin were not affected. Heat stress reduced ($P < 0.05$) blood pressure and heart rates and increased ($P < 0.05$) left ventricular wall thickness, but neither heat stress nor ractopamine resulted in cardiac arrhythmias. Muscle glucose uptake did not differ among groups, but insulin-stimulated glucose oxidation was reduced ($P < 0.05$) in muscle from heat-stressed lambs. These findings demonstrate that chronic heat stress impairs growth, metabolism, and wellbeing even when the impact of feed intake is eliminated by pair-feeding, and that systemic inflammation and hypercatecholaminemia are likely contributors to these deficits. Moreover, ractopamine improved muscle growth indicators without worsening the effects of heat stress.

INTRODUCTION

Environmental heat stress diminishes growth efficiency and jeopardizes wellbeing of livestock, particularly in feedlot animals (Renaudeau et al., 2012). Engineering interventions such as construction of artificial pen shade, water misters, and pen sprinklers are effective approaches for cooling pens, but the benefits are somewhat inconsistent and the financial cost to producers can be prohibiting (Boyd et al., 2015a; Hagenmaier et al., 2016). We postulate that a better understanding of the physiological mechanisms mediating health and performance deficits could lead to more effective strategies for offsetting the detrimental effects of heat stress on livestock. Much of the impact of heat stress is facilitated by reduced energy intake (Brown-Brandl et al., 2003;

Brown-Brandl et al., 2017), but our research team recently found that skeletal muscle-specific glucose metabolism was also impaired in lambs exposed to chronic heat stress (Barnes et al., 2019b). Similar findings were reported in heat-stressed pigs (Zhao et al., 2018), which also had impaired skeletal muscle fatty acid metabolism. Inflammatory factors are regulators of muscle growth and metabolism (Cadaret et al., 2017; Cadaret et al., 2019a), and our earlier study revealed evidence of systemic inflammation as a component of chronic heat stress (Barnes et al., 2019b). Thus, the present study sought to determine the role of systemic inflammation and other stress responses in heat stress-induced deficits when the effect of reduced feed intake was eliminated by pair-feeding. In addition, feedlot cattle are often supplemented with the β1 adrenergic agonist ractopamine HCl during the last 28 to 40 d of the feedlot phase, which stimulates muscle growth and reduces adiposity (Bittner et al., 2016; Bittner et al., 2017). Such growth promoters help producers fulfill the US and global demands for high-quality protein, which by proxy benefits the economic sustainability of the beef industry. However, studies in cattle and pigs indicate that ractopamine may (James et al., 2013; Hagenmaier et al., 2017) or may not (Baszczak et al., 2006; Mendoza et al., 2017) modify the animal's response to external stressors. Moreover, although transient episodes of tachycardia and arrhythmias have been noted in humans when beginning or changing dosages of certain β agonists for respiratory ailments (Sears, 2002), the effects of ractopamine on cardiovascular function in livestock have not been fully elucidated. It is worth noting that ruminant cardiac tissues express moderate amounts of β1 adrenergic receptors (Odore et al., 2007) and that ractopamine has some affinity for the more common β2 adrenergic receptors (Colbert et al., 1991). Because of the social and economic importance of a

supplement that has the ability to produce more lean meat from fewer animals, it is imperative to define the additional risk, if any, of supplementing ractopamine to livestock during exposure to environmental heat stress. The objective of this study was to comprehensively assess the individual and interacting effects of 30-d heat stress and daily ractopamine supplementation on growth, metabolic efficiency, and wellbeing, and to identify mechanisms for these effects in the absence of differential feed intake in finishing lambs, which are an effective model for studying ruminant growth and metabolism in place of cattle (Sewell et al., 2009; Lundy et al., 2015).

MATERIALS AND METHODS

Animals and experimental design.

This study was approved by the Institutional Animal Care and Use Committee at the University of Nebraska-Lincoln (UNL). Studies were performed at the UNL Animal Science Complex, which is accredited by AAALAC International. Twenty-six crossbred Rambouillet wethers averaging 43 ± 1 kg were utilized in this study. Lambs were purchased from a commercial feedlot and given a 21-d acclimation period during which they were housed at 25°C and transitioned to a pelleted lamb grower/finisher diet (Complete B30; Purina Animal Nutrition, St. Louis, MO) that contained 30 g/ton lasalocid. Lambs were then individually penned and randomly assigned to thermoneutral conditions of 18°C, 15% relative humidity (controls; n = 14) or heat stress conditions of 40°C, 35% relative humidity for 12 h and 30°C, 35% relative humidity for 12 h/d (heat-stressed; n = 12) conditions for a period of 30 d. High temperatures within the thermally-regulated environmental chambers were transitioned to 32°C on d -1 and then to 40°C

thereafter. Heat-stressed lambs were fed *ad libitum* and controls were pair-fed to the average of the heat-stressed group beginning on d -5. In a 2 x 2 factorial, lambs also received an oral capsule bolus containing no supplement (n = 14) or ractopamine HCl (60 mg/d; n = 12) delivered once daily at 0700 via gavage. Daily dry matter intake and water intake were determined as previously described (Barnes et al., 2019b). Lambs were weighed on d -7, 0, 14, 21, and 30, and these bodyweights (BW) were used to calculate average daily gain and gain-to-feed ratios. Respiratory rates and rectal temperatures were determined at 0700, 1300, and 1900 on d -1, 2, 7, 14, 21, and 30. To estimate carcass characteristics, ultrasonic measurements were performed on d 0, 14, and 30. On d 31, lambs were euthanized by double barbiturate overdose and necropsied in random order. Loin and rib cutouts were taken from the left side of the carcass. Loins were removed between the 12th rib and the connection point of the hip bone and weighed. Four-rib cutouts were trimmed at the leading edge of the 9th rib and the distal edge of the 12th rib, weighed, and dissected into muscle, fat, and bone components. Organs were weighed and tissue samples of liver, kidney, heart, adrenal, lung, bladder, rumen, and ileum along with all feet were examined for pathologies by the UNL Veterinary Diagnostics Center.

Ultrasonic carcass measurements.

Back fat (subcutaneous) thickness, loin depth, loin-eye area, and body wall thickness between the 12th and 13th ribs were estimated by ultrasonography using Ultrasound Guidelines Council techniques as previously described (Emenheiser et al., 2010; Tait, 2016). Ultrasonic images were captured from each lamb's left loin area with an Aloka SSD-500 ultrasound (Corometrics Medical Systems, Wallingford, CT) that was

outfitted with a 17-cm, 3.5-mHz linear transducer probe and a Superflab wave guide standoff pad (Mick Radio-Nuclear Instruments, Inc., Mt. Vernon, NY). Vegetable oil was used as a couplant. Measurements were interpreted from single images frozen in real time.

Cardiovascular measurements.

Heart rates, cardiac rhythms, and blood pressures were determined on d -1, 2, 7, 14, 21, and 30 with a multi-parameter veterinary monitor (Cardell 9500, Midmark, Dayton, OH) as previously described (Lopes et al., 2016). Briefly, lambs remained in their pens and were manually restrained by the head, which they had been conditioned to during the acclimation period. Alligator clips were used to attach the leads to shorn areas of the skin. Lead 1 was attached over the cervical vertebrae, lead 2 was attached on the fore flank, and the ground lead was attached on the rear flank. One-min electrocardiograms were recorded based on the manufacturer's recommendations for waveform speed and sensitivity and were professionally evaluated for arrhythmias by a veterinarian. Heart rates were averaged across the 1-min period. An SV 4 vinyl cuff (Midmark) was placed around the metacarpal area of the forelimb equidistance between the knee and fetlock, and 5 consecutive blood pressure readings were recorded at 1-min intervals, per manufacturer's recommendations. Values for systolic blood pressure, diastolic blood pressure, and mean arterial blood pressure were averaged across these five readings. At necropsy, hearts were dissected as previously described (Antolic et al., 2015) and left ventricle thickness, right ventricle thickness, and septum thickness were measured using a digital caliper (Traceable, Webster, TX).

Blood parameters.

Blood was collected via jugular venipuncture into EDTA vacutainer tubes (~6 ml) and heparinized syringes (~0.5 ml) for analysis of blood components as previously described (Barnes et al., 2019b). Samples collected in EDTA tubes were used to quantify total and differential white blood cell (WBC) concentrations using HemaTrue Veterinary Hematology Analyzer (Heska, Loveland, CO). Samples collected into heparinized syringes were used to quantify blood glucose, lactate, pH, O_2, CO_2, HCO_3, Ca^{2+}, Na^{+}, and K^{+} using an ABL 90 FLEX blood gas analyzer (Radiometer, Copenhagen, Denmark). Plasma was isolated from the remaining blood in the EDTA tube by centrifugation at 14,000 x *g* for 5 min, and commercial ELISA kits were used to determine plasma concentrations of insulin (Alpco Diagnostics, Windham, NH), epinephrine (LDN, Nordhorn, Germany), TNFα (Wuhan Fine Biotech, Wuhan, China), and cortisol (Oxford Biomedical Research, Riviera Beach, FL) in duplicate. Intra-assay and inter-assay coefficients of variance were less than 12% for all ELISA. Plasma concentrations of urea nitrogen, cholesterol, high-density lipoprotein cholesterol, and triglycerides were determined by a Vitros-250 Chemistry Analyzer (Ortho Clinical Diagnostics, Linden, NJ) by the University of Nebraska Biomedical and Obesity Research Core.

Skeletal muscle glucose metabolism.

Sartorius muscle isolation. At necropsy, *Sartorius* muscles were collected intact (i.e. tendon to tendon) from both hindlimbs at necropsy. Longitudinal strips (~400 mg) were used to measure *ex vivo* skeletal muscle glucose uptake and oxidation rates as

previously described (Cadaret et al., 2017; Yates et al., 2019). Briefly, muscle strips were stratified by mass and placed in 6-well tissue culture plates. Muscle strips were then pre-incubated at 37°C for 1 h in gassed (95% O_2, 5% CO_2) Krebs-Henseleit bicarbonate buffer (KHB) containing 0 (basal) or 5 mU/ml insulin (Humulin-R, Ely Lilly, Indianapolis, IN). Pre-incubation media also contained 5 mM glucose, 35 mM mannitol, and 0.1% bovine serum albumin (Sigma-Aldrich, St. Louis, MO). Muscle strips were then washed in the respective basal or insulin-spiked KHB media with no glucose, 40mM mannitol, and 0.1% bovine serum albumin at 37°C for 20 min.

Ex Vivo Glucose Uptake. Glucose uptake was measured in one subset of *Sartorius* muscle strips by determining the incorporation rate of radiolabeled 2-deoxglucose as previously described (Cadaret et al., 2017; Cadaret et al., 2019a). After the wash incubation, these muscle strips were incubated in their respective basal or insulin-spiked KHB media containing 1 mM [^{3}H]2-deoxyglucose (300 μCi/mmol) and 39mM [1-^{14}C] mannitol (1.25 μCi/mmol) at 37°C for 20 min. Plates containing the muscle strips were then cooled at -20°C for 2 min and placed on ice. Muscle strips were washed 3 times in ice-cold phosphate-buffered saline (PBS; 7.4 pH) and lysed in 2 M NaOH at 37°C for 1 h. UltimaGold scintillation fluid (Perkin-Elmer; Waltham, MA) was added to the lysate, and specific activity of ^{3}H and ^{14}C was measured by liquid scintillation with a BC 1900 TA LC counter (Beckman-Coulter; Brea, CA). Specific activity ^{3}H was used to determine the amount of 2-deoxyglucose that had accumulated and ^{14}C was used to estimate the amount of extracellular fluid in the sample, as mannitol is not taken up by muscle. Specific activity of the media was also determined from the average of six 10-μl aliquots

mixed with 500 µl distilled water and scintillation fluid. Glucose uptake rates over the 20-min period were normalized to the mass of the muscle strip.

Ex Vivo Glucose Oxidation. Glucose oxidation was measured in the 2nd subset of *Sartorius* muscle strips by determining the production rate of radiolabeled CO_2 from radiolabeled glucose as previously described (Cadaret et al., 2017; Cadaret et al., 2019a). After the wash incubation, muscle strips were placed in one side of sealed dual-well chambers and incubated in their respective basal or insulin-spiked KHB media containing 5 mM [^{14}C-U] D-glucose (0.25 µCi/mmol; Perkin-Elmer) at 37°C for 2 h. In the adjacent well, 2 M NaOH was placed to capture CO_2. After 2 h, chambers were cooled at -20°C for 2 min and 2 M HCl was added to the media through the seal. The chambers were then incubated at 4°C for 1 h. Afterward, NaOH was collected from the chamber and mixed with UltimaGold scintillation fluid to determine specific activity of $^{14}CO_2$ via liquid scintillation. Glucose oxidation in pmol was calculated from dpm counts for $^{14}CO_2$ using the specific activity of the media, which was determined as described above. Glucose oxidation rates over the 2-h period were normalized to the mass of the muscle strip.

Statistical Analysis.

All data except histopathology data were analyzed using the mixed procedure of SAS 9.4 (SAS Institute, Cary, NC) to determine the effects of environmental condition, supplement, and their interaction in a 2 x 2 factorial design. Lamb was considered the experimental unit, and repeated measures (day) were used for serial measurements such as BW and blood components. *Ex vivo* data were analyzed with environmental condition and supplement as main effects and media insulin level as a repeated measure. Glucose

uptake and oxidation rates were each measured in 6 technical reps/media condition for each lamb, which were then averaged. Histopathological data were analyzed for differences due to environmental conditions or dietary supplement by Chi-squared test using the frequency procedure of SAS. Fisher's exact test was used for frequency analysis in which more than 25% of cells contained expected frequencies of less than 5. These data are presented as frequency of occurrence (%). All other data are presented as means ± standard error. The threshold for significance was $P \leq 0.05$, and tendencies are noted when $P \leq 0.10$.

RESULTS

Growth and body composition.

No environmental condition x supplement interactions were observed for dry matter intake, average daily gain, or gain-to-feed ratios over any of the measured timeframes. Dry matter intake did not differ between thermoneutral controls and heat stressed lambs (1.09 ± 0.03 vs. 1.12 ± 0.08 kg/d, respectively) due to pair feeding and did not differ between supplement groups. Average daily gain and gain-to-feed ratios between d 0 and d 7 were reduced ($P < 0.05$) in heat-stressed lambs when compared to controls but did not differ subsequently (**Table 1**). No interactions were observed among environmental conditions, supplement, or day for any ultrasonic measurements. Ultrasonic measurements for back fat thickness were initially less ($P < 0.05$) for ractopamine-supplemented lambs than unsupplemented lambs at the start of the study, but the changes over time did not differ between environmental conditions or supplements. Ultrasonic measurements for loin-eye area and loin depth indicated reduced

($P < 0.05$) growth in heat-stressed lambs over the course of the 30-d study, regardless of supplementation (**Table 2**). Ultrasonic measurements for body wall thickness did not differ between environmental conditions or supplements.

Loin weights and loin weight / BW did not differ between environmental conditions but were greater ($P < 0.05$) in ractopamine-supplemented lambs than in unsupplemented lambs (**Table 3**). Four-rib weights and four-rib weight / BW were less ($P < 0.05$) in heat-stressed lambs than controls, regardless of supplement. Bone weights and fat weights from the dissected four-rib cut-out were less ($P < 0.05$) for heat-stressed lambs than for controls, and fat weights were greater ($P < 0.05$) in ractopamine-supplemented compared to unsupplemented lambs. Dissected four-rib muscle weights did not differ among any groups, but an environmental condition x supplement interaction was observed ($P < 0.05$) for four-rib muscle weight / BW, which were reduced ($P < 0.05$) in all heat-stressed lambs compared to unsupplemented controls but not ractopamine-supplemented controls. Proximate analysis of the longissimus dorsi revealed environmental condition x supplement interactions ($P < 0.05$) for moisture, protein, and fat percentages. Moisture percentage was greater ($P < 0.05$) in muscle from unsupplemented heat-stressed lambs than ractopamine-supplemented heat-stressed lambs or any controls. Protein percentage was greater ($P < 0.05$) in muscle from all heat-stressed lambs than controls, but was also greater ($P < 0.05$) in ractopamine-supplemented heat-stressed lambs compared to unsupplemented heat-stressed lambs. Fat percentage was less ($P < 0.05$) in all heat-stressed lambs compared to controls but was also less ($P < 0.05$) in unsupplemented heat-stressed lambs compared to ractopamine-supplemented heat-stressed lambs.

Organ size and pathology.

Adrenal weights (5.1 ± 0.7 vs. 2.6 ± 0.6 g), adrenal weight / BW (0.026 ± 0.006 vs. 0.049 ± 0.007 g/kg), lung weights (893 ± 91 vs. 566 ± 80 g), and lung weight / BW (8.7 ± 1.0 vs. 5.5 ± 0.9 g/kg) were greater ($P < 0.05$) in heat-stressed lambs than in controls, regardless of ractopamine supplementation. Spleen weights (170 ± 21 vs. 250 ± 18 g) and spleen weight / BW (1.6 ± 0.2 vs. 2.4 ± 0.2 g/kg) were less ($P < 0.05$) in heat-stressed lambs than in controls, regardless of supplement. Kidney weights, kidney weight / BW, liver weights, liver weight / BW, heart weights, and heart weight / BW did not differ among groups.

Histopathological diagnoses of hepatic multifocal neutrophilic aggregates, renal nephrosis or tubule mineralization, illeal Peyer's patch lymphoid follicles, and myocardial, adrenal, or pulmonary focal lymphoid infiltrates did not differ in frequency between heat-stressed lambs and controls or between ractopamine-supplemented and unsupplemented lambs. No pathological conditions were diagnosed in bladder or rumen tissues in any lambs. Hoof wall overgrowth was more prevalent ($P < 0.05$) in heat-stressed lambs than controls (91% vs. 7%) but did not differ in prevalence between ractopamine-supplemented and unsupplemented lambs. Laminitis of the hoof did not differ in prevalence among any groups.

Rectal temperature, respiration rates, and water consumption.

An environmental condition x supplement x day interaction was observed ($P < 0.05$) for morning rectal temperatures and environmental condition x day interactions

were observed ($P < 0.05$) for evening rectal temperatures, evening water consumption, and respiratory rates at all three times of day. In general, rectal temperatures were greater ($P < 0.05$) in heat-stressed lambs than controls on all days excluding d -1 when measured in the morning (**Figure 1A**), at midday (**Figure 1B**), or in the evening (**Figure 1C**). Midday rectal temperatures were also lower ($P < 0.05$) in all ractopamine-supplemented lambs compared to unsupplemented lambs, regardless of environmental condition. Morning rectal temperatures on d 14, 21, and 30 were lower ($P < 0.05$) in heat-stressed lambs when supplemented ractopamine but did not differ between supplemented and unsupplemented controls. Evening rectal temperatures did not differ between supplements on any day. Morning, mid-day, and evening respiratory rates (**Figure 1D**, **1E**, and **1F**, respectively) did not differ between groups on d -1 but were greater ($P < 0.05$) in heat-stressed lambs than controls on all other days, regardless of supplement. Morning and mid-day water consumption rates (**Figures 1G** and **1H**, respectively) were greater ($P < 0.05$) in heat-stressed lambs than controls, regardless of supplement. Evening water consumption rates were greater ($P < 0.05$) in heat-stressed lambs compared to controls, but only on d 2 and 14 (**Figure 1I**).

Blood metabolites and hormones.

Blood plasma epinephrine concentrations were greater ($P < 0.05$) in heat-stressed lambs compared to controls, regardless of ractopamine supplementation (**Figure 2A**). An environmental condition x supplement x day interaction was observed ($P < 0.05$) for plasma TNFα concentrations, which were greater ($P < 0.05$) in unsupplemented heat-stressed lambs than controls on d 14 and in all heat-stressed lambs than controls on d 30

(**Figure 2B**). Plasma cortisol and insulin concentrations did not differ among any groups (**Figures 2C** and **2D**, respectively). Blood glucose concentrations (4.25 ± 0.09 vs. 4.46 ± 0.04 mM) tended to be less ($P = 0.07$) in heat-stressed lambs than controls, and an environmental condition x day interaction was observed ($P < 0.05$) for glucose-to-insulin ratios. These ratios (5.8 ± 1.1 vs. 9.0 ± 1.3 mmol/U) were less ($P < 0.05$) in heat-stressed lambs than controls on d 30 but did not differ on any other days. Blood lactate concentrations did not differ among any groups. Blood plasma urea nitrogen concentrations (**Figure 3A**) were reduced ($P < 0.05$) and plasma triglyceride, cholesterol, and HDL-cholesterol concentrations (**Figures 3B**, **3C**, and **3D**, respectively) were greater ($P < 0.05$) in heat-stressed lambs than in controls, regardless of supplement.

Blood gasses and electrolytes.

Blood O_2 partial pressures and hemoglobin-bound O_2 (**Figures 4A** and **4B**, respectively) were greater ($P < 0.05$) in heat-stressed lambs compared to controls and were greater ($P < 0.05$) in ractopamine-supplemented compared to unsupplemented lambs. Hemoglobin-bound CO_2 was greater ($P < 0.05$) in heat-stressed lambs compared to controls and was less ($P < 0.05$) in ractopamine-supplemented compared to unsupplemented lambs (**Figure 4D**). Environmental condition x day interactions were observed ($P < 0.05$) for blood CO_2 partial pressures (**Figure 4C**), HCO_3 concentrations (**Figure 4E**), and base excesses, which did not differ among groups on d -1 but were greater ($P < 0.05$) for heat-stressed lambs than controls on all other days, regardless of supplementation. Blood pH did not differ among any groups. Blood Na^+ concentrations (**Figure 4F**) did not differ among any groups, but blood K^+ concentrations (**Figure 4G**)

were greater ($P < 0.05$) in heat-stressed lambs regardless of supplement, and blood Ca^{++} concentrations (**Figure 4H**) were less ($P < 0.05$) in ractopamine-supplemented lambs regardless of environmental condition.

Hematology.

Blood WBC, monocyte, and granulocyte concentrations (**Figures 5A**, **5B**, and **5C**, respectively) were greater ($P < 0.05$) in blood from heat-stressed lambs than from controls (**Figure 5**). An environmental condition x supplement x day interaction was observed ($P < 0.05$) for blood lymphocyte concentrations (**Figure 5D**), which did not differ among any groups on d -1, 7, 21, or 30 but were greater ($P < 0.05$) in all heat-stressed lambs and ractopamine-supplemented controls compared to unsupplemented controls on d 2 and 14. Blood hematocrit, hemoglobin, mean corpuscular volume, mean corpuscular hemoglobin concentration, mean packed cell volume, red blood cell concentrations, and platelet concentrations did not differ among any groups. An environmental condition x supplement x day interaction was observed ($P < 0.05$) for red blood cell distribution width, which did not differ among any groups on d -1, 2, 7, and 14 but was less ($P < 0.05$) in ractopamine-supplemented and unsupplemented heat-stressed lambs and in ractopamine-supplemented controls compared to unsupplemented controls on d 21 (18.3 ± 0.3, 17.8 ± 0.5, 18.1 ± 0.4, and 19.2 ± 0.4 %, respectively) and d 30 (18.6 ± 0.3, 17.8 ± 0.4, 18.9 ± 0.4, and 19.5 ± 0.6 %, respectively).

Cardiovascular parameters.

Representative electrocardiograms are reported in **Figure 6A**. No pathological abnormalities in cardiac rhythms were detected from electrocardiograms. No interactions among environmental condition, supplement, and day were observed for blood pressures. Systolic arterial blood pressures (**Figure 6B**) and mean arterial blood pressures (93.0 ± 1.1 vs. 97.8 ± 1.1 mmHg)) were less ($P < 0.05$) in heat-stressed lambs and diastolic arterial blood pressures (**Figure 6C**) tended to be less ($P = 0.09$) in heat-stressed lambs compared to controls. An environmental condition x day interaction was observed ($P < 0.05$) for pulse rate (**Figure 6D**), which did not differ among groups on d -1, 2, or 30 but was less ($P < 0.05$) in heat-stressed lambs compared to controls on d 7, 14, and 21.

Environmental condition x supplement interactions were observed ($P < 0.05$) for left ventricular thickness and left ventricle / septum, which were greater ($P < 0.05$) in all heat-stressed lambs than controls but were greatest ($P < 0.05$) in unsupplemented heat-stressed lambs (**Table 4**). Right ventricular thickness, septum thickness, and left ventricle / right ventricle tended to be greater ($P < 0.05$) in heat-stressed lambs, regardless of supplement. Right ventricle / septum did not differ among groups.

Skeletal muscle glucose metabolism.

No interactions between environmental condition, supplement, and media condition were observed for *ex vivo* skeletal muscle glucose uptake rates (**Figure 7A**), which were greater ($P < 0.05$) in insulin-spiked media compared to basal media but did not differ among any groups. An environmental condition x supplement x media condition interaction was observed ($P < 0.05$) for *ex vivo* skeletal muscle glucose

oxidation rates (**Figure 7B**). In basal media, glucose oxidation was greater ($P < 0.05$) in muscle from ractopamine-supplemented controls than from unsupplemented controls or heat-stressed lambs. In insulin-spiked media, glucose oxidation was less ($P < 0.05$) in muscle from heat-stressed lambs than controls, regardless of supplement.

DISCUSSION

In this study, we found that heat stress sustained over a 30-d period reduced muscle growth, impaired metabolism, and altered cardiovascular function in feedlot lambs independently of its effects on dietary intake. Heat-stressed lambs exhibited reduced weight gain and growth efficiency compared to their pair-fed thermoneutral counterparts early in the feeding period and presented smaller ultrasound-estimated ribeye areas and loin depths by the end of the feeding period. At necropsy, they possessed lighter primal cuts with less intramuscular fat content. Muscle strips isolated from heat-stressed lambs were impaired in their capacity for glucose metabolism, which coincided with hyperlipidemia throughout the study. Heat stress produced predictable physiological responses including hyperthermia, hyperventilation, adrenal hypertrophy, and hypercatecholaminemia but also induced chronic systemic inflammation that was characterized by increased circulating leukocytes and TNFα. Surprisingly, circulating cortisol concentrations were similar between heat-stressed and thermoneutral lambs throughout the study, perhaps because pair-feeding equalized nutritional status. It appeared that heat-stressed lambs developed left-ventricular hypertrophy despite having lower peripheral blood pressures and heart rates throughout the study. Although heat stress did not increase the prevalence of any internal organ lesions, it did increase adrenal

and lung size, reduce spleen size, and was associated with greater prevalence of hoof wall overgrowth. Daily supplementation of the β1 adrenergic agonist ractopamine resulted in moderate improvements in muscle growth but did not improve growth efficiency or metabolic function, although the fact that feed intake was reduced may have contributed to its limited effectiveness. Importantly, ractopamine did not cause cardiovascular abnormalities, internal organ lesions, or hoof pathologies. It also did not worsen the negative effects of heat stress on wellbeing or performance and in some cases moderated them. Together, these findings demonstrate that heat stress impairs growth and efficiency in feedlot lambs via physiological mechanisms that are independent of and in addition to reduced feed intake. Two such mechanisms indicated by the results of this study are sustained systemic inflammation and hypercatecholaminemia. In addition, ractopamine supplementation during heat stress did not present a greater apparent risk to animal wellbeing than supplementation under thermoneutral conditions.

By pair-feeding thermoneutral controls to heat-stressed lambs, we were able to demonstrate that the early deficits in growth efficiency and the sustained deficits in muscle growth occurred in the absence of reduced dietary intake. The consequences of heat-stressed livestock going off of feed are well-characterized across many species (Mader, 2003; Wheelock et al., 2010; Zhao et al., 2018), and in an earlier study, we observed that heat stress caused lambs to consume about 21% less of a high-concentrate diet when offered *ad libitum* (Barnes et al., 2019b). However, growth rates in our previous study were reduced by 36%, which led us to speculate that mechanisms other than just eating less were contributing to heat stress-induced growth restriction. In the present study, growth indicators were indeed impaired when reduced feed intake was

eliminated as a cause. In fact, heat-stressed lambs lost weight over the 1st week of this study, although their average daily gain and feed efficiency recovered and stabilized thereafter, indicating at least some acclimation to heat-stress conditions. It is important to note that this study does not discount the role of reduced feed intake in heat stress-induced growth deficits but in fact confirms its importance, as growth restriction here was less severe than in the previous study. Moreover, the rates of gain in our pair-fed thermoneutral lambs were only a fraction of those observed in *ad libitum*-fed thermoneutral lambs from our previous study (Barnes et al., 2019b). Nevertheless, muscle growth rates estimated by ultrasonic loin measurements progressively worsened over the 30-d period, resulting in diminished size of primal cuts by the end of the study and demonstrating conclusively the existence of direct mechanisms of heat stress.

Daily supplementation of the β1 adrenergic agonist ractopamine at the recommended dosages did not impact the rate of gain or feed efficiency for lambs housed under either environmental condition, but it did increase indicators of muscle growth. When we failed to observe effects on growth or efficiency from ractopamine or zilpaterol included in the feed rations in our previous study (Barnes et al., 2019b), we speculated that it was due to potential variation among lambs in the amount or timing of supplement consumed or to the short (21-d) duration of the feeding period. However, administering exact doses of ractopamine by gavage and extending the feeding period to 30 d in the present study likewise did not affect weight gain or growth efficiency. Recent studies have reported that supplementing ractopamine to bulls (Consolo et al., 2016; Antonelo et al., 2017) or steers (Bittner et al., 2017) for around 30 d increased average daily gain by up to 28% and feed efficiency by up to 30%, which makes it tempting to speculate that

sheep are less responsive to β1 agonists than cattle. However, ractopamine did increase loin growth by about 15% in our lambs, which was comparable to the gain in loin muscle size observed in cattle. It also partially mitigated the loss of intramuscular fat due to heat stress, which was unexpected considering that it reduces marbling in cattle under normal environmental conditions (Gonzalez et al., 2010; Bittner et al., 2016).

Muscle isolated from heat-stressed lambs at the end of the feeding period exhibited intrinsically-impaired capacity for insulin-stimulated glucose oxidation, although glucose oxidation rates under unstimulated conditions were normal. Impaired responsiveness of glucose oxidation to insulin confirmed our previous observations in lambs heat stressed for 21 d (Barnes et al., 2019b) as well as a recent study in pigs heat stressed for 7 d (Zhao et al., 2018). Moreover, this impairment occurred in the presence of normal insulin-responsiveness glucose uptake, which demonstrates inefficient glucose metabolism that presumably contributes to poor growth efficiency. Although we did not assess skeletal muscle glucose uptake rates in our previous study, we did observe indicators of compensatory insulin sensitivity in muscle from heat-stressed lambs (Barnes et al., 2019b). Moreover, skeletal muscle gene expression in pigs after 8-d heat stress indicated that oxidative metabolism capacity was reduced despite normal or even greater insulin-responsive glucose clearance (Sanz Fernandez et al., 2015; Victoria Sanz Fernandez et al., 2015). Thus, we speculate that impaired insulin-stimulated glucose oxidation by heat stress is not a product of disruption in canonical insulin signaling and in fact may be the impetus for enhanced insulin sensitivity. Daily supplementation of ractopamine had no effect on glucose uptake or oxidation rates, which is not surprising

considering our previous observations of its effects on skeletal muscle metabolism (Cadaret et al., 2017).

Heat stress induced persistent systemic inflammation over the 30-d period, which helps to explain the observed reductions in muscle growth and metabolic function. Muscle growth requires incorporation of stem cells called myoblasts as well as favorable rates of protein synthesis relative to protein degradation (Rhoads et al., 2016). We were not able to assess myoblast function or protein synthesis in the present study, but we previously observed that enhanced inflammatory tone impaired myoblasts isolated from fetal sheep (Posont et al., 2018a). Moreover, a recent study in mice showed that chronic inflammation disrupts anabolic protein turnover patterns (Ceelen et al., 2018). In a separate study, we reported that *in vitro* exposure to inflammatory cytokines did not affect insulin-responsive glucose uptake of muscle from adult rats, but that insulin-responsive glucose oxidation was impaired despite a stimulatory response to cytokines in the absence of insulin (Cadaret et al., 2017). We also reported that chronic inflammatory conditions *in utero* reduced glucose oxidation rates in fetal muscle without affecting glucose uptake (Cadaret et al., 2019a; Cadaret et al., 2019b).

Systemic inflammation and hypercatecholaminemia may also explain hyperlipidemia in our heat-stressed lambs. Catecholamines stimulate fat mobilization (Ferlay and Chilliard, 2018), and high circulating epinephrine in our heat-stressed lambs coincided with elevated circulating cholesterol and triglycerides. Although we did not detect a reduction in back fat thickness via ultrasound, heat-stressed lambs had reduced fat content in primal cuts and reduced intramuscular fat percentages in the loin muscle, perhaps due to greater rates of mobilization. In addition, inflammation disrupts β

oxidation of fatty acids (Remels et al., 2010; Remels et al., 2013), which may have resulted in lipid accumulation in the bloodstream. Blood plasma urea nitrogen concentrations were reduced by heat stress in this study, which was consistent with our previous findings in *ad libitum*-fed sheep near the end of a 21-d heat-stress period (Barnes et al., 2019b) but not with other heat stress studies of shorter duration (Mahjoubi et al., 2015; Mahjoubi et al., 2016; Yazdi et al., 2016). Blood urea nitrogen concentrations are typically increased by undernutrition and high circulating cortisol concentrations (Hammon et al., 2003; Buntyn et al., 2016; Santana et al., 2019), neither of which were present in our study. Daily supplementation of ractopamine had no impact on skeletal muscle glucose metabolism, fat mobilization, or blood urea nitrogen but reduced circulating Ca^{2+} levels by about 5%, similar to findings in feedlot cattle (Abney et al., 2007).

Heat stress induced cardiovascular changes and was associated with organ hypertrophy/atrophy and hoof overgrowth, which demonstrates its detrimental effects on wellbeing. Ractopamine supplementation, however, did not diminish any wellbeing indicators and did not exacerbate any of the ill-effects of heat stress. Heart rates were not initially affected by heat stress but were reduced beginning one week into the study, despite the presence of adrenal hypertrophy and hypercatecholaminemia. The literature indicates that heat stress typically increases heart rate, especially during acute periods (Crandall and Gonzalez-Alonso, 2010; Iguchi et al., 2012). However, chronic hyperventilation by our heat-stressed lambs caused them to be hyperoxemic, which in humans has been shown to reduce heart rates by about 10% (Siński et al., 2016). Moreover, a study in Angus bulls reported that mild heat stress (28.6 to 31.4°C) for 10 d

caused hyperventilation and reduced heart rates by about 7% (Valente et al., 2013). Heat stress also reduced peripheral blood pressures in the present study, which has been observed in humans exposed to acute heat stress (Crandall et al., 2008; Crandall and Gonzalez-Alonso, 2010; Iguchi et al., 2012) and is associated with hyperoxemia (Jones et al., 1984). Relative hypertrophy of the left ventricle in heat-stressed lambs was not consistent with the observed hyperoxemia or hypotension and was most likely the product of sustained systemic inflammation (Sani et al., 2018) and hypercatecholaminemia (Kelm et al., 1996; Kinugawa et al., 1999). Ractopamine supplementation moderated heat stress-induced left ventricular hypertrophy, perhaps by reducing adrenergic sensitivity in cardiac tissues (Odore et al., 2007; Badino et al., 2008). Heat-stressed lambs also had larger lungs, which were likely a product of sustained hyperventilation (Faridy and Yang, 1989), and smaller spleens, which were likely the product of inflammation (Ohtsu et al., 2015). The hoof overgrowth observed in heat-stressed lambs was perhaps due to greater peripheral blood flow (Wheeler et al., 1972).

From these results, we conclude that heat stress reduces growth and metabolic efficiency in finishing lambs at least in part through chronic inflammatory and adrenergic responses, as we previously postulated (Barnes et al., 2019b). Even when the effect of reduced feed intake was eliminated by pair-feeding, heat stress reduced skeletal muscle growth, impaired glucose metabolism, and compromised wellbeing in feedlot lambs. Daily supplementation of the β1 agonist ractopamine did not compromise wellbeing and in fact moderated the impact of heat stress on several growth and health indicators. Ractopamine also increased some indicators of muscle growth independent of heat stress but did not affect any metabolic indicators. Achieving optimal growth without

compromising wellbeing remains a priority for the livestock industry, even as it faces the challenges of climate change, greater production demands, and expanding scrutiny from consumers. Heat stress-induced reductions in feed intake are difficult for producers to address, but the findings of this study demonstrate that additional mechanisms independent of nutritional intake contribute to deficits in growth and efficiency associated with heat stress. Identifying physiological targets such as systemic inflammation and hypercatecholaminemia is a fundamental step for new strategies to improve outcomes in heat-stressed livestock.

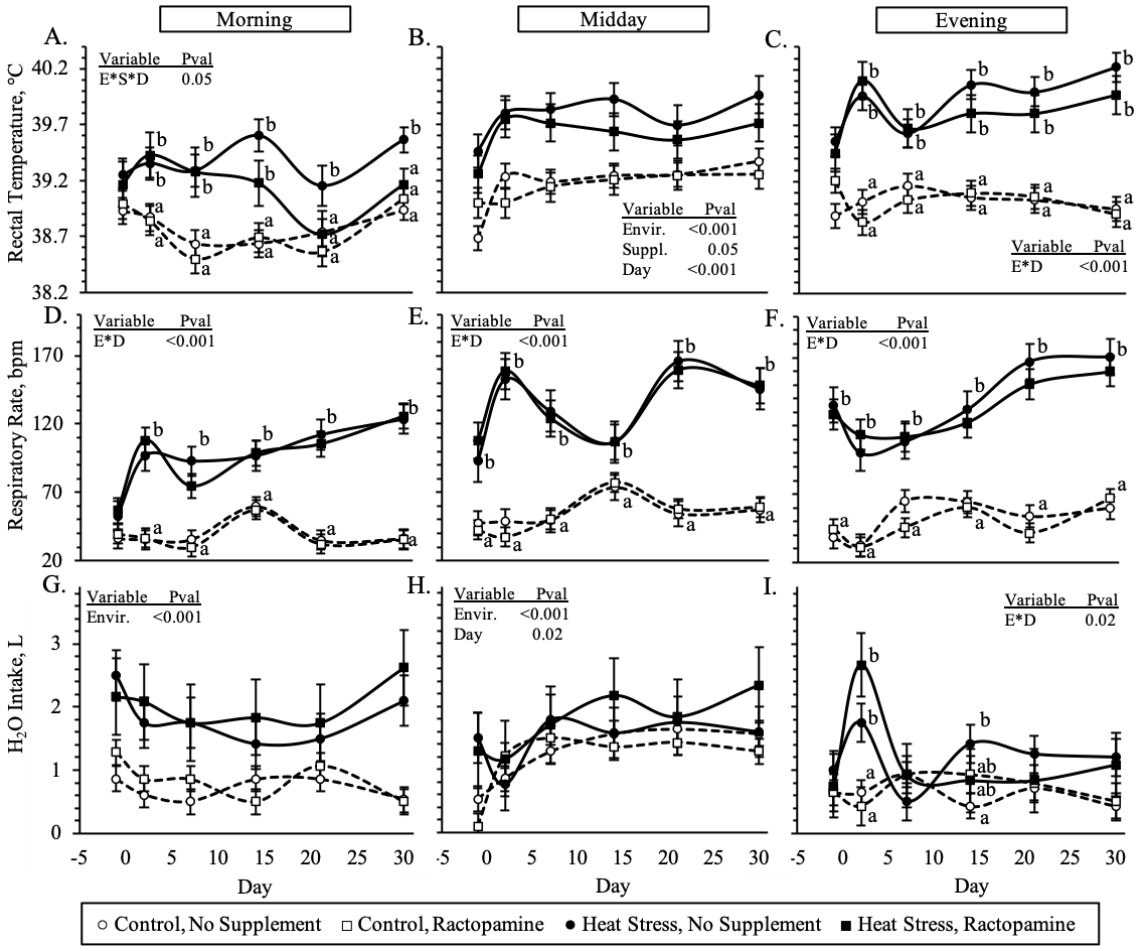

Figure 1. Physiological responses in heat-stressed lambs fed concentrate diets supplemented with ractopamine for 30 d. Data are shown for rectal temperatures (A., B., C.), respiratory rates (D., E., F.), and H_2O intake (G., H., I.) Assessments were performed in the morning (0700), at midday (1300), and in the evening (1900). Lambs were fed in a 2 x 2 factorial: unsupplemented controls (n = 7), controls supplemented with 60 mg/d ractopamine HCl (n = 7), unsupplemented heat-stressed lambs (n = 6), and heat-stressed lambs supplemented with ractopamine HCl (n = 6). Effects of environmental condition (Envir.), dietary supplement (Suppl.), day, and the interaction (E*S*D) are noted when significant ($P < 0.05$).

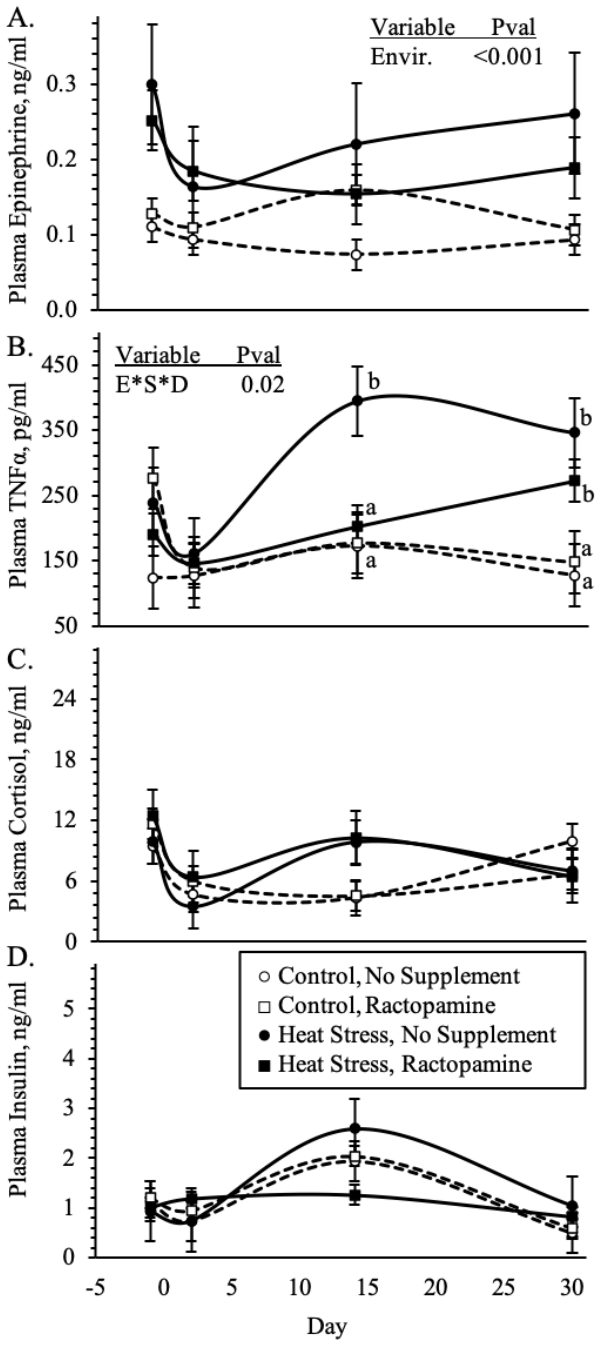

Figure 2: Circulating hormone concentrations in heat-stressed lambs fed concentrate diets supplemented with ractopamine for 30 d. Data are shown for plasma epinephrine (A.), TNFα (B.), cortisol (C.), and insulin (D.) concentrations. Lambs were fed in a 2 x 2 factorial: unsupplemented controls (n = 7), controls supplemented with 60 mg/d ractopamine HCl (n = 7), unsupplemented heat-stressed lambs (n = 6), and heat-stressed lambs supplemented with ractopamine HCl (n = 6). Effects of environmental condition (Envir.), dietary supplement (Suppl.), day, and the interaction (E*S*D) are noted when significant ($P < 0.05$). Where interactions were observed, means with differing superscripts differ ($P < 0.05$) within each day.

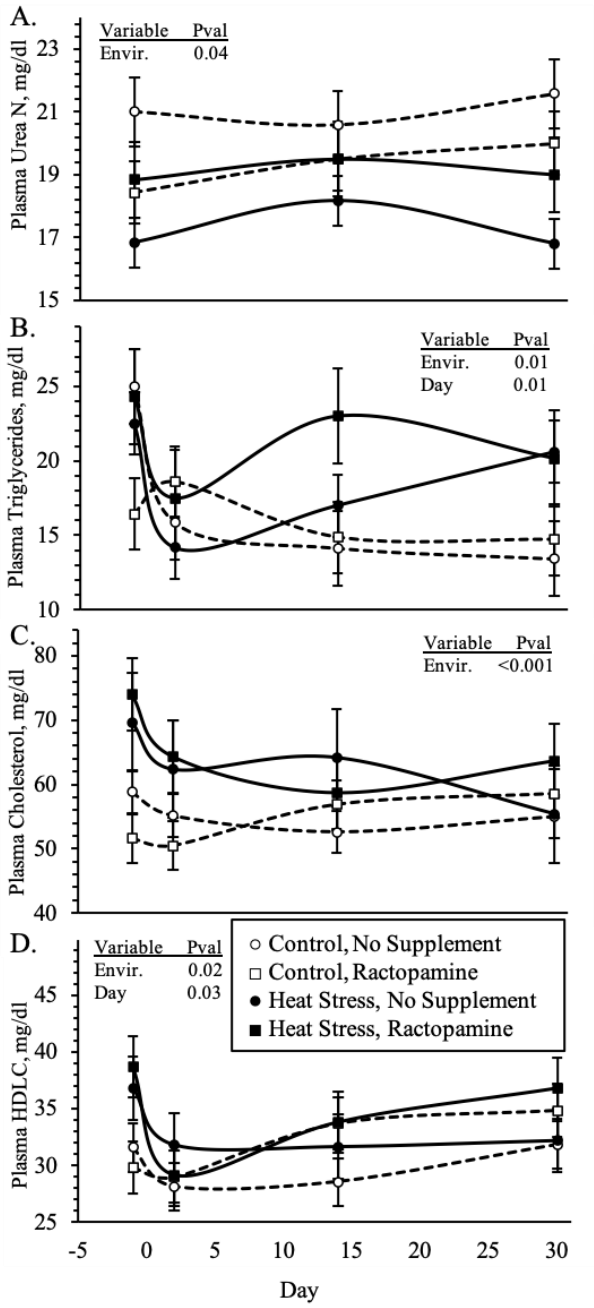

Figure 3: Circulating metabolites in heat-stressed lambs fed concentrate diets supplemented with ractopamine for 30 d. Data are presented for blood plasma urea nitrogen (A.), triglyceride (B.), cholesterol (C.), and HDL-cholesterol (D.) concentrations. Lambs were fed in a 2 x 2 factorial: unsupplemented controls (n = 7), controls supplemented with 60 mg/d ractopamine HCl (n = 7), unsupplemented heat-stressed lambs (n = 6), and heat-stressed lambs supplemented with ractopamine HCl (n = 6). Effects of environmental condition (Envir.), dietary supplement (Suppl.), day, and the interaction (E*S*D) are noted when significant ($P < 0.05$).

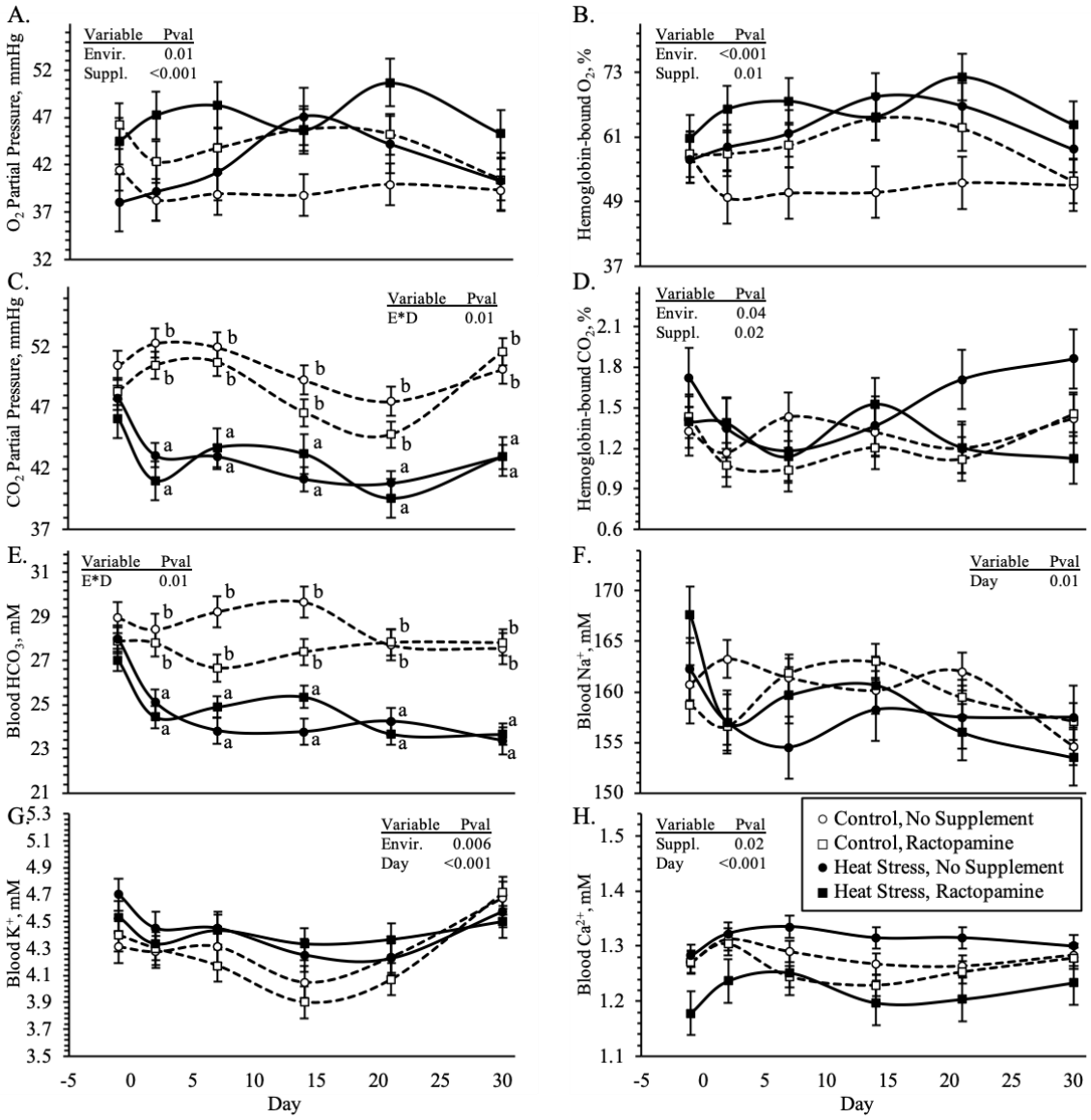

Figure 4: Blood gas and electrolyte concentrations in heat-stressed lambs fed concentrate diets supplemented with ractopamine for 30 d. Data are presented for O_2 partial pressure (A.), hemoglobin-bound O_2 (B.), CO_2 partial pressure (C.), hemoglobin-bound CO_2 (D.), HCO_3 concentrations (E.), Na^+ concentrations (F.), K^+ concentrations (G.), and Ca^{2+} concentrations (H.). Lambs were fed in a 2 x 2 factorial: unsupplemented controls (n = 7), controls supplemented with 60 mg/d ractopamine HCl (n = 7), unsupplemented heat-stressed lambs (n = 6), and heat-stressed lambs supplemented with ractopamine HCl (n = 6). Effects of environmental condition (Envir.), dietary supplement (Suppl.), day, and the interaction (E*S*D) are noted when significant ($P < 0.05$). Where interactions were observed, means with differing superscripts differ ($P < 0.05$) within each day.

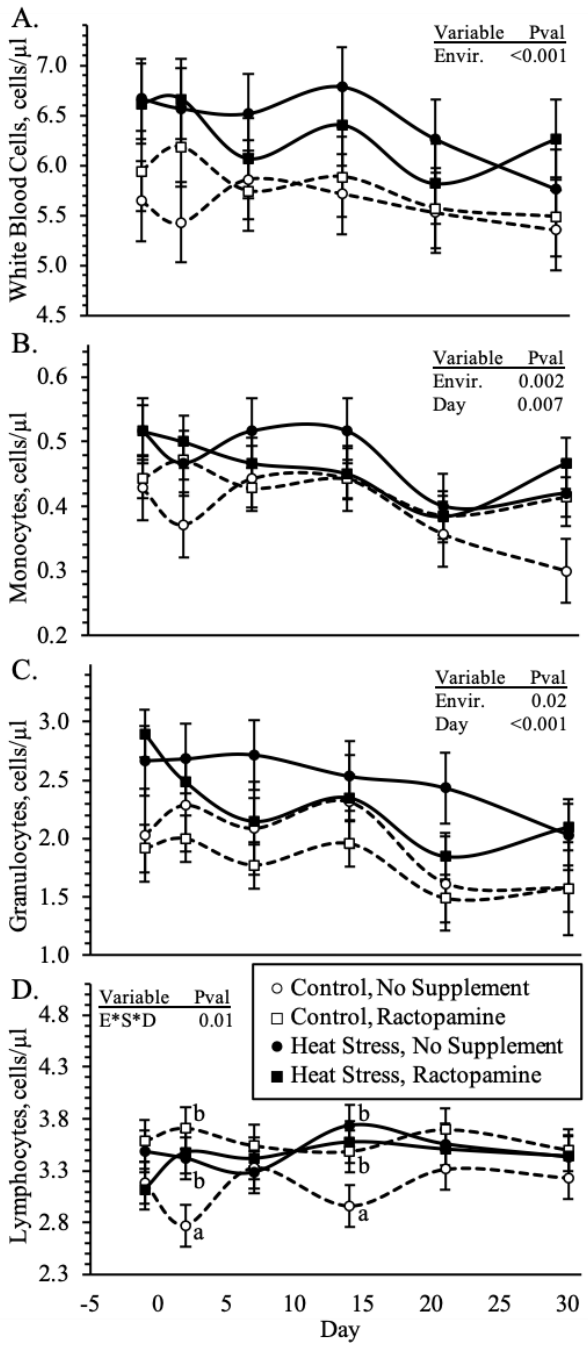

Figure 5: Circulating leukocyte concentrations in heat-stressed lambs fed concentrate diets supplemented with ractopamine for 30 d. Data are presented for total white blood cell (A.), monocyte (B.), granulocyte (C.), and lymphocyte (D.) concentrations in whole blood. Lambs were fed in a 2 x 2 factorial: unsupplemented controls (n = 7), controls supplemented with 60 mg/d ractopamine HCl (n = 7), unsupplemented heat-stressed lambs (n = 6), and heat-stressed lambs supplemented with ractopamine HCl (n = 6). Effects of environmental condition (Envir.), dietary supplement (Suppl.), day, and the interaction (E*S*D) are noted when significant ($P < 0.05$). Where interactions were observed, means with differing superscripts differ ($P < 0.05$) within each day.

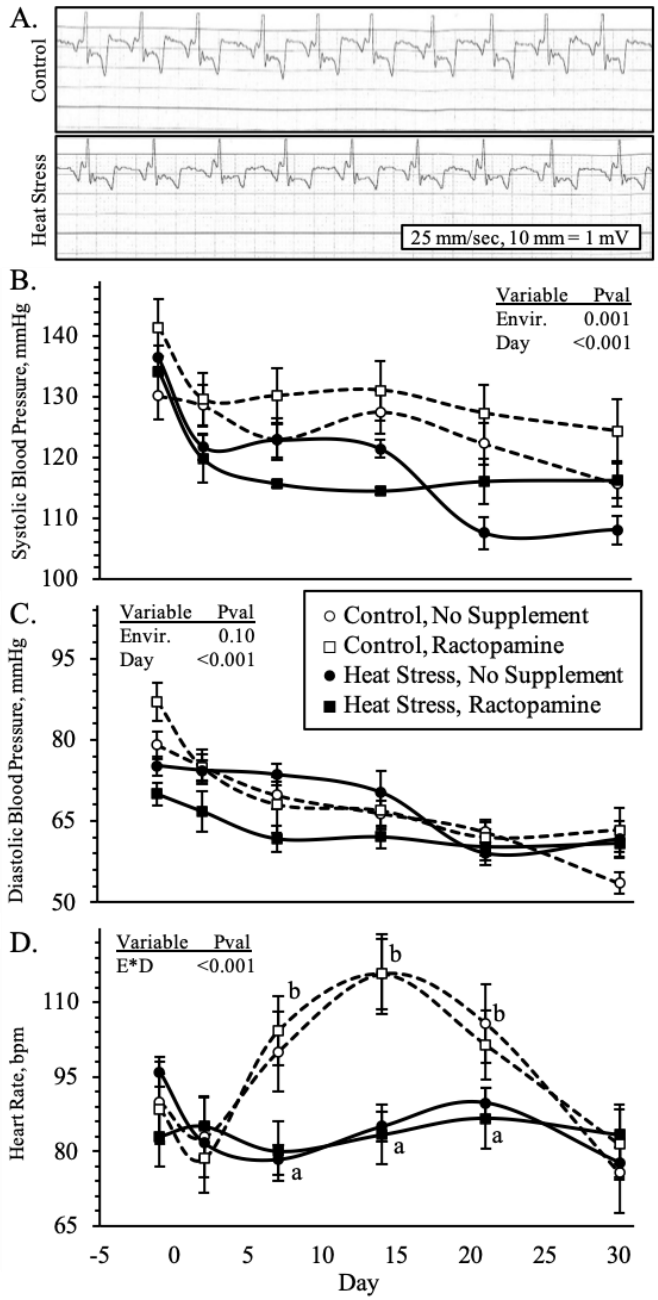

Figure 6: Cardiovascular responses in heat-stressed lambs fed concentrate diets supplemented with ractopamine for 30 d. Representative electrocardiograms are shown for controls and heat-stressed lambs (A.). Data are presented for systolic blood pressure (B.), diastolic blood pressure (C.), and heart rates (D.). Lambs were fed in a 2 x 2 factorial: unsupplemented controls (n = 7), controls supplemented with 60 mg/d ractopamine HCl (n = 7), unsupplemented heat-stressed lambs (n = 6), and heat-stressed lambs supplemented with ractopamine HCl (n = 6). Effects of environmental condition (Envir.), dietary supplement (Suppl.), day, and the interaction (E*S*D) are noted when significant ($P < 0.05$). Where interactions were observed, means with differing superscripts differ ($P < 0.05$) within each day.

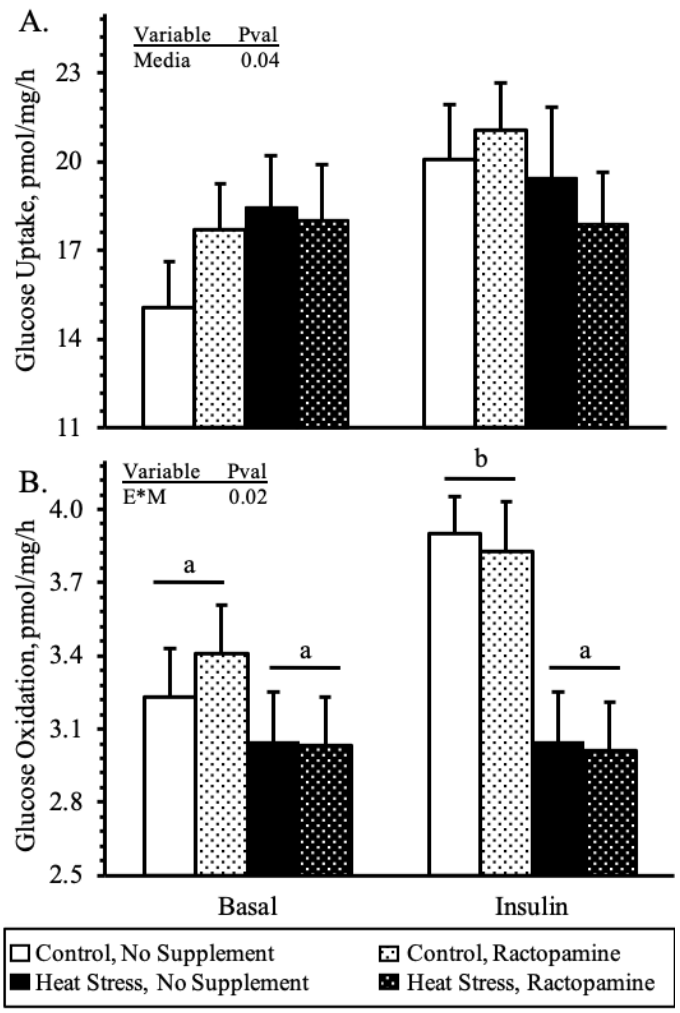

Figure 7: Ex vivo glucose metabolism in skeletal muscle from heat-stressed lambs fed concentrate diets supplemented with ractopamine for 30 d. Data are presented for glucose uptake (A.) and glucose oxidation (B.) rates in intact sartorius muscle strips incubated with 0 (basal) or 5 mU/ml insulin. Lambs were fed in a 2 x 2 factorial: unsupplemented controls (n = 7), controls supplemented with 60 mg/d ractopamine HCl (n = 7), unsupplemented heat-stressed lambs (n = 6), and heat-stressed lambs supplemented with ractopamine HCl (n = 6). Effects of environmental condition (Envir.), dietary supplement (Suppl.), media, and the interaction (E*S*M) are noted when significant ($P < 0.05$). Where interactions were observed, means with differing superscripts differ ($P < 0.05$).

Table 1. Growth and efficiency metrics in heat-stressed lambs fed concentrate diets supplemented with ractopamine for 30 d.[1]

Variable	Control		Heat Stress		*P*-value		
	No Suppl.	Ractopamine	No Suppl.	Ractopamine	Enviro.	Suppl.	E*S
Dry matter intake,[2] kg/d							
d 0 to 7	0.89 ± 0.04	0.96 ± 0.02	1.07 ± 0.09	1.05 ± 0.09	0.06	NS	NS
d 7 to 14	1.05 ± 0.05	1.06 ± 0.04	1.07 ± 0.17	1.08 ± 0.08	NS	NS	NS
d 14 to 21	1.19 ± 0.03	1.19 ± 0.03	1.06 ± 0.21	1.16 ± 0.11	NS	NS	NS
d 21 to 30	1.21 ± 0.02	1.15 ± 0.06	1.25 ± 0.04	1.19 ± 0.10	NS	NS	NS
Average daily gain,[2] kg/d							
d 0 to 7	0.019 ± 0.037	0.024 ± 0.067	-0.122 ± 0.071	-0.144 ± 0.097	0.04	NS	NS
d 7 to 14	0.093 ± 0.022	0.127 ± 0.031	0.155 ± 0.038	0.095 ± 0.063	NS	NS	NS
d 14 to 21	0.084 ± 0.015	0.100 ± 0.020	0.106 ± 0.039	0.089 ± 0.043	NS	NS	NS
d 21 to 30	0.053 ± 0.009	0.090 ± 0.026	0.080 ± 0.029	0.047 ± 0.036	NS	NS	NS
Gain-to-feed ratios[2]							
d 0 to 7	0.018 ± 0.036	0.029 ± 0.072	-0.120 ± 0.061	-0.165 ± 0.106	0.03	NS	NS
d 7 to 14	0.091 ± 0.024	0.126 ± 0.029	0.117 ± 0.027	0.078 ± 0.056	NS	NS	NS
d 14 to 21	0.069 ± 0.026	0.091 ± 0.026	0.073 ± 0.030	0.074 ± 0.028	NS	NS	NS
d 21 to 30	0.044 ± 0.007	0.030 ± 0.027	0.062 ± 0.023	0.032 ± 0.027	NS	NS	NS

[1] Ractopamine HCl (60 mg/d) was bolused daily.

[2] Daily averages over each period.

NS = not significant.

Table 2. Ultrasound-estimated loin growth in heat-stressed lambs fed concentrate diets supplemented with ractopamine for 30 d.[1]

Variable	Control		Heat Stress		*P*-value		
	No Suppl.	Ractopamine	No Suppl.	Ractopamine	Enviro.	Suppl.	E*S
Loin-eye Area, cm^2							
d 0	12.9 ± 0.3	12.0 ± 0.4	13.9 ± 0.7	13.0 ± 0.7	0.07	NS	NS
d 14	13.3 ± 0.5	12.9 ± 0.3	13.6 ± 0.6	13.2 ± 0.8	NS	NS	NS
Δd 0 to 14	+0.4 ± 0.6	+0.9 ± 0.5	-0.3 ± 0.4	+0.2 ± 0.4	NS	NS	NS
d 30	14.5 ± 0.6	13.1 ± 0.5	12.5 ± 0.6	13.1 ± 0.7	0.09	NS	NS
Δd 0 to 30	+1.6 ± 0.7	+1.2 ± 0.6	-1.4 ± 0.7	+0.1 ± 0.4	0.002	NS	NS
Loin depth, mm							
d 0	24.9 ± 0.5	24.5 ± 0.1	26.8 ± 1.0	26.7 ± 0.8	0.008	NS	NS
d 14	25.7 ± 0.7	25.1 ± 0.5	25.9 ± 1.0	26.2 ± 1.0	NS	NS	NS
Δd 0 to 14	+0.8 ± 0.7	+0.6 ± 0.5	-0.9 ± 0.6	-0.5 ± 0.9	0.05	NS	NS
d 30	26.1 ± 0.5	25.5 ± 0.7	24.6 ± 0.6	24.9 ± 0.8	0.09	NS	NS
Δd 0 to 30	+1.2 ± 0.5	+1.0 ± 0.7	-2.2 ± 0.6	-1.8 ± 1.0	<0.001	NS	NS

[1] Ractopamine HCl (60 mg/d) was bolused daily.

Δ = change over time; NS = not significant.

Table 3. Primal cut metrics and composition in heat-stressed lambs fed concentrate diets supplemented with ractopamine for 30 d.[1]

Variable	Control		Heat Stress		*P*-value		
	No Suppl.	Ractopamine	No Suppl.	Ractopamine	Enviro.	Suppl.	E*S
4-Rib[2]							
Mass, g	488.8 ± 18.8	506.4 ± 24.1	422.7 ± 20.2	447.6 ± 17.1	0.006	NS	NS
Mass / BW	13.6 ± 0.7^{a}	10.2 ± 0.8^{b}	7.1 ± 0.9^{c}	8.3 ± 0.7^{c}	-	-	0.009
Bone Mass, g	120.1 ± 5.4	113.2 ± 5.1	88.4 ± 9.1	99.5 ± 6.6	0.003	NS	NS
Fat Mass, g	135.7 ± 7.3	155.6 ± 6.5	109.4 ± 5.0	117.4 ± 2.9	<0.001	0.02	NS
Lean Mass, g	219.3 ± 6.1	218.9 ± 11.1	209.8 ± 13.1	203.0 ± 7.5	NS	NS	NS
Loin							
Mass, g	526.1 ± 19.5	583.1 ± 14.7	497.4 ± 19.3	594.4 ± 43.5	NS	0.009	NS
Mass / BW	11.1 ± 0.5	12.9 ± 0.5	10.2 ± 0.6	12.9 ± 0.5	NS	<0.001	NS
% Moisture	71.6 ± 0.3^{a}	71.5 ± 0.1^{a}	72.7 ± 0.2^{b}	71.3 ± 0.1^{a}	-	-	0.003
% Protein	20.2 ± 0.1^{a}	20.0 ± 0.1^{a}	20.5 ± 0.1^{b}	21.0 ± 0.1^{c}	-	-	0.006
% Fat	7.0 ± 0.3^{a}	7.1 ± 0.1^{a}	5.3 ± 0.2^{b}	6.4 ± 0.2^{c}	-	-	0.03

[1] Ractopamine HCl (60 mg/d) was bolused daily.

[2] 9th to 12th rib section.

^{a,b,c} Means with different superscripts differ ($P < 0.05$).

BW = bodyweight; NS = not significant.

Table 4. Heart metrics in heat-stressed lambs fed concentrate diets supplemented with ractopamine for 30 d.

Variable	Control		Heat Stress		*P*-value		
	No Suppl.	Ractopamine	No Suppl.	Ractopamine	Enviro.	Suppl.	E*S
Heart Mass, g	282 ± 32	274 ± 32	351 ± 38	318 ± 34	0.10	NS	NS
Heart Mass / BW	2.7 ± 0.3	2.7 ± 0.3	3.3 ± 0.4	3.1 ± 0.3	NS	NS	NS
L. Ventricular Wall, mm	16.7 ± 0.9^{a}	18.1 ± 1.3^{ab}	22.9 ± 0.8^{c}	20.3 ± 0.6^{b}	-	-	0.03
R. Ventricular Wall, mm	7.5 ± 0.6	7.1 ± 0.6	9.4 ± 0.7	7.6 ± 0.6	0.08	NS	NS
Septum, mm	17.5 ± 1.1	17.4 ± 0.9	18.9 ± 0.9	19.4 ± 0.9	0.09	NS	NS
L. Ventricle / Septum	0.96 ± 0.02	1.04 ± 0.06	1.24 ± 0.05	1.05 ± 0.04	-	-	0.01
R. Ventricle / Septum	0.43 ± 0.01	0.41 ± 0.02	0.51 ± 0.08	0.40 ± 0.05	NS	NS	NS
L. Ventricle / R. Ventricle	2.25 ± 0.15	2.53 ± 0.15	2.59 ± 0.18	2.75 ± 0.16	0.09	NS	NS

1 Ractopamine HCl (60 mg/d) was bolused daily.

a,b,c Means with different superscripts differ ($P < 0.05$).

BW = bodyweight; L. = left; NS = not significant; R. = right

CHAPTER 3

Atypical cyclicity at puberty in beef cows is associated with early deficits in muscling, metabolic indicators, and myoblast function in offspring but does not impact feedlot performance.

ABSTRACT

Cow fertility and calf post-natal growth efficiency are imperative to successful beef cattle production systems. In the University of Nebraska-managed beef herd, a subset of cows have been identified as having irregular reproductive cyclicity patterns or being non-cyclic from weaning to 1st breeding. These cows exhibiting irregular patterns of cyclicity are predicted to be identified as having high concentrations of androstenedione in their follicular fluid. Cows with high concentrations of androstenedione in their follicular fluid are sub-fertile, however, when they do become pregnant and wean a calf, their calves are heavier compared to control counterparts. These cows' phenotype is similar to women diagnosed with polycystic ovary syndrome, which is characterized by hyperandrogenism, anovulation or polycystic ovaries. We hypothesized calves from cows exhibiting irregular cyclicity patterns from weaning to 1st breeding have enhanced growth efficiency compared to calves from cows with normal cyclicity. To test this hypothesis, we evaluated growth and metabolism pre-weaning and, in the feedlot, myoblast function, and carcass characteristics at harvest in calves from cows with typical, start-stop and non-cycling reproductive cyclicity patterns from weaning to 1st breeding. Start-stop and non-cyclic cows from weaning to 1st breeding calved later in the season compared to typical cycling cows ($P \leq 0.05$). There were no differences in birth weight among calves.

Weaning weight tended to be decreased (P=0.09) among calves from start-stop and non-cyclic cows, however, differences were diminished when adjusting weaning weights to a standard 205-d. There were no differences for initial body weight, final body weight, average daily gain, or dry matter intake during the growing or finishing phase in the feedlot. Feed efficiency in the feedlot tended to be increased (P=0.09) in calves from non-cyclic cows, however, differences were diminished when calf performance was normalized by carcass. Indicators of body composition showed a tendency for reduced (P=0.10) muscle mass in calves from non-cyclic and start-stop cows. There were no differences in hot carcass weight, dressing percentages, ribeye area, 12th-rib fat thickness or marbling scores. Blood glucose-to-insulin ratios tended to be increased (P=0.09) in calves from non-cyclic cows. Plasma cholesterol, high-density lipoprotein cholesterol, and triglycerides were reduced (P≤0.05) in calves from non-cyclic cows. Plasma blood urea nitrogen was not different. Rates of myoblast proliferation isolated from calves were increased (P≤0.05) in calves from start-stop and non-cyclic cows. Glycolysis rates from the same myoblasts were decreased (P≤0.05) in calves from non-cyclic cows. These data independently and combined suggest calves from cows with irregular cyclicity from weaning to 1st breeding have chronic inflammation. While the specific mechanism of inflammation is unknown, further research may allow mediators of abnormal cyclicity and offspring inflammation-induced dysfunction to be elucidated.

INTRODUCTION

Efficient reproduction and growth in beef cattle are critical in meeting the increasing dietary demands of the growing population. Age at puberty, plane of nutrition,

breed, and number of estrus cycles prior to 1st breeding are contributing factors in a cow's reproductive success throughout her lifetime (Perry et al., 1991). Moreover, earlier age at 1st calving in beef cows is associated with increased growth efficiency in her subsequent offspring (Bourdon and Brinks, 1982). In the University of Nebraska-managed beef herd, we have identified a subset of females that exhibited irregular cyclicity patterns between weaning and their 1st breeding season, which we postulate are predictive markers for sub-fertility and may be associated with high concentrations of androstenedione in their follicular fluid (Cupp et al., 2019). Cows with high follicular fluid androstenedione were previously shown to have decreased fertility, but those that do become pregnant wean calves that average 10 to 17 kg heavier compared to the heard average (Summers et al., 2014). Furthermore, these cows have similar characteristics with women diagnosed with polycystic ovary syndrome, which is a leading cause of infertility in reproductive-aged women that is characterized by hyperandrogenism, anovulation, or follicular arrest (Summers et al., 2014; Cupp et al., 2019). We hypothesized that calves from cows that exhibited irregular cyclicity between weaning and their 1st breeding season would have superior growth efficiency, which we expected to be characterized by better myoblast function, muscling, and metabolic efficiency. The objective of this study was to test this hypothesis by evaluating growth and metabolic parameters of calves prior to weaning and in the feedlot, as well as carcass characteristics at harvest. To accomplish this, we compared calves from cows that were designated as having typical pubertal cyclicity, start-stop pubertal cyclicity, or non-cyclic puberty.

MATERIALS AND METHODS

Animals and Experimental Design.

This study was approved by the Institutional Animal Care and Use Committee at the University of Nebraska-Lincoln, which is accredited by AAALAC International. Studies were performed at the University of Nebraska-Lincoln Eastern Nebraska Research and Extension Center (Ithaca, NE) and the University of Nebraska-Lincoln Animal Science Complex (Lincoln, NE). Steer calves from crossbred Red Angus dams were utilized in this study. Dams had been classified into one of three pubertal groups based on the progesterone pattern they exhibited between weaning and their 1st breeding season as previously described (Cupp et al., 2019). Briefly, weekly blood samples were collected from weaning to breeding and plasma progesterone concentrations were measured by radioimmunoassay to determine puberty. Heifers were considered pubertal when their plasma progesterone concentrations were greater than 1 ng/ml. Heifers achieving and maintaining this progesterone concentration until breeding were classified as having typical pubertal cyclicity. Heifers reaching this progesterone concentration and then subsequently falling below 1 ng/ml were classified as having start-stop pubertal cyclicity. Heifers never reaching this progesterone concentration were classified as being non-cycling at puberty. Females in the start-stop and non-cycling pubertal classifications were previously shown to have higher rates of *ex vivo* androstenedione production by their ovarian cortex tissues (Cupp et al., 2019). In two consecutive years, steer calves were randomly selected from each of the three maternal pubertal groups (n = 10/group/yr) to evaluate offspring growth and performance. Calves were spring-born and

reared by dams on native Nebraska pasture until weaning at about 7 mo of age. Calves were weighed within 24 h of birth and again at weaning. Blood samples and semitendinosus muscle biopsies were collected at 3 mo of age.

Feedlot Performance.

After weaning, calves were placed in pens with a shaded Calan-gate feeding system and evaluated over an 85-d growing period in which they were supplied *ad libitum* a corn-based grower diet (**Table 1**) that met NRC requirements and *ad libitum* water. Initial and final bodyweights were recorded on the 1st and last day, respectively, of the growing phase, and average daily gain was calculated. Dry matter intake was recorded daily, and feed efficiency over the growing period was calculated as average daily gain divided by the average dry matter intake. Calves remained in Calan-gated pens and were transitioned to a corn-based finishing diet (**Table 2**) for a 168-d feeding period. Initial bodyweight, final shrunk bodyweight, average daily gain, dry matter intake, and feed efficiency were recorded for the finishing period.

Carcass Merit.

Steers were harvested at a commercial abattoir (Greater Omaha Packing Company, Omaha, NE). At slaughter, hot carcass weights were recorded, and dressing percentages were calculated as hot carcass weight divided by final live shrunk bodyweight. Following a 48-h chill, 12th-rib fat was measured and USDA-called marbling scores and ribeye areas were recorded. Following harvest, carcass-adjusted final bodyweight, average daily gain, and feed efficiency were calculated as previously described (Oney et al., 2018).

Blood parameters.

Blood was collected via jugular venipuncture into EDTA tubes (6 ml). Plasma was isolated from whole blood by centrifugation (14,000 x *g*, 5min). Plasma glucose concentrations were measured via glucose meter (One Touch, Lifescan, Milpitas, CA). Plasma insulin concentrations were determined in duplicate with a commercial ELISA kit (Bovine Insulin, Alpco Diagnostics, Windham, NH). Intra-assay and inter-assay coefficients of variance were less than 20%. Concentrations of blood plasma urea nitrogen, cholesterol, high-density lipoprotein cholesterol, triglycerides, and lipase were determined with a Vitros-250 Chemistry Analyzer (Ortho Clinical Diagnostics, Linden, NJ) by the University of Nebraska Biomedical Obesity Research Core.

Body Composition Estimates.

Biological impedance analysis was performed at the time muscle biopsies were collected to estimate body composition as previously described by (Gibbs et al., 2019) with some modifications. One set of electrode terminals were placed 10 cm behind the scapula and electrodes were 2.5 cm apart. The 2nd set of electrodes was placed 10 cm in front of the point of the hip and electrodes were 2.5 cm apart. Measurements were averaged and analyzed for body composition differences among groups.

Myoblast function and metabolism.

Myoblast isolation. Calves were immobilized in a flip chute, and the area over the semitendinosus muscle was anesthetized using injectable lidocaine 2% (VetOne, MWI Animal Health, Boise, ID). The area was closely shorn and cleaned with alternating

cycles of betadine and 70% ethanol. A 6-cm vertical incision was made through the skin and adipose, and a 5-g sample of the semitendinosus muscle was removed. Fat was trimmed away, and the muscle samples were stored in Belzer Cold Storage solution (Bridge to Life, Columbia, SC) for transport to the laboratory, typically within 4 h. The muscle was washed in ice-cold PBS and then hand minced. Myoblasts were liberated from minced muscle and isolated via serial centrifugation as previously described (Yates et al., 2014) with some modifications. Minced tissue was placed into 50-mL tubes, washed in ice-cold PBS, and pelleted. The pellet was re-suspended in PBS with Protease type XIV from *Strept. Griseus* (1.25 mg/mL; Sigma-Aldrich, St Louis, MO), digested at 37$_{o}$C for 1 h, and then re-pelleted. Myoblasts were then isolated by serial centrifugation and incubated for 2 h in DMEM + 10% FBS to purify isolate and then grown in complete growth media (DMEM + 20% FBS +1% AbAm (Antibiotic-Antimycotic; Gibco) + 0.5% gentamicin (Gibco) + 0.4% Fungizone (Abmphotericin B; Gibco) on fibronectin-coated tissue culture plates (10 mg/ml; Sigma-Aldrich) overnight. Myoblasts were then washed with PBS and grown for 5 d in complete growth media, replacing the media every other d. Myoblasts were lifted from the plate using Accutase (Sigma-Aldrich), incubated for 2 h in DMEM + 10% FBS to further purify isolate, and then slowly frozen in complete growth media + 10% dimethyl sulfoxide (Sigma-Aldrich) before being stored in liquid nitrogen.

Myoblast proliferation. Myoblasts were rapidly thawed and seeded on a 6-well fibronectin-coated tissue culture plate at a density of 5,000 cells per well. They were grown in complete growth media for 3 d and then incubated in un-spiked or 10μm androstenedione-spiked complete growth media for 24 h. Ethanol carrier was added to

un-spiked wells at equal volumes. After 24 h incubation, myoblasts were pulsed with EdU for 2 h, cooled on ice, and fixed in suspension with 4% paraformaldehyde. Myoblasts were stained in suspension using Click-iT EdU staining kit (ThermoFisher, Waltham, MA). Click-iT EdU cocktail was added at 50 µM and incubated for 2 h at 37$_{o}$C. Myoblasts replicating during the 2-h period were identified with AlexaFluor555 and counted via flow cytometry (zEPI; Orflo Technologies, Hailey, ID).

Myoblast Oxidative Metabolism. Myoblasts were seeded on a 24-well fibronectin-coated Seahorse XF Mito Stress Test (Aligent, Santa Clara, CA) plate at a density of 20,000 cells per well in complete growth media and incubated (37$_{o}$C, 5% CO_2) overnight. Complete growth media was then replaced with XF Assay media (Aligent) supplemented with 5.5 mM D-glucose (Sigma-Aldrich), 1.0 mM sodium pyruvate (Sigma-Aldrich) and 2 mM L-glutamine (Sigma-Aldrich). Assay media was adjusted to pH of 7.4, filter sterilized, and spiked with 0 or 5 mU/ml insulin (Humulin-R, Eli Lilly, Indianapolis, IN). Media was pre-heated to 37$_{o}$C, and one-half of the wells from each animal received basal media whereas the other one-half received insulin-spiked media. The plate was placed in a non-CO_2 incubator for 1 h prior to assay. To estimate oxidative metabolism, oxygen consumption rates (OCR) under normal (baseline) conditions and for proton leak, maximal respiration, non-mitochondrial respiration, respiration for ATP production, coupling efficiency, and spare respiratory capacity were measured via sequential injection of 1 µM oligomycin, 1 µM carbonyl cyanide-p-trifluoromethoxyphenylhydrazone (FCCP), and 0.5 µM rotenone as recommended by the manufacturer. To estimate glycolytic metabolism, extracellular acidification rates (ECAR) for non-glycolytic acidification, glycolysis, and glycolytic capacity were

measured during the same sequential injections. Following the OCR/ECAR assay, cells were lysed with RIPA buffer (Thermo Scientific) and protein was quantified using Pierce BCA Protein Assay Kit (Thermo Scientific) to normalize Seahorse XF OCR/ECAR values.

Statistical Analysis

Data were analyzed using SAS 9.4 (SAS Institute, Cary, NC). Growth data, blood components, and bioelectrical impedance data were analyzed for differences among maternal pubertal groups by ANOVA using the mixed procedure, and Fisher's LSD was used for mean separation. Data from *ex vivo* myoblast studies were likewise analyzed using the mixed procedure, with incubation condition as the repeated measure. For myoblast proliferation studies, three technical reps/media condition for each calf were averaged and the mean is reported. Similarly, three technical reps/media condition were averaged for OCR/ECAR assays, and the mean is reported. Oxygen consumption data are reported as both rates and as percentages of total OCR (i.e. OCR component / total OCR). Calf is the experimental unit for all outputs, and data are presented as mean ± standard error. Significant differences were declared at $\alpha \leq 0.05$ and tendencies at $\alpha \leq 0.10$.

RESULTS

Growth performance.

Cows that were non-cyclic or exhibited start-stop cyclicity between weaning and their 1st breeding season had greater ($P \leq 0.05$) number of days between the start of the calving season and the day on which they calved compared to cows with typical cyclicity

prior to their 1st breeding. Birth weights did not differ among groups. Weaning weight tended to be reduced ($P = 0.09$) in calves of both start-stop and non-cycling cows compared to calves of typical cycling cows (**Figure 1A**). However, 205-d adjusted weaning weights did not differ among groups (**Figure 1B**). Average daily gain from birth to weaning did not differ among groups. Initial body weight, final body weight, average daily gain, and dry matter intake in both the growing phase and finishing phase of the feeding period did not differ among groups. Feed efficiency tended to be increased ($P = 0.10$) among calves of non-cycling cows compared to calves of typical cycling cows in both the growing and finishing phases. However, carcass-adjusted final live bodyweight, average daily gain, and feed efficiency did not differ among groups.

Body composition estimates and carcass characteristics.

Fat-free mass estimated by bioelectrical impedance assay tended to be decreased ($P = 0.10$) among calves of start-stop and non-cycling cows compared to calves of typical cycling cows. Fat-free soft tissue estimates tended to be decreased ($P = 0.09$) among calves of start-stop and non-cycling cows compared to calves of typical cycling cows. The estimated sum of muscles and lean trim, sum of muscles, and sum of hind-quarter muscles all tended to be decreased ($P = 0.10$) in calves of start-stop and non-cycling cows compared to calves of typical cycling cows. Moisture, protein, and fat estimates did not differ among groups. At harvest, dressing percentages, hot carcass weights, ribeye areas, 12th-rib fat thicknesses, and marbling scores did not differ among groups.

Blood analysis.

Blood glucose concentration and plasma insulin concentrations did not differ among groups at the time muscle biopsies were collected, but glucose-to-insulin ratios tended to be increased ($P = 0.09$) in calves of non-cycling cows compared to calves of typical cycling cows (**Figure 2A**). Blood plasma urea nitrogen and lipase concentrations did not differ among groups at the time of muscle biopsies. Plasma cholesterol tended to be decreased ($P = 0.09$) in calves from non-cycling cows compared to calves from typical and start-stop cycling cows (**Figure 2B**). Plasma high-density lipoprotein cholesterol also tended to be decreased ($P = 0.09$) in calves from non-cycling cows compared to calves from typical and start-stop cycling cows (**Figure 2C**). Plasma triglyceride concentrations were decreased ($P \leq 0.05$) in calves from non-cycling cows compared to calves from typical and start-stop cycling cows (**Figure 2D**).

Myoblast proliferation and metabolism.

No maternal group x media condition interactions were observed for myoblast proliferation or any of the myoblast metabolic measurements. Rates of proliferation were increased ($P \leq 0.05$) in myoblasts isolated from the calves of start-stop and non-cycling cows compared to calves of typical cycling cows but did not differ between basal and androstenedione-spiked media (**Figure 3A**). Baseline respiration rates of myoblasts did not differ among groups or between basal and insulin-stimulated conditions. Proton leak rates and protein leak / total OCR did not differ among groups and only a tendency for increased ($P = 0.10$) insulin sensitivity for proton leak rates was observed in myoblasts from calves of typical and non-cycling cows compared to calves of start-stop cows.

Maximum respiration rates were increased ($P \leq 0.05$) and maximum respiration / total OCR tended to be increased ($P = 0.07$) in myoblasts from calves of non-cycling cows compared to calves of start-stop and typical cows, but there were no differences among groups for insulin sensitivity of maximum respiration. Spare respiratory capacity was decreased ($P \leq 0.05$) in myoblasts from calves of start-stop cows compared to calves of typical and non-cycling cows and spare respiratory capacity / total OCR tended to be increased ($P = 0.07$) in calves of non-cycling cows compared to calves of typical and start-stop cows. Non-mitochondrial oxygen consumption rates and non-mitochondrial consumption / total OCR did not differ among groups, but insulin sensitivity of non-mitochondrial oxygen consumption tended to be decreased ($P = 0.10$) in calves of start-stop and non-cycling cows compared to calves of typical cows. Rate, percentage of total OCR, and insulin sensitivity for oxygen consumption for ATP production and for coupling efficiency did not differ among groups. Non-glycolytic acidification did not differ among groups, but glycolysis rates were reduced ($P \leq 0.05$) in myoblasts from calves of non-cycling cows compared to calves of typical and start-stop cows. Glycolysis rates were increased ($P \leq 0.05$) in myoblasts incubated in insulin-spiked media compared to basal media, regardless of maternal pubertal group. Glycolytic capacity was decreased ($P \leq 0.05$) in myoblasts from calves of non-cycling cows compared to calves of typical and start-stop cows (**Figure 3B**).

DISCUSSION

In this study, we found that irregular and delayed reproductive cyclicity from weaning to 1st breeding in heifers did not affect subsequent growth rates of their offspring

pre-weaning or in the feedlot. However, calves born to cows expressing these irregularities had reduced estimated muscling prior to weaning. Coincidently, myoblasts isolated from these calves had increased proliferation rates but decreased glycolytic capacity. Similar adjusted 205-d weaning weights combined with indicators of diminished muscle mass suggest that body composition is affected in calves from cows with irregular or delayed cyclicity. Moreover, the observed differences in unadjusted weaning weights were presumably due to the later calving dates for cows with irregular and delayed cyclicity, as their well-established poor fertility (Summers et al., 2014; Cupp et al., 2019) lead them to become pregnant later in the breeding season. Surprisingly, we observed no differences in growth traits among calves during the feeding period even when adjusted for carcass metrics. This perhaps indicates the capacity for catch-up growth in the calves of cows with irregular or delayed cyclicity, although it is not clear from this study what the mechanism might be. We recognize that additional experimental units might clarify our findings for the feedlot phase and that this is a limitation of this study. This is particularly true considering that enhanced cellular replication coupled with indicators of impaired glucose metabolism in muscle stem cells from calves of cows with altered early cyclicity would indicate poor functional efficiency prior to entering the feedlot. Nevertheless, these findings show that delayed puberty and atypical reproductive cyclicity patterns of beef cows between weaning and their 1st breeding season coincided with unusual myoblast function and metabolism as well as altered body composition in their offspring but not pre-weaning rates of gain, feedlot performance, or carcass merit.

The timing and pattern of reproductive cyclicity exhibited by beef cows in the months between weaning and their 1st breeding did not affect their calves' birthweights or

their rates of weight gain prior to weaning or in the feedlot. Although the bodyweights of calves from dams with abnormal cyclic patterns leading up to 1st breeding were lighter at the actual time of weaning, the standard practice of adjusting weaning weights to 205 d eliminated these differences. Moreover, we found that cows with start-stop cyclicity or that were non-cyclic prior to their 1st breeding season gave birth at later dates compared to cows that had achieved puberty normally and that their calves were simply younger on the day of weaning. Phillippo et al. (1987) have shown that irregular reproductive cyclicity results in delayed conception and in turn birth, and therefore less time for the offspring to grow before being weaned. Thus, the observed differential weaning weights were almost certainly attributable to the later calving dates in our cows with altered cyclicity, which were presumably a product of their subfertility (Cupp et al., 2019).

In contrast to bodyweights, indicators of pre-weaning muscle mass were diminished in calves from cows designated as start-stop and non-cycling at puberty, leading us to speculate that these calves had altered body composition compared to calves from cows with normal cyclicity patterns at puberty. Bodyweights change relatively quickly over short periods of time, which makes age-adjustment appropriate, but body composition remains fairly static in the absence of major diet or environmental changes. For example, Meyer et al. (2005) showed via ultrasonography that back fat thickness and ribeye areas in beef calves measured at 118 d of age were not statistically different from measurements in the same animals at 90 d of age. Thus, we postulate that the reduction in estimated muscling in the present study was not due to later calving dates. Rather, it may be attributable to inflammation, as our previous work in sheep has indicated that greater inflammatory tone decreases muscle growth and increases fat deposition (Gibbs et al.,

2019). Despite less estimated muscle mass, proliferation rates were increased in myoblasts isolated from young offspring born to cows that exhibited irregular reproductive cyclicity patterns, which would seem to be paradoxical. However, greater proliferation can be indicative of an inability of myoblasts to exit the cell cycle and differentiate (Yang et al., 2012; Contreras et al., 2018), which is a necessary step for muscle growth. Because apparent muscle mass was reduced, we speculate that myoblast differentiation capacity was likely decreased, which we will determine in future experiments. If impaired myoblast differentiation is indeed the case, it could also be explained by heightened inflammation, since inflammatory factors have been shown by us and others to down-regulate proteins associated with differentiation and to decrease the rate at which myoblasts exit the cell cycle (Langen et al., 2004; Posont et al., 2018b). Poor differentiation of myoblasts has also been associated with decreased muscle mass (Dogra et al., 2006). Alternatively, myoblasts from calves born to cows with irregular pubertal cyclicity may be showing a compensatory enhancement in proliferation in culture if the *in vivo* physiological conditions within these calves are indeed suppressive. In other words, myoblast proliferation may be inhibited by chronic factors within the calves, leading to hyper-responsiveness that manifests when removed from the suppressive environment. Surprisingly, myoblast proliferation was not impacted when incubation media was spiked with androstenedione, indicating that hyperandrogenism is unlikely to be the direct cause for altered myoblast function. However, hyperandrogenism associated with polycystic ovary syndrome in women is linked to chronic inflammation (González et al., 2009). Of course, these findings do not confute the possibility that differences in muscling are due to later birth dates and less time for muscle growth.

Moreover, calves from cows that were atypically-cycling at puberty reached the same growth milestones in the feedlot as calves from typical females, and their carcass characteristics were not affected at harvest. Although it is not clear from our findings how this catch-up growth is achieved, it may simply be attributable to the substantially increased nutrient density that awaits calves when they are weaned from cows and transitioned into the feedlot. Thus, the potential impact of abnormal pubertal cyclicity of a cow on her offspring may not be great enough to affect its growth once the calves are nutritionally-independent from maternal influence. It is worth noting that a limitation of our growth assessments in this study is the need for greater power through additional experimental units. Estimated body composition data and feedlot performance data are only currently available for animals in year 1 of this study. We anticipate data from animals in year 2 of this study to eventually become available, which will provide greater power for experimental comparisons.

Total oxidative metabolism in myoblasts isolated from offspring was not affected by pubertal cyclicity patterns in cows. However, glycolysis was reduced in myoblasts from calves born to cows that were non-cycling at puberty, which demonstrates a shift in glucose-specific metabolism. Aldoretta and Hay (1999) have previously shown that although total oxidative metabolic rates may not differ, substrate-specific oxidation rates are in flux. Thus, changes in oxidation rates for one substrate are usually offset by conversely proportional changes in oxidation rates for another. Glycolysis is most commonly diminished when circulating glucose levels are chronically depleted (Cohen et al., 1995). However, plasma glucose concentrations measured during pre-weaning muscle biopsies were normal for all calves in the present study, indicating a low likelihood that

they were chronically hypoglycemic. Furthermore, glucose-to-insulin ratios were greater for calves from cows that were non-cyclic prior to 1st breeding, which would indicate greater insulin sensitivity that would presumably stimulate greater glucose metabolism. Although it is not fully clear why glycolysis was diminished in the myoblasts from these offspring, impaired glucose metabolism by muscle is yet another outcome of this study that could perhaps be explained by inflammation, as we have previously observed inflammation-induced disruptions in glucose uptake and insulin-stimulated oxidation (Cadaret et al., 2017; Posont et al., 2019).

Circulating lipid and lipoprotein concentrations were reduced in offspring from cows that were non-cyclic prior to 1st breeding, which we speculate is attributable to a reduction in mobilization from fat stores and/or increased lipid utilization by tissues. Reduced high-density lipoprotein cholesterol can be a product of inflammation, which acutely increases circulating cholesterol but downregulates hepatic enzymes related to cholesterol synthesis and causes a decrease in high-density lipoprotein cholesterol (Feingold et al., 1993; Feingold and Grunfeld, 2016). Moreover, Kalra et al. (2006) showed an association between impaired insulin responsiveness and dyslipidemia including reduced high-density lipoprotein cholesterol in women with polycystic ovary syndrome. Although, reduced high-density lipoprotein cholesterol is often presented with increased triglycerides, long-term inflammation can also cause dysregulation of fatty acid release from adipocytes into circulation (Maurizi et al., 2018). Nevertheless, these data demonstrate that dyslipidemia is present in young calves from cows that were non-cyclic at puberty. Conversely, blood urea nitrogen levels were not different among calves, likely indicating normal amino acid metabolism.

In conclusion, these data confirm an association between atypical maternal cyclicity at puberty and early metabolic changes in offspring that coincided with indicators of reduced muscle mass. Although the underlying cause is unclear, many of our findings would be consistent with heightened inflammatory activity in calves from cows that exhibited irregular cyclicity between weaning and their 1st breeding season. Thus, there is a clear need to further investigate the potential role of inflammation and to identify possible mediators that link irregular pubertal cyclicity in dam to these conditions in their offspring. Identifying the mechanisms for this link, inflammation or otherwise, will allow for management strategies to address early muscle growth and metabolic deficits in these calves.

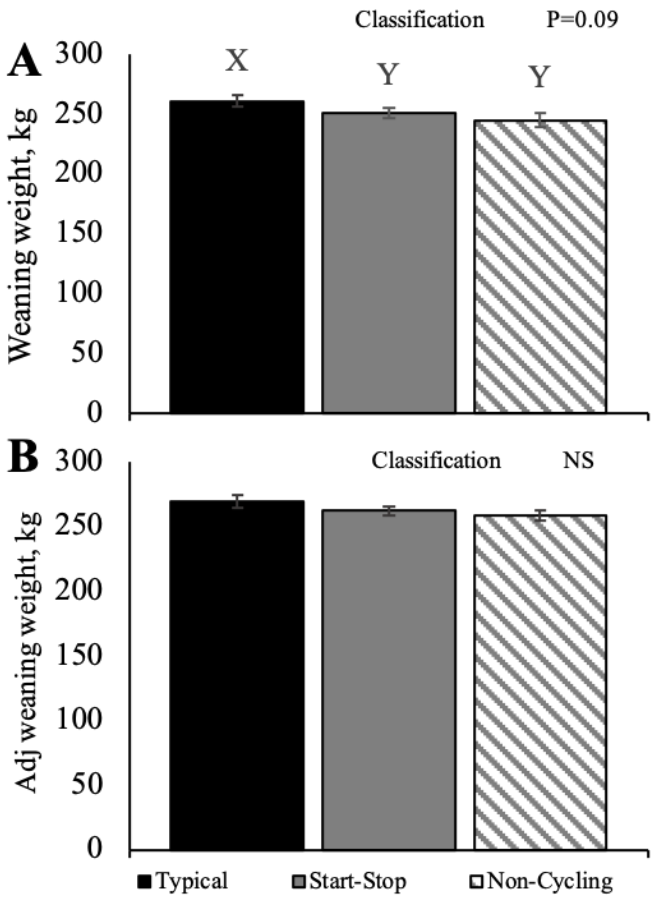

Figure 1: Data are shown for weaning weight (A) and 205-d adjusted weaning weight (B) for calves from cows with differing cyclicity from weaning to breeding: typical, start-stop cyclicity, and non-cycling. Effects of puberty cyclicity classification are noted when significant ($P<0.05$).

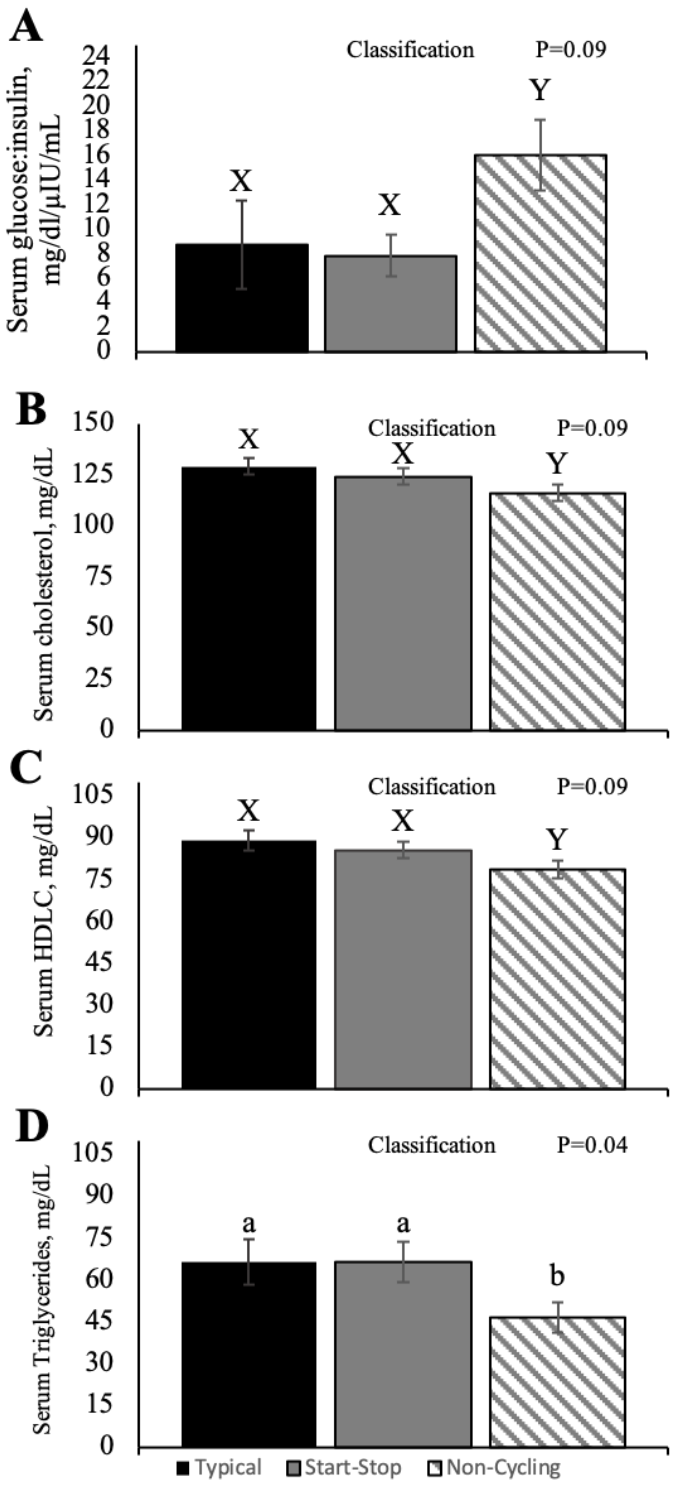

Figure 2: Data are shown for blood glucose-to-insulin (A), serum cholesterol (B), serum high-density lipoprotein cholesterol (C), and serum triglycerides (D) for calves from cows with differing cyclicity from weaning to breeding: typical, start-stop cyclicity, and non-cycling. Effects of puberty cyclicity classification are noted when significant ($P<0.05$).

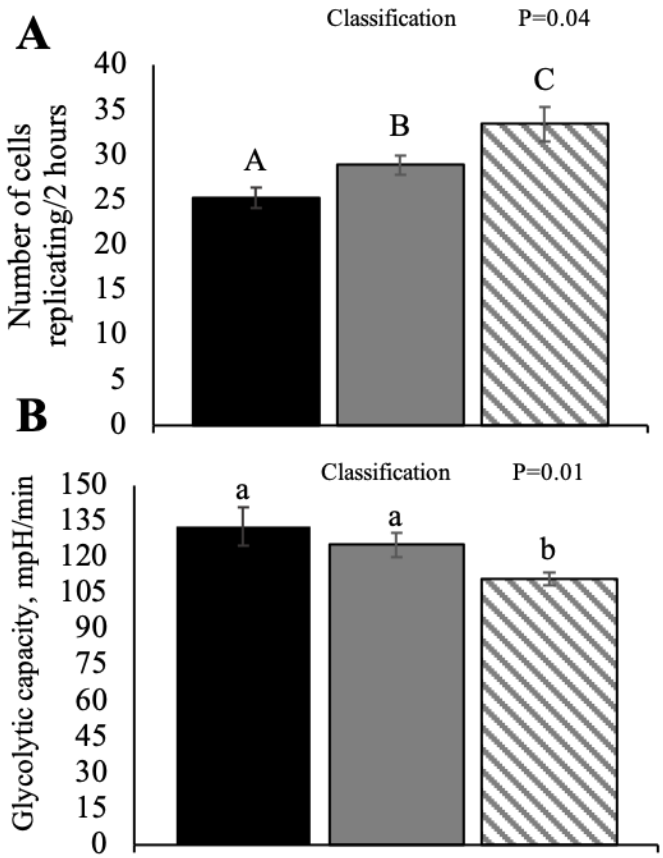

Figure 3: Data are shown for myoblast rate of proliferation (A) and, myoblast glycolytic capacity (B) for calves from cows with differing cyclicity from weaning to breeding: typical, start-stop cyclicity, and non-cycling. Effects of puberty cyclicity classification are noted when significant ($P<0.05$).

Feedlot Growing Phase Diet Ingredients
Corn silage
Soybean Meal
Supplement
NexCorn

Table 1: Dietary ingredients fed to calves during the growing phase in the feedlot.

Feedlot Finishing Phase Diet Ingredients
Grass Hay
Steam Flaked Corn
High Moisture Corn
Dry rolled corn
Modified dried distillers grains
Supplement

Table 2: Dietary ingredients fed to calves during the finishing phase in the feedlot.

REFERENCES

Abney, C. S., J. T. Vasconcelos, J. P. McMeniman, S. A. Keyser, K. R. Wilson, G. J. Vogel, and M. L. Galyean. 2007. Effects of ractopamine hydrochloride on performance, rate and variation in feed intake, and acid-base balance in feedlot cattle. J Anim Sci 85(11):3090-3098. doi: 10.2527/jas.2007-0263

Aldoretta, P. W., and J. William W. Hay. 1999. Effect of glucose supply on ovine uteroplacental glucose metabolism. American Journal of Physiology-Regulatory, Integrative and Comparative Physiology 277(4):R947-R958. doi: 10.1152/ajpregu.1999.277.4.R947

Allen, R. E., R. A. Merkel, and R. B. Young. 1979. Cellular aspect of muscle growth: myogenic cell proliferation. Journal of animal science 49(1):115-127.

Antolic, A., X. Feng, C. E. Wood, E. M. Richards, and M. Keller-Wood. 2015. Increased maternal nighttime cortisol concentrations in late gestation alter glucose and insulin in the neonatal lamb. Physiol Rep 3(9)doi: 10.14814/phy2.12548

Antonelo, D. S., M. R. Mazon, K. E. Z. Nubiato, J. F. M. Gomez, D. J. Brigida, R. C. Gomes, A. S. Netto, P. R. Leme, and S. L. Silva. 2017. Effects of immunocastration and beta-adrenergic agonists on the performance and carcass traits of feedlot finished Nellore cattle. Anim. 11(11):2103-2110. doi: 10.1017/S1751731117000842

Auchus, R. J., T. C. Lee, and W. L. Miller. 1998. Cytochrome b 5 augments the 17, 20-lyase activity of human P450c17 without direct electron transfer. Journal of Biological Chemistry 273(6):3158-3165.

Aurousseau, B., A. Connell, D. Revell, H. Rocha, and G. Lobley. 1993. Hind-limb protein metabolism in growing sheep; effects of acute and chronic infusion of clenbuterol by close arterial and systemic routes. Comparative Biochemistry and Physiology Part C: Pharmacology, Toxicology and Endocrinology 106(2):529-535.

Azziz, R., E. Carmina, D. Dewailly, E. Diamanti-Kandarakis, H. F. Escobar-Morreale, W. Futterweit, O. E. Janssen, R. S. Legro, R. J. Norman, and A. E. Taylor. 2009. The Androgen Excess and PCOS Society criteria for the polycystic ovary syndrome: the complete task force report. Fertility and sterility 91(2):456-488.

Badino, P., R. Odore, S. Pagliasso, A. Schiavone, C. Girardi, and G. Re. 2008. Steroid and beta-adrenergic receptor modifications in target organs of broiler chickens fed with a diet containing beta2-adrenergic agents. Food Chem Toxicol 46(6):2239-2243. doi: 10.1016/j.fct.2008.02.025

Barnes, T. L., C. N. Cadaret, K. A. Beede, T. B. Schmidt, J. L. Petersen, and D. T. Yates. 2019a. Hypertrophic muscle growth and metabolic efficiency were impaired by chronic heat stress, improved by zilpaterol supplementation, and not affected by ractopamine supplementation in feedlot lambs. Journal of animal science 97(10):4101-4113.

Barnes, T. L., C. N. Cadaret, K. A. Beede, T. B. Schmidt, J. L. Petersen, and D. T. Yates. 2019b. Hypertrophic muscle growth and metabolic efficiency were impaired by chronic heat stress, improved by zilpaterol supplementation, and not affected by ractopamine supplementation in feedlot lambs1. J Anim Sci 97(10):4101-4113. doi: 10.1093/jas/skz271

Baszczak, J. A., T. Grandin, S. L. Gruber, T. E. Engle, W. J. Platter, S. B. Laudert, A. L. Schroeder, and J. D. Tatum. 2006. Effects of ractopamine supplementation on behavior of British, Continental, and Brahman crossbred steers during routine handling. J Anim Sci 84(12):3410-3414. doi: 10.2527/jas.2006-167

Beatty, D. T., A. Barnes, E. Taylor, D. Pethick, M. McCarthy, and S. K. Maloney. 2006. Physiological responses of Bos taurus and Bos indicus cattle to prolonged, continuous heat and humidity1. Journal of Animal Science 84(4):972-985. doi: 10.2527/2006.844972x

Beermann, D. 2002. Beta-adrenergic receptor agonist modulation of skeletal muscle growth. Journal of Animal Science 80(E-suppl_1):E18-E23.

Beermann, D., W. Butler, D. Hogue, V. Fishell, R. Dalrymple, C. Ricks, and C. Scanes. 1987. Cimaterol-induced muscle hypertrophy and altered endocrine status in lambs. Journal of Animal Science 65(6):1514-1524.

Biolo, G., X.-J. Zhang, and R. R. Wolfe. 1995. Role of membrane transport in interorgan amino acid flow between muscle and small intestine. Metabolism 44(6):719-724.

Bittner, C., D. Burken, A. Shreck, J. MacDonald, G. Erickson, and N. Pyatt. 2015. Effect of 300 or 400 mg Daily of Ractopamine Hydrochloride on Growth Performance and Carcass Characterisitics of Finishing Steers During the Last 14, 28, or 42 Days. 2015 Nebraska Beef Cattle Report, University of Nebraska-Lincoln Extension, MP101

Bittner, C. J., G. I. Crawford, L. L. Berger, S. Holt, R. R. Pritchard, W. J. Platter, M. T. Van Koevering, N. A. Pyatt, and G. E. Erickson. 2016. Effect of ractopamine hydrochloride (Optaflexx) dose and duration on growth performance and carcass characteristics of finishing steers. J Anim Sci 94(12):5382-5392. doi: 10.2527/jas.2016-0807

Bittner, C. J., M. A. Greenquist, D. B. Burken, A. L. Shreck, J. C. MacDonald, T. J. Klopfenstein, W. J. Platter, M. T. Van Koevering, N. A. Pyatt, and G. E. Erickson. 2017. Evaluation of ractopamine hydrochloride (Optaflexx) on growth performance and carcass characteristics of finishing steers across different feeding durations. J Anim Sci 95(1):485-498. doi: 10.2527/jas.2016.0806

Bourdon, R., and J. Brinks. 1982. Genetic, environmental and phenotypic relationships among gestation length, birth weight, growth traits and age at first calving in beef cattle. J. Anim. Sci. 55(3):543-553.

Boyd, B. M., S. D. Shackelford, K. E. Hales, T. M. Brown-Brandl, M. L. Bremer, M. L. Spangler, T. L. Wheeler, D. A. King, and G. E. Erickson. 2015a. Effects of shade and feeding zilpaterol hydrochloride to finishing steers on performance, carcass quality, heat stress, mobility, and body temperature. J Anim Sci 93(12):5801-5811. doi: 10.2527/jas.2015-9613

Boyd, B. M., S. D. Shackelford, K. E. Hales, T. M. Brown-Brandl, M. L. Bremer, M. L. Spangler, T. L. Wheeler, D. A. King, and G. E. Erickson. 2015b. Effects of shade and feeding zilpaterol hydrochloride to finishing steers on performance, carcass quality, heat stress, mobility, and body temperature1. Journal of Animal Science 93(12):5801-5811. doi: 10.2527/jas.2015-9613

Brown-Brandl, T. M., C. G. Chitko-McKown, R. A. Eigenberg, J. J. Mayer, T. H. Welsh, J. D. Davis, and J. L. Purswell. 2017. Physiological responses of feedlot heifers

provided access to different levels of shade. Anim. 11(8):1344-1353. doi: 10.1017/S1751731116002664

Brown-Brandl, T. M., J. A. Nienaber, R. A. Eigenberg, G. L. Hahn, and H. Freetly. 2003. Thermoregulatory responses of feeder cattle. Journal of Thermal Biology 28(2):149-157. doi: 10.1016/s0306-4565(02)00052-9

Bruusgaard, J. C., I. B. Johansen, I. M. Egner, Z. A. Rana, and K. Gundersen. 2010. Myonuclei acquired by overload exercise precede hypertrophy and are not lost on detraining. Proceedings of the National Academy of Sciences 107(34):15111-15116. doi: 10.1073/pnas.0913935107

Buntyn, J. O., N. C. Burdick Sanchez, T. B. Schmidt, G. E. Erickson, S. E. Sieren, S. J. Jones, and J. A. Carroll. 2016. The metabolic, stress axis, and hematology response of zilpaterol hydrochloride supplemented beef heifers when exposed to a dual corticotropin-releasing hormone and vasopressin challenge. J Anim Sci 94(7):2798-2810. doi: 10.2527/jas.2015-0192

Burghen, G. A., J. R. GIVENS, and A. E. KITABCHI. 1980. Correlation of hyperandrogenism with hyperinsulinism in polycystic ovarian disease. The Journal of Clinical Endocrinology & Metabolism 50(1):113-116.

Byrem, T., T. Robinson, Y. Boisclair, A. Bell, W. Schwark, and D. Beermann. 1992. Analysis and pharmacokinetics of cimaterol in growing Holstein steers. Journal of animal science 70(12):3812-3819.

Byrem, T. M., D. H. Beermann, and T. F. Robinson. 1996. Characterization of dose-dependent metabolic responses to close arterial infusion of cimaterol in the hindlimb of steers. Journal of animal science 74(12):2907-2916.

Cadaret, C. N., K. A. Beede, H. E. Riley, and D. T. Yates. 2017. Acute exposure of primary rat soleus muscle to zilpaterol HCl (β2 adrenergic agonist), TNFα, or IL-6 in culture increases glucose oxidation rates independent of the impact on insulin signaling or glucose uptake. Cytokine 96:107-113. doi: 10.1016/j.cyto.2017.03.014

Cadaret, C. N., E. M. Merrick, T. L. Barnes, K. A. Beede, R. J. Posont, J. L. Petersen, and D. T. Yates. 2019a. Sustained maternal inflammation during the early third-trimester yields intrauterine growth restriction, impaired skeletal muscle glucose metabolism, and diminished beta-cell function in fetal sheep1,2. J Anim Sci 97(12):4822-4833. doi: 10.1093/jas/skz321

Cadaret, C. N., R. J. Posont, K. A. Beede, H. E. Riley, J. D. Loy, and D. T. Yates. 2019b. Maternal inflammation at midgestation impairs subsequent fetal myoblast function and skeletal muscle growth in rats, resulting in intrauterine growth restriction at term. Transl Anim Sci 3(2):867-876. doi: 10.1093/tas/txz037

Capper, J. L., and D. J. Hayes. 2012. The environmental and economic impact of removing growth-enhancing technologies from US beef production. Journal of Animal Science 90(10):3527-3537.

Carnagarin, R., A. M. Dharmarajan, and C. R. Dass. 2015. Molecular aspects of glucose homeostasis in skeletal muscle–A focus on the molecular mechanisms of insulin resistance. Molecular and cellular endocrinology 417:52-62.

Ceelen, J. J. M., A. Schols, A. E. M. Kneppers, R. Rosenbrand, M. M. Drozdz, S. J. van Hoof, C. C. de Theije, M. Kelders, F. Verhaegen, and R. C. J. Langen. 2018. Altered protein turnover signaling and myogenesis during impaired recovery of

inflammation-induced muscle atrophy in emphysematous mice. Sci Rep 8(1):10761. doi: 10.1038/s41598-018-28579-4

Chaze, T., B. Meunier, C. Chambon, C. Jurie, and B. Picard. 2008. In vivo proteome dynamics during early bovine myogenesis. Proteomics 8(20):4236-4248.

Cohen, N., L. Rossetti, P. Shlimovich, M. Halberstam, M. Hu, and H. Shamoon. 1995. Counterregulation of hypoglycemia: skeletal muscle glycogen metabolism during three hours of physiological hyperinsulinemia in humans. Diabetes 44(4):423-430.

Colbert, W. E., P. D. Williams, and G. D. Williams. 1991. β-Adrenoceptor Profile of Ractopamine HCl in Isolated Smooth and Cardiac Muscle Tissues of Rat and Guinea-pig. Journal of Pharmacy and Pharmacology 43(12):844-847. doi: 10.1111/j.2042-7158.1991.tb03192.x

Consolo, N. R., B. S. Mesquita, F. D. Rodriguez, V. G. Rizzi, and L. F. Silva. 2016. Effects of ractopamine hydrochloride and dietary protein content on performance, carcass traits and meat quality of Nellore bulls. Anim. 10(3):539-546. doi: 10.1017/s1751731115001895

Contreras, O., M. Villarreal, and E. Brandan. 2018. Nilotinib impairs skeletal myogenesis by increasing myoblast proliferation. Skeletal muscle 8(1):5.

Crandall, C., T. Wilson, J. Marving, T. Vogelsang, A. Kjaer, B. Hesse, and N. Secher. 2008. Effects of passive heating on central blood volume and ventricular dimensions in humans. The Journal of physiology 586(1):293-301. doi: 10.1113/jphysiol.2007.143057

Crandall, C. G., and J. Gonzalez-Alonso. 2010. Cardiovascular function in the heat-stressed human. Acta Physiol (Oxf) 199(4):407-423. doi: 10.1111/j.1748-1716.2010.02119.x

Cupp, A. S., S. Nafziger, M. Abedal-Majed, S. Tenley, M. Hart, A. Snider, J. Bergman, S. Kurz, J. Davis, and J. Wood. 2019. 206 Attainment and maintenance of pubertal cyclicity may predict high A4 cows with reduced fertility. Journal of Animal Science 97(Supplement_2):118-119.

Dávila-Ramírez, J., U. Macías-Cruz, N. Torrentera-Olivera, H. González-Ríos, S. Soto-Navarro, R. Rojo-Rubio, and L. Avendaño-Reyes. 2014. Effects of zilpaterol hydrochloride and soybean oil supplementation on feedlot performance and carcass characteristics of hair-breed ram lambs under heat stress conditions. Journal of Animal Science 92(3):1184-1192.

DeFronzo, R. A., and D. Tripathy. 2009. Skeletal Muscle Insulin Resistance Is the Primary Defect in Type 2 Diabetes. Diabetes Care 32(suppl 2):S157-S163. doi: 10.2337/dc09-S302

Dogra, C., H. Changotra, S. Mohan, and A. Kumar. 2006. Tumor Necrosis Factor-like Weak Inducer of Apoptosis Inhibits Skeletal Myogenesis through Sustained Activation of Nuclear Factor-κB and Degradation of MyoD Protein. Journal of Biological Chemistry 281(15):10327-10336. doi: 10.1074/jbc.M511131200

Eigenberg, R., T. Brown-Brandl, J. A. Nienaber, and G. L. Hahn. 2005. Dynamic response indicators of heat stress in shaded and non-shaded feedlot cattle, Part 2: Predictive relationships. Biosystems Engineering 91(1):111-118.

Eisemann, J. H., and D. G. Bristol. 1998. Change in Insulin Sensitivity or Responsiveness Is Not a Major Component of the Mechanism of Action of Ractopamine in Beef Steers. The Journal of Nutrition 128(3):505-511. doi: 10.1093/jn/128.3.505

Emenheiser, J. C., S. P. Greiner, R. M. Lewis, and D. R. Notter. 2010. Validation of live animal ultrasonic measurements of body composition in market lambs. J. Anim. Sci. 88(9):2932-2939. doi: 10.2527/jas.2009-2661

Exton, J. H., N. Friedmann, E. H.-A. Wong, J. P. Brineaux, J. D. Corbin, and C. R. Park. 1972. Interaction of glucocorticoids with glucagon and epinephrine in the control of gluconeogenesis and glycogenolysis in liver and of lipolysis in adipose tissue. Journal of Biological Chemistry 247(11):3579-3588.

Faridy, E. E., and W. Z. Yang. 1989. Role of hyperventilation in hypoxia on lung growth in rats. Respir Physiol 76(2):179-190. doi: 10.1016/0034-5687(89)90096-0

Feingold, K. R., and C. Grunfeld. 2016. Effect of inflammation on HDL structure and function. Curr Opin Lipidol 27(5):521-530. doi: 10.1097/mol.0000000000000333

Feingold, K. R., I. Hardardottir, R. Memon, E. Krul, A. Moser, J. Taylor, and C. Grunfeld. 1993. Effect of endotoxin on cholesterol biosynthesis and distribution in serum lipoproteins in Syrian hamsters. Journal of lipid research 34(12):2147-2158.

Feingold, K. R., I. Staprans, R. Memon, A. Moser, J. Shigenaga, W. Doerrler, C. Dinarello, and C. Grunfeld. 1992. Endotoxin rapidly induces changes in lipid metabolism that produce hypertriglyceridemia: low doses stimulate hepatic triglyceride production while high doses inhibit clearance. Journal of lipid research 33(12):1765-1776.

Ferlay, A., and Y. Chilliard. 2018. Responses of body fat mobilization to isoproterenol or epinephrine challenge in adult cows: influence of energy level, breed, and body fatness. J Anim Sci 96(1):331-342. doi: 10.1093/jas/skx020

Gerich, J. E., M. Langlois, C. Noacco, V. Schneider, and P. H. Forsham. 1974. Adrenergic modulation of pancreatic glucagon secretion in man. The Journal of clinical investigation 53(5):1441-1446.

Gibbs, R. L., C. N. Cadaret, R. M. Swanson, K. A. Beede, R. J. Posont, T. B. Schmidt, J. L. Petersen, and D. T. Yates. 2019. Body composition estimated by bioelectrical impedance analyses is diminished by prenatal stress in neonatal lambs and by heat stress in feedlot wethers. Translational Animal Science 3(Supplement_1):1691-1695.

González, F., N. S. Rote, J. Minium, and J. P. Kirwan. 2009. Evidence of proatherogenic inflammation in polycystic ovary syndrome. Metabolism 58(7):954-962.

Gonzalez, J. M., S. E. Johnson, A. M. Stelzleni, T. A. Thrift, J. D. Savell, T. M. Warnock, and D. D. Johnson. 2010. Effect of ractopamine–HCl supplementation for 28 days on carcass characteristics, muscle fiber morphometrics, and whole muscle yields of six distinct muscles of the loin and round. Meat Science 85(3):379-384. doi: https://doi.org/10.1016/j.meatsci.2010.02.004

Günther, S., J. Kim, S. Kostin, C. Lepper, C.-M. Fan, and T. Braun. 2013. Myf5-positive satellite cells contribute to Pax7-dependent long-term maintenance of adult muscle stem cells. Cell stem cell 13(5):590-601.

Hagenmaier, J. A., C. D. Reinhardt, S. J. Bartle, and D. U. Thomson. 2016. Effect of shade on animal welfare, growth performance, and carcass characteristics in large

pens of beef cattle fed a beta agonist in a commercial feedlot. J Anim Sci 94(12):5064-5076. doi: 10.2527/jas.2016-0935

Hagenmaier, J. A., C. D. Reinhardt, M. J. Ritter, M. S. Calvo-Lorenzo, G. J. Vogel, C. A. Guthrie, M. G. Siemens, K. F. Lechtenberg, D. J. Rezac, and D. U. Thomson. 2017. Effects of ractopamine hydrochloride on growth performance, carcass characteristics, and physiological response to different handling techniques. J Anim Sci 95(5):1977-1992. doi: 10.2527/jas.2016.0936

Hahn, G. 1999a. Dynamic responses of cattle to thermal heat loads. Journal of animal science 77(suppl_2):10-20.

Hahn, G. L. 1999b. Dynamic responses of cattle to thermal heat loads. J Anim Sci 77 Suppl 2(suppl_2):10-20. doi: 10.2527/1997.77suppl_210x

Hammon, H. M., S. N. Sauter, M. Reist, Y. Zbinden, C. Philipona, C. Morel, and J. W. Blum. 2003. Dexamethasone and colostrum feeding affect hepatic gluconeogenic enzymes differently in neonatal calves. J Anim Sci 81(12):3095-3106. doi: 10.2527/2003.81123095x

Hogeveen, K. N., P. Cousin, M. Pugeat, D. Dewailly, B. Soudan, and G. L. Hammond. 2002. Human sex hormone–binding globulin variants associated with hyperandrogenism and ovarian dysfunction. The Journal of Clinical Investigation 109(7):973-981. doi: 10.1172/JCI14060

Ibanez, L., N. Potau, P. Chacon, C. Pascual, and A. Carrascosa. 1998. Hyperinsulinaemia, dyslipaemia and cardiovascular risk in girls with a history of premature pubarche. Diabetologia 41(9):1057-1063.

Iguchi, M., A. E. Littmann, S. H. Chang, L. A. Wester, J. S. Knipper, and R. K. Shields. 2012. Heat stress and cardiovascular, hormonal, and heat shock proteins in humans. J Athl Train 47(2):184-190. doi: 10.4085/1062-6050-47.2.184

Jaillard, S., V. Houfflin-Debarge, Y. Riou, T. Rakza, S. Klosowski, P. Lequien, and L. Storme. 2001. Effects of catecholamines on the pulmonary circulation in the ovine fetus. American Journal of Physiology-Regulatory, Integrative and Comparative Physiology 281(2):R607-R614.

James, B. W., M. D. Tokach, R. D. Goodband, J. L. Nelssen, S. S. Dritz, K. Q. Owen, J. C. Woodworth, and R. C. Sulabo. 2013. Effects of dietary L-carnitine and ractopamine HCl on the metabolic response to handling in finishing pigs. J Anim Sci 91(9):4426-4439. doi: 10.2527/jas.2011-4411

Jasuja, R., P. Ramaraj, R. P. Mac, A. B. Singh, T. W. Storer, J. Artaza, A. Miller, R. Singh, W. E. Taylor, M. L. Lee, T. Davidson, I. Sinha-Hikim, N. Gonzalez-Cadavid, and S. Bhasin. 2005. Δ-4-Androstene-3,17-Dione Binds Androgen Receptor, Promotes Myogenesis in Vitro, and Increases Serum Testosterone Levels, Fat-Free Mass, and Muscle Strength in Hypogonadal Men. The Journal of Clinical Endocrinology & Metabolism 90(2):855-863. doi: 10.1210/jc.2004-1577

Jones, R., W. M. Zapol, and L. Reid. 1984. Pulmonary artery remodeling and pulmonary hypertension after exposure to hyperoxia for 7 days. A morphometric and hemodynamic study. Am J Pathol 117(2):273-285.

Kalra, A., S. Nair, and L. Rai. 2006. Association of obesity and insulin resistance with dyslipidemia in Indian women with polycystic ovarian syndrome. Indian journal of medical sciences 60(11):447-453.

Karlsson, H. K., and J. R. Zierath. 2007. Insulin signaling and glucose transport in insulin resistant human skeletal muscle. Cell biochemistry and biophysics 48(2-3):103-113.

Kelm, M., S. Schafer, S. Mingers, M. Heydthausen, M. Vogt, W. Motz, and B. E. Strauer. 1996. Left ventricular mass is linked to cardiac noradrenaline in normotensive and hypertensive patients. J Hypertens 14(11):1357-1364. doi: 10.1097/00004872-199611000-00015

Kinugawa, T., M. Mori, K. Ogino, A. Endo, M. Kato, T. Kato, S. Osaki, A. Ohtahara, O. Igawa, I. Hisatome, and C. Shigemasa. 1999. Hypertensive left ventricular hypertrophy is linked to an enhanced catecholamine response to submaximal exercise. Eur J Clin Invest 29(7):594-602. doi: 10.1046/j.1365-2362.1999.00501.x

Knochenhauer, E. S., T. J. Key, M. Kahsar-Miller, W. Waggoner, L. R. Boots, and R. Azziz. 1998. Prevalence of the Polycystic Ovary Syndrome in Unselected Black and White Women of the Southeastern United States: A Prospective Study1. The Journal of Clinical Endocrinology & Metabolism 83(9):3078-3082. doi: 10.1210/jcem.83.9.5090

Kohn, R. A., M. M. Dinneen, and E. Russek-Cohen. 2005. Using blood urea nitrogen to predict nitrogen excretion and efficiency of nitrogen utilization in cattle, sheep, goats, horses, pigs, and rats1. Journal of Animal Science 83(4):879-889. doi: 10.2527/2005.834879x

Koohmaraie, M., S. Shackelford, N. Muggli-Cockett, and R. Stone. 1991. Effect of the β-adrenergic agonist L644, 969 on muscle growth, endogenous proteinase activities, and postmortem proteolysis in wether lambs. Journal of Animal Science 69(12):4823-4835.

Kuang , S., S. B. Chargé , P. Seale , M. Huh , and M. A. Rudnicki 2006. Distinct roles for Pax7 and Pax3 in adult regenerative myogenesis. Journal of Cell Biology 172(1):103-113. doi: 10.1083/jcb.200508001

Kuang, S., K. Kuroda, F. Le Grand, and M. A. Rudnicki. 2007. Asymmetric self-renewal and commitment of satellite stem cells in muscle. Cell 129(5):999-1010.

Langen, R. C., J. L. Van Der Velden, A. M. Schols, M. C. Kelders, E. F. Wouters, and Y. M. Janssen-Heininger. 2004. Tumor necrosis factor-alpha inhibits myogenic differentiation through MyoD protein destabilization. FASEB J 18(2):227-237. doi: 10.1096/fj.03-0251com

Lefkowitz, R. J., J. M. Stadel, and M. G. Caron. 1983. Adenylate cyclase-coupled beta-adrenergic receptors: structure and mechanisms of activation and desensitization. Annual review of biochemistry 52(1):159-186.

Legro, R. S., A. R. Kunselman, and A. Dunaif. 2001. Prevalence and predictors of dyslipidemia in women with polycystic ovary syndrome. The American journal of medicine 111(8):607-613.

Lopes, D. I., M. G. Sousa, A. T. Ramos, and V. M. Maruo. 2016. Cardiotoxicity of Senna occidentalis in sheep (Ovis aries). Open Vet J 6(1):30-35. doi: 10.4314/ovj.v6i1.5

López-Carlos, M., R. Ramírez, J. Aguilera-Soto, A. Plascencia, H. Rodríguez, C. Aréchiga, R. Rincón, C. Medina-Flores, and H. Gutierrez-Bañuelos. 2011. Effect of two beta adrenergic agonists and feeding duration on feedlot performance and carcass characteristics of finishing lambs. Livestock Science 138(1-3):251-258.

Lundy, E. L., D. D. Loy, and S. L. Hansen. 2015. Influence of distillers grains resulting from a cellulosic ethanol process utilizing corn kernel fiber on nutrient digestibility of lambs and steer feedlot performance. J Anim Sci 93(5):2265-2274. doi: 10.2527/jas.2014-8572

Mader, T. L. 2003. Environmental stress in confined beef cattle1. J. Anim. Sci. 81(14_suppl_2):E110-E119. doi: 10.2527/2003.8114_suppl_2E110x

Mahjoubi, E., H. Amanlou, M. Hossein Yazdi, N. Aghaziarati, G. R. Noori, C. I. Vahl, B. J. Bradford, and L. H. Baumgard. 2016. A supplement containing multiple types of gluconeogenic substrates alters intake but not productivity of heat-stressed Afshari lambs. J Anim Sci 94(6):2497-2505. doi: 10.2527/jas.2015-9697

Mahjoubi, E., M. H. Yazdi, N. Aghaziarati, G. R. Noori, O. Afsarian, and L. H. Baumgard. 2015. The effect of cyclical and severe heat stress on growth performance and metabolism in Afshari lambs. J Anim Sci 93(4):1632-1640. doi: 10.2527/jas.2014-8641

Maurizi, G., L. Della Guardia, A. Maurizi, and A. Poloni. 2018. Adipocytes properties and crosstalk with immune system in obesity-related inflammation. J Cell Physiol 233(1):88-97. doi: 10.1002/jcp.25855

Mendoza, S. M., R. D. Boyd, C. E. Zier-Rush, P. R. Ferket, K. D. Haydon, and E. van Heugten. 2017. Effect of natural betaine and ractopamine HCl on whole-body and carcass growth in pigs housed under high ambient temperatures. J Anim Sci 95(7):3047-3056. doi: 10.2527/jas.2017.1622

Mersmann, H. J. 1998. Overview of the effects of β-adrenergic receptor agonists on animal growth including mechanisms of action. Journal of animal science 76(1):160-172.

Meyer, D. L., M. S. Kerley, E. L. Walker, D. H. Keisler, V. L. Pierce, T. B. Schmidt, C. A. Stahl, M. L. Linville, and E. P. Berg. 2005. Growth rate, body composition, and meat tenderness in early vs. traditionally weaned beef calves. J Anim Sci 83(12):2752-2761. doi: 10.2527/2005.83122752x

Miller, W. L., and R. J. Auchus. 2011. The molecular biology, biochemistry, and physiology of human steroidogenesis and its disorders. Endocrine reviews 32(1):81-151.

Mitlöhner, F. M., J. L. Morrow, J. W. Dailey, S. C. Wilson, M. L. Galyean, M. F. Miller, and J. J. McGlone. 2001. Shade and water misting effects on behavior, physiology, performance, and carcass traits of heat-stressed feedlot cattle1. Journal of Animal Science 79(9):2327-2335. doi: 10.2527/2001.7992327x

Moloney, A., P. Allen, D. Ross, G. Olson, and E. Convey. 1990. Growth, feed efficiency and carcass composition of finishing Friesian steers fed the β-adrenergic agonist L-644,969. journal of Animal Science 68(5):1269-1277.

Muroya, S., I. Nakajima, and K. Chikuni. 2002. Sequential expression of myogenic regulatory factors in bovine skeletal muscle and the satellite cell culture. Animal Science Journal 73(5):375-381. doi: 10.1046/j.1344-3941.2002.00052.x

Nienaber, J., and G. Hahn. 2007. Livestock production system management responses to thermal challenges. International Journal of Biometeorology 52(2):149-157.

Norman, A. W., and H. L. Henry. 2014. Hormones. Elsevier Science.

Nourozi, M., M. Abazari, M. Raisianzadeh, M. Mohammadi, and A. ZareShahne. 2008. Effect of terbutaline and metaproterenol (two beta-adrenergic agonists) on

performance and carcass composition of culled Moghani ewes. Small Ruminant Research 74(1-3):72-77.

O'Connor, R., W. Butler, K. Finnerty, D. Hogue, and D. Beermann. 1991. Acute and chronic hormone and metabolite changes in lambs fed the beta-agonist, cimaterol. Domestic Animal Endocrinology 8(4):537-548.

O'Brien, M., R. Rhoads, S. Sanders, G. Duff, and L. Baumgard. 2010. Metabolic adaptations to heat stress in growing cattle. Domestic animal endocrinology 38(2):86-94.

Odore, R., P. Badino, R. Barbero, B. Cuniberti, S. Pagliasso, C. Girardi, and G. Re. 2007. Regulation of tissue beta-adrenergic, glucocorticoid and androgen receptors induced by repeated exposure to growth promoters in male veal calves. Res Vet Sci 83(2):227-233. doi: 10.1016/j.rvsc.2006.12.011

Ohtsu, H., M. Yamazaki, H. Abe, H. Murakami, and M. Toyomizu. 2015. Heat Stress Modulates Cytokine Gene Expression in the Spleen of Broiler Chickens. The Journal of Poultry Science 52(4):282-287. doi: 10.2141/jpsa.0150062

Oney, C., M. Streeter, W. Nichols, B. Dicke, R. Cooper, D. Jordon, T. Scott, E. Larson, S. McNeley, and R. Raymond. 2018. Effects of using increasingly aggressive implant protocols on feedlot performance and carcass characteristics of calf-fed steers. The Professional Animal Scientist 34(4):379-389.

Perry, R., L. Corah, R. Cochran, J. Brethour, K. Olson, and J. Higgins. 1991. Effects of hay quality, breed, and ovarian development on onset of puberty and reproductive performance of beef heifers. Journal of production agriculture 4(1):13-18.

Phillippo, M., W. Humphries, T. Atkinson, G. Henderson, and P. Garthwaite. 1987. The effect of dietary molybdenum and iron on copper status, puberty, fertility and oestrous cycles in cattle. The Journal of Agricultural Science 109(2):321-336.

Picard, B., L. Lefaucheur, C. Berri, and M. J. Duclos. 2002. Muscle fibre ontogenesis in farm animal species. Reproduction Nutrition Development 42(5):415-431.

Posont, R., K. Beede, S. Limesand, and D. Yates. 2018a. Changes in myoblast responsiveness to TNFa and IL-6 contribute to decreased skeletal muscle mass in intrauterine growth restricted fetal sheep. Translational Animal Science 2(Suppl1):S44-S47. doi: 10.1093/tas/txy038

Posont, R. J., K. A. Beede, S. W. Limesand, and D. T. Yates. 2018b. Changes in myoblast responsiveness to TNFα and IL-6 contribute to decreased skeletal muscle mass in intrauterine growth restricted fetal sheep. Translational animal science 2(suppl_1):S44-S47.

Posont, R. J., C. N. Cadaret, K. A. Beede, J. K. Beard, R. M. Swanson, R. L. Gibbs, J. L. Petersen, and D. T. Yates. 2019. Maternal inflammation at 0.7 gestation in ewes leads to intrauterine growth restriction and impaired glucose metabolism in offspring at 30 d of age. Translational Animal Science 3(Supplement_1):1673-1677.

Rasmussen, B. B., E. Volpi, D. C. Gore, and R. R. Wolfe. 2000. Androstenedione does not stimulate muscle protein anabolism in young healthy men. The Journal of Clinical Endocrinology & Metabolism 85(1):55-59.

Reeds, P., S. Hay, P. Dorward, and R. Palmer. 1988. The effect of β-agonists and antagonists on muscle growth and body composition of young rats (Rattus sp.).

Comparative Biochemistry and Physiology Part C: Comparative Pharmacology 89(2):337-341.

Rehfeldt, C., I. Fiedler, G. Dietl, and K. Ender. 2000. Myogenesis and postnatal skeletal muscle cell growth as influenced by selection. Livestock Production Science 66(2):177-188.

Remels, A. H., H. R. Gosker, J. Bakker, D. C. Guttridge, A. M. Schols, and R. C. Langen. 2013. Regulation of skeletal muscle oxidative phenotype by classical NF-kappaB signalling. Biochim Biophys Acta 1832(8):1313-1325. doi: 10.1016/j.bbadis.2013.03.018

Remels, A. H., H. R. Gosker, P. Schrauwen, P. P. Hommelberg, P. Sliwinski, M. Polkey, J. Galdiz, E. F. Wouters, R. C. Langen, and A. M. Schols. 2010. TNF-alpha impairs regulation of muscle oxidative phenotype: implications for cachexia? FASEB J 24(12):5052-5062. doi: 10.1096/fj.09-150714

Renaudeau, D., A. Collin, S. Yahav, V. de Basilio, J. L. Gourdine, and R. J. Collier. 2012. Adaptation to hot climate and strategies to alleviate heat stress in livestock production. Anim. 6(5):707-728. doi: 10.1017/s1751731111002448

Rhoads, R. P., L. H. Baumgard, S. W. El-Kadi, and L. D. Zhao. 2016. PHYSIOLOGY AND ENDOCRINOLOGY SYMPOSIUM: Roles for insulin-supported skeletal muscle growth. J Anim Sci 94(5):1791-1802. doi: 10.2527/jas.2015-0110

Rosenson, R. S., D. A. Wolff, A. L. Huskin, I. B. Helenowski, and A. W. Rademaker. 2007. Fenofibrate therapy ameliorates fasting and postprandial lipoproteinemia, oxidative stress, and the inflammatory response in subjects with hypertriglyceridemia and the metabolic syndrome. Diabetes Care 30(8):1945-1951.

Sani, C. M., E. P. L. Pogue, J. B. Hrabia, A. G. Zayachkowski, M. M. Zawadka, A. G. Poniatowski, D. Dlugosz, W. Lesniak, O. Kruszelnicka, B. Chyrchel, and A. Surdacki. 2018. Association between low-grade chronic inflammation and depressed left atrial compliance in heart failure with preserved ejection fraction: A retrospective analysis. Folia Med Cracov 58(2):45-55. doi: 10.24425/fmc.2018.124657

Santana, P. F., V. R. Rocha Junior, J. R. M. Ruas, F. P. Moncao, L. A. Borges, T. E. S. Sousa, F. V. E. Silva, W. O. Rabelo, C. Carvalho, and E. C. J. Sales. 2019. Nutritional efficiency of feed-restricted F1 Holstein/Zebu cows during the middle third of lactation. Asian-Australas J Anim Sci:236-244. doi: 10.5713/ajas.18.0795

Sanz Fernandez, M. V., S. K. Stoakes, M. Abuajamieh, J. T. Seibert, J. S. Johnson, E. A. Horst, R. P. Rhoads, and L. H. Baumgard. 2015. Heat stress increases insulin sensitivity in pigs. Physiol Rep 3(8)doi: 10.14814/phy2.12478

Sears, M. R. 2002. Adverse effects of β-agonists. Journal of Allergy and Clinical Immunology 110(6):S322-S328. doi: 10.1067/mai.2002.129966

Sewell, J. R., L. L. Berger, T. G. Nash, M. J. Cecava, P. H. Doane, J. L. Dunn, M. K. Dyer, and N. A. Pyatt. 2009. Nutrient digestion and performance by lambs and steers fed thermochemically treated crop residues. J Anim Sci 87(3):1024-1033. doi: 10.2527/jas.2008-0974

Shappell, N., V. Feil, D. Smith, G. Larsen, and D. McFarland. 2000. Response of C2C12 mouse and turkey skeletal muscle cells to the β-adrenergic agonist ractopamine. Journal of animal science 78(3):699-708.

Singh, R., J. N. Artaza, W. E. Taylor, N. F. Gonzalez-Cadavid, and S. Bhasin. 2003. Androgens Stimulate Myogenic Differentiation and Inhibit Adipogenesis in C3H 10T1/2 Pluripotent Cells through an Androgen Receptor-Mediated Pathway. Endocrinology 144(11):5081-5088. doi: 10.1210/en.2003-0741

Siński, M., J. Lewandowski, A. Dobosiewicz, J. Przybylski, P. Abramczyk, and Z. Gaciong. 2016. The effect of hyperoxia on central blood pressure in healthy subjects. Archives of medical science : AMS 12(5):992-999. doi: 10.5114/aoms.2015.49038

Smith, S., D. Garcia, and D. Anderson. 1989. Elevation of a specific mRNA in longissimus muscle of steers fed ractopamine. Journal of animal science 67(12):3495-3502.

Smith, S. B., S. K. Davis, J. J. Wilson, R. T. Stone, F. Wu, D. K. Garcia, D. K. Lunt, and A. M. Schiavetta. 1995. Bovine fast-twitch myosin light chain 1: cloning and mRNA amount in muscle of cattle treated with clenbuterol. American Journal of Physiology-Endocrinology and Metabolism 268(5):E858-E865.

St-Pierre, N., B. Cobanov, and G. Schnitkey. 2003. Economic losses from heat stress by US livestock industries. Journal of dairy science 86:E52-E77.

Summers, A. F., W. E. Pohlmeier, K. M. Sargent, B. D. Cole, R. J. Vinton, S. G. Kurz, R. M. McFee, R. A. Cushman, A. S. Cupp, and J. R. Wood. 2014. Altered theca and cumulus oocyte complex gene expression, follicular arrest and reduced fertility in cows with dominant follicle follicular fluid androgen excess. PLoS One 9(10):e110683. doi: 10.1371/journal.pone.0110683

Tait, R. G. 2016. Ultrasound Use for Body Composition and Carcass Quality Assessment in Cattle and Lambs. Veterinary Clinics of North America: Food Animal Practice 32(1):207-218. doi: https://doi.org/10.1016/j.cvfa.2015.09.007

Valente, E. E. L., M. L. Chizzotti, C. V. R. Oliveira, M. C. Galvão, S. S. Domingues, A. C. Rodrigues, M. M. Ladeira, R. A. Ferreira, and F. H. M. Chizzotti. 2013. Effect of heat stress on intake and metabolism of Bos taurus (Angus) and Bos indicus (Nellore). In: J. W. Oltjen, E. Kebreab and H. Lapierre, editors, Energy and protein metabolism and nutrition in sustainable animal production: 4th International Symposium on Energy and Protein Metabolism and Nutrition Sacramento, California, USA 9-12 September 2013. Wageningen Academic Publishers, Wageningen. p. 291-292.

van Hooff, M. H., F. J. Voorhorst, M. B. Kaptein, R. A. Hirasing, C. Koppenaal, and J. Schoemaker. 1999. Endocrine features of polycystic ovary syndrome in a random population sample of 14-16 year old adolescents. Hum Reprod 14(9):2223-2229. doi: 10.1093/humrep/14.9.2223

Victoria Sanz Fernandez, M., J. S. Johnson, M. Abuajamieh, S. K. Stoakes, J. T. Seibert, L. Cox, S. Kahl, T. H. Elsasser, J. W. Ross, S. C. Isom, R. P. Rhoads, and L. H. Baumgard. 2015. Effects of heat stress on carbohydrate and lipid metabolism in growing pigs. Physiol Rep 3(2)doi: 10.14814/phy2.12315

Voet, D., J. G. Voet, and C. W. Pratt. 2013. Fundamentals of biochemistry: life at the molecular level.

Wallace, M. B., J. LIM, A. CUTLER, and L. BUCCI. 1999. Effects of dehydroepiandrosterone vs androstenedione supplementation in men. Medicine & Science in Sports & Exercise 31(12):1788.

Wheeler, J., J. Bennett, and J. Hutchinson. 1972. Effect of ambient temperature and daylength on hoof growth in sheep. The Journal of Agricultural Science 79(1):91-97.

Wheeler, T., and M. Koohmaraie. 1992. Effects of the β-adrenergic agonist L644, 969 on muscle protein turnover, endogenous proteinase activities, and meat tenderness in steers. journal of Animal Science 70(10):3035-3043.

Wheelock, J. B., R. P. Rhoads, M. J. VanBaale, S. R. Sanders, and L. H. Baumgard. 2010. Effects of heat stress on energetic metabolism in lactating Holstein cows1. Journal of Dairy Science 93(2):644-655. doi: https://doi.org/10.3168/jds.2009-2295

Wolfe, R. R. 2006. The underappreciated role of muscle in health and disease. Am. J. Clin. Nutr. 84(3):475-482. doi: 10.1093/ajcn/84.3.475

Yang, H., F. Li, X. Kong, X. Yuan, W. Wang, R. Huang, T. Li, M. Geng, G. Wu, and Y. Yin. 2012. Chemerin regulates proliferation and differentiation of myoblast cells via ERK1/2 and mTOR signaling pathways. Cytokine 60(3):646-652.

Yang, Y. T., and M. A. McElligott. 1989. Multiple actions of beta-adrenergic agonists on skeletal muscle and adipose tissue. Biochemical Journal 261(1):1.

Yates, D. T., L. E. Camacho, A. C. Kelly, L. V. Steyn, M. A. Davis, A. T. Antolic, M. J. Anderson, R. Goyal, R. E. Allen, K. K. Papas, W. W. Hay, Jr., and S. W. Limesand. 2019. Postnatal beta2 adrenergic treatment improves insulin sensitivity in lambs with IUGR but not persistent defects in pancreatic islets or skeletal muscle. J Physiol 597(24):5835-5858. doi: 10.1113/JP278726

Yates, D. T., D. S. Clarke, A. R. Macko, M. J. Anderson, L. A. Shelton, M. Nearing, R. E. Allen, R. P. Rhoads, and S. W. Limesand. 2014. Myoblasts from intrauterine growth-restricted sheep fetuses exhibit intrinsic deficiencies in proliferation that contribute to smaller semitendinosus myofibres. The Journal of Physiology 592(14):3113-3125. doi: 10.1113/jphysiol.2014.272591

Yazdi, M. H., H. R. Mirzaei-Alamouti, H. Amanlou, E. Mahjoubi, A. Nabipour, N. Aghaziarati, and L. H. Baumgard. 2016. Effects of heat stress on metabolism, digestibility, and rumen epithelial characteristics in growing Holstein calves. J Anim Sci 94(1):77-89. doi: 10.2527/jas.2015-9364

Yildirim, B., N. Sabir, and B. Kaleli. 2003. Relation of intra-abdominal fat distribution to metabolic disorders in nonobese patients with polycystic ovary syndrome. Fertility and Sterility 79(6):1358-1364. doi: 10.1016/S0015-0282(03)00265-6

Zhao, L., R. P. McMillan, G. Xie, S. Giridhar, L. H. Baumgard, S. El-Kadi, J. Selsby, J. Ross, N. Gabler, M. W. Hulver, and R. P. Rhoads. 2018. Heat stress decreases metabolic flexibility in skeletal muscle of growing pigs. Am J Physiol Regul Integr Comp Physiol 315(6):R1096-r1106. doi: 10.1152/ajpregu.00404.2017