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Evaluation of the Interaction of Beta-Adrenergic Agonists Supplementation and Heat Stress on Growth Performance and Carcass Composition in Feeder Lambs

Lauren Elisabeth Kett
University of Nebraska-Lincoln, lkett@huskers.unl.edu

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EVALUATION OF THE INTERACTION OF BETA - ADRENERGIC AGONISTS

SUPPLEMENTATION AND HEAT STRESS ON GROWTH PERFORMANCE AND

CARCASS COMPOSITION IN FEEDER LAMBS

by

Lauren E. Kett

A THESIS

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EVALUATION OF THE INTERACTION OF BETA - ADRENERGIC AGONISTS
SUPPLEMENTATION AND HEAT STRESS ON GROWTH PERFORMANCE AND
CARCASS COMPOSITION IN FEEDER LAMBS

Lauren Elisabeth Kett, M.S.

University of Nebraska, 2018

Advisor: Ty B. Schmidt

Forty-nine crossbred feeder lambs (wethers, n = 49; 53.3 ± 3.7 kg BW) were utilized to evaluate the interaction of β - adrenergic agonist (βAA) supplementation and heat stress on growth performance and carcass composition. Utilizing and 3 x 2 factorial design, lambs were randomly assigned to one of three βAA supplementation: 1) Control, CON, 2) Ractopamine Hydrochloride at 40 mg/hd/d, RHCL, and Zilpaterol Hydrochloride at 2.5 mg/hd/d, ZHCL for a period of 20 d and one of two environmental conditions (Thermal Neutral: TN and Heat Stress: HS). The TN environment had a constant thermal heat index (THI) of 16.6°C. Within the HS environment, a cyclic design was utilized to achieve a THI of 29.5ºC from 10:00 to 20:00 h and a THI of 24.5ºC from 22:00 to 08:00 h. Starting at 08:01 and continuing to 09:59 h, temperature and RH were gradually increased to achieve a THI of 29.5ºC at 10:00 h and reduction of temperature and RH from 20:01 to 21:59 h to achieve a THI of 24.5ºC at 22:00 h. Regardless of βAA supplementation ($P = \geq 0.09$), lambs exposed to the HS environment had reduced DMI ($P < 0.001$), ADG ($P = 0.002$), and final BW ($P = 0.03$). In addition, exposure to the HS environment (regardless of βAA supplementation; $P = \geq 0.07$)

decreased HCW ($P < 0.001$), percent change in LM area ($P = 0.004$) and percent change in LM depth ($P = 0.005$). There was a βAA x environment interaction associated with RHCL supplementation and heat stress ($P = 0.003$). Lambs supplemented RHCL in the HS environment had reduced (P = 0.003) respiration rates, when compared to CON and ZHCL supplemented lambs. Supplementation of ZHCL decreased adipose tissue ($P = 0.05$) and increased percent fat free lean ($P = 0.01$), when compared to RHCL and CON lambs. Within the current study, both heat stress and βAA supplementation had an impact on growth performance and carcass composition. However, the data does not indicate that there was any significant interaction between βAA supplementation within a heat stress environment on growth performance or carcass composition in feeder lambs.

DEDICATION

I would like to dedicate this thesis to my parents John and Andrea Kett, as well as my mentor Dr. Jane Ann Boles. My parents are a huge driving force in my life and always push me to try my hardest and achieve my goals. Without them I would never have made it to my first 4-H meeting where my passion for agriculture began. Through 4-H and FFA my passion for agriculture grew and lead me to Montana State University where I got to know Dr. Jane Ann Boles. Thanks to Jane Ann I found my passion for meat science. She pushed me to not only know the information long enough to pass one of her exams, but to understand, remember, and apply the information I learned in class to use in life. Without her I would not have made it to graduate school, where my passion for agriculture continued to grow. So, to Dad, Mom, and Jane Ann thank you for your strong personalities that I look up to daily, and I love you all for your guidance and wisdom on my adventure through life.

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From the valley of California, to the mountains in Montana, to the corn fields of Nebraska, I never imagined that I would move to the Midwest. However, my passions for agriculture lead me to the land of corn and cattle. I came to Nebraska with the idea of working with cattle (which was an amazing thought); however, that did not quite happen. After working with sheep, swine, and carcasses I have to say my time in Nebraska has been an educational adventure. The University of Nebraska - Lincoln has brought me so many experiences, opportunities, and connections that I could not have gotten elsewhere.

I would like to thank my committee for all their assistance, guidance, help, and laughs throughout my time here. I have had the opportunity to work with a variety of professors diverse in the world of animal science. My committee includes Dr. Ty Schmidt, Dr. Jessica Petersen, and Dr. Steve Jones. I would like to thank Dr. Schmidt for taking me on as his student for the past two years. Working with Dr. Schmidt has brought many opportunities of collaboration with new and exciting work. He is one person who is full of ideas and continues to keep pushing forward in the world of animal science. Second, I would like to thank Dr. Petersen for all her help during the time on and off our research project, our gossip about current TV shows, and her long list of jokes. Lastly, I would like to thank Dr. Jones for all his talks that made me miss the mountains, and for taking me in as a teaching assistant in his undergraduate classes where I broadened my knowledge on animal production, harvest, and fabrication.

During my time at UNL I have had time to meet and work with numerous graduate students, undergraduates, staff, and faculty. With their knowledge, advice, guidance, and help I was able to build lifelong relationships, and can leave UNL with a

broad view on animal science. I would also like to thank all the students and staff who

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days of dissecting carcasses. Without their jokes, smiles, encouragement, and help I

might still be dissecting lamb carcasses today. My time at UNL will be one to

remember! GO BIG RED!

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

CHAPTER I

INTRODUCTION

To improve growth and efficiency of livestock different growth enhancement technologies are utilized, including the use of β – adrenergic agonists (βAA). Two βAA approved for use in livestock are ractopamine hydrochloride (RHCL), a β_1 – adrenergic agonist, and zilpaterol hydrochloride (ZHCL), a β_2 - adrenergic agonist. β – adrenergic agonists are phenethanolamines, similar to the endogenous catecholamines epinephrine and norepinephrine that function as energy repartitioning agents (Pearson and Dutson, 1991). Supplementation of a βAA increases final live weight, increases ADG, improves G:F, and increases HCW when supplemented to feedlot cattle (Lean et al., 2014). Additionally, supplementation of RHCL to finishing swine and supplementation of RHCL or ZHCL in lambs resulted in improved growth performance and feed efficiency (Garbossa et al., 2013; Lopez-Carlos et al., 2010). With improvements in growth performance and carcass merit βAA can serve as a valuable tool for the efficiency and sustainability of livestock production not only in the US, but worldwide.

Heat stress is the result of an imbalance between heat load and heat dissipation of an object and its environment. A homeostatic imbalance during heat stress causes heat load to be greater than the amount of heat loss (Mahesh Singh et al., 2016). Decreases in performance due to heat stress include decreased feed intake to decrease metabolic heat production, which allows the animal to cope with the surrounding environmental heat (Mitlöhner et al., 2001). Belasco et al. (2015) reported a 10% decrease in ADG and a 9.9% increase in G:F as cattle spent more time within feedlots when temperatures were at

extremes. When exposed to heat stress, mortality rate in cattle within feedlots increased

by 0.5% and cattle profits resulted in a $78/hd loss (Belasco et al., 2015). In 1999, high

heat and humidity in Nebraska resulted in more than 5,000 cattle deaths and a $21.5 to

$35 million loss in cattle production (Hungerford et al., 2000). Due to this negative

economic impact it is important to find ways to monitor and alleviate heat stress to ensure

there is a positive impact on growth and animal well-being. Therefore, the following

literature review evaluates the overall effects of βAA supplementation and heat stress on

performance and production.

CHAPTER II

LITERATURE REVIEW

I. Muscle Growth:

The rate and efficiency at which skeletal muscle grows is vital to ensure biological functionality within the living animal and the sustainable production of high-quality meat. Skeletal muscle growth can be achieved through two distinct growth phases, hyperplasia and hypertrophy. Muscle hyperplasia is the proliferation of muscle cells and occurs primarily during prenatal development (te Pas et al., 2004). Prenatal hyperplasia ultimately determines the number of muscle fibers present at birth. Muscle hypertrophy is the enlargement (length and circumference) of individual muscle fibers. Being a postmitotic tissue, the majority of muscle growth postnatally is achieved via hypertrophy (te Pas et al., 2004). Satellite cells are mitotically active cells that when activated proliferate and fuse to existing myofibres to cause an increase in muscle volume (Moss and LeBlond, 1971). Muscle hypertrophy is also a result of alterations in protein accretion when the rate of protein synthesis exceeds the rate of protein degradation. During maturational hypertrophy, or hypertrophy in response to various stimuli, this change in protein accretion is controlled through changes in the circulating concentrations of anabolic hormones.

The rates of protein synthesis and protein degradation are important to the regulation of protein turnover (Demling, 2005). Anabolic hormones are key hormones during energy and protein regulation. Major regulation hormones include, but are not limited to, insulin, growth hormone, and insulin-like growth factor – I (Demling, 2005).

Insulin is produced by the β cells within the islets of Langerhans located within the pancreas (Swatland, 1994). Insulin secretion is stimulated by an increased concentration of glucose in blood (Nelson and Cox, 2013). While insulin receptors are located throughout the body, the primary target locations include the liver, muscle tissue, and adipose tissue (Norman and Henry, 2015). When insulin binds to insulin receptors on muscle and liver this stimulates the uptake of glucose and increases production of glycogen (Nelson and Cox, 2013). Binding of insulin to the insulin receptors on muscle tissue also stimulates the uptake and utilization of amino acids to stimulate protein synthesis (Swatland, 1994; Demling, 2005).

Growth hormone (GH) is a peptide hormone produced by somatotroph cells in the anterior pituitary that stimulate the production of insulin-like growth factor I (IGF-I) to stimulate satellite cell proliferation and increases lipolysis and protein synthesis (Norman and Henry, 2015; te Pas et al., 2004). Insulin-like growth factor – I is a peptide hormone that causes proliferation and differentiation during prenatal development, and hypertrophy in postnatal development (te Pas et al., 2004). Neural pathways control secretion of GH and are stimulated by growth hormone releasing hormone, and are inhibited by somatostatin (Norman and Henry, 2015). When secreted into circulation, GH binds to growth hormone receptors (GHR) located on the membranes of tissues such as the liver, muscle, and adipose (Demling, 2005). When GH binds to the liver, IGF – I is synthesized and secreted into circulation. Approximately 98% of circulating IGF-I bind to IGF binding proteins causing a stimulation of amino acid uptake, increased protein synthesis and decreased protein degradation (Norman and Henry, 2015; Demling, 2005; te Pas et al., 2004).

II. Adrenergic Receptors:

For more than 50 years, growth enhancement technology has been investigated and utilized to repartition energy to improve growth in livestock. Ahlquist (1948) was one of the first to introduce two classes of adrenergic receptors: the α - and β-adrenergic receptors. He suggested that the α – receptor associates with excitatory responses like vasoconstriction, while the β – receptor associates with inhibitory responses like vasodilation. Thus, each adrenergic response is highly depended on the sub-type and location of the receptor (Beerman, 2002). Adrenergic receptors are divided into two classes, α (αAR) and β (βAR). α – adrenergic receptors consist of two subclasses, α_1 and α_2, and βAR consist of three subclasses, β_1, β_2, and β_3 (Pearson and Dutson, 1991). β – adrenergic receptors are located on most mammalian cell plasma membranes; however, some tissues have a greater affinity for specific βAR. β_1 – adrenergic receptors are prominent in cardiac tissue, β_2AR in bronchial, skeletal muscle, and adipose tissue, and β_3AR in brown adipose (Mersmann, 1998).

Adrenergic receptors are ubiquitous receptors belonging to the seven-transmembrane receptor superfamily which signals through a heterotrimeric G-protein (Rasmussen et al, 2011). The seven-transmembrane structure includes seven hydrophobic domains and exposed hydrophilic loops, composed of amino acids, which anchor into the cells plasma membrane (Norman and Henry, 2015). G-proteins are heterotrimeric and consist of three subunits (α, β, and γ) that mediate cellular responses (Rasmussen et al., 2011). Cellular responses are dependent on the specificity of the G_α-protein: either $G_s\alpha$, the stimulatory response, or $G_i\alpha$, the inhibitory response. These

specific G_α- proteins are responsible for the stimulation or inhibition of adenylate cyclase and the production of cyclic adenosine monophosphate (cAMP) from ATP (Norman and Henry, 2015).

The three subtypes of βAR work in a similar manner when bound to natural or synthetic substances to signal a response. β – adrenergic agonists enter the body through oral ingestion and travel through the circulatory system. Once a βAA binds to a βAR of the target cell, guanosine diphosphate (GDP) releases and guanosine triphosphate (GTP) binds causing the α-subunit to dissociate from the β and γ subunits of the G_s-protein (Norris and Carr, 2013). The dissociated $G_s\alpha$ subunit binds to the catalytic portion of the enzyme adenylyl cyclase to produce cAMP from ATP (Norris and Carr, 2013). Cyclic adenosine monophosphate is a secondary messenger that initiates intracellular responses to amplify a signal from the first messenger at the receptor of the G-protein (Norris and Carr, 2013). When concentrations of cAMP increase, protein kinase A is activated releasing different catalytic subunits to phosphorylate intracellular proteins to elicit cell responses (Mersmann, 1995; Mills, 2002; Norman and Henry, 2015). Protein kinase A is a cytosolic enzyme that phosphorylates enzymes in the cell to activate enzymatic breakdown of glycogen to glucose-phosphate, along with activation of hormone-sensitive lipase in adipose cells. This process provides energy for muscle cells and liver cells and production of non-esterified fatty acids from fat cells (Norris and Carr, 2013). In livestock production this mode of action is to increase lean muscle mass through increased protein accretion and decrease adipose tissue through increased adipose degradation (Mersmann, 1998).

III. β - Adrenergic Agonists:

β – adrenergic agonists are synthetic phenethanolamines similar to the neurotransmitter norepinephrine and adrenal medullary hormone epinephrine (Pearson and Dutson, 1991). Norepinephrine is synthesized by sympathetic postganglionic fibers, while epinephrine is produced by the adrenal medulla. Neuroendocrine cells in the adrenal gland, also known as chromaffin cells, produce 80% of circulating epinephrine, and 20% of circulating norepinephrine (Costanzo, 2015). Once produced, chromaffin granules, storage vesicles located in sympathetic nerve endings, store epinephrine and norepinephrine until signaled for release (Sherwood et al., 2013). Both epinephrine and norepinephrine are important during stress responses, for circulation control, and energy metabolism. The affinity for epinephrine and norepinephrine to bind to adrenergic receptors depends on the type and location of the receptor. Epinephrine binds to α_1, α_2, β_1, and β_2 receptors, while norepinephrine binds to β_1 receptors, along with α_1 and α_2 receptors with greater affinity than epinephrine (Sherwood et al., 2013). The binding of catecholamines increases vasoconstriction through α_1-receptors, while epinephrine increases vasodilation through β_2 – receptors (Sherwood et al., 2013). Therefore, a cellular response is dependent on what substrate binds to a specific receptor on a target cell.

Two βAA have been identified and approved by the United States Food and Drug Administration (FaD) for use in livestock production, ractopamine hydrochloride (RHCL) and zilpaterol hydrochloride (ZHCL). Ractopamine hydrochloride is a β_1 AA approved for use in swine under the tradename Paylean®, and for cattle under the

tradename Optaflexx® (*Elanco Animal Health,* Greenfield, IN). Ractopamine

hydrochloride was first approved in 1999 for the use in finishing swine for 14 – 28 d at a

rate of 4.5 – 9 g/ton of feed to improve feed efficiency, increase weight gain, and carcass

leanness (FaD: NADA, 1999). In 2003, RHCL was approved for the use in confinement

fed cattle during the last 28 to 42 d at a rate of 8.2 – 24.6 g/ton of feed to improve feed

efficiency, increase weight gain and improve carcass leanness (FaD: NADA, 2003).

Zilpaterol hydrochloride is a β_2AA approved for use in cattle under the tradename

Zilmax® (*Merck Animal Health*, Madison, NJ). In 2006, ZHCL was approved for the use

in confinement fed cattle during the last 20 – 40 d at a rate of 6.8 g/ton of feed to improve

feed efficiency, increase weight gain and carcass leanness in cattle (FaD: NADA, 2006).

Currently RHCL is approved in 26 countries for use in swine and cattle; while ZHCL is

approved in 16 countries (with eight in progress) for use in cattle (globalfarmernetwork.

org 2012; zilmax.com).

IV. β - Adrenergic Agonists Impact on Growth Performance and Carcass Composition:

Extensive research has been done looking at the impact of both RHCL and ZHCL

on performance and carcass merit in livestock. With extensive amount of research, Lean

et al. (2014) conducted a meta-analysis to evaluate the impact of βAA supplementation

on feedlot cattle utilizing data extracted from 47 trials for ZHCL, and 54 trials for RHCL.

Results from the meta-analysis indicated that the average d of RHCL supplementation

was 30.8 ± 5.3 d and 26.6 ± 9.0 d for ZHCL supplementation (Lean et al., 2014). In

regard to changes in performance when compared to control cattle, RHCL

supplementation decreased DMI by 0.003 ± 0.001 kg/d, increased ADG by 0.19 ± 0.8

kg/d and G:F by 0.02 ± 0.02. Zilpaterol hydrochloride supplementation, when compared

to controls, decreased DMI by 0.12 ± 0.5 kg/hd/d, increased ADG by 0.15 ± 0.9 kg/d and

improved G:F by 0.03 ± 0.02 kg/kg (Lean et al., 2014). β - adrenergic agonist

supplementation increased final BW by 8 ± 0.4 kg for both RHCL and ZHCL, while

HCW increased 6 and 15 ± 1.3 kg with RHCL and ZHCL, respectively. When

comparing the two types of βAA and HCW, this data puts ZHCL to have a 9 kg increase

in HCW when compared to RHCL. With an increase in final BW and HCW, RHCL

increased dressing percentage by 0.3%, while ZHCL increased dressing percentage by

1.7 ± 2.2 % (Lean et al., 2014). Overall, the meta – analysis suggested that both βAA

(RHCL and ZHCL) improve feedlot performance, dressing percentage, and HCW;

however, cattle supplemented ZHCL had larger longissimus muscle area (8.0 ± 2.3 cm^2

vs. 1.8 cm^2), and a larger decrease in 12th rib fat thickness (0.11 ± 0.7 cm vs 0.0003 cm)

when compared to cattle supplemented RHCL (Lean et al., 2014). Additional research

has been conducted since the meta-analysis in 2014 to evaluate the effects of

supplementation of RHCL and ZHCL on livestock performance and well-being. More

recent research continues to investigate the utilization of βAA supplementation and

reports similar results.

Steers supplemented RHCL at concentrations of 200 – 400 mg/hd/d for 30 d

resulted in an average of 0.23 kg increase in ADG, and a 0.02 increase in G:F when

compared to control steers (Arp et al., 2014). Within the same study steers supplemented

ZHCL at a concentration of 7.5 mg/kg/d for 23 d with a three d withdrawal, increased

ADG and G:F by 0.48 kg and 0.03, respectively, when compared to both control and

RHCL steers (Arp et al., 2014). When steers were supplemented ZHCL or RHCL there

was a tendency for DMI to decrease when compared to controls. Supplementation of

RHCL at 300 or 400 mg/kg/d when compared to controls increased HCW by 4 kg and 6.3

kg, respectively. Supplementation of ZHCL increased HCW by 11.1 kg and improved

dressing percentage by 1.4% (Arp et al., 2014). Arp et al. (2014) also reported that

supplementation of RHCL and ZHCL improved LM area within steers by 1.4 cm^2 and 6.7

cm^2, respectively. Utilization of βAA was reported to decrease marbling score, while

improving yields of the round and loin sub-primal cuts. Arp et al.'s (2014) summarized

the utilization of βAA to increase steer growth performance and carcass yield when

compared to controls.

Two studies in 2015 utilized the supplementation of ZHCL at 8.33 mg/kg DM to

finishing steers. Boyd et al. (2015) supplemented ZHCL for 21 d with a three d

withdrawal in steers. Van Bibber – Krueger et al. (2015) supplemented ZHCL for 23 d

with a three d withdrawal. Both studies reported improvement in HCW, dressing

percentage, and LM area; while having no effect on ADG and G:F. Van Bibber –

Krueger et al. (2015) reported an 8% decrease in DMI of steers supplemented ZHCL

when compared to controls. Boyd et al. (2015) found no differences in DMI between

supplement and control steers. Hot carcass weight improved by 14 kg with a 2% increase

in dressing percentage for steers supplemented ZHCL when compared to controls (Boyd

et al., 2015; Van Bibber – Krueger et al., 2015). Additionally, LM area increased by 16.4

and 10.6 cm^2 for ZHCL steers when compared to controls (Boyd et al., 2015; Van Bibber

– Krueger et al., 2015). Control steers had increased USDA yield grades; however, there

were no differences between control steers and ZHCL steers for final live weight (Boyd

et al., 2015; Van Bibber – Krueger et al., 2015). Within the two studies it was concluded

that supplementation of βAA improved the growth performance and carcass measurements in steers when compared to controls.

Bittner et al. (2016) recommended that the ideal supplementation of RHCL to finishing steers is at 200 mg/hd/d for 28 d. Utilizing a dosage gradient of 0 to 200 mg/hd/d over a 28 – 42 d the study reported improvements in DMI, ADG, G:F, HCW, and LM area as dosage concentrations increased (Bittner et al., 2016). Dry matter intake decreased slightly from 10.9 kg/d to 10.6 kg/d with increasing dosage of RHCL. Average daily gain improved with an increased dosage of RHCL from 100 and 200 mg/hd/d by 3.4% and 10.7%, respectively. Additionally, G:F improved by 5% for steers supplemented 100 mg RHCL/hd/d and 13% for steers supplemented 200 mg RHCL/hd/d when compared to control steers (Bittner et al., 2016). Ractopamine hydrochloride supplementation has been reported to improve HCW, and when supplemented at 100 mg/hd/d and 200 mg/hd/d improved HCW by 2.2 kg and 4.1 kg, with no effect on dressing percentage (Bittner et al., 2016). In relation to the study by Arp et al. (2014), Bittner et al. (2016) reported an increase in LM are by 3.0 cm^2 with a dosage of 200 mg RHCL/hd/d. Marbling scores did decrease by 6 units with the 200 mg RHCL/hd/d; however other carcass characteristics like back fat and yield grade were not different between control steers and RHCL steers (Bittner et al., 2016). As the studies continue, the results remain similar in a sense that supplementation of a βAA improves most growth characteristics.

Two main factors that can alter the way a βAA affects cattle is through dosage concentration as well as supplementation time. Bittner et al. (2017) analyzed the changes in growth and carcass characteristics in finish steers supplemented RHCL at

concentrations of 0 to 400 mg/hd/d during a period of 28 – 42 d. Growth factors such as

DMI, ADG, G:F, and final body weight differed dependent on dose and duration. When

steers were supplemented RHCL at 200 mg/hd/d or 400 mg/hd/d there was no effect on

DMI; however, at a dose of 300 mg/hd/d DMI decreased by 3.3% (Bittner et al., 2017).

Supplementation of 200 mg/hd/d for 28 d improved ADG by 10.7% and G:F by 11.6%;

while a 300 mg RHCL/hd/d steer saw no improvements in ADG with a 5.7%

improvement in G:F. The effect of RHCL on final live weight has been reported to have

no effect with βAA supplementation, or has been reported to increase (Lean et al., 2014).

Bittner et al. (2017) reported that RHCL increased final live weight from 7.5 kg to 13 kg,

dependent on dosage and feeding duration. The best combination for increased live

weight was in steers supplemented 300 mg/hd/d for 35 d with a 12 kg increase in live

weight when compared to controls (Bittner et al., 2017). With a supplementation of

RHCL at 400 mg/hd/d, Hagenmaier et al. (2017) reported improvements in ADG by

21.2%, G:F by 20%, with a 7 kg increase in HCW and 4 cm^2 increase in LM area.

Additionally, HCW was increased between 7.1 kg and 10.7 kg when steers were

supplemented 400 mg RHCL/hd/d, with no differences in other carcass characteristics

such as dressing percentage, marbling, LM area, and 12th rib back fat (Bittner et al.,

2017). These studies come to show that selecting the correct dosage and duration for

feeding is important to both growth characteristics as well as carcass characteristics in

finishing steers.

      While supplementation of βAA is approved for the use in cattle and swine, it is

not approved for utilization in sheep. Yet, there is extensive research done that utilize

sheep as a model for future cattle work. Supplementation of RCHL to finishing male

lambs at 20 ppm for 32 d resulted in a 0.03 kg/d increase in live weight gain; however, there was no effect on final weight, G:F, or carcass characteristics when compared to controls (Robles – Estrada et al., 2009). Lopez – Carlos et al. (2010) supplemented lambs different dosages of RHCL (0.35 to 1.05 mg/kg/d) for 42 d and reported a 4.5% increase in G:F, as well as a 7.1% increase in total weight gain when compared to controls. There was no effect between supplement and control lambs on DMI; however, as dosage of RHCL increased DMI linearly decreased (Lopez – Carlos et al., 2010). When comparing the differences between RHCL and ZHCL on live and carcass performance, ZHCL has been reported to have stronger results (Lopez – Carlos et al., 2010; Lopez – Carlos et al., 2011).

Supplementation of ZHCL to lambs at doses between 0.1 and 0.3 mg/kg/d for 42 d resulted in a 2 kg increase in HCW with a 5% improvement in dressing percentage when compared to controls (Lopez – Carlos et al., 2010). Zilpaterol hydrochloride also decreased 12th rib fat thickness by 1.9% when compared to control lambs, which was larger than the 0.9% decrease from RHCL. Lopez – Carlos et al. (2010) also reported that ZHCL supplementation improved carcass conformation in lambs leading to a 2 cm^2 increase in LM area. Additionally, male lambs supplemented ZHCL at 6 mg/kg/d for 32 d, G:F was improved by 20.5% (Robles – Estrada et al., 2009). When comparing ZHCL (6 mg/kg DM) to RHCL (20 mg/kg DM) supplemented lambs, ZHCL improved HCW by 3.9%, DP by 3.8%, and reduced fat thickness by 20.6% (Lopez-Carlos et al., 2011). Use of βAA in sheep production results in increased feed efficiency and growth which leads to improvement in protein synthesis and decreased adipose deposition; similar to what is

reported in cattle (Robles – Estrada et al., 2009). Researchers can thus utilize sheep as a small ruminant model for future cattle work.

V. Stress and the Hypothalamic–Pituitary–Adrenal Axis:

Stress is described in the literature as a condition caused by a combination of factors (stressors) that alters the balance of biological systems homeostasis. Two categories of stress include eustress and distress. Eustress is stress that is not ideally detrimental to biological systems thus does not affect homeostasis. Distress is stress caused by a stressor that poses a threat to biological systems and becomes detrimental to homeostasis (Moberg and Mench, 2000). Stressors are the units that cause the stress and can be classified as physical, chemical, social, physiological or psychological (Sherwood et al., 2013). The response to a stressor depends on the degree of stress, which can be described as, but not limited to, acute or chronic stress (Sherwood et al., 2013). Acute or short-term stress relies on the release of catecholamines to mobilize energy resources to respond to quick disturbances to bring the body back to homeostasis. Chronic or long-term stress increases synthesis of glucocorticoids to respond to and resist a stressor (Sherwood et al., 2013). The "fight or flight" response is associated with the sympathetic nervous system and is activated when exposure to a stressful situation occurs. Neural signals are sent from the brain to the adrenal medulla where endogenous catecholamines, epinephrine and norepinephrine, are released (Nelson and Cox, 2013).

A key component during stress is the hypothalamic-pituitary-adrenal (HPA) axis. The HPA axis is responsible for regulating the secretion of different glucocorticoid hormones from the anterior pituitary gland (Moberg and Mench, 2000). Cannon (1929) first introduced the regulation of the HPA axis as an important system to return the body

back to a state of homeostasis. The hypothalamus receives a stress signal causing the

release of corticotropin – releasing hormone (CRH) which acts on the anterior pituitary

section of the pituitary gland. Adrenocorticotropic hormone (ACTH) is then released

which stimulates the adrenal gland complex to produce the glucocorticoid, cortisol

(Sherwood et al, 2013). Glucocorticoids are important for the conversion of glucose to

energy, and concentrations are regulated in order to maintain homeostasis (Moberg and

Mench, 2000). Every animal reacts differently to stressors; however, the biological

functions to react to the stimulus and elicit a cell response are similar (Salak-Johnson and

McGlone, 2007; Everly and Lating, 2013). As exposure to stressors continues

throughout production, animals adapt which allows a quicker return to homeostasis. Yet,

if the stressor continues and exceeds threshold limits, signals are sent to trigger a stress

response (Hahn et al., 2009).

VI. Environmental Stress:

Environmental conditions can have a significant impact on the health,

performance, and well – being of livestock. Environmental stress (heat stress or cold

stress) alter the animals ability to maintain thermal regulation. Cold stress is a result due

to a hypothermic response, while heat stress is a result due to a hyperthermic response.

Hypothermic responses occur when heat loss due to environmental temperatures exceeds

heat production resulting in decreased body temperatures (Khounsy et al., 2012). A

hyperthermic response occurs when heat load exceeds heat loss, enabling the animal's

ability to dissipate heat, resulting in increased body temperature (Srikandakumar et al.,

2003). Both occurrences can impact the heath, performance, and well-being of livestock.

Factors affecting heat stress include, but are not limited to, temperature, humidity, radiation, wind speed, species, and breed (Lara and Rostagno, 2013; Scharf et al., 2010). Cold stress is not as detrimental to cattle production due to the animal's ability to use metabolic heat production to maintain body temperatures, while during heat stress the animal must dissipate heat in order to regulate body temperature. In confinement fed cattle, heat stress occurs when external environmental conditions exceed the homeostatic tolerance range of individuals, resulting in the inability to cope and activation of a stress response (Gaughan et al., 2008).

The temperature humidity index (THI) is a standard tool utilized by production managers to evaluate thermal environments based upon ambient temperature and relative humidity (Hahn et al., 2009; Mader et al., 2006). The Livestock Weather Safety Index applies the THI to classify heat stress categories as: $\leq$ 74 units, normal; 74 – 79 units, alert; 79 – 84 units; danger; and $\geq$ 84 units, emergency (Mader et al, 2006). When exposed to heat stress physiological and behavioral changes in cattle occur resulting in increased mortality and a decrease in overall production (Belasco et al., 2015). Decreases in performance are largely due to decreased feed intake in order to decrease metabolic heat production to cope with the surrounding environmental heat (Mitlöhner et al., 2001).

VII. Impact of Heat Stress on Performance:

When exposed to heat stress, mortality rates of cattle within feedlots increased by 0.5% and cattle profits resulted in a $78/hd loss (Belasco et al., 2015). Production factors including health, feed efficiency, growth, and milk production are negatively affected as exposure to heat stress increases. Changes in DMI are strong indicators of stress when exposed to heat. O'Brien et al. (2010) reported that heat stressed cattle in environmental

conditions between 29°C and 40°C decreased DMI by 12% with an increase in water intake by 2.85 L/d. Cattle exposed to increased temperatures (20.3°C to 29.3°C) decreased feed performance by 11% in DMI, 15% in ADG, and 6% in feed to gain (F:G; Morrison and Lofgreen, 1979). Mitlöhner et al. (2001) also reported decreases in DMI by 7%, resulting in a 27 kg/hd loss in final body weight and a 16 kg loss in HCW. Dairy cattle in heat stress condition between 29.7 and 39.2°C decreased DMI by 35% which resulted in a 35% decrease in milk production (Rhoads et al., 2008). When the ability to dissipate heat decreases, due to increased environmental heat conditions, production decreases leading to decreased overall profit for producers. Thus, recognition of heat stressed animals and utilization of different methods to mitigate stress is important.

Animal affected by heat stress alter physiological responses such as respiration rate, body temperature, and heart rate to adjust to heat stress. Gaughan et al. (2008) reported that when environmental temperatures reached $\geq$ 25°C respiration rates increased, based upon noticeable and subjective increased panting scores. In order to cope with heat stress, physiological functions increase in order to dissipate heat load as a means of returning the body back to homeostasis (Lowe et al., 2002). Both increased respiration rate and increased rectal temperature correlate with an increase in the THI (Lowe et al., 2002). Cattle can alter respiration rates as a biological mechanism to maintain a core body temperature. Ruminants are homoeothermic animals, leading to a constant core temperature, and need to balance heat from metabolism with heat lost to heat gained from the environment (NRC, 1981; Singh et al., 2016). In addition to physiological functions, factors such as genetics, coat color, current health, and the ability

for coping with the environment are influential in the response an animal has to heat stress (Gaughan et al., 2008).

Production systems can utilize different methods to alleviate the effects of heat stress, like shade and misting, to improve cattle performance (Mitlöhner et al., 2001). Heifers exposed to misting decreased rectal temperatures by 0.8°C and respiration rate by nine breaths/min, while shade decreased respiration rates by 13 breaths/min (Mitlöhner et al., 2001). Heat stress as a result of increased temperatures and humidity elicits a stress response once above threshold which in turn decreases production performance and health (Mader et al., 2006). For every animal that is affected from heat stress, either through death or severe injury leads to a $5,000 loss in production (Mader, 2003). Extreme environmental conditions are a concern for producers due to decreased production and animal health, which could lead to decreased income as well as increased chances of death.

VIII. Conclusion:

By utilizing technologies to manage growth as well as environmental influences, producers can continue to increase production during times of stress. A possible way to alleviate effects of heat stress is the use of βAA. Supplementation of βAA results in vasodilation, increasing the amount of nutrient flow to the body, skeletal muscle, and adipose tissue (Mersmann, 1998). Administration of ZHCL decreases body temperature in steers, and vaginal temperatures in heifers (Boyd et al., 2015; Buntyn et al., 2016). Boyd et al. (2015) also reported increased respiration rate associated with ZHCL supplementation, which is consistent with the FDA feed label for ZHCL. Combinations of increased respiration rates and decreased temperatures due to supplementation of a

βAA could result in increased heat loss during heat stress, although there is no direct evidence reported in literature. During severe heat stress ewe lambs supplemented 10 mg ZHCL/ewe/d resulted in 2.3 kg increase in HCW, 2.1 increase in CCW, 7.8% improvement in DP, and an increased LM area by 3.4 cm^2 when compared to controls (Macias-Cruz et al., 2010). Previous research demonstrated that the use of βAA alleviates symptoms of stress as well as being able to improve performance during times of stress. However, a recent study suggested that there is an association between supplementation of βAA and heat stress events that resulted in increased mortality rates in feedlot cattle (Loneragen et al., 2014). Therefore, the objective for this study is to evaluate the impact and or interaction of βAA supplementation on growth performance and carcass composition of feeder lambs exposed to a heat stress challenge.

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CHAPTER III

THE EFFECT ON PRODUCTION PERFORMANCE AND CARCASS

COMPOSITION OF LAMBS SUPPLEMENTATED BETA – ADRENERGIC

AGONISTS WITHIN A CONTROLED HEAT STRESS CHALLENGE

ABSTRACT

Forty-nine crossbred feeder lambs (wethers, n = 49; 53.3 ± 3.7 kg BW) were
utilized to evaluate the interaction of β - adrenergic agonist (βAA) supplementation and
heat stress on growth performance and carcass composition. Utilizing and 3 x 2 factorial
design, lambs were randomly assigned to one of three βAA supplementation: 1) Control,
CON, 2) Ractopamine Hydrochloride at 40 mg/hd/d, RHCL, and Zilpaterol
Hydrochloride at 2.5 mg/hd/d, ZHCL for a period of 20 d and one of two environmental
conditions (Thermal Neutral: TN and Heat Stress: HS). The TN environment had a
constant thermal heat index (THI) of 16.6°C. Within the HS environment, a cyclic
design was utilized to achieve a THI of 29.5ºC from 10:00 to 20:00 h and a THI of
24.5ºC from 22:00 to 08:00 h. Starting at 08:01 and continuing to 09:59 h, temperature
and RH were gradually increased to achieve a THI of 29.5ºC at 10:00 h and reduction of
temperature and RH from 20:01 to 21:59 h to achieve a THI of 24.5ºC at 22:00 h.
Regardless of βAA supplementation ($P = \geq 0.09$), lambs exposed to the HS environment
had reduced DMI ($P < 0.001$), ADG ($P = 0.002$), and final BW ($P = 0.03$). In addition,
exposure to the HS environment (regardless of βAA supplementation; $P = \geq 0.07$)
decreased HCW ($P < 0.001$), percent change in LM area ($P = 0.004$) and percent change
in LM depth ($P = 0.005$). There was a βAA x environment interaction associated with

RHCL supplementation and heat stress ($P = 0.003$). Lambs supplemented RHCL in the HS environment had reduced (P = 0.003) respiration rates, when compared to CON and ZHCL supplemented lambs. Supplementation of ZHCL decreased adipose tissue ($P = 0.05$) and increased percent fat free lean ($P = 0.01$), when compared to RHCL and CON lambs. Within the current study, both heat stress and βAA supplementation had an impact on growth performance and carcass composition. However, the data does not indicate that there was any significant interaction between βAA supplementation within a heat stress environment on growth performance or carcass composition in feeder lambs.

Keywords: β – agonist, heat stress, growth performance, carcass composition

INTRODUCTION

A major concern at times of elevated heat and relative humidity is the onset of the negative impact/danger of heat stress. Heat stress is an environmental stressor that results in the heat load exceeding heat loss, and can be influenced by factors such as temperature, humidity, radiation, wind speed, species, and breed (Lara and Rostagno, 2013; Scharf et al., 2010; Srikandakumar et al., 2003). Due to increased heat loads and a reduction in the ability to dissipate heat, heat stress negatively alters homeostasis which affects performance characteristics, economical value, and animal well – being. St. Pierre et al. (2003) reported an estimated economic loss of $1.7 billion to the livestock industry due to increased mortality and decreased growth performance. Heat stress has a significant impact on ruminants, Ruminants are susceptible to heat stress, Dixon et al., (1999) reported a 9% decrease in dry matter intake (DMI) and reduced body weight (25 g/d) (Dixon et al., 1999). Mitlöhner et al., (2001) reported that cattle exposure to heat stress resulted in 7% reduction in DMI and a 27 kg/hd reduction in body weight. .

The Livestock Weather Safety Index (LWSI) serves as the guidelines for estimating the danger presented to livestock. The LWSI calculations are based upon ambient temperature and relative humidity (Mader et al., 2006) and applies the THI to classify heat load into four categories: No Stress = ≤ 74°F (≤ 23.3°C), Alert = 74 – 79°F (23.3 – 25.6°C), Danger = 79 – 84°F (26.1 – 28.3°C) and Emergency = ≥ 84°F (≥ 28.9°C; Figure 1; LCI, 1970; Mader et al, 2006). As the THI exceeds 26.1°C, categories Danger into Emergency, traits such as growth performance and carcass composition begin to decrease, with increased rates of mortality (Morrison and Lofgreen, 1979; Rhoads et al., 2008; O'Brien et al., 2010).

In the state of Nebraska alone during times of increased environmental temperatures and increased relative humidity, heat stress was detrimental to producers in the years of 1999, 2009, and 2013. In total during these three heat events producers lost around 13,000 hd. of cattle which could estimate an economic loss of around $22 million dollars (Hungerford et al., 2000; Lincoln Journal Star, 2009; Brown-Brandl, 2013). A current example in Nebraska includes a 3 d heat event (June 27 – June 30, 2018). Environmental temperature and relative humidity reached a high of 34°C and 40% RH, resulting in a temperature humidity index (THI) of 29.4°C, in the Emergency category (Table 1).

In a meta-analysis of feedlot mortality conducted by Loneragen et al. (2014) there was a suggested association between increased rates of mortality in feedlot cattle and supplemented a β – adrenergic agonists (βAA) during heat stress events. A survey done in 2015 reported that within the United States cattle industry approximately 85% of producers used a type of βAA in cattle finishing diets (Samuelson et al., 2016). With approximately 85% of finishing cattle supplemented a type of βAA it is important to understand the possible interaction between βAA and heat stress. β – adrenergic agonists act as energy repartitioning agents to improve growth and carcass composition in livestock (Etherton, 2009). Once a βAA bind to β – adrenergic receptors (βAR) a cascade of events occurs causing phosphorylation of intracellular proteins. This cascade of events leads to increased protein synthesis with decreased protein degradation in muscle, as well as increased lipolysis with decreased lipogenesis in adipose tissue (Mersmann, 1998). Two approved βAA for use in livestock are a β_1AA, ractopamine hydrochloride (RHCL), and a β_2AA, zilpaterol hydrochloride (ZHCL). Ractopamine

hydrochloride and ZHCL improve growth performance and carcass composition in finishing cattle, resulting in increased profit for producers (Lean et al., 2014). While there is literature related to the separate impact of βAA and heat stress on the growth performance and carcass composition of livestock, there is limited data regarding the interaction between βAA and heat stress. Therefore, the objective of this study was to evaluate the impact of different βAA, heat stress, and the interaction of βAA and heat stress on the growth performance and carcass composition of feeder lambs.

MATERIALS AND METHODS

Animal and Experimental Design

All experimental procedures were in compliance with the *Guide for the Care and Use of Agricultural Animals in Research and Teaching* and approved by the University of Nebraska – Lincoln's Institutional Animal and Care and Use Committee (IACUC #1300).

Forty-nine crossbred feeder lambs (wethers, 53.3 ± 3.7 kg) were sourced and transported to the University of Nebraska – Lincoln's Animal Science Complex. Upon arrival, lambs were weighed, rectal temperatures recorded, ear tagged with individual ID's, metaphylacticaly treated [Ivomec®; 10 mL/hd (Merial, Duluth, GA) and Draxxin®; 1 mL/hd (Zoetis, Parsippany, NJ)]. Based upon initial BW, lambs were assigned to one of two blocks (block one, 39.99 ± 1.92 kg, n = 24; block two, 37.35 ± 1.92 kg, n = 25). Lambs were then placed into four group pens (two groups/block) with ad libitum access to water. Upon receiving lambs were received a receiving ration and then transitioned to a 90% diet, which the lambs were fed for the remainder of the study (block 1 lambs were transitioned over a period of 81 d and block 2 lambs were transitioned over a period of 109 d; Table 2).

For each block, lambs were randomly allocated into one of six treatments groups: Control / Thermal Neutral (CON/TN; n = 9), Ractopamine Hydrochloride / Thermal Neutral (RHCL/TN; n = 8), Zilpaterol Hydrochloride / Thermal Neutral (ZHCL/TN; n = 8), Control / Heat Stress (CON/HS; n = 8), Ractopamine Hydrochloride / Heat Stress (RHCL/HS; n = 8), and Zilpaterol Hydrochloride / Heat Stress (ZHCL/HS; n = 8). Six d prior to the start of the trial, lambs were moved into the assigned environments. Within the TN environment, lambs were placed into individual stalls (1.829 m x 0.914 m), each equipped with an individual feed bunk and waterer. For the HS environment, lambs were placed into individual stalls (1.524 m x 0.914 m) within the thermal chamber, and each stall was equipped with an individual feed bunk and waterer. For both environments, lights were controlled through a light/dark cycle of 16 h of light starting at 0630 h followed by 8 h of dark.

Utilizing the Livestock Weather Safety Index (LCI, 1970, Mader et al. 2006), a constant THI of 18.3°C was targeted for the TN environment. For the HS environment, a cyclic temperature design was utilized to achieve a day time THI of 30°C (LCI, 1970; Mader et al. 2006; NOAA Heat Index of 55°C) from 1000 – 2000 h, and a night time THI of 23.9°C (LCI, 1970; Mader et al. 2006; NOAA Heat Index of 32°C) from 2200 – 0800 h. The cyclic design incorporated a 2 h heat up period from 08:01 – 09:59 h and a 2 h cool down period from 20:01 – 21:59 h. The THI (°F) was calculated using ambient temperature (T, °C) and relative humidity (RH, %) in the Temperature – Humidity Index equation reported by the LCI (1970) and Mader et al. (2006; $\left\{ (8.0 \; x \; T) + \left[\left(\frac{\% \, RH}{100} \right) x (T - 14.4) \right] + 46.4 \right\} =$ THI (°F). The THI was then converted to °C. For environments, ambient temperature and relative humidity was monitored by Hobo[®]

Temp/RH 3.5% Data Logger (Model UX100 – 003; Onset Computer Corporation, Bourne, MA). Hobos were programed to record ambient temperature and relative humidity in 15 min intervals.

Ractopamine hydrochloride was supplemented at 40 mg/hd/d and ZHCL was supplemented at 2.5 mg/hd/d. Proper dosage for use of βAA in sheep was calculated to mimic the supplementation dose for cattle at 200 mg/hd/d of RHCL and 6 mg/hd/d of ZHCL. For both βAA treatment groups, βAA was supplemented via a ground corn carrier incorporated into the daily offering of feed. Lambs within the CON treatment groups received 200 g of fine ground corn with no addition of βAA, RHCL lambs received 199.96 g of fine ground corn with 0.04 g of RHCL, and ZHCL lambs received 199.9975 g of fine ground corn with 0.0025 g of ZHCL. Daily orts, feed left over from the day before, were recorded at 0730 h and utilized to determine adjustment to daily allotment. Orts collection began six days before supplementation began. Beginning on d 1, the 200 g sample of βAA supplements were hand mixed into 0.91 kg of feed and offered at 0800 h to ensure consumption of supplementation. The remaining allotment of feed was provided at 1400 h.

Each d at 0800, 1400, and 2000 h, water disappearance, rate of respiration, and rectal temperature were recorded. Water disappearance was determined utilizing an 18.9 L bucket with a graduated scale in 1 L increments. Amount of water in each bucket was recorded and then filled to 14 L to determine disappearance. Respiration rate was determined via one visual observation of respiration for a period of 15 sec ever check, then multiplied by 4 to determine respirations/min. Rectal temperatures were measured once every time point, using a ReliOn 8 second thermometer (Bentonville, AR).

Ultrasound Analysis

Real-time ultrasound images were collected to evaluate the loin eye area, loin eye depth, back fat thickness of the 12th/13th rib, and body wall thickness. Ultrasound was conducted by a trained ultrasound technician who was certified through the National Sheep Improvement Program from June 2010 to June 2014. Longitudinal ultrasonic scans were taken by placing the transducer head in the center of the last costae. A Classic scanner 200 (Classic Medical Co., Tequesta, FL) equipped with a 3.5 – Mhz, 18 cm linear array transducer was used to collect images. Real-time images were captured and evaluated to record measurements on d 1, 10 and 21. Ultrasound measurements and prediction equations were utilized to calculate predicted values of fat free carcass lean (FFL, kg), % FFL, total dissected carcass lean (TDL, kg), and % TDL (Berg et al. 1996).

Harvest and Fabrication

On d 21, lambs were relocated to the University of Nebraska – Lincoln Loeffel Meat Lab facility for harvest. Harvest order was determined by randomly assigning lambs within treatment. Live weight (prior to harvest) and hot carcass weights were collected and then carcasses were chilled (2°C) for 48 h. After a 48 h chill, carcasses were ribbed between the 12th/13th rib, separated into the fore-saddle, hind-saddle, and medially separated into left and right sides. Following fabrication, the left side of each carcass was fabricated into major and minor primal cuts according the USDA Institutional Meat Purchasing Specifications (IMPS): Square Cut Shoulder (IMPS 207), Rack (IMPS 204), Loin (IMPS 232), Leg (IMPS 233), breast (209) and plate/flank/fore-shank. Major and minor primal cuts were trimmed to an external fat thickness of 3.1 mm weighed and dissected to obtain lean muscle, adipose tissue, and bone. Carcass were

dissected in the same order harvested to determine lean muscle mass, adipose tissue, and bone were weighed and recorded for the fore-saddle and hind-saddle. At the conclusion of the trial, all products/by-products were retained and incinerated.

Statistical Analysis

Data were analyzed as a completely randomized block design using the GLIMMIX procedure of SAS (SAS Inst. Inc., Cary, NC USA). The fixed effects were defined as supplementation of βAA, environment conditions, and the interaction of βAA x environment conditions. Block was utilized as a random effect and the experimental unit was defined as individual lamb. Analysis of block as a random variable indicated there was no block effect, and random statement was removed from the analysis. When main effects or interaction of the main effects were significant ($P \leq 0.05$), specific treatment comparisons were made using PDIFF SAS. Data is reported as the LSMeans ± SD.

RESULTS

Lambs within in the TN environment were exposed to a constant to LWSI category of *No Stress* (THI of 16.6°C; Table 3). Within the HS environment, from 10:00 – 20:00 h, lambs were exposed to a LWSI category of *Emergency* (THI of 29.5°C) and a LWSI category of *Alert* (THI of 24.5°C (Table 3).

Physiological Response

There was an effect of environment ($P < 0.001$) on water disappearance and rectal temperature (Table 4). There was no interaction of βAA x environment ($P \geq 0.08$), or an effect of βAA supplementation ($P \geq 0.39$). Regardless of βAA supplementation, lambs

within the HS environment had greater water disappearance (0.72 L), when compared to lambs within the TN environment (1.98 ± 0.89 and 1.27 ± 0.56 L). There was a similar response in regard to increased rectal temperature in HS environment lambs by 0.65°C when compared to TN environment (39.78 ± 1.04 and 39.13 ± 0.61 °C). An interaction of βAA x environment was observed ($P = 0.003$) for respiration rate (Figure 2). Within the TN environment there was no effect due to supplementation treatments ($P = 0.75$), however, within the HS environment, respiration rate decreased for lambs supplemented RHCL (140.2 ± 46.6 breaths/min) when compared to lambs supplemented ZHCL ($P = 0.007$; 160.7 ± 48.7 breaths/min) and non-supplemented CON lambs ($P = 0.02$; 158.7 ± 50.5 breaths/min).

Growth Performance

There was an effect of environment ($P \leq 0.002$) for DMI and ADG; however, there was no interaction of βAA x environment ($P \geq 0.48$), or an effect of βAA supplementation ($P \geq 0.13$; Table 4). Dry matter intake decreased by 0.29 kg ($P < 0.001$) in HS environment lambs (1.10 ± 0.16 kg) compared to TN environment (1.39 ± 0.22 kg). Average daily gain was decreased 0.08 kg/d ($P = 0.002$) in HS environment lambs (0.14 ± 0.06 kg/d) compared to TN environment (0.18 ± 0.09 kg/d). There was no interaction of βAA x environment ($P = 0.63$), or an effect of environment ($P = 0.15$), or βAA supplementation ($P = 0.09$) for G:F.

For initial live BW measured on d 1, there was no interaction of βAA x environment ($P = 0.97$), or an effect for environment ($P = 0.25$; Table 4), or βAA supplementation ($P = 0.33$). At the end of the trial, there was an effect of environment ($P = 0.01$) on final live BW (d 20), however, there was no interaction of βAA x environment

($P = 0.91$), or an effect for βAA supplementation ($P = 0.70$). Final live BW decreased

2.71 kg ($P = 0.01$) in HS environment lambs (51.77 ± 2.97 kg) compared to TN

environment (54.48 ± 3.03 kg). Due to the changes in live BW over time the percent

change in BW was determined for the overall study. For percent change in live BW

overall, there was an effect for environment ($P = 0.003$), with no interaction of βAA x

environment ($P = 0.45$), or an effect of βAA supplementation ($P = 0.11$). Percent change

in live BW overall was decreased by 3.45% in HS environment lambs ($P = 0.003$; $6.12 \pm$

4.52%) compared to TN environment (9.62 ± 4.52%)

Pre-harvest body composition predicted by ultrasound measurements

Ultrasound measurements were taken during the study to evaluate 12th/13th rib BF

thickness, LM area, LM depth, and body wall thickness over 20 d. For all measurements

on d 1 there was no interaction of βAA x environment ($P \geq 0.07$), or an effect of

environment ($P \geq 0.13$), or βAA supplementation ($P \geq 0.27$). However, on d 20 there

was an effect for βAA supplementation ($P = 0.04$) on 12th/13th rib BF thickness, as well

as an effect of environment ($P < 0.001$) on LM area, and LM depth (Table 5). There was

no interaction of βAA x environment ($P = 0.90$) on body wall thickness, or an effect

associated with environment ($P = 0.21$), or βAA supplementation ($P = 0.67$).

For 12th/13th rib BF thickness, there was an effect for βAA supplementation ($P =$

0.04), but there was no interaction of βAA x environment ($P = 0.31$), or an effect of

environment ($P = 0.11$). Supplementation of ZHCL within the TN environment

increased 12th/13th rib BF thickness when compared to supplementation of non-

supplemented CON lambs on d 21 (0.54 ± 0.18 and 0.36 ± 0.09 cm). For LM area and

LM depth, there was an effect of environment ($P < 0.001$), however, there was no interaction of βAA x environment ($P \geq 0.16$), or an effect for βAA supplementation ($P \geq 0.25$). Loin muscle area decreased in size with HS environment lambs when compared to TN environment (16.94 ± 0.34 and 18.58 ± 0.28 cm^2). Loin muscle depth decreased in size with HS environment lambs when compared to TN environment (2.94 ± 0.12 vs. 3.18 ± 0.07 cm). For percent change overall, form initial measurements on d1 to the final measurement on d21, there was no interaction of βAA x environment ($P \geq 0.25$), or an effect of βAA supplementation ($P \geq 0.07$) for any ultrasound measurement. There was an environmental effect resulting in decreased LM area ($P = 0.03$) and LM depth ($P = 0.005$) in HS environment lambs when compared to TN environments (9.39 ± 10.02 and 15.52 ± 9.97 cm^2 with 4.62 ± 5.35 and 9.42 ± 5.14 cm, respectively).

Predicted values of total dissected lean (TDL) and fat free lean (FFL), there was an effect for environment ($P = 0.02$), however, there was no interaction of the βAA x environment ($P \geq 0.93$), or an effect of βAA supplementation ($P \geq 0.85$; Table 6). Weight of TDL decreased in HS environment lambs (12.48 ± 0.82 kg) when compared to lambs within the TN environment (12.98 ± 0.89 kg). Similarly, predicted weight of FFL decreased in HS environment lambs (11.68 ± 0.79 kg) when compared to TN environment (12.15 ± 0.86 kg). For predicted percent of TDL and FFL, there was an effect of βAA supplementation ($P = 0.04$); however, there was no interaction of βAA x environment ($P \geq 0.62$), or an effect of environment ($P \geq 0.06$). Lambs supplemented ZHCL ($52.02 \pm 0.74\%$) resulted in decreased percent TDL compared to non-supplemented CON lambs ($P = 0.01$; $52.46 \pm 0.55\%$). Additionally, lambs supplemented

ZHCL (48.60 ± 0.72%) resulted in decreased percent FFL compared to non-supplemented CON lambs ($P = 0.01$; 49.04 ± 0.47%).

Post-harvest carcass characteristics and composition

There was an effect of environment ($P < 0.001$) on HCW and left side carcass weight, while there was no interaction of βAA x environment ($P \geq 0.69$), or an effect of βAA supplementation ($P \geq 0.39$; Table 4; Table 7). Hot carcass weight decreased 2.08 kg in HS environment lambs (27.64 ± 1.90 kg) when compared to lambs within TN environment (29.72 ± 1.49 kg). Similarly, left side carcass weights decreased 1.08 kg in HS environment carcasses compared to TN environment (13.43 ± 1.07 and 14.51 ± 0.87 kg). The percent fore-saddle and percent hind-saddle of the carcass resulted in no interaction of βAA x environment ($P \geq 0.28$), and no effect for environment ($P \geq 0.35$), or βAA supplementation ($P \geq 0.66$).

When evaluating carcass composition there was an effect for βAA supplementation ($P \leq 0.05$) on percentage of adipose tissue and lean muscle, however, there was no interaction of βAA x environment ($P \geq 0.18$), or an effect of environment ($P \geq 0.16$; Table 7). Adipose tissue percentage was decreased with supplementation of ZHCL in comparison to supplementation of RHCL (23.94 ± 3.12 and 26.83 ± 4.50%). Additionally, lean muscle mass was increased with supplementation of ZHCL (53.62 ± 2.95%), in comparison to non-supplementation CON ($P = 0.03$; 51.52 ± 2.02%) and RHCL lambs ($P = 0.003$; 50.63 ± 3.17%). For percentage of bone, there was no interaction of βAA x environment ($P = 0.71$), and no effect for environment ($P = 0.09$), or βAA supplementation ($P = 0.47$).

DISCUSSION

The Livestock Weather Safety Index (LWSI) utilizes the THI to classify weather stress categories (°C) as *No Stress*: THI ≤ 23.3, *Alert*: 23.3 – 25.6, *Danger*: 26.1 – 28.3, and *Emergency*: ≥ 28.9. Based upon the LWSI, lambs within the HS environment were exposed to an Emergency heat stress THI (THI = 29.5°) from 10:00 – 20:00 h and an *Alert* heat stress (THI 24.5°C) from 22:00 – 08:00 h. Lambs within the TN environment were exposed to a constant *No Stress* THI (THI = 16.6°C). As a THI increases between 26.1 – 28.3°C (category Danger) researchers have reported growth performance in ruminants as THI increases reaches the LSWI category of *Emergency* (THI = 28.9°C) feedlot cattle mortality rate increased (Hahn and Mader, 1997). For the current trial, the environment within the HS remained within the Emergency category from 10:00 h thru 20:00 h and within the Alert category from 22:00 h – 08:00 h. To combat the challenge of heat stress, ruminants must divert energy for maintenance and growth toward physiological means of dissipating excessive heat load gained from the environment. (Baumgard and Rhoads, 2012).

Supplementation of βAA has drawn recent scrutiny with regards of concerns of animal well-being concerns. Thomson et al. (2015) reported a potential link between the supplementation of ZHCL and lameness of cattle at the time of harvest. In addition, Lonergan et al. (2015) utilized a meta-analysis of feedlot close-out summaries to evaluate possible interaction between βAA supplementation and changes in feedlot mortality rates. Results of this meta-analysis suggested a potential association between the βAA supplementation and environmental conditions. To date there has been no controlled environmental trials to investigate this potential association of βAA and heat stress. To

the authors knowledge, there has been no reported controlled studies conducted that directly evaluated this potential interaction. Within our controlled study, no interaction between βAA supplementation and exposure to the HS environment. There was however and intriguing physiological response related to lambs' supplementation RHCL within the HS environment. Lambs supplemented RHCL respiration rates within the HS environment were 13% less than those of both the lambs within the CON and ZHCL. This change in respiration may be associated with the physiological action of βAA in regard to vasodilator. When βAA's bind to the βAR on smooth muscle cells the response is to initiate a relaxation of muscle and associated tissue to allow for increased blood flow (Mersmann, 1998; Alquist, 1948). Supplementation of a βAA (RHCL) in the present study, could relate to improvements of respiration rate due to increased blood and subsequence increased air capacity/respiration. In times of heat stress improved respiration rate may be seen as beneficial in preventing hyperthermia due to alterations in moisture levels within the respiratory tract (da Silva et al., 2017).

The attempt to maintain homeostasis during increased heat loads in cattle and sheep impact factors such as water intake and rectal temperatures (El – Tarabany et al., 2017; O'Brien et al., 2010; Shirley, 1985). Within the current trial, lambs in the HS environment had increased water disappearance levels and had increased rectal temperatures when exposed to the HS environment. Lowe et al. (2002) reported a 1°C increase in rectal temperature in lambs exposed to a heat stress environment. When rectal temperature rises even 1°C, livestock performance can be negatively affected, which was observed in the present study (Kadzere et al., 2002). Overall, results of the current study indicated a similar heat stress response related to physiological responses

where lambs within the HS environment had a 0.72 L increase in water disappearance and a 1.2ºC increase in body temperature when compared to the lambs within the TN environment.

Numerous research trials have reported an improvement in feed efficiency and carcass composition in both cattle and lambs supplemented a βAA (Lean et al., 2014; Lopez – Carlos et al., 2010). In the current trial, supplementation of βAA did not result in significant changes in feed efficiency. Additionally, there were no differences in G:F, body wall thickness, percent foresaddle, percent hindsaddle, and percent bone in regard to the environment or supplementation of βAA.

Heat stress leads to a compromised feed intake and feed efficiency in cattle and sheep (Hagenmaier et al., 2016; Barnesa et al., 2004). A decrease in DMI thus negatively affects growth performance and carcass composition (Macias – Cruz et al., 2010; O'Brien et al., 2010; Morrison and Lofgreen, 1979). Similarly, during the current 20 d heat stress challenge there was a negative impact due on DMI and ADG, and growth performance, final live weight and HCW. During times of heat stress sheep reduced DMI by 13%, while cattle had decreased DMI 7 – 12% and ADG around 11 – 15% (O'Brien et al., 2010; Mitlöhner et al., 2001; Shafie et al., 1994; Morrison and Lofgreen1979). Mitlöhner et al. (2001) reported a 27 kg loss in final body weight and 16 kg loss in HCW in heat stressed cattle when compared to cattle not exposed to heat stress. Lambs within the HS environment consumed less feed, which could be a cause for the decreased weight performance throughout the study.

Heat stress events limit performance due to redistribution of energy toward physiological alterations to reduce heat load, thus limiting energy for maintenance and

growth (Gowane et al., 2017; Belhadj Slimen et la., 2015). Mitlöhner et al. (2001) reported that cattle carcasses exposed to heat stress resulted in an 8.31% decrease in fat thickness measurements. In the current study, ultrasound measurements showed similar results for decreased growth of the LM area and LM depth in HS environment lambs. However, HS environment appear to affect fat thicknesses when compared to TN environment lambs. The greater the length of exposure to a heat stress environment did not affect the LM area of lambs, however, it did results in a linear decrease in dressing percentages up to 7.83% with 8 h of exposure (Rana et al., 2014). Using the ultrasound measurements and prediction equations from Berg et al. (1996) there was an observed decrease in predicted weight values of TDL and FFL for lambs exposed to a HS environment. Decreased TDL and FFL values continue to follow suit with decreased production performance due to a decrease in feed intake and nutrient utilization.

β – adrenergic agonist supplementation positively impacted carcass composition of lambs in the current study. Supplementation of ZHCL resulted in increased percentages of FFL when compared to RHCL and non-supplemented CON lambs. Zilpaterol hydrochloride also decreased percentages of adipose tissue compared to RHCL lambs. Lopez – Carlos et al. (2010) reported similar observations of alterations in carcass characteristics through the utilization of both RHCL and ZHCL supplementation in feeder lambs. Within the current study, supplementation of ZHCL also had a greater dressing percentage and muscle area, with decreased fat thicknesses when compared to RHCL lambs (Lopez – Carlos et al., 2010). Cattle supplemented 200 mg RHCL had a linear decrease in yield grade when compared to cattle supplemented 0 mg (Bittner et al.,

2016). Through the supplementation of βAA, skeletal muscle mass increases while body

fat decreases due to the shift in energy utilization (Mersmann, 1998).

CONCLUSION

Data from the current study would indicate that heat stress has a negative effect

on the growth performance of feeder lambs. Similar data is reported in other studies that

utilize sheep exposed to increased environmental temperatures having a negative impact

on physiological responses and growth performance (Dixon et al., 1999; Marai et al.,

2007). With decreased feed intake and efficiency characteristics like weight gain and

HCW are also negatively affected. In addition to the effect of heat stress,

supplementation of βAA, specifically ZHCL, improved carcass composition of feeder

lambs with increased percentages of lean muscle, and decreased percentages of adipose

tissue. Supplementation of βAA in times of climate change has been reported to have an

association with increased mortality rates in cattle (Loneragen et al., 2014). However,

within the current controlled heat stress challenge there was no interaction observed

between supplementation of βAA and heat stress that would affect growth performance

or carcass composition. A lack of an interaction in the controlled study concludes that

there are no detrimental effects on production and animal well-being though the

utilization of βAA supplementation during heat stress events.

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Table 1. Environmental summary of a 4 d heat event (June 27 – June 30, 2018) around Lincoln, NE, and the association of heat and humidity on the Temperature Humidity Index (THI) stress category

	°C	%RH	THI[1]	Stress Category
27-Jun	32	51	81	Danger
28-Jun	34	60	85	Emergency
29-Jun	37	40	85	Emergency
30-Jun	32	70	84	Emergency

Temperature – humidity index (THI, °F) = {0.8 x T + [(% RH/ 100) x (ambient temperature – 14.4)] + 46.4} (Temperature (T; °C) and relative humidity (RH; %); LCI, 1970; Mader et al., 2006)

Table 2. Composition of diets fed to control (CON), ractopamine hydrochloride (RHCL), or zilpaterol hydrochloride (ZHCL) treatment groups as a percent of DM basis during a 20 d controlled heat stress challenge

| | Dietary Rations[1] | | | | | | | 90% | |
| | | | | | | | Con | RHCL[2] | ZHCL[2] |
Ingredients	Receiving	10%	20%	40%	60%	80%	Con	RHCL[2]	ZHCL[2]
SweetBran®, %	54.8	54.3	53.8	52.8	51.8	50.8	49.0	49.0	49.0
Dry – Rolled Corn, %	-	3.8	7.5	15.1	22.7	30.2	37.8	37.8	37.8
Chopped Alfalfa, %	41.1	97.8	34.5	27.9	21.4	14.9	8.3	8.3	8.3
Mineral Supplement[3], %	4.1	4.1	4.1	4.1	4.1	4.1	4.1	4.1	4.1
Treatment Suppl., g[2]	-	-	-	-	-	-	200.0	200.0	200.0

[1] For both blocks the receiving diet was fed for 12 d, followed by the 10% for 10 d for block 1 and 33 d for block 2. Block 1 continued with a step-up schedule of 20% for 5 d, 40% for 60 d, and the 80% for 8 d. The 90% concentrate was fed in block 1 for 33 d. After being held on 10% for 33 d fed at 2.2% of body weight per group pen, block 2 followed the same step up schedule.

[2] Lambs received RHCL and ZHCL for a 20-d period accounting for 0.8 % of diet. The CON contained only fine ground corn. Ractopamine hydrochloride supplementation contained 40 mg/hd/d Type A medication and was fed at 0.04 g with fine ground corn. Zilpaterol hydrochloride supplementation contained 2.5 mg/hd/d Type A medication and was fed at 0.0025 g with fine ground corn.

[3] Mineral supplements were comprised of 2.1% limestone, 2% Producers Pride General Purpose Mineral, and 20 g/ton of Rumensin.

Table 3. Environmental analysis of the average ambient temperature, relative humidity, Temperature Humidity Index (THI), and Heat Index (HI) of feeder lambs supplemented 0 mg (CON), 40 mg ractopamine hydrochloride (RHCL), or 2.5 mg zilpaterol hydrochloride (ZHCL) during a 20 d controlled heat stress challenge

	Thermal Neutral			Heat Stress		
	°C	%RH	THI[2]	°C	%RH	THI
10:01 – 20:00 h	18.18	28.75	16.67	40.10	25.51	29.47
20:01 – 22:00 h	18.19	28.25	16.66	34.12	38.44	27.35
22:01 – 08:00 h	18.14	25.53	16.58	29.12	42.64	24.46
08:01 – 10:00 h	18.16	31.43	16.72	34.83	34.20	27.22

[1] Temperature (T, °C) and relative humidity (%) were measured every 15 min.
[2] Temperature Humidity Index (THI, °F) = {0.8 x T + [(% RH / 100) x (ambient temperature – 14.4)] + 46.4} (LCI, 1970; Mader et al., 2006)

Table 4. Growth performance of feeder lambs supplemented 0 mg (CON), 40 mg ractopamine hydrochloride (RHCL), or 2.5 mg zilpaterol hydrochloride (ZHCL) during a 20 d controlled heat stress challenge

Variable	βAA Supplementation				Environment			P-value		
	CON	RHCL	ZHCL	SD	TN	HS	SD	βAA	Enviro.	Interaction
Water Disappearance (L)[1]	1.61	1.66	1.59	0.84	1.27[a]	1.98[b]	0.56	0.94	< 0.001	0.88
Respiration (breaths/min)	109.68	109.82	112.13	49.24	69.14[a]	153.24[b]	26.94	0.75	< 0.001	0.003
Rectal Temperature (°C)	102.91	102.99	103.13	0.96	102.43[a]	103.61[b]	0.61	0.39	< 0.001	0.08
DMI (kg)	1.25	1.24	1.25	0.22	1.39[a]	1.10[b]	0.22	0.97	< 0.001	0.49
ADG (kg/d)	0.22	0.15	0.18	0.07	0.23[a]	0.14[b]	0.07	0.13	0.002	0.48
G:F	0.19	0.13	0.16	0.06	0.18	0.15	0.06	0.09	0.15	0.63
Initial live weight, kg	48.42	49.62	49.92	2.95	49.84	48.80	2.97	0.33	0.25	0.97
Final live weight, kg	52.97	52.67	53.72	3.67	54.48[a]	51.77[b]	3.13	0.70	0.01	0.91
% Δ Overall[2]	9.38	6.54	7.60	2.99	9.57[a]	6.12[b]	2.99	0.10	0.003	0.45
HCW, kg	28.45	28.43	29.17	0.60	29.73[a]	27.64[b]	1.49	0.39	< 0.001	0.81

[1] Water disappearance is the amount of water consumed over a certain period of time
[2] Percent Δ Overall is the difference in initial live weight and final live weight [(Initial live weight – Final live weight)/Initial live weight]

Table 5. Ultrasonic measurement analysis of feeder lambs supplemented 0 mg (CON), 40 mg ractopamine hydrochloride (RHCL), or 2.5 mg zilpaterol hydrochloride (ZHCL) during a 20 d controlled heat stress challenge

	βAA Supplementation				Environment			P-value		
Variable	CON	RHCL	ZHCL	SD	TN	HS	SD	βAA	Enviro.	β x E
Back Fat, cm										
d 1	0.28	0.34	0.33	0.09	0.33	0.31	0.11	0.27	0.57	0.72
d 20	0.37[a]	0.41[ab]	0.48[b]	0.09	0.45	0.39	0.10	0.04	0.11	0.31
Overall % Δ[1]	34.67	32.46	46.05	25.42	41.45	33.70	28.43	0.44	0.38	0.57
Loin Eye Area, cm²										
d 1	15.73	16.26	15.67	1.63	16.10	15.67	1.60	0.54	0.38	0.07
d 20	17.36	17.64	18.28	1.59	18.58[a]	16.94[b]	1.38	0.25	< 0.001	0.16
Overall % Δ	10.83	9.39	17.14	8.89	15.52[a]	9.39[b]	9.97	0.07	0.03	0.25
Loin Eye Depth, cm										
d 1	2.84	2.82	2.85	0.14	2.91	2.83	0.15	0.41	0.13	0.29
d 20	3.02	3.09	3.08	0.18	3.18[a]	2.94[b]	0.14	0.46	< 0.001	0.51
Overall % Δ	6.39	6.49	8.17	5.40	9.42[a]	4.62[b]	5.14	0.60	0.005	0.27
Body Wall, cm										
d 1	1.57	1.69	1.66	0.18	1.64	1.64	0.26	0.46	0.93	0.92
d 20	1.94	2.02	2.03	0.20	2.05	1.94	0.26	0.67	0.21	0.90
Overall % Δ	25.64	20.32	23.39	11.54	26.64	19.60	15.72	0.70	0.17	0.82

[1] Overall % Δ was calculated as the difference in initial d 1 ultrasound body measurements and final d 20 ultrasound body measurements: [(d1 value – d 20 value) / d 1 value]

Table 6. Predicted carcass composition[1] based upon ultrasound measurements collected prior to harvest of feeder lambs supplemented 0 mg (CON), 40 mg ractopamine hydrochloride (RHCL), or 2.5 mg zilpaterol hydrochloride (ZHCL) during a 20 d controlled heat stress challenge

Variable	βAA Supplementation				Environment			P-value		
	CON	RHCL	ZHCL	SD	TN	HS	SD	βAA	Enviro.	β x E
Predicted TDL, kg[1]	12.67	12.70	12.87	0.89	12.98[a]	12.48[b]	0.82	0.85	0.02	0.94
Predicted TDL, %	52.46[a]	52.23[ab]	52.02[b]	0.55	52.10	52.37	0.58	0.04	0.06	0.67
Predicted FFL, kg[2]	11.87	11.89	11.99	0.86	12.15[a]	11.68[b]	0.79	0.87	0.02	0.93
Predicted FFL, %	49.04[a]	48.80[ab]	48.60[b]	0.47	48.92	48.71	0.55	0.04	0.13	0.62

[1] Predictions equations of Total Dissectible Lean (TDL) = 0.694 + (0.213 x Live Weight (LW)) – (0.789 x Back Fat (BF)) + (1.12 x Loin Muscle depth (LM)); % TDL = 58.22 – (0.095 x LW) – (11.1 x BF) + (0.349 x LM depth)
[2] Fat free carcass lean weight (FFL) = 0.422 + (0.207 x LW) – (1.24 x BF) + (1.05 x LM depth); % FFL = 53.23 – (0.054 x LW) – (12.01 x BF) + (0.0341 x LM depth)

Table 7. Carcass composition analysis of feeder lambs supplemented 0 mg (CON), 40 mg ractopamine hydrochloride (RHCL), or 2.5 mg zilpaterol hydrochloride (ZHCL) during a 20 d controlled heat stress challenge

| Variable | βAA Supplementation | | | | Environment | | | P-value | | |
	CON	RHCL	ZHCL	SD	TN	HS	SD	βAA	Enviro.	β x E
Left Side Wt., kg	13.88	13.84	14.19	1.06	14.51[a]	13.43[b]	0.87	0.56	0.001	0.69
Foresaddle, %[1]	46.92	46.94	46.68	0.94	46.48	47.21	2.60	0.95	0.35	0.28
Hindsaddle, %[2]	44.37	43.98	44.80	0.88	44.50	44.23	2.22	0.66	0.73	0.94
FFL, %[3]	51.52[a]	50.63[a]	53.62[b]	2.02	52.48	51.36	2.31	0.01	0.16	0.36
Adipose, %[4]	24.88[ab]	26.83[a]	23.94[b]	2.07	25.40	25.04	2.43	0.05	0.71	0.18
Bone, %[5]	23.60	22.54	22.45	1.62	22.12	23.60	2.32	0.47	0.09	0.71

[1] Fore-saddle % is the percent of the left side of the carcass that makes up the fore-saddle section
[2] Hind-saddle % is the percent of the left side of the carcass that makes up the hind-saddle section
[3] Fat Free Lean (FFL) % is the percent of fat free lean tissue obtained from the left side of the carcass
[4] Adipose % is the percent of adipose tissue obtained from the left side of the carcass
[5] Bone % is the percent of bone obtained from the left side of the carcass

Figure 1. Environmental stress categories based upon the Livestock Weather Safety Index Temperature Humidity[1] Index utilized in a 20 d controlled heat stress challenge with feeder lambs supplemented 0 mg (CON), 40 mg ractopamine hydrochloride (RHCL), or 2.5 mg zilpaterol hydrochloride (ZHCL).

		Ambient Temperature (°C)																	
		18	20	22	24	26	28	30	32	34	36	38	40	42	44	46	48	50	52	54
Percent Relative Humidity	5%	16.1	17.2	17.8	18.9	20.0	20.6	21.1	22.8	23.9	24.4	25.6	26.7	27.2	28.3	29.4	30.0	31.1	32.2	33.3
	15%	16.1	17.2	18.3	19.4	20.6	21.7	22.8	23.9	25.0	25.6	26.7	27.8	28.9	30.0	31.1	32.2	33.3	34.4	35.6
	25%	16.7	17.8	18.9	20.0	21.1	22.2	23.3	24.4	26.1	27.2	28.3	29.4	30.6	31.7	32.8	33.9	35.0	36.1	37.8
	35%	16.7	17.8	19.4	20.6	21.7	23.3	24.4	25.6	26.7	28.3	29.4	30.6	32.2	33.3	34.4	36.1	37.2	38.3	39.4
	45%	16.7	18.3	19.4	21.1	22.2	23.9	25.0	26.7	27.8	29.4	30.6	32.2	33.3	35.0	36.1	37.8	38.9	40.6	41.7
	55%	17.2	18.3	20.0	21.7	23.3	24.4	26.1	27.8	28.9	30.6	32.2	33.3	35.0	98.0	38.3	39.4	41.1	42.8	43.9
	65%	17.2	18.9	20.6	22.2	23.9	25.6	27.2	28.3	30.0	31.7	33.3	35.0	36.7	38.3	40.0	41.7	43.3	44.4	46.1
	75%	17.8	19.4	21.1	22.8	24.4	26.1	27.8	29.4	31.1	32.8	35.0	36.7	38.3	40.0	41.7	43.3	45.0	46.7	48.3
	85%	17.8	19.4	21.1	23.3	25.0	26.7	28.9	30.6	32.2	34.4	36.1	37.8	39.4	41.7	43.3	45.0	47.2	48.9	50.6

[1] White cells THI value classification of "No Stress", Yellow cells THI value classification of "Alert", Orange cells THI value classification of "Danger", and Red cells THI value classification of "Emergency"

Figure 2. Interaction of β - adrenergic agonist supplementation during a controlled heat stress challenge on respiration rate of feeder lambs supplemented 0 mg (CON), 40 mg ractopamine hydrochloride (RHCL), or 2.5 mg zilpaterol hydrochloride (ZHCL)
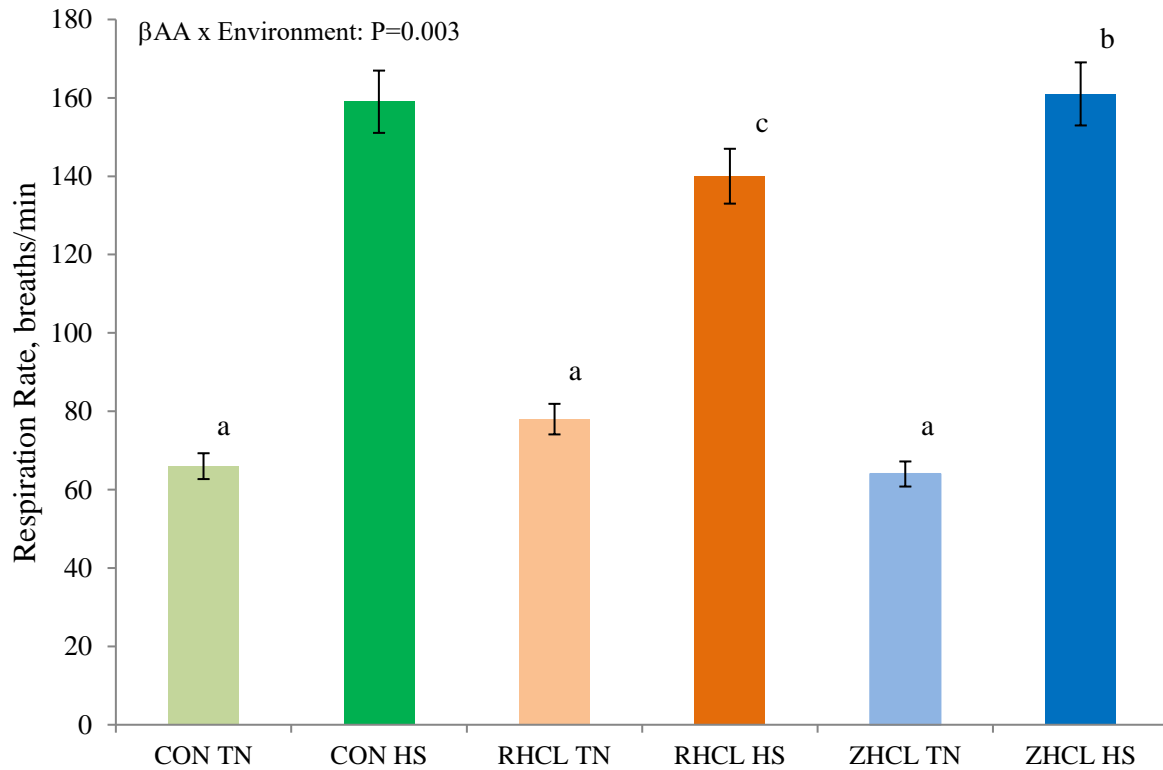
Treatment Groups: Control / Thermal Neutral (CON TN); Control / Heat Stress (CON HS); Ractopamine Hydrochloride / TN (RHCL TN); Ractopamine Hydrochloride / Heat Stress (RHCL HS); Zilpaterol Hydrochloride / Thermal Neutral (ZHCL TN); Zilpaterol Hydrochloride / Heat Stress (ZHCL HS)