2018

The effect of copper supplementation on performance and carcass characteristics of cattle utilizing growth promoting technologies

Elizabeth Marie Messersmith
Iowa State University

The effect of copper supplementation on performance and carcass characteristics of cattle utilizing growth promoting technologies

by

Elizabeth Marie Messersmith

A thesis submitted to the graduate faculty

in partial fulfillment of the requirements for the degree of

MASTER OF SCIENCE

Major: Animal Science

Program of Study Committee:
Stephanie Hansen, Major Professor
Grant Dewell
Daniel Loy

The student author, whose presentation of the scholarship herein was approved by the program of study committee, is solely responsible for the content of this thesis. The Graduate College will ensure this thesis is globally accessible and will not permit alterations after a degree is conferred.

Iowa State University

Ames, Iowa

2018

TABLE OF CONTENTS

NOMENCLATURE

ADG	Average daily gain
BA	Beta agonist
β-AR	Beta-adrenergic receptor
βARK	β-adrenergic receptor kinase
BF	Back fat
BSC	bovine satellite cells
BW	Body weight
Ca	Calcium
cAMP	Cyclic adenosine monophosphate
CCS	Copper chaperone for superoxide dismutase
Cd	Cadmium
Co	Cobalt
Cp	Ceruloplasmin
CP	Crude Protein
Cu	Copper
d	Day
DDGS	Dry distillers grains solubles
DM	Dry matter
DMI	Dry matter intake
DMT1	Divalent metal transporter 1
DP	Dressing percent
E2	Estradiol
ECM	Extracellular matrix
EGFR	Epidermal growth factor receptor
Fe	Iron
G:F	Gain:feed

GH	Growth hormone
GPCR	G protein coupled receptor
GPER-1	G protein coupled estrogen receptor 1
GRK	G protein coupled receptor kinases
GSH	Reduced glutathione
h	Hour
hbEGF	Heparin binding epidermal growth factor-like growth factor
HCW	Hot carcass weight
hLOXL-2	Human lysyl oxidase like protein 2
IGF-I	Insulin like growth factor I
KPH	Kidney pelvic heart fat
LOX	Lysyl oxidase
LOXL1-4	Lysyl oxidase like proteins
MDGS	Modified distillers grains solubles
Mg	Magnesium
MMP2/9	Matrix metalloproteinases 2 and 9
Mn	Manganese
Mo	Molybdenum
N	Nitrogen
NASEM	The National Academies of Sciences, Engineering, and Medicine
NDF	Neutral detergent fiber
NEFA	Non-esterified fatty acids
PD	Parkinson's disease
PDE	Phosphodiesterase
PKA	Protein kinase A
RAC	Ractopamine hydrochloride
REA	Ribeye area

S	Sulfur
SEM	Standard error of the mean
SOD	Superoxide dismutase
TBA	Trenbolone acetate
TMR	Total mixed ration
YG	Yield grade
ZH	Zilpaterol hydrochloride
Zn	Zinc

ACKNOWLEDGMENTS

I would like to express my gratitude to my major professor and mentor, Dr. Stephanie Hansen for pushing me to become a better scientist and uncovering my interest in mineral nutrition. Your guidance and answering of my countless questions were greatly appreciated over the past two years as I grew as a researcher. I also want to express my thanks to my committee members Drs. Dan Loy and Grant Dewell and teaching mentor Brad Skaar for their time and willingness to share their knowledge of the industry with me.

To my fellow graduate students, office mates, past Hansen lab members, and Hansen lab undergrads especially Remy Carmichael, Erin Deters, Katie VanValin, Trey Jackson, Erika Lundy, Emma Niedermayer, Sarah Hartman, Claire Andreson, Katie Hochmuth, and Katie Heiderscheit thank you for your support and guidance. Your friendship and endless jokes were much appreciated during the long hours spent at the farm and in the lab.

I would also like to thank my friends and family for their support and encouragement throughout the past two years. To my husband, Noah, I am forever grateful for your support in all that I do. I appreciate the countless meals you have prepared and laundry you have folded so that I could focus on school. To my parents, thank you for encouraging me to follow my dreams. Your support has been unwavering as I have ventured out on my own. Thank you, mom for sharing your love of learning and dad for sharing your passion for cattle with me. I am truly blessed.

ABSTRACT

Growth promoting technologies such as beta agonists and hormone implants are used extensively throughout the feedlot industry (Samuelson et al., 2016). These technologies are known to improve performance and efficiency of growth and thus are vital to sustained profitability for cattle producers. The ability to further improve the effectiveness of these technologies would improve productivity of cattle and may be accomplished through the strategic supplementation of trace minerals such as Cu. Although industry recommendations for Cu are two times NASEM (2016) recommendations, little research has evaluated trace mineral supplementation in addition to growth promoting technologies. Therefore, the effects of greater concentrations of minerals such as Cu in the diet of cattle utilizing growth promoting technologies has not yet been validated. The research trials described in this thesis were designed to 1) determine the effect of Cu status of finishing beef steers on beta agonist induced growth performance and 2) examine the impacts of industry rates of Cu supplementation on the implant growth response of finishing feedlot steers. Through our first research objective, the supplementation of varying concentrations of Cu to finishing feedlot cattle differentially impacted the beta agonist induced performance response of cattle. Within cattle receiving the beta agonist treatment, performance was greatest at Cu supplementation at NASEM (2016) recommendations. These performance differences were observed in Cu supplemented cattle with adequate liver Cu status suggesting further classification of Cu status for optimal growth is needed. Through our second study performance was impacted by an interaction between implant and Cu in which cattle not

supplemented with Cu experienced a 24.7% increase in ADG due to implant while industry recommendations of Cu supplementation resulted in a 15.3% improvement in ADG due to implant. Additionally, liver Cu concentrations tended to increase and liver Mn concentrations decreased 14 days after implantation, while liver Zn and plasma Zn revealed trends for decreases due to implant. Together these studies indicate Cu may impact the physiological response to growth promoting technologies and that implants may alter mineral metabolism. Additionally, these data revealed that supplementation of Cu beyond NASEM (2016) recommendations may not promote optimal performance from cattle utilizing growth promoting technologies. Collectively, the studies presented in this thesis find that concentrations of Cu commonly supplemented within the industry are not merited. This work warrants additional research to better understand the role of Cu in growth induced through technologies such as beta agonists and hormone implants.

CHAPTER 1. GENERAL INTRODUCTION

Growth promoting technologies within the feedlot have substantially improved the productivity of the beef industry. Both hormonal implants and beta agonists (**BA**) are common technologies used in the industry today. Hormonal implants have been utilized for more than 50 years and nearly 70 and 85 percent of cattle represented in a survey of consulting nutritionists are implanted with a two implant protocol or fed a BA, respectively (Samuelson et al., 2016). Such practices indicate the importance of growth promoting technologies to maximize production and efficiencies throughout all stages of production in the feedlot sector.

Enhanced growth seen in cattle receiving hormonal implants and BA may lead to increased nutritional requirements such as minerals. Particularly, copper (**Cu**) may be required in this state of increased growth due to its support of extracellular matrix formation through the Cu dependent enzyme lysyl oxidase (**LOX**) that forms crosslinking of collagen and elastin supporting the structural integrity of the cell (Rucker et al., 1998). Further, implanted steers have been observed to have lesser liver Cu status 14 days after reimplantation (20 mg E_2 and 200 mg TBA) than nonimplanted counterparts suggesting Cu may be important in hormonal implant induced growth (Niedermayer et al., 2018). Additionally, Cu appears to have a potential role in the BA pathway through its inhibition of phosphodiesterase (**PDE**) in cultured adipocytes (Krishnamoorthy et al., 2016).

Therefore, a combination of strategic trace mineral supplementation in addition to growth promoting technologies may be required to optimize beef production. In two studies, this research examines the impacts of varying concentrations of dietary Cu

provided to beef finishing steers either receiving the BA ractopamine hydrochloride or with or without a potent steroid hormone implant.

Thesis Organization

The following chapter, Chapter II, will present a detailed review of the literature regarding the mechanisms of BA and hormonal implants in addition to the importance of the biological processes of Cu in growing cattle. The following two chapters, chapters III and IV, will present research that is intended to be published in the *Journal of Animal Science* or the *Professional Animal Scientist*. The research presented in chapter III will focus on the effect of initial liver copper status in finishing steers fed three dietary concentrations of copper with or without ractopamine hydrochloride on liver copper status, growth performance, and carcass characteristics. Chapter IV will concentrate on the effect of supplementation of copper to steers that either received a potent combination implant or did not receive an implant on performance and carcass characteristics. This thesis will then conclude in Chapter V with overall research findings and proposals for further research.

CHAPTER 2. LITERATURE REVIEW

Beta Agonists

The use of BA in the cattle industry is prominent due to the desirable growth efficiencies observed with use in feedlot cattle. Nearly 85% of cattle represented in surveys of either consulting nutritionists or feedlot managers are fed a BA (Birch, 2015; Samuelson et al., 2016). A meta-analysis of cattle fed either zilpaterol hydrochloride (**ZH**; tradename Zilmax) or ractopamine hydrochloride (**RAC**; tradename Optaflexx or Actogain45) indicated that the use of BA improved live BW 8 kg and ADG by 0.15 to 0.19 kg/day for both ZH and RAC (Lean et al., 2014). Further analysis revealed that HCW was 15 kg heavier for cattle fed ZH with RAC fed cattle having improved HCW over control animals but to a lesser degree than ZH (Lean et al., 2014). This improvement in growth is animal dependent, as animals are born with a finite number of muscle fibers indicating that growth elicited from BA use is a result of hypertrophy as fiber DNA content does not increase (Mills, 2002; Johnson et al., 2014). Growth is elicited through the beta-adrenergic receptor (**β-AR**) belonging to a family of G-protein coupled receptors (**GPCR**; Johnson et al., 2014).

There are three known β-AR, including β_1AR, β_2AR, and β_3AR that are found in different proportions throughout body tissues (Johnson et al., 2014). In cattle, skeletal muscle contains over 99% β_2 receptors while adipose tissue contains greater than 90% β_2 receptors (Johnson et al., 2014). The importance of receptor type is due to the affinity of BA to these specific receptors. Currently the only BA available in the U.S., RAC is a β_1 agonist with partial β_2 agonist activity (Johnson et al., 2014). In contrast, ZH, while having an affinity for both β_1 and β_2 receptors, is predominantly a β_2 agonist (Verhoeckx

et al., 2005). In addition to receptor types, there are differences in the types of BA. Some BA are full agonists that allow maximum stimulation after binding to the β-AR while others are classified as partial agonists for their incomplete activity (Rosenbaum et al., 2009; Warne et al., 2011; Manglik et al., 2015). Furthermore, a third type of ligand called an antagonist can bind to the β-AR but does not elicit a response, therefore blocking the receptor site from an active agonist (Rosenbaum et al., 2009; Warne et al., 2011; Manglik et al., 2015). The biological importance of these agonists and antagonists are demonstrated in the BA pathway.

Beta agonist pathway

Beta agonists do not directly act upon muscle fibers to elicit growth, but instead trigger a cascade of effects by binding to β-AR on the plasma membrane of most mammalian cells (Mersmann, 1998). These GPCR are composed of seven hydrophobic transmembrane domains with hydrophilic segments in between these domains alternating intracellularly and extracellularly (Strader et al., 1989; Mersmann, 1998; Rosenbaum et al., 2009). These domains are continuous structures with each hydrophobic region containing 20 to 25 amino acids with variable lengths of hydrophilic regions between each domain spanning in total over 400 amino acids in length (Strader et al., 1989; Mersmann, 2002). The two ends of the membrane protein amino acid chain are on inverse sides of the plasma membrane. The amino-terminus is located on the extracellular portion while the carboxy-terminus is situated intracellularly (Strader et al., 1989; Strosberg and Pietri-Reuxel, 1996). The extracellular segments of these domains play a critical role in the binding site of the BA as amino acids from several of the transmembrane loops interact with the ligand as it binds to the receptor (Mersmann, 1998). The seven extracellular domains are arranged in

a circular formation with extracellular portions of transmembrane domains 3, 4, 5, 6, and 7 forming the main ligand binding pocket, while transmembrane domains 1, 2, 3, and 7 form the shallow minor binding pocket on the extracellular side of the GPCR (Schwartz et al., 2006).

Examination of the full agonists carmoterol and isoprenaline alongside the partial agonists salbutamol and dobutamine show that these four agonists bind to the ligand-binding pocket of the β-AR in a similar fashion. A secondary amine and β-hydroxyl group form potential hydrogen bonds with aspartic acid 121 and asparagine 329 residues except for in dobutamine which lacks the β-hydroxyl group (Warne et al., 2011). Another hydrogen bond is also formed with the asparagine 310 residue of the β-AR (Warne et al., 2011). In addition, all agonists form hydrogen bonds with serine 211 and induce a conformational change in serine 212 to allow for a hydrogen bond to form with asparagine 310 (Warne et al., 2011). When full agonists are bound to the β-AR, conformation changes in the side chains of serine 212 and serine 215 weaken the interactions between transmembrane domains 4 and 5 while strengthening the interaction between transmembrane 5 and 6 with an added hydrogen bond (Warne et al., 2011). Both the weakening and strengthening of these bonds may facilitate the movement of transmembrane 5 and 6 as has been noted during rhodopsin activation (Park et al., 2008; Warne et al., 2011). This movement of transmembrane domains is thought to be due to allosteric coupling of the ligand binding pocket and the cytoplasmic domain of the β-AR (Manglik et al., 2015). In addition to the binding of the agonist to the ligand binding pocket, the G-protein, G_s is required for the β-AR to elicit a response to the agonist subsequently resulting in the outward movement of transmembrane 6 (Manglik et al., 2015). Further, the

binding of G_s to β-AR stabilizes the cytoplasmic region of transmembrane 7 and elicits a change in the structure of the extracellular loop between transmembrane 4 and 5 (Devree et al., 2016). A β_2-AR-G_s complex has been shown to have two aromatic residues, phenylalanine 193 and tyrosine 308, that move approximately 2-2.5 Å closer to each other to form a lid-like structure over the β-AR ligand binding site (Devree et al., 2016). This lid provides protection to the binding site from other potential ligands as well as from the dissociation of a bound ligand (Devree et al., 2016). Movement of the β-AR transmembrane domains may be enhanced by the methylation of phospholipids in the plasma membrane (Hirata et al., 1979). The stimulation of rat reticulocytes with L-isoproterenol resulted in increased phospholipid methylation and thus fluidity of the membrane while also increasing the activation of adenylyl cyclase from the β-AR activity (Hirata et al., 1979). This increased fluidity of the plasma membrane is believed to enhance the β-AR coupling to adenylyl cyclase by allowing the domains to move more easily through the membrane to obtain their active form (Hirata et al., 1979).

The G proteins necessary to couple agonist activation of β-AR to adenylyl cyclase activity are guanine nucleotide binding regulatory proteins (Gilman, 1984). Two G proteins have a role in this response. The G_s protein is a stimulating protein while G_i inhibits the coupling of β-AR to adenylyl cyclase for activation (Rasenick et al., 1994; Rosenbaum et al., 2009). Both of these proteins are heterotrimeric with α, β, and γ subunits that are all required for activation (Rasenick et al., 1994). Following the agonist binding to the β-AR a G protein is bound intracellularly catalyzing a reaction leading to the release of GDP from the α subunit of the G protein (Devree et al., 2016). Intracellular GTP then binds the G protein rendering it in the active state (Strader et al., 1989; Sprang, 1997). The G_s

subunit dissociates from the $G_{\beta\gamma}$ subunit and is responsible for the activation of adenylyl cyclase to generate cAMP (Tang and Gilman, 1992). The production of cAMP can have various biological effects as it is a secondary intracellular messenger (Robison et al., 1968). Within the BA pathway, cAMP activates protein kinase A (**PKA**; Beavo et al., 1974). The structure of PKA is composed of two catalytic subunits as well as 2 regulatory subunits; cAMP binds directly to the regulatory subunits allowing for the release of the active catalytic subunits (Zhang et al., 2012). The catalytic subunits of PKA are responsible for the phosphorylation of specific target proteins such as hormone sensitive lipase in the BA pathway (Mersmann, 1998; Zhang et al., 2012). The use of hormone sensitive lipase knock out mice given the universal BA isoproterenol demonstrated that activation of hormone sensitive lipase is responsible for the lipolysis response from BA use as knock out mice had lesser circulating concentrations of free fatty acids (Haemmerle et al., 2002).

Beyond the stimulation of lipolysis, BA positively impact muscle accretion in part by decreasing protein degradation (Mersmann, 1998). The supplementation of clenbuterol to healthy rats increased muscle weight while also increasing the rate in which protein and RNA accumulated in the muscles (Reeds and Hay, 1986). Further, the treatment of bupivacaine hydrochloride injured muscle cells with the BA fenoterol resulted in greater restoration of muscle than saline treated rats signifying that BA promote regenerative muscle growth as well as new muscle accretion in healthy tissue (Beitzel et al., 2004). In addition to muscle tissue repair, cAMP dependent muscle hypertrophy was observed in muscle $G_{s\alpha}$ knock out mice as muscle mass of these mice was less than wild type mice indicating that $G_{s\alpha}$ activation of muscle hypertrophy is necessary (Chen et al., 2009). Further, the BA clenbuterol was observed to produce a dose dependent decline in muscle

proteolysis of treated rats (Navegantes et al., 2000). Testing the effects of clenbuterol on proteolysis, cAMP inhibitors DBcAMP and IBMX were each incubated with the BA (Navegantes et al., 2000). The addition of inhibitors to clenbuterol resulted in no difference in proteolysis activity between treatment, although values were still lesser than in control rats (Navegantes et al., 2000). This suggests that the proteolytic effects of BA are mediated through cAMP which likely activates a protein kinase that phosphorylates and thus inactivates the mechanism for muscle proteolysis (Navegantes et al., 2000). Presumably, this mechanism is the cAMP facilitated production of calpastatin that leads to decreased muscle proteolysis through the inhibition of calpain (Cong et al., 1998).

Further work has indicated that BA muscle accretion response is not only due to activation of the cAMP pathway. The Akt/mTOR pathway has been proposed to be stimulated by the BA clenbuterol with the β and γ subunits of the G_s protein mediating this activation (Sneddon et al., 2001; Kline et al., 2007). Supplementation of clenbuterol to rats resulted in an increase in p70 S6 kinase and 4E-BP1, the downstream metabolites of the Akt/mTOR pathway that result in protein synthesis (Sneddon et al., 2001; Kline et al., 2007). Further treating rats with the mTOR inhibitor rapamycin resulted in a lessening of the clenbuterol protein accretion effect (Kline et al., 2007). Although it is known that BA promote protein accretion, the specific mechanism by which stimulated G proteins activate this hypertrophy is not fully understood. Although increased muscle accretion due to BA is readily accepted, additional studies are warranted to fully understand how both the Akt/mTOR and cAMP pathway mediate BA-induced growth.

Beta agonist regulation

Following the activation of β-AR, the receptor must be regulated to manage the signal. Only three families of proteins are known to interact directly with agonist activated GPCRs including G-protein-coupled-receptor-kinases (**GRK**), β-arrestins, and G-proteins (Lefkowitz and Shenoy, 2005). The β-adrenergic receptor kinase (**βARK**) is considered part of the GRK family. Through the stimulation of purified hamster lung β-AR with isoproterenol, βARK was determined to phosphorylate agonist occupied β-AR as receptor phosphorylation increased up to 10-fold with stimulation in comparison to the antagonist bound β-AR (Benovic et al., 1986). This phosphorylation is the beginning of the regulation process. The βARK is initiated once the β-AR is bound by an agonist, after which βARK is translocated from the cytosol to the plasma membrane to phosphorylate the β-AR (Strasser et al., 1986). Serine and threonine residues on either the carboxy-terminal tail region or the third cytoplasmic loop of β-AR are the target sites of phosphorylation for GRK (Reiter and Lefkowitz, 2006; Ma and Pei, 2007). Both β_1-AR and β_2-AR cytoplasmic tails contain 10-11 serine and threonine for such phosphorylation (Hausdorff et al., 1990). In addition to βARK, PKA, a downstream product of GPCR activation, can phosphorylate the β-AR and uncouple the G-protein (Baillie et al., 2003). Both PKA and βARK are required to work in concert to cause desensitization of β-AR, but may work individually under long term exposure to BA (Hausdorff et al., 1990). In the case of mutated β_2-AR from Chinese hamster fibroblasts either lacking the βARK or PKA phosphorylation site stimulation with 10 nM of isoproterenol revealed insight into β-AR phosphorylation (Hausdorff et al., 1989). Cells lacking the βARK sites experienced 80% more phosphorylation over basal rates (Hausdorff et al., 1989). In

contrast, receptors lacking either PKA or both PKA and βARK phosphorylation sites only experienced 30-40% phosphorylation over basal levels (Hausdorff et al., 1989). These data suggest that at low isoproterenol stimulation phosphorylation is preferred at the carboxyl-terminal βARK sites.

Following phosphorylation, β-arrestins are recruited to the agonist bound β-AR with high affinity as an essential cofactor to βARK (Hausdorff et al., 1990; Reiter and Lefkowitz, 2006; Deshpande et al., 2008). Both the agonist induced receptor conformation alteration and the phosphorylation of the β-AR lead to the recruitment of β-arrestins to the β-AR (Gurevich and Benovics, 1993; Lefkowitz, 1998). It is suggested that β-arrestin engages with the β-AR through a two-step mechanism that first involves the interaction between the phosphorylated carboxy-terminal tail of the receptor and the amino-terminal domain of the β-arrestin followed by the insertion of the finger loop within the core of the β-AR (Shukla et al., 2014). Through chemical cross-linking and modeling, this core interaction is thought to be between the lysine 235 residue on the third intracellular loop of the β-AR, specifically in β_2V_2R and the lysine 77 residue in the finger loop of β-arrestin1 (Kumari et al., 2016). Further, superimposing the structures of β-AR bound β-arrestin and β-AR bound $G_{s\alpha}$ suggests steric hindrance by β-arrestin due to the overlapping presence of both β-arrestin and $G_{s\alpha}$ (Kumari et al., 2016). This steric hindrance blocks the interaction of G-proteins with the β-AR, therefore uncoupling the G-protein from the β-AR and discontinuing the BA activated GPCR cascade of effects (Deshpande et al., 2008; Shenoy and Lefkowitz, 2011). This uncoupling of the G-protein leads to the desensitization of the β-AR (Shenoy et al., 2001). The effectiveness of β-arrestin regulation of GPCR activity can be demonstrated through β-arrestin2 knock out

airway smooth muscle cells derived from mice that were stimulated with isoproterenol for 10 minutes (Deshpande et al., 2008). A greater accumulation of cAMP was seen in β-arrestin2 knock out cells in comparison to wild type cells treated with the same isoproterenol (Deshpande et al., 2008). However this short duration study was unable to measure time to desensitization for each cell treatment (Deshpande et al., 2008).

The binding of β-arrestin to the β-AR begins the process of internalization, as β-arrestins are vital to the trafficking of β-AR to intracellular vesicles for degradation or recycling (Shenoy et al., 2001; Reiter and Lefkowitz, 2006). Specifically, these β-arrestins act as scaffolding proteins to bind proteins responsible for the movement of a specific β-AR (Ma and Pei, 2007). The majority of β-AR are internalized through clathrin mediated vesicles that form clathrin-coated pits on the plasma membrane awaiting the arrival of phosphorylated β-AR-β-arrestin complexes (Reiter and Lefkowitz, 2006). Both clathrin and adaptor protein 2 interact with β-arrestins for this specific route of endocytosis (Laporte et al., 2000). Clathrin binds to a conserved motif, while adaptor protein 2 interacts with arginine 394 and 396 on the carboxyl-terminal tail of β-arrestin (Laporte et al., 2000). However, through florescence microscopy of HEK293 cell β-AR-β-arrestin complexes lacking the clathrin binding motif it has been demonstrated that clathrin binding is not important for translocating these complexes to clathrin-coated pits for endocytosis (Laporte et al., 2000). The formation of a vesicle from these clathrin-coated pits is mediated by the GTPase dynamin that pinches the edges of the plasma membrane together to form the vesicle (Zhang et al., 1996). In the case of class A receptors such as β_2-AR, β-arrestin is only loosely associated with the receptor at the plasma membrane and dissociates into the cytosol following the formation of clathrin-

coated vesicles (Oakley et al., 1999; Zhang et al., 1999). Although studied extensively, a comprehensive understanding of how β-AR-β-arrestin complexes relocate to the clathrin-coated pits for endocytosis is unclear.

Once engulfed in an intracellular vesicle, phosphorylated β-AR are dephosphorylated by a membrane associated phosphatase (Krueger et al., 1997). Prior to the association of phosphatase to the phosphorylated receptor, the pH of the vesicle becomes acidic leading to a conformational change to the β-AR allowing the phosphatase to interact (Krueger et al., 1997). The acidic nature of the vesicle is vital for dephosphorylation as phosphorylated β-AR treated with NH_4Cl halted the dephosphorylation and thus re-sensitization of these receptors (Krueger et al., 1997). Following the dephosphorylation of these β-AR, they are returned to the plasma membrane as functional receptors (Pippig et al., 1995). However, not all β-AR are recycled, some are degraded (Shenoy et al., 2001). Chronic desensitization of β-AR can be overcome with the help of β-arrestins trafficking unrecyclable β-AR to intracellular vesicles for degradation so new receptors can be synthesized (Shenoy and Lefkowitz, 2003). Whether a β-AR will be recycled or degraded is also affected by the duration of BA exposure; the longer the exposure the more likely β-AR will be degraded rather than recycled (Shenoy et al., 2001). The duration effect is a limiting factor in the use of BA in cattle and is the reasoning behind the feeding of BA for only the final 28 to 42 days on feed.

Phosphodiesterase

In addition to GRK and β-arrestin regulation, β-AR activity is also mediated through phosphodiesterases (**PDE**) which are responsible for degrading intracellular

cAMP (Shakur et al., 2001). High intracellular concentrations of cAMP can only be obtained through the activation of adenylyl cyclase or the inhibition of PDE (Hamza and Zhan, 2009). As a secondary messenger in the BA pathway, cAMP binds to the R subunit of PKA with high affinity through salt bridges and H bonds (Krishnamurthy et al., 2014). The only known means of dissociating cAMP from PKA to recycle back to the inactive holoenzyme is through PDE regulation (Wang et al., 2007; Hamza and Zhan, 2009). This cAMP dissociation from PKA is in addition to degradation of free cytosolic cAMP further preventing the activation of substrates such as PKA (Conti and Jin, 1999; Shakur et al., 2001). Within the PDE family there are 11 varieties that are specific to substrate and appear to be selectively located to specific tissues (Omori and Kotera, 2007). Varieties known for the hydrolysis of cAMP include PDE4, PDE7, and PDE8 (Shakur et al., 2001; Omori and Kotera, 2007). Additionally, PDE1, PDE2, PDE3, PDE10, and PDE11 are capable of hydrolyzing both cAMP and cGMP with the remainder of the 11 PDE proteins only equipped for the hydrolysis of cGMP (Shakur et al., 2001; Omori and Kotera, 2007). Further, PDE3 and PDE4 varieties appear to be most relevant to a BA response in cattle as PDE3 varieties are found in adipocytes and PDE4 is the largest family of PDEs expressed highly in most tissues (Beavo, 1995).

The structure of PDE is fairly conserved across family members with the C-terminal sequence having 20 to 45% homology between PDE families and up to 80% homology within a PDE family (Francis et al., 2000). In contrast to the conserved C-terminal, the N-terminal domain of each PDE family is distinct and regulates the activity of these proteins (Handa et al., 2008; Maurice et al., 2014). Within the C-terminal lies the catalytic domain (Francis et al., 2000). The catalytic domain of PDE4 is comprised of 17

α helices split into three subdomains (Xu et al., 2000). The first of these subdomains has the N-terminal tail and is a bundle of H3, H5, H6, and H7 with two short helices (H2 and H4) interconnecting these (Xu et al., 2000). In addition, this first subdomain also contains H0 and H1 which are poorly conserved across PDE families implying that their presence is not pertinent to catalytic function (Xu et al., 2000). The second subdomain is much smaller, consisting of only two short α helices (H8 and H9) that sit perpendicular to the antiparallel α helices H10 and H11 (Xu et al., 2000). Lastly, the third domain includes α helices H12, H13, H14, H15, and H16 (Xu et al., 2000). This subdomain is recognized by its β hairpin loop that extends between H12 and H13 as well as the partially conserved proline 453 residue forming a kink within the H15 helix and the C-terminal tail (Xu et al., 2000).

The deep binding pocket lies within the third subdomain and extends to incorporate both the first and second subdomains (Xu et al., 2000). Interestingly, 21 residues are fully conserved throughout all PDE families, 20 of these are positioned near this pocket, while 12 of them are directly in this binding pocket (Xu et al., 2000; Hamza and Zhan, 2009). As PDE4 is known to bind cAMP, the atomic structure of PDE4 shows that only this pocket is large enough to house the secondary messenger and its degraded product, AMP (Xu et al., 2000). The pocket itself is lined with hydrophobic and negatively charged residues and contains two divalent metal binding sites at the bottom (Xu et al., 2000; Hamza and Zhan, 2009). These metal atoms have been identified as Zn^{2+} and Mg^{2+} and are held in place by residues from all three subdomains (Hamza and Zhan, 2009). The Zn^{2+} residue helps to stabilize the structure of the catalytic domain locking the three subdomains together (Xu et al., 2000). Additionally, the Zn^{2+} metal binding site contains two conserved histidine and

aspartic acid residues that are part of a signature recognition sequence (Hamza and Zhan, 2009). Further, the deletion of the first 298 amino acids in the N-terminal determined that Mg^{2+} is located in the catalytic domain and not on the N-terminal as this deletion still elicited Mg^{2+} dependent PDE activity (Alvarez et al., 1995). It has since been observed that both Mn^{2+} and Co^{2+} can be just as effective in this catalytic role (Francis et al., 2000).

In addition to the direct regulatory role PDE plays in intracellular messaging, PDE4 specifically has been recruited by β-arrestin to β_2-AR for the control of PKA at the membrane because phosphorylation of β-AR by PKA switches its predominant coupling from G_s to inhibitory G_i protein (Baillie et al., 2003). This control of PKA ties back to the ability of PDE to deactivate PKA by degrading the bound cAMP as noted previously so the attenuated β-AR signal can proceed (Wang et al., 2007; Hamza and Zhan, 2009). In a negative feedback loop fashion, the phosphorylation of PDE4 on the N-terminal by PKA leads to the activation of PDE in rats (Sette and Conti, 1996). Specifically the serine 54 residue in the N-terminal is vital to the phosphorylation of PDE4 in rats as the substitution of this serine residue with alanine proved unfruitful (Sette and Conti, 1996). This mechanism leads to a tight regulation of PKA in the cell. The recruitment of PDE by β-arrestin to sites of high PKA activity in HEK293 cells provides further evidence for this PDE and β-arrestin relationship (Perry et al., 2002). In addition to the activation of PDE through phosphorylation by PKA, PDE can be activated through Ca^{2+}/calmodulin binding to specific sites on the N-terminal (Francis et al., 2000). Between PDE families the N-terminal provides unique activation sites that are responsible for the direct function of the specific enzyme (Houslay et al., 2007). These unique sites range from Ca^{2+}/calmodulin binding sites in PDE1 to more common PKA phosphorylation sites in PDE3 and PDE2

isoenzymes (Francis et al., 2000). The differences in activation and regulation between PDE families is most likely dependent on location, although overall function of PDE remains focused on hydrolysis of cAMP or cGMP. Through the hydrolysis of cAMP, PDE is a potent inhibitor of the β-AR signaling cascade in addition to regulation through β-arrestins. Although thought of as separate pathways of regulation, review of both families of proteins reveal that both PDEs and β-arrestins work together to modulate β-AR signaling. These proteins provide tight regulation of secondary messenger signals. In the absence of such homeostatic measures secondary messengers such as cAMP would lead to the over stimulation of some pathways. Specifically, in the BA pathway this prolonged stimulation could lead to the depletion of lipid stores due to the activation of hormone sensitive lipase mediated through cAMP activated PKA (Beavo et al., 1974; Mersmann, 1998; Zhang et al., 2012). Subsequently, this could result in loss of body condition and furthermore interrupt the supply of lipids for the structural integrity of phospholipid bilayers in cells (Dobretsov et al., 1977). Therefore, this regulation is vital to ensure there is no over or under stimulation of such potent cell signaling molecules.

Performance and carcass attributes of beta agonist use

The previously discussed mechanism by which BA elicit growth responses indicate that muscle accretion should be augmented and fat deposition decreased. Moreover, the use of BA in feedlot cattle has been shown to improve the efficiency of muscle accretion (Johnson et al., 2014). In the feedlot industry both RAC and ZH have commonly been used with similar yet distinct results. A meta-analysis of both RAC and ZH use in cattle indicates that ZH generally induces a greater performance response than RAC (Lean et al., 2014). Live BW of BA-fed cattle increased 8 kg while the ZH cattle

maintained more of this weight in HCW averaging 15 kg heavier than control animals (Lean et al., 2014). This disproportionate improvement in HCW over live BW in ZH-fed cattle is a direct result of an increase in DP of 1.7%; additionally improved DP in RAC-fed cattle is also observed (Lean et al., 2014). Evidence of lessened fat accretion is provided by the 1 mm decrease in back fat thickness of ZH cattle and decreased marbling observed in both ZH and RAC cattle (Lean et al., 2014). Considering the potency of ZH in comparison to RAC, fat depletion may be more likely to occur intramuscularly before back fat is affected as these results would suggest.

Over the past several years RAC has become the only commercially available BA for use in cattle in the United States and is labeled to be fed the last 28 to 42 days of the feeding period at 70 to 430 mg·animal^{-1}·day^{-1} with no withdrawal time. Variations of the labeled dose and duration have been examined with the overall consensus that RAC improves feed efficiency, final live BW, and HCW (Lean et al., 2014). However, an optimum combination of dose and duration may exist. Supplementation of RAC at 200 mg·animal^{-1}·day^{-1} during the final 28 days of the feeding period has been shown to improve ADG and feed efficiency while also improving the HCW and REA of British, Continental crossbred, and Brahman crossbred calf-fed steers, demonstrating that the BA response is produced regardless of breed type (Gruber et al., 2007). Additional studies have confirmed the improvement of ADG, feed efficiency, and HCW from RAC supplementation (Walker et al., 2006; Winterholler et al., 2007), although these performance related enhancements are not always observed (Gonzalez et al., 2009; Hales et al., 2016). Whether there is a lack in statistical power or biological reasoning behind the lack of performance from RAC is unclear. To further understand the BA response of

RAC, combinations of RAC dose and duration have been examined to determine a potentially optimum feeding protocol. Testing the efficacy of three doses of RAC: 0, 100, or 200 mg·animal^{-1}·day^{-1} with three durations of use: 28, 35, and 42 days were fed to 360 crossbred steers weighing approximately 545 kg (Abney et al., 2007). Results suggested that of these treatments feeding 200 mg·animal^{-1}·day^{-1} for 35 days provided the most favorable performance (Abney et al., 2007). As RAC dose increased, final BW, ADG, feed efficiency, and HCW all increased linearly while REA tended to linearly increase signifying a dose dependent response to RAC (Abney et al., 2007). Furthermore, as the duration of treatment increased, ADG quadratically increased with 35 day ADG showing a 14.8% improvement over 28 day ADG, although no further improvement in ADG was observed at 42 days on RAC treatment (Abney et al., 2007). In addition, 1,509 crossbred steers weighing approximately 530 kg fed either 0, 100, or 200 mg·animal^{-1}·day^{-1} of RAC for 28, 35, or 42 days elicited similar performance results for RAC, although no differences in performance or carcass characteristics were observed from duration of RAC treatment (Bittner et al., 2016). Although mixed, these results suggest that prolonged exposure to RAC may dampen the BA response, which would be indicative of desensitization of the BA pathway as previously discussed.

Hormonal Implants

Hormonal implants have been used in cattle production for decades offering greater growth rates, improved feed efficiency, and increased muscle deposition in comparison to non-implanted animals (Samber et al., 1996; Duckett et al., 1997). With approximately 70% of cattle represented by a survey of consulting nutritionists receiving a two implant protocol, the opportunity to further improve cattle performance through

progress in hormonal implant programs is vast (Samuelson et al., 2016). There are a wide variety of approved implants available for use in cattle ranging from suckling calves to finishing cattle in the feedlot. The active ingredients of these implants are variations of naturally occurring and synthetic androgens and estrogens, most commonly trenbolone acetate (TBA), estradiol (E_2), or estradiol benzoate (Montgomery et al., 2001). These hormones are often used in combination to allow for additive performance as TBA and E_2 combinations are more effective than either on their own (Frey et al., 1995). This additive performance may be a result of the different mode of action each hormone uses to elicit muscle deposition.

Hormonal implant mechanisms of action

Hormonal implants are known to improve growth as well as feed efficiency, but the exact mechanisms by which these occur is yet to be fully understood. It appears that both androgens and estrogens elicit their signal through distinct yet similar routes. Although the mechanisms of stimulation may be varied, both improve muscle growth. Animals are born with a finite number of muscle fibers therefore growth must occur through hypertrophy (Mills, 2002). This hypertrophy is a result of DNA accumulation in the cell as myoblasts are the primary source of muscle growth (Chen et al., 2005). Myoblasts are not always active; when they are in the quiescent state they are called satellite cells (Chen et al., 2005). These satellite cells are located between the basal lamina and the sarcolemma of the muscle fiber (Moss and Leblond, 1971). Following activation satellite cells become myoblasts that begin to proliferate and differentiate into myotubes before fusing with existing muscle fibers (Moss and Leblond, 1971; Chen et al., 2005). These satellite cells are thought to make up between 2 and 10% of nuclei

within a myofiber and are responsible for the hypertrophy of the individual fiber (Thompson et al., 1989).

It has been observed that satellite cells are more abundant during early development when animals are more prone to rapid growth and begin to decline with age (Chen et al., 2005). Evidence of this decline has been observed in pigs when nuclei accumulation was evident through 80 days postnatal (Swatland, 1977). Following this time period nuclei presence began to decline in individual fibers of porcine muscle as expected following this period of rapid growth (Swatland, 1977). Additionally, in steers fed to different end weights muscle DNA accumulation began to plateau once steers reached 360 kg (Trenkle et al., 1978). Further, the lag phase of satellite cells retrieved from 12 and 24 month old rats was significantly longer than in cells from 3 month old rats, thus growth is not as prolific with age (Dodson and Allen, 1987). Together these studies provide evidence that growth rate begins to decline as animals age in part due to alterations in satellite cell function.

Muscle growth can be positively impacted through the use of hormonal implants. Culturing of satellite cells isolated from steers implanted with Revalor-S, a combination implant (120 mg TBA and 24 mg E_2), revealed that cells from implanted steers proliferated more rapidly than satellite cells from nonimplanted steers between 24 and 72 hours of culture (Johnson et al., 1998a). Interestingly, at 72 hours the satellite cells from nonimplanted steers had comparable rates of proliferation to those of implanted steers (Johnson et al., 1998a). These data suggest that satellite cells that were exposed to TBA and E_2 from an implant were activated more quickly than satellite cells from steers that were not implanted (Johnson et al., 1998a).

Additionally, when steers were implanted with Revalor-S circulating insulin like growth factor I (**IGF-I**) was increased by 6 days after implantation (Johnson et al., 1998a). It appears that IGF-I is important in both the proliferation and differentiation of skeletal muscle myoblasts due to its vast expression in skeletal muscle (Florini et al., 1996; Layne and Farmer, 1999). Therefore the sustained elevation of IGF-I in circulation between day 0 and 40 of implant is consistent with the profuse growth observed during this time period (Johnson et al., 1998a). Furthermore, in steers implanted with Revalor-S for 40 days longissimus dorsi IGF-I mRNA was 68% greater than in nonimplanted steers (Johnson et al., 1998b). Additionally, both E_2 (25.7 mg) and combination TBA (120 mg) and E_2 (25.7 mg) implants resulted in 78 and 65% increases in IGF-I mRNA, respectively in longissimus dorsi muscle 28 days after implantation in comparison to nonimplanted controls (Pampusch et al., 2008). Thus, the use of hormonal implants for the stimulation of satellite cells offers opportunity to further enhance the efficiency at which growth occurs.

Although often administered concurrently with an androgen, E_2 can directly mediate muscle growth. In proliferating BSC cultures stimulated with 0.01, 0.1, 1, and 10 nM of E_2 IGF-I mRNA increased in a dose dependent manner (Kamanga-Sollo et al., 2004). Further, steers treated with E_2 (25.7 mg) had increased muscle IGF-I mRNA expression (Pampusch et al., 2008). These findings again suggest that IGF-I is an important factor in hormone mediated growth. The impact of E_2 on IGF-I expression is thought to be facilitated through G protein coupled receptor 30 that has since been renamed as G protein coupled estrogen receptor-1 (**GPER-1**) because of its direct role with E_2 (Kamanga-Sollo et al., 2008a; Kamanga-Sollo et al., 2008b; Filardo and Thomas,

2012). However, the treatment of BSC with G1 (100 nM), an antagonist of GPER-1, resulted in a significant increase in IGF-I mRNA, but did not affect proliferation after the 48 hour treatment (Kamanga-Sollo et al., 2008a). Further, the treatment of BSC with 10 nM of ICI, an E_2 receptor inhibitor, did not hinder the E_2 stimulation of IGF-I expression, but rather when increased to 100 nM for 48 hours resulted in a 93% increase in IGF-I mRNA of BSC (Kamanga-Sollo et al., 2008a). The combined failed repression of IGF-I expression through ICI inhibition of the E_2 receptor in addition to G1 not being able to suppress E_2 receptors still suggests that GPER-1 is involved in E_2 mediated stimulation of IGF-I expression even though G1 suppression of GPER-1 did not yield the expected IGF-I inhibition (Bologa et al., 2006; Kamanga-Sollo et al., 2008a). One possibility for this is the G1 suppression directed E_2 to utilize other receptors to elicit the IGF-I response in its absence (Kamanga-Sollo et al., 2008a).

In addition to GPER-1 mediated E_2 stimulation of muscle growth, E_2 receptor-α is believed to provide another mechanism to stimulate hypertrophy. Treating BSC with 0.001 nM E_2 resulted in an increase of E_2 receptor-α mRNA by 2.3 times that of control treated BSC cultures (Kamanga-Sollo et al., 2004). Interestingly, higher doses of E_2 did not elicit this increase in E_2 receptor-α mRNA (Kamanga-Sollo et al., 2004). Perhaps this lack of response in receptors to further E_2 stimulation is a mechanism of homeostatic regulation. In culture conditions in which E_2 treatment does not increase the expression of IGF-I or the type I IGF receptor, E_2 treatment still stimulates the proliferation of BSC in culture (Kamanga-Sollo et al., 2008b). This uninterrupted proliferation of BSC indicates that proliferation is stimulated through other receptors such as E_2 receptor-α (Kamanga-Sollo et al., 2008a; Kamanga-Sollo et al., 2008b). Proliferation of BSC due to E_2

receptor-α stimulation by E_2 was confirmed in BSC cultured in conditions where E_2 does not stimulate IGF-I mRNA expression (Kamanga-Sollo et al., 2013).

Additionally, the activation of matrix metalloproteinases 2 and 9 (**MMP2/9**) may be involved in the E_2 stimulation of BSC proliferation through the release of heparin binding epidermal growth factor-like growth factor (**hbEGF**) from the cell surface that binds to epidermal growth factor receptor (**EGFR**) mediating the downstream pathways that regulate cell proliferation (Filardo et al., 2000; El-Shewy et al., 2004). The inhibition of MMP2/9 suppresses E_2 stimulated BSC proliferation and supports the hypothesis that E_2 binding to GPER-1 activates these metalloproteinases resulting in the proteolytic release of membrane bound hbEGF and subsequent activation of the EGFR (Kamanga-Sollo et al., 2014). The MMP2/9 role may go beyond proteolytic release of hbEGF as these MMP2/9 are involved in extracellular matrix alterations that are involved in the proliferation and impending migration of satellite cells (Montarras et al., 2013).

Further, protein accretion through satellite cell proliferation is believed to be the direct target of androgens due to the expression of androgen receptors on the plasma membrane of satellite cells (Doumit et al., 1996). The binding of an androgen to the androgen receptor forms a complex that can bind to target genes for the regulation of transcription (Chen et al., 2005). However, this ligand bound receptor complex does not bind directly to the target gene but rather to androgen response elements (Freedman, 1992). Specifically, an androgen response element has been verified in the promoter region of the IGF-I gene (Wu et al., 2007). This implies that the activation of an androgen receptor leads to the production of IGF-I and contributes to the circulating pool of this protein. In accordance with this claim, the treatment of BSC collected from the

semimembranosus muscle of steers with either 1 or 10 nM of TBA increased IGF-I mRNA to 1.7 times that observed in control BSC thereby validating this androgen receptor stimulation of IGF-I transcription (Kamanga-Sollo et al., 2004). Furthermore, treating BSC with flutamide, an androgen receptor inhibitor, completely repressed the ability of TBA (10 nM) to increase IGF-I expression in culture (Kamanga-Sollo et al., 2008a). Additionally, androgens have been observed to increase the presence of androgen receptors in the skeletal muscle of castrated rats when treated with dihydrotestosterone (50 $mg \cdot kg^{-1} \cdot day^{-1}$ for 7 days; Antonio et al., 1999). Incremental upregulation of androgen receptors in porcine satellite cells has been observed with the treatment of 10^{-7} M testosterone for 6, 12, and 24 hours (Doumit et al., 1996). The amplification of androgen receptors and IGF-I in response to androgen treatment signifies the importance of these receptors in the androgen-containing implant response.

In addition to androgen receptors mediating the androgen response from hormonal implants, androgen activation of a GPCR has been demonstrated to increase muscle growth (Heinlein and Chang, 2002). The inhibition of GPCR with GDPβS, a GPCR inhibitor, completely suppressed TBA stimulated proliferation of BSC indicating the necessity of GPCR to mediate a TBA proliferation response (Thornton et al., 2015). Additionally, the individual inhibition of MMP2/9, hbEGF, EGFR, receptor tyrosine kinase erbB2, and IGF-I receptor suppressed the TBA-induced stimulation of proliferation suggesting that all of these factors are involved in muscle growth through the stimulation of satellite cell proliferation (Thornton et al., 2015; Thornton et al., 2016). Although protein synthesis rate is impacted by the inhibition of GPCR, MMP2/9, hbEGF, erbB2, and IGF-IR protein degradation is not affected by all of these (Thornton et al.,

2016). Inhibiting EGFR and erbB2 in BSC stimulated with 10 nM of TBA resulted in a decrease in the protein degradation rate suggesting the involvement of both EGFR and erbB2 in protein degradation (Thornton et al., 2016). These studies suggest that both E_2 and TBA are more similar in their stimulation of BSC proliferation than previously thought due to their use of a GPCR and the subsequent activation of MMP2/9 leading to EGFR stimulation and therefore increased proliferation of satellite cells.

Further, it has also been suggested that hypertrophy as a result of TBA stimulation involves altering the responsiveness of satellite cells to factors such as IGF-I and fibroblast growth factor (Thompson et al., 1989). Through body composition analysis of rats treated with a TBA injection (80 μg/ 100 g BW), the weight gain thereafter was a result of both muscle and skeletal tissue growth (Thompson et al., 1989). It was further found that rats treated with TBA had greater accumulation of DNA per muscle fiber than control rats demonstrating the important role satellite cells have in muscle hypertrophy (Thompson et al., 1989). Through the evaluation of muscle cells cultured from TBA treated rats, it was concluded that these androgen treated rats were more responsive to IGF-I and fibroblast growth factor due to their increase in hypertrophy in comparison to control rats (Thompson et al., 1989).

It appears that in addition to androgen, E_2, and GPCR receptors the type I IGF receptor is involved in the proliferation of BSC. This receptor consists of 2 α and 2 β subunits that are conjoined through disulfide bonds (Kamanga-Sollo et al., 2013). The α subunits are completely extracellular and contain the IGF binding sites while the β subunits transverse the cell membrane and contain a tyrosine kinase domain that is essential for receptor function (Steele-Perkins et al., 1988). In E_2 stimulated BSC, the

treatment of LR3-IFGI (an analogue of IGF-I) with AG1024, an inhibitor of type I IGF receptor β tyrosine kinase, resulted in the hindrance of BSC proliferation mediated through LR3-IGFI as well as the ability of E_2 to stimulate proliferation of BSCs (Kamanga-Sollo et al., 2013). Further, type I IGF receptor siRNA is capable of completely hindering the stimulatory effects of LR3-IGFI and E_2 on BCS proliferation (Kamanga-Sollo et al., 2013). These results suggest that type I IGF receptor is vital in the ability of E_2 to stimulate cell proliferation (Kamanga-Sollo et al., 2013).

With both androgen and E_2 stimulation the secretion of growth hormone (**GH**) from the pituitary gland is also involved in the subsequent growth response (Velloso, 2008). In order to affect growth, GH stimulates the production of IGF-I both in the liver and tissues such as muscle (Velloso, 2008). Production of IGF-I occurs in the liver and forms a ternary complex with the binding protein IGFBP-3 and the acid labile subunit for transport in the plasma (Fan et al., 2009). This complex allows for the controlled release of IGF-I in the plasma for delivery to tissues in need of IGF-I action (Fan et al., 2009). It is not fully understood what growth benefits are due to circulating IGF-I or direct GH action (Fan et al., 2009). Additionally, IGF-I is responsible for the negative feedback of GH as high circulating IGF-I stunts the release of GH from the pituitary gland (Velloso, 2008). Although in IGF-I knock out mice growth was less restricted in comparison to both IGF-I and GH receptor knock out mice, suggesting that growth from GH is also mediated through an IGF-I independent pathway (Lupu et al., 2001). Although the mechanism for hormonal implant induced growth is not fully understood, it appears that each of these receptors and proteins may play a critical role in mediating this growth response in a collective manner.

Effect of hormonal implant on cattle performance

Hormonal implants are known to enhance the growth rate of cattle. A multitude of implant strategies can be utilized. Therefore, the specific combination of hormones and timing may be of value in determining an implant strategy. In a test of hormone efficacy many studies have looked at treatments with only TBA or E_2 in comparison to combinations of the two. Additionally, studying the effect of hormonal implants on intact animals has also improved the understanding of this growth promoting technology. In a study of both steers and bulls implanted once with 120 mg TBA, 120 mg TBA and 24 mg E_2, or no implant produced a 22% increase in ADG over control counterparts during the growing phase of production (Hunt et al., 1991). However, there was no difference in ADG or feed efficiency in steers treated with TBA only (Hunt et al., 1991). Interestingly, this combination implant in steers produced an ADG response that was comparable to bulls of any treatment (Hunt et al., 1991). The lack of differences between implant treatments in bulls is likely due to greater circulating testosterone in comparison to steers suggesting that bulls are growing at their maximum rate prior to the administration of hormonal implants. Further examination into biological differences in sex hormones was conducted in a combination implant study using steers and heifers. The utilization of a combination implant with 200 mg TBA and 28 mg estradiol benzoate (Synovex Plus) resulted in an overall 36% greater ADG for heifers and 16% greater in steers although differences in BW between treatments were not observed until later in the feeding period (Smith et al., 2007). These differences between heifers and steers may be specific to this trial as in general it appears that steers respond better to hormonal implants than heifers due to their higher growth potential.

Although most implants are combinations of both TBA and E_2, some less potent implants are comprised of E_2 only. In steers an E_2 only implant (30 mg) has been shown to increase ADG by 7% and tended to improve feed efficiency by 3% over non-implanted steers (Bartle et al., 1992). However, it is thought these performance results are to some extent diluted due to the long duration of both trials that comprised this study (140-168 days) in comparison to the lasting effects of the E_2 implant (Bartle et al., 1992). In addition, the benefits of a TBA and E_2 combination implant were observed in this study where steer performance linearly increased as both hormones were increased from 20 mg TBA and 4 mg of E_2 to 140 mg TBA and 28 mg E_2 (Bartle et al., 1992). The 140 mg TBA and 28 mg E_2 combination implant was determined to be the most effective as ADG was improved by 18% and feed efficiency by 10% over non-implanted controls (Bartle et al., 1992). Further, steers implanted with Revalor-S (120 mg TBA and 24 mg E_2) had 28% greater ADG and 23% greater feed efficiency in comparison to nonimplanted steers (Johnson et al., 1998b). Interestingly, in yearling steers implanted with 120 mg TBA and 24 mg E_2 ADG and feed efficiency were improved by 18 and 13% in the first 40 days following implantation, respectively, while no differences in ADG or feed efficiency were observed for the overall 143 day trial (Johnson et al., 1996). Through these studies it is established that hormonal implant growth responses are dose dependent and are enhanced through the administration of higher doses of both androgens and estrogens.

As the end-product is of economic concern to producers, understanding the effects of hormonal implants on the carcass is vital. In yearling steers implanted with 120 mg TBA and 24 mg E_2 HCW was improved by 26 kg in steers that were fed to 143 days from the time of implantation (Johnson et al., 1996). Additionally, Angus steers implanted with

140 mg TBA and 28 mg E_2 had a 20 kg advantage in HCW in comparison to nonimplanted Angus steers (Perry et al., 1991). Further studies with steers and heifers revealed a 21 kg increase in HCW for steers implanted with 200 mg TBA and 28 mg estradiol benzoate, implanted heifers only tended to increase HCW (Smith et al., 2007). In an effort to determine the effects of implants on carcass traits throughout the feeding period steers were serial slaughtered at 40, 115, and 143 days post implantation of 120 mg TBA and 24 mg E_2 (Johnson et al., 1996). At day 40 there were no differences, but by day 115 REA was larger for implanted steers while percent kidney pelvic heart fat (**KPH**) was decreased in implanted steers at day 143 (Johnson et al., 1996). This larger REA agrees with previous work that showed a linear increase in REA with increased combination implant hormone dose as well as a 23% larger REA in heifers implanted with a high potency implant (Bartle et al., 1992; Smith et al., 2007). However, this incremental increase in hormone dosage also resulted in an linear decrease in marbling scores (Bartle et al., 1992). However, marbling scores are not always decreased with implants, often feeding implanted cattle up to two weeks longer than nonimplanted cattle allows for the fast growing muscle to deposit fat, therefore feeding cattle to a common fat cover appears to alleviate any depression in marbling (Johnson et al., 1996; Guiroy et al., 2002).

Overall, it appears that use of hormonal implants in beef production results in greater performance and feed efficiency especially in the first 40 days following implantation. Additionally, a two implant protocol utilizing two combination implants results in a 7.5% increase in HCW and 9.0% increase in REA in comparison to

nonimplanted control cattle (Duckett and Pratt, 2014). Through the performance benefits of utilizing hormonal implants cattle can be grown in a more efficient manner.

Copper

The formation of hemoglobin was first noted to be Cu dependent in rats and signified that Cu is a vital component to proper biological function (Hart et al., 1928). Since this early discovery, Cu has been identified to have a critical role in growth through the Cu dependent enzyme lysyl oxidase that is responsible for cross-linking in connective tissue and formation of the extracellular matrix (**ECM**; Rucker et al., 1998). The ECM is of great importance in growth as it is composed of both structural and functional proteins that are responsible for the integrity of the cell and contributes to cell regeneration and tissue development (Gentili and Cancedda, 2009). In addition, Cu is essential for iron (**Fe**) oxidation via the proteins hephaestin and ceruloplasmin (Vulpe et al., 1999; Eisenstein, 2000; Vashchenko and MacGillivray, 2013). As ferroxidases, both hephaestin and ceruloplasmin are crucial to the oxidation of Fe to facilitate Fe passage to the Fe transporter, transferrin, to be distributed throughout the body (Cherukuri et al., 2005; Vashchenko and MacGillivray, 2013). Without adequate Cu status to support function of these ferroxidases, a decrease in hephaestin within cells as well as an increase in Fe concentrations in the cell has been observed further indicating that Fe transport has declined due to the critical role of Cu in ferroxidases (Suttle, 2010). An antioxidant role has also been associated with Cu through its catalytic function in Cu Zn superoxide dismutase in which the Cu molecule is partly exposed within the active site for the binding of reactive oxygen molecules (Tainer et al., 1983). One of the more easily visualized functions of Cu in the body is its role in tyrosinase to convert tyrosine to melanin (Suttle, 2010). The improper function of

tyrosinase results in the depigmentation of hair follicles resulting in the speckled gray patches of hair often seen near the eyes of severely Cu deficient cattle (Suttle, 2010). The many roles of Cu in the body clearly indicate the importance of this mineral on biological functions.

Copper absorption and trafficking

Copper absorption in the enterocyte is mainly facilitated through the transport protein CTR1 on the apical membrane as demonstrated by a decrease in Cu accumulation in peripheral tissues and poor growth in transgenic mice lacking intestinal epithelial cell CTR1 (Nose et al., 2006). The reliance on CTR1 is further indicated by resorption of CTR1 knockout mice embryos, signifying that the absence of CTR1 is embryonically lethal (Lee et al., 2001). Structurally, CTR1 is made up of three transmembrane domains as discovered through hydropathy analysis of the gene sequence (Eisses and Kaplan, 2002). These domains are fairly conserved across species with transmembrane domain 1 having the most variability (Puig et al., 2002). Imaging of CTR1 further expanded understanding of the transport protein by indicating that CTR1 is a trimer with each monomer consisting of three transmembrane domains to form a pore for Cu transport through the plasma membrane (Aller and Unger, 2006). This discovery implies that CTR1 more closely resembles a channel rather than a transport protein.

The extracellular portion of the protein is considered the N-terminal or ecto-domain, whereas the cytosolic portion is the C-terminal (Ohrvik and Thiele, 2014). As a transport protein, CTR1 is responsible for the uptake of Cu into the enterocyte as discovered in work focused on the biological function of both mouse and human CTR1 (Lee et al., 2000; Lee et al., 2002a). This singular directional movement of Cu indicates

that the extracellular and cytosolic terminals must be specific to this pull of Cu into the cell. Genetic analysis of Cu transport proteins across yeast, mice, and humans indicated that the N-terminal has three to five methionine motifs arranged as MXXM or MXM; where M represents methionine and X any amino acid (Puig et al., 2002). In *Saccharomyces cerevisiae* CTR1, the last methionine before transmembrane domain 1 is labeled methionine 127; to analyze the importance of this methionine, it was replaced with cysteine or serine resulting in significant decreases in Cu absorption (Puig et al., 2002). The decline in Cu absorption suggests that methionine 127 is integral in the coordination of Cu transport through CTR1. The N-terminal of human CTR1 is comprised of known Cu ligands methionine and histidine as well as serine (Zhou and Gitschier, 1997). The abundance of Cu ligands suggests that methionine and histidine may be essential in scavenging for Cu to import. On the cytosolic side of the plasma membrane, the C-terminal of yeast CTR1 is composed of two cysteine-X-cysteine motifs (Xiao and Wedd, 2002). The energetics of Cu passage, although not fully understood, have been evaluated. Cell culture work has indicated that Cu movement through CTR1 is ATP independent and is unaffected by an inhibitor of Na^+/K^+ ATPase (Lee et al., 2002b). This suggests Cu uptake through CTR1 is passive.

In addition to CTR1, the Fe^{2+} transporter divalent metal transporter 1 (**DMT1**) was evaluated for its ability to transport Cu across the apical membrane of enterocytes. Utilizing Caco-2 cells in ascorbate infused medium, DMT1 transported Cu across the apical membrane (Arredondo et al., 2003). The addition of ascorbate to the medium was utilized from preliminary data that suggested Cu could not be transported through DMT1 without a change in oxidation state. The successful transport of Cu through DMT1 in the presence

of ascorbate indicates that Cu is in the reduced state (Cu^{1+}) due to the reducing power of ascorbate. In addition, Cu was also noted to be increasingly transported through DMT1 in Fe deficient rats (Jiang et al., 2013). These studies indicate that Cu can indeed be transported by DMT1, but also that Cu transport can be affected by the presence of other minerals.

Following absorption through the apical membrane of both the enterocyte and hepatocyte, Cu is met with chaperones to further distribute the mineral throughout the cell to fulfil Cu dependent functions and proteins. The two cysteine residues of the CTR1 C-terminal may be integral in the transfer of Cu^{1+} from CTR1 to the chaperone protein (Xiao and Wedd, 2002). Copper is capable of damaging proteins, lipids, and nucleic acids due to its redox potential in which an accumulation of unbound Cu can lead to the formation of oxygen radicals (Rosenzweig et al., 1999; Gaetke and Chow, 2003). The use of chaperone proteins minimizes the risk of potential harm to the cell by ensuring that the Cu molecule is escorted through the cell at all times. There are three chaperone proteins that are essential for Cu intracellular transport, including CCS, Cox17, and Atx1.

The Cu chaperone for SOD (**CCS**) transports Cu from the CTR1 C-terminal to Cu, Zn SOD1 (Casareno et al., 1998). Three domains make up the chaperone, each with distinct features. Domain I is considered the amino-terminal region with a Cu binding site similar to the Cu chaperone Atx1 (Rosenzweig et al., 1999). Although equipped with a Cu binding site, domain I Cu binding is only necessary under conditions of severe deficiency (Schmidt et al., 1999). Domain II of CCS is somewhat homologous to SOD1, through yeast cell testing this domain appears to have a role in forming a complex with SOD1 to facilitate the transfer of Cu (Schmidt et al., 2000). Further experiments with yeast cells indicate that

domain II is important for interacting with SOD1, but not capable of transferring Cu on its own (Schmidt et al., 2000). The final portion of CCS, domain III, has a CXC copper binding site that is required for the transfer of Cu from the chaperone into the SOD1 structure as noted in vivo with yeast CCS (Schmidt et al., 1999). Considering domain I is limited to Cu binding during periods of Cu deficiency, domain III would be the primary binder of Cu to thus interact with SOD1.

The first evidence of the Cu chaperone Cox17 was discovered in yeast cells with defective cytochrome c oxidase, determined to be caused by a mutation in the Cox17 gene (Glerum et al., 1996a). Cytochrome c oxidase is located in the inner membrane of the mitochondria and is comprised of 13 subunits (Tsukihara et al., 1996). The enzyme has two Cu centers, Cu_A and Cu_B, in which three Cu ions are necessary for function of the enzyme and thus the respiratory chain (Tsukihara et al., 1996). Upon binding Cu from CTR1, Cox17 transports Cu to the intermembrane space of mitochondria where it transfers Cu to both Sco1 and Cox11 (Horng et al., 2004). Following the transfer of Cu ions from Cox17, Sco1 and Cox11 provide Cu to the Cu_A center in Cox2 and the Cu_B center in Cox1 of cytochrome c oxidase, respectively (Horng et al., 2004). In addition to the protein Sco1, Sco2 has been noted to have overlapping functions, but is not homologous to Sco1 indicating that both are needed for proper Cu incorporation into cytochrome c oxidase (Glerum et al., 1996b).

In addition to CCS and Cox17, Cu imported through CTR1 can also bind to the Cu chaperone Atx1/Atox1 through a bond with two cysteine residues and a sulfur atom of the Atx1 molecule for secure transport (Pufahl et al., 1997). The human homologue to Atx1, is HAH1, and its function is conserved across cell type (Hamza et al., 1999). The Atx1 Cu

loaded molecule diffuses through the cytoplasm of the hepatocyte to the target ATP7B ATPase located on the golgi apparatus where it interacts with the cytosolic Atx1-like domains (Klomp et al., 1997; Hung et al., 1998). Further, the import of Cu into the trans golgi network leads to the activation of Ceruloplasmin (**Cp**), a multi-Cu ferroxidase (Terada et al., 1995). Upon binding to the golgi apparatus, Cu is transported down a thermodynamic gradient produced by the ATPase ATP7A or ATP7B in enterocyte or liver cells, respectively (Pufahl et al., 1997). Specifically, ATP7A has been found through fluorescent imaging to localize to the trans golgi network to accumulate Cu and then move to the plasma membrane when cellular Cu is high for the efflux of Cu out of the enterocyte (Petris et al., 1996). This indicates that the P-type ATPases are important in the transport of Cu to the portal vein for further compartmentalization in the liver.

Liver absorption of Cu from the portal vein is similar to the enterocyte with the exception that a metalloreductase is not necessary considering Cu is already in the reduced state (Kim et al., 2008). The aforementioned Cu chaperones CCS, Cox17, and Atx1 bind to free Cu as it enters the cell to direct the Cu to specific cuproproteins or pathways (Glerum et al., 1996a; Lutsenko, 2010). In addition to Cu Zn SOD, cytochrome c oxidase, and Cp among others, Cu can be stored within the hepatocytes bound to the protein metallothionein which can hold 11 to 12 Cu ions at one time (Luza and Speisky, 1996). The role of metallothionein in the liver goes beyond temporary storage for cytoplasmic Cu, it alternatively is a protective mechanism from Cu-induced cytotoxicity (Linder and Hazegh-Azam, 1996; Luza and Speisky, 1996). This protective system may be why metallothionein has a greater affinity for Cu than either Cd or Zn and the release of Cu from the storage protein requires extremely low pH values (Luza and Speisky, 1996). Further information

on the mobilization of Cu from metallothionein storage during a time of deficiency is limited with more in-depth work needed to understand this mechanism.

As Cu needs of the body are met, excess Cu must be released to avoid over accumulation of the metal; biliary excretion represents the majority of Cu removal (Luza and Speisky, 1996). This biliary excretion is facilitated through ATP7B moving from the trans-Golgi network to lysosomes when Cu concentrations are high; ATP7B is responsible for the transport of Cu into lysosomes for further release into the bile canaliculus powered by ATP hydrolysis (Polishchuk et al., 2014; Ogórek et al., 2017). In Wilson's disease ATP7B is impaired or absent, thus resulting in the build-up of Cu within hepatocytes as ATP7B is the only known means of Cu export (Poujois et al., 2017). Both metallothionein and reduced glutathione (**GSH**) bind free Cu^{+}, this may in part be a mechanism to cope with an increase in hepatocyte Cu concentrations (Luza and Speisky, 1996; Aliaga et al., 2016). As the available metallothionein and GSH reserves are depleted, the hepatocytes experience oxidative stress leading to cell apoptosis (Zischka and Lichtmannegger, 2014). Free Cu released from the cells binds to serum albumin, as free Cu in the body is dangerous, and travels to the brain, eyes, and occasionally the kidneys and heart where it will accumulate (Zischka and Lichtmannegger, 2014). This accumulation in the eyes, known as the Kayser-Fleischer ring, is seen in approximately 99% of Wilson's disease patients, although less severe or early stages of the disease may only result in increased hepatic concentrations of Cu (Brewer, 2000; Liu et al., 2002).

In addition to metallothionein's role in prevention of cytotoxicity from Cu, GSH can function as both a nucleophile as well as a reductant in an effort to protect nucleic acids and protein from harmful interactions with free Cu (Freedman et al., 1989; Hanna and

Mason, 1992; Pompella et al., 2003). Most functions of GSH are related to the thiol group of the protein acting as a reductant as noted in plant cell work, further exemplified by GSH having the greatest intracellular availability of cysteine residues for the binding of Cu (Nagalakshmi and Prasad, 2001; Aliaga et al., 2016). This tripeptide is mainly expressed in the cytosol, accounting for 80 to 85 percent of cellular expression, while also having an antioxidant role within the mitochondria (Aliaga et al., 2016). The antioxidant capacity of GSH is diverse, it can either scavenge for reactive oxygen species or serve as a substrate for glutathione transferase enzymes during the conjugation of potentially pro-oxidant molecules (Deponte, 2013). Furthermore GSH is a cofactor for glutathione peroxidase that specifically reduces the reactive oxygen species hydrogen peroxide and lipid peroxides resulting in the oxidized form GSSG (Aliaga et al., 2016). The ratio of GSH and GSSG within the cell is of great importance for the redox potential as a high GSH:GSSG ratio is necessary for optimum protein synthesis (Nagalakshmi and Prasad, 2001). Regardless of the redox state, both GSH and GSSG are capable of forming complexes with Cu; although GSH tends to form compounds more readily due to its greater intracellular concentration (Aliaga et al., 2016). The study of the Cu reaction with GSH has led to the understanding that one Cu^{+} ion is bound to two GSH molecules to form a stable Cu (I)- $[GSH]_2$ complex (Aliaga et al., 2016). This complex may be an important component of Cu acquisition from the Cu transport protein Ctr1. Through the utilization of HEK293 cells treated with L-buthionine-sulfoximine for the reduction of cellular GSH, it has been demonstrated that the rate of Cu influx is dependent upon GSH as the initial rate of Cu entry into the cell declined under these conditions of low GSH (Maryon et al., 2013). The slowed rate of Cu entry was reversed by the supplementation of GSH to the cell, indicating that GSH may have a role

in the acquisition of Cu from Ctr1 (Maryon et al., 2013). This finding changes our current understanding of intracellular Cu trafficking. It has been assumed that Cu chaperones CCS, Cox17, and Atx1 directly interact with Ctr1 to acquire Cu, although this study reveals that these chaperones may in fact rely on GSH to load Cu for intracellular trafficking (Maryon et al., 2013).

Ceruloplasmin

It appears that Cp has many functions including Cu transport, antioxidant capabilities, amine oxidase activity, and most notably is a multi-Cu ferroxidase with a role in Fe mobilization (Zaitseva et al., 1996). Ceruloplasmin belongs to a family of multi-Cu oxidases including ascorbate oxidase and laccase that all have three spectroscopically different forms of Cu: type I, type II, and type III (Askwith et al., 1994). X-ray imaging of human serum Cp at 3.1 Å revealed that the protein is comprised of six domains arranged in a triangular formation (Zaitseva et al., 1996). Through comparison to family member protein ascorbate oxidase, the six domains were detected to be linked by disulfide bridges in accordance with structural similarities to ascorbate oxidase (Messerschmidt and Huber, 1990). Further, the x-ray crystallography of the holo protein indicated the six domains were comprised of three homologous regions that were made up of two parts each (Linder, 2016). Six to eight Cu ions are associated with the structure of Cp, six of which bind to the major Cu binding sites across the six domains (Sato and Gitlin, 1991). Within Cp there are three type I Cu binding sites at which a transfer of charge between S and Cu results in the intense blue coloration of the protein (Hellman and Gitlin, 2002). These type I Cu-binding sites have a cysteine, two histidine amino acids, and a methionine as ligands for Cu on domains IV and VI (Zaitseva et al., 1996). On domain II, the methionine is substituted with

a leucine residue analogous to fungal laccases (Zaitseva et al., 1996; Bento et al., 2007). Only one type II Cu binding site is represented on the protein in addition to two type III Cu binding sites (Hellman and Gitlin, 2002). The type II and III Cu binding sites are close together forming a trinuclear type II-type III Cu cluster that is important in the redox properties of Cp (Calabreses et al., 1983).

In addition to the six major Cu binding sites, Cp is also host to two metal ion-binding sites on the protein surface of domains IV and VI and are home to the oxidation of Fe, Cu, and Co upon binding (Bento et al., 2007; Lindley et al., 1997). In observance of rat Cp a new metal ion-binding site was discovered on domain II with three protein ligands, histidine 290, aspartic acid 292, and lysine 512 acting in coordination with a molecule of water to bind Cu to the labile site (Samygina et al., 2017). This newly found labile Cu binding site is found on only three other species not including human or bovine Cp and is likely the result of a single amino acid substitution as human Cp has a leucine in place of the histidine found in the new binding site (Samygina et al., 2017). This single substitution of amino acid residues indicates that Cp is variable between species and with better resolution imaging to come further understanding of the intricate Cp protein will be learned. In addition, a Ca^{2+} binding site on domain I was confirmed through the improved resolution of x-ray imaging (Bento et al., 2007). The interaction between Cp and red blood cells is a Ca^{2+} dependent process in which the binding of Cp to the plasma membrane of the red blood cell is the limiting step thought to be mediated by Ca^{2+} (Saenko and Yaropolov, 1990). Furthermore, the rigid structure of human Cp is in part due to the discovery of Na^{+} at the base of each pair of domains (Bento et al., 2007). In addition to the new labile Cu binding site, it was also found that Zn can bind to major Cu binding sites as

picked up by x-ray fluorescence at a rate of 40-50% substitution (Samygina et al., 2017). However, 100% substitution of Cu by Zn at the six major binding sites rendered the protein inactive in both in vivo and in vitro studies (Samygina et al., 2017).

As aforementioned, the role of Cp as a ferroxidase is amongst the most prominent functions of this protein. Indication of potential ceruloplasmin activity to oxidize Fe from the ferrous (Fe^{2+}) to ferric (Fe^{3+}) form was found in serum samples (Osaki et al., 1966; Roeser et al., 1970). This transformation makes Fe available to be bound to the Fe transport protein transferrin for transport through the serum (Aisen et al., 2001). The importance of the ferroxidase activity of Cp is further indicated by the accumulation of Fe in the basal ganglia of the brain as seen in a hereditary Cp deficiency of Japanese siblings (Morita et al., 1995). In addition, the administration of Cp to Cp knockout mice resulted in improved Cp concentration with restored ferroxidase activity leading to the disappearance of accumulated Fe in the brain (Zanardi et al., 2018).

An acute phase response is elicited by acute phase proteins such as Cp that act as part of host defense system against stress, inflammation, trauma, or infection (Cray et al., 2009). Ceruloplasmin's role as an acute phase protein has been documented in many species. Feed deprivation in broilers elicited an acute phase response that included an increase in Cp after 30 hours (Najafi et al., 2016). A positive correlation between fasting glucose and Cp was found in a case study enrolling initially healthy men and women in Sweden while recording blood parameters throughout their lives following a diabetes diagnosis, but no direct link between Cp and diabetes was detected (Muhammad et al., 2016). Further, Cp has been shown to mimic superoxide dismutase by inhibiting the reduction of ferricytochrome c and other lipid bound molecules (Goldstein et al., 1979).

An acute phase response can result in a 2- to 3-fold increase in Cp in response to a stressor (Giurgea et al., 2005). Although functioning as an acute phase protein Cp has a substantially lower oxygen radical scavenging activity in comparison to circulating superoxide dismutase (Goldstein et al., 1979). Taken together these studies indicate that Cp does have a role in acute phase responses, but to what degree is yet to be determined.

A link between estrogen and the Cu dependent protein Cp has been known since the 1960's when estrogenic oral contraceptives were shown to increase circulating Cp (Carruthers et al., 1966). Later the connection between Cp and estrogen was discovered to be independent of liver Cu status as mice fed a diet high in antagonistic Zn still secreted Cp, regardless of low liver Cu status (Evans et al., 1970). Injecting male and ovariectomized female rats with 50 μg/day of estradiol-17β for up to 42 days revealed increases in both serum Cp and Cu that began to plateau after 28 days of injections (Sunderman et al., 1971). Further increasing the dose of estradiol-17β to 140 μg/day led to a slow rise in serum Cp concentrations that were 70% greater than control rats on day 7 and almost 3 times as great on day 14 (Middleton and Linder, 1993). Moreover, the study of mammary epithelial cells in differing concentrations of Cu medium and at varying states of cellular differentiation: resting, lactating, and suckling, indicate that Cp transcription and secretion is increased in lactating and suckling cells (Freestone et al., 2016). This increase in both apo and holo Cp during lactation and suckling is understood to be a hormonal response present when Cu concentrations were adequate (Freestone et al., 2016). This increase in circulating Cp in response to estradiol-17β suggests that the Cu requirement of the animal may be increased due to the estradiol-17β treatment

providing a possible linkage between Cu supplementation and hormonal implant use in livestock production.

Lysyl oxidase

Lysyl oxidase (**LOX**) is most notably known for its ability to catalyze covalent cross-linking structures within the ECM (Zhang et al., 2015). The oxidative function of the protein modifies the ε- amino groups of lysine side chains in both collagen and elastin to aldehydes (Oldfield et al., 2018).The reactive aldehydes left behind form covalent crosslinks with nearby amines and other aldehydes, thus stabilizing the collagen and elastin fibers within the ECM (Smith-Mungo and Kagan, 1998). The catalytic function of LOX is Cu dependent as the removal of Cu with a high urea concentration and use of a Cu chelator inhibited LOX activity (Gacheru et al., 1990). In addition to dependence on Cu, LOX catalytic domain requires a quinone component, lysyl tyrosylquinone for catalytic function (Wang et al., 1996). The incorporation of both Cu and a quinone into the protein for catalytic function link the LOX family with the Cu amine oxidase family with the proposed mechanism for LOX based on this family of proteins (Finney et al., 2014; Moon et al., 2014). However, sequence analysis of both protein families indicate there is no obvious homology (Zhang et al., 2018); indicating that the LOX mechanism of action may be distinct from the Cu amine oxidase family.

Within the LOX family there are four LOX like proteins (**LOXL1-4**) of which the Cu-binding and catalytic domains are highly conserved (Csiszar, 2001). Limited structural analysis of LOX has led to a better understanding of protein function through the combination of structural knowledge from each protein type within the family. These LOX proteins have three conserved histidine residues within their catalytic site, which

are believed to be the binding site of Cu^{2+} (Moon et al., 2014). These three conserved histidine residues are thought to form an octahedral configuration with Cu^{2+} within the catalytic domain (Gacheru et al., 1990). Further work utilizing a 3-point mutation model and gene recombination found that of these histidine residues histidine 303 is the catalytic base of LOX proteins (Oldfield et al., 2018). The incorporation of other amino acid residues in place of histidine 303 yielded negative results as no Cu incorporation or lysyl tryosylquinone formation was detected (Oldfield et al., 2018).

Differences between the LOX family proteins arise from structural components. Both LOX and LOXL1 contain an N-terminal pro-peptide, which is removed through proteolytic cleavage in the ECM during protein maturation (Moon et al., 2014). Further, LOXL2-4 each consist of 4 scavenger receptor cysteine-rich (SRCR) domains which are believed to facilitate exchanges between ECM proteins (Hohenester et al., 1999). The cysteine dense nature of these domains and the catalytic domain has created difficulties in the structural analysis of LOX proteins (Moon et al., 2014). However, human LOXL-2 (**hLOXL-2**) protein has been extensively analyzed in a Zn bound precursor state in which the catalytic domain has been shown to have both a core and extra segments (Zhang et al., 2018). The core is comprised of three β-sheets; two smaller and one larger in which disulfide bonds stabilize the structure (Zhang et al., 2018). The extra segments surround the core of the catalytic domain with only one segment linked to the core through a bent α-helix link and a disulfide bridge (Zhang et al., 2018). Another extra segment has a β-hairpin structure that accommodates the highly conserved Cu-binding motif and is linked by a disulfide bridge to the first extra segment for stability (Zhang et al., 2018). Further the overall structure of hLOXL-2 was found to be triangular in nature with SRCR3,

SRCR4, and the catalytic domain each making up a corner of this structure (Zhang et al., 2018). The SRCR3 domain is linked to the catalytic domain through hydrogen bonds while the SRCR4 domain does not have direct contact with the catalytic domain (Zhang et al., 2018) Further distinctions between LOX proteins may be their predominant tissue expression. The LOXL-3 gene when first isolated was found in the heart, liver, smooth muscle, kidney, pancreas, and brain (Jourdan-Le Saux et al., 2001). Through immunofluorescence staining, LOXL3 was found primarily in the palate and tongue of mice (Zhang et al., 2015). This finding was verified when LOXL3 knock out mice were born with cleft palates and some with spinal deformities (Zhang et al., 2015).

The LOX family of proteins can be regulated through various biological factors. The removal of Cu was thought to render the enzyme inactive, but interestingly mature LOX depleted of Cu was found to maintain around half of its catalytic function (Tang and Klinman, 2001). The collagen associated protein fibromodulin directly influences site specific collagen cross-linking (Kalamajski et al., 2016). Furthermore, fibromodulin has also been shown to increase enzymatic activity of LOX, indicating the importance of this protein in LOX regulation (Kalamajski et al., 2016). In addition, estrogen has been shown to decrease LOX activity when engineered ligament sections were treated with physiologically high doses of 17β-estradiol (500 pg/ml) for 24 to 48 hours (Lee et al., 2015). This estrogen response appears to affect the ability of present LOX to effectively form cross-links within the collagen, understanding of how this estrogen response occurs is awaiting further research (Lee et al., 2015). Furthermore a prostaglandin derivative also decreased LOX activity in amniotic tissue leading to decreased cross-linking stabilization through the breakdown of exposed non-crosslinked structures that often

results in preterm labor (Liu et al., 2016). With much of the current research disregarding sex differences, fully understanding the effects of sex specific hormones may lead to a more in-depth understanding of enzyme responses beyond LOX.

Cuproproteins

Although Cp and LOX have important roles in the body, there are other cuproproteins into which Cu can be incorporated. The Cu dependent enzyme Cu Zn superoxide dismutase (Cu Zn SOD) damages free radicals produced through oxidative metabolism (Keller et al., 1991). With a dimer structure, each subunit contains one of the key components; either Cu or Zn (Keller et al., 1991). The absence of the Cu molecule renders the enzyme catalytically inactive, while Zn provides only a structural role (Tainer et al., 1983; Kim et al., 2008). Previously, it was thought that the Cu chaperone CCS was the sole provider of Cu to Cu Zn SOD (Casareno et al., 1998). However CCS knockout mice still exhibit some Cu Zn SOD activity (Wong et al., 2000). This activity reveals that a secondary Cu chaperone must exist for the delivery of Cu to the SOD enzyme (Wong et al., 2000). Not all organisms rely on CCS to deliver Cu to the SOD enzyme, the nematode *Caenorhabditis elegans* has an alternative mechanism (Jensen and Culotta, 2005). Through the expression of this nematode enzyme in *S. cerevisiae* Atx1, Cox17, and metallothionein were ruled out leaving GSH as the secondary Cu chaperone (Jensen and Culotta, 2005). A depletion of GSH in yeast cells void of CCS resulted in a loss of Cu Zn SOD activity that was restored through GSH supplementation (Jensen and Culotta, 2005). Although both mechanisms are functional for Cu transport it appears that CCS is still the primary Cu chaperone as Cu Zn SOD activation does not depend on GSH when CCS is present in normal conditions (Jensen and Culotta, 2005). These findings were

further investigated in a mouse model in which CCS was absent. As previously seen in yeast cells, once depleted of GSH, these CCS knockout mice were void of Cu Zn SOD activity (Carroll et al., 2006). This discovery expands our understanding of Cu trafficking beyond the three previously known Cu chaperones.

As an antioxidant Cu Zn SOD has a notable impact on the viability of cells. The use of Cu Zn SOD knockout mice revealed that the disfunction of Cu Zn SOD leads to a shorter lifespan (Elchuri et al., 2005). Tumors began to form on the livers of knockout mice as young as 20 months old; upon further examination of the livers, signs of oxidative damage were seen in the cytoplasm as well as the nucleus and mitochondria to a lesser extent (Elchuri et al., 2005). To represent the muscle wasting disorder, sarcopenia, Cu Zn SOD knockout mice with increased oxidative stress were utilized (Shi et al., 2014). These mice exhibited minimal growth and strength in their hind limbs in comparison to wild-type counterparts (Shi et al., 2014). Assessment of the nervous system showed that nerve stimulation in the hind limb muscle was dampened in these knockout mice due to apparent reactive oxygen species damage to the muscle from the lack of Cu Zn SOD sequestration (Shi et al., 2014). These studies among others show that the presence of active Cu Zn SOD provides a multitude of benefits to an organism, as the dysfunction of the enzyme leads to disarray in the cell from the accumulation of unregulated reactive oxygen species.

Beyond Cu Zn SOD, tyrosinase is also a Cu dependent enzyme. This reliance on Cu was tested in mouse melanoma tissue devoid of Cu by cyanide exposure (Lerner et al., 1950). Tyrosinase activity in these cells was nearly depleted until the supplementation of Cu to the tissue resulted in near 90 percent return of tyrosinase activity in these

melanoma cells (Lerner et al., 1950). This apparent need for Cu is rooted in the two Cu binding sites that reside within the catalytic site of enzyme (Oetting, 2000; Ismaya et al., 2011). The corresponding Cu atoms are secured through six histidine residues (Oetting, 2000). Provided Cu is present at the catalytic site, tyrosinase is responsible for the rate limiting step of melanin production (Cooksey et al., 1997). Tyrosinase does not convert tyrosine directly to melanin, but rather produces a quinone product, DOPA quinone, that is further converted to melanin (Cooksey et al., 1997; Matoba et al., 2006; Ismaya et al., 2011).

Mutations in a multitude of possible genes can result in the absence of melanin in the skin, hair, and eyes of those affected by albinism due to the lack of tyrosinase activity (Montoliu et al., 2014). The early understanding of this condition believed tyrosinase was not present in the cell due to observing untouched radio labeled carbon tyrosine molecules in both albino skin and hair samples (Fitzpatrick and Kuikita, 1956). Further work by Kugelman and Van Scott with hair bulb samples from mutation free and albino animal and human subjects indicated that tyrosinase activity can occur in albino hair follicles free of inhibitory agents (Kugelman and Van Scott, 1961). These results contradict Fitzpatrick and Kuikita's work, although natural inhibitory factors of tyrosinase are found in the skin and may cause partial inactivation of the enzyme leading to the varied results of these studies (Kugelman and Van Scott, 1961). The variety of genes now associated with albinism may also help to link these findings together, as not all mutations elicit the same response (Montoliu et al., 2014). In addition to its relationship with albinism, Parkinson's disease (**PD**) has also been associated with tyrosinase (Hasegawa and Takafumi, 2010). To determine the impact of tyrosinase in

neural cells, tyrosinase was overexpressed leading to increased dopamine and reactive oxygen species (Hasegawa and Takafumi, 2010). This dopamine acts as a substrate for tyrosinase to subsequently produce neuromelanin in the substantia nigra (Hasegawa and Takafumi, 2010). The dopaminergic neurons of the substantia nigra are of concern in PD, due to the common movement problems associated with the disease occurring with the loss of these cells (Greggio et al., 2005). Furthermore, the depigmentation of the substantia nigra region is a key attribute of PD, indicating the importance of tyrosinase in maintaining neuromelanin (Hasegawa et al., 2008). An over-abundance of dopamine in the cell can result in cytotoxicity; the conversion of dopamine to the more stable neuromelanin in part is a protective mechanism against cellular damage if tyrosinase was markedly active (Hasegawa and Takafumi, 2010). Overall the role of tyrosinase goes beyond simple pigmentation with its function in neuromelanin formation within the substantia nigra to aid in maintaining a homeostatic environment within neural cells.

Copper status and dietary antagonists

Understanding the role of Cu in the body depends on the ability to evaluate the Cu status of the animal. In ruminant animals the liver is widely accepted as the main site of Cu accumulation in the body (Kincaid, 2000). The Cu status of an animal can also be obtained by analyzing the Cu or Cp content of the plasma. Plasma parameters have proven to be less accurate than liver stores because plasma Cu is known to remain constant (Claypool et al., 1975). As liver stores supply the plasma with Cu, the liver must be depleted before changes in plasma Cu are observed (Naylor and Ralston, 1991). An analysis between liver and plasma Cu concentrations showed that a drop in plasma Cu was only expected when the liver Cu concentration dropped to approximately 40 mg/kg DM or lesser (Claypool et al.,

1975). Other studies have indicated that plasma Cu is unaffected until liver Cu concentrations reach below 20 or 30 mg/kg DM (Mills, 1987). These ranges in liver Cu suggest that in some situations the beginning state of Cu deficiency may differ. These variations may be dependent upon either Cu source, animal breed, or the presence of an antagonist. Similarly, ceruloplasmin can be measured in the plasma as an indicator of Cu status, but measurements are variable outside of severe deficiency (Milne, 1998). Inconsistency in Cp measurements as a status indicator are a result of Cp functioning as an acute phase protein which may indicate unrealistically high Cu status in comparison to liver Cu stores during an immune response deeming the sole use of this status indicator unreliable (Denko, 1979). A combination of all three Cu status indicators can lead to well-rounded observations on the Cu status of a ruminant animal.

Although measurements of status can be taken, various factors as alluded to earlier can influence the uptake or status of an animal. Within cattle, breed has been discovered to impact Cu homeostatic mechanisms as Simmental cattle excreted twice as much biliary Cu as Angus (Gooneratnet et al., 1994). Additionally, duodenal Cu transporter Ctr1 mRNA was decreased and Atp7A mRNA tended to be less in Simmental cows in comparison to Angus (Fry et al., 2013). Interestingly, the liver and plasma Cu concentrations between these breeds were not different even though Cu transporters were not as apparent in the Simmental cows (Fry et al., 2013). The maintained Cu status of Simmental cattle in comparison to the Angus breed suggests that there are breed differences in the efficiency at which Cu can be absorbed or maintained in the body. Further biological differences may impact Cu homeostasis and status but are unknown at this time. Beyond breed differences, the dietary allowance of Cu can also impact the status of the animal. Cattle fed adequate

dietary Cu, 10 mg Cu/kg DM, have greater liver Cu concentrations in comparison to cattle receiving a Cu deficient diet indicating that liver Cu status directly reflects dietary Cu content when significant antagonists are not present (Mills, 1987; Dias et al., 2013; Fry et al., 2013; NASEM, 2016). Recognizing the potential for breaches in Cu homeostasis may be as simple as testing the content of Cu and potent Cu antagonists in the diet to better understand what the status of the animal may resemble.

However, the sole reliance on dietary Cu content as a preliminary status indicator may not be sufficient due to dietary interactions. The supplementation of Cu to meet the physiological needs of beef cattle during all stages of production is recommended at 10 mg Cu/kg DM in the diet (NASEM, 2016). Although this concentration of dietary Cu is recognized to maintain growth and prevent symptoms of deficiency, other factors must be considered for overall supplementation strategies. The effectiveness of 10 mg Cu/kg DM at preventing deficiency symptoms is impacted by the concentration of additional minerals in the diet. A connection between Cu and molybdenum (**Mo**) was demonstrated in cases of Cu pine in calves grazing in the United Kingdom. By supplementing Cu sulfate, symptoms of un-thriftiness and depigmentation were alleviated from the calves which appeared to suffer from Cu deficiency likely caused by high Mo in the grass (Jamieson and Allcroft, 1949). This indication of a relationship between Cu and Mo was also seen in grazing sheep in Australia in which small quantities of Mo resulted in decreased Cu accumulation in the liver (Dick and Bull, 1945). The supplementation of 5 mg Mo/kg DM was shown to decrease plasma Cu in cattle not supplemented Cu by day 28 of the trial (Ward and Spears, 1997). Upon analysis of feedstuffs, a dietary Mo concentration in excess of 1-3 mg Mo/kg DM is considered to have an antagonistic role, while maintaining a Cu to Mo ratio above

4.5:1 can prevent adverse effects of Mo on Cu utilization (Mortimer et al., 1999). However, Mo (molybdate) has little affinity for Cu ions on its own (Alvarez et al., 2010). The addition of S to the diet can further exacerbate Cu deficiency resulting in the formation of thiomolybdates which are insoluble complexes that render Cu unavailable (Allen and Gawthornet, 1987; Arthington, 2003). These thiomolybdates are formed through the reaction of molybdate with sulfide in the rumen as proven through the tracking of labeled Mo contributions and are primarily in the tri- or tetra-thiomolybdate form when found in ruminal digesta (Price et al., 1987). By feeding a combination of 2 mg/kg DM Mo and 0.3% S in the diet of steers with no supplemental Cu for approximately 90 days liver Cu concentrations can be decreased to nearly 90 percent of initial values, proving the potency of this antagonism (Hartman et al., 2018). These thiomolybdates are responsible for an increase in biliary excretion of Cu from liver stores, a reduction in available circulating Cu due to the induction of strong bonds with the carrier albumin, as well as the removal of Cu from metalloenzymes (Suttle, 1991).

In addition to the effects of S and Mo, Zn has been shown to hinder Cu absorption and decrease liver Cu concentrations when fed above 100 mg/kg in the diet (Naylor and Ralston, 1991). The national recommendation for Zn is 30 mg Zn/kg DM although greater concentrations of Zn are fed throughout the industry averaging 109 mg Zn/kg DM (NASEM, 2016; Samuelson et al., 2016). Utilizing rats, the association between dietary Zn and Cu absorption was explored. The supplementation of excess Zn resulted in depressed Cu absorption, when both Zn (1 mg) and Cu (1 μg) were supplemented directly to the intestinal segment being measured (Van Campen, 1967). By noting impaired Cu absorption, the liver as a point of Zn interference was ruled out (Van Campen, 1967).

Further studies utilized fractionated mucosal cells to determine that Cu and Zn were most likely binding to the protein metallothionein (Fischer et al., 1981). In addition, with greater Zn supplementation, the amount of Cu bound to metallothionein increased, leading to greater Cu retention in the mucosal cells (Fischer et al., 1981). The trend in supplementing Zn beyond national recommendations may impact Cu absorption. However, a theoretical increase in Cu supplementation could offset absorption problems associated with high dietary Zn, but the ratio at which these minerals should be included is not well understood.

Performance attributes from copper supplementation

As the previous sections have eluded to, Cu plays an essential role in cattle growth as a component of various enzymes. Although measuring these specific enzymes may demonstrate the importance of Cu, further performance driven results often express the holistic significance of the mineral to a broader audience. Through the supplementation of varying concentrations of dietary Cu some performance differences have been observed. A comparison of Cu sulfate and Cu lysine at 5 mg Cu/kg DM in a 6.2 mg Cu/kg DM basal diet led to improved gain in Cu sulfate fed steers in comparison to both Cu lysine and control steers during the 21 day receiving period (Ward et al., 1993). However, during the 98 day finishing study performance was unaffected by dietary treatment (Ward et al., 1993). Additionally, when steers were injected with 90 mg Cu as glycinate prior to weaning and supplemented 5 mg Cu/kg DM in a 2.9 mg Cu/kg DM basal diet throughout the growing and finishing trial Cu supplementation resulted in greater ADG and improved feed efficiency during the finishing period (Ward and Spears, 1997). In contrast, Cu supplementation at 20 or 40 mg Cu/kg DM from Cu sulfate or 20 mg Cu/kg DM from Cu citrate, Cu proteinate, or tribasic Cu chloride (basal diet, 4.9 mg

Cu/kg DM) depressed ADG, feed intake and feed efficiency during the finishing period (Engle and Spears, 2000a). However, these differences in performance due to Cu supplementation are not consistent with further research. The supplementation of Cu at 5, 10, 20, or 40 mg Cu/kg DM from Cu sulfate or 5 mg Cu/kg DM from Cu lysine in basal diets containing between 4.9 and 9.8 mg Cu/kg DM resulted in no differences in ADG, DMI, or feed efficiency (Ward et al., 1993; Engle and Spears, 2000b; Engle and Spears, 2001). It appears that cattle with maintained adequate Cu status (plasma >0.5 mg/L, liver > 40 mg Cu/kg DM) do not benefit from further Cu supplementation in regards to growth (Ward et al., 1993; Kincaid, 2000). Further, performance may have been positively impacted if cattle were Cu deficient prior to the start of Cu supplementation. Overall, these studies suggest that Cu supplementation alone does not strongly affect cattle performance. However, these studies do not factor in the potential importance of Cu with the use of growth promoting technologies. Therefore, additional work to determine the effects of Cu on performance of cattle utilizing growth promoting technologies is warranted.

Beyond live animal performance, the supplementation of Cu has been shown to have variable effects on carcass characteristics. Cattle supplemented with Cu had leaner carcasses with decreased back fat thickness in comparison to cattle on a control diet (Ward and Spears, 1997). In steers supplemented with either 10 or 20 mg Cu/kg DM from Cu sulfate or Availa Cu (amino acid-bound Cu) back fat was depressed in a concentration dependent manner in which cattle supplemented with 20 mg Cu/kg DM regardless of source had decreased back fat measurements in comparison to steers supplemented with 10 mg Cu/kg DM, with control steers having the greatest back fat

measurements (Johnson and Engle, 2003). The effect of dietary Cu on back fat was also observed when feeding either 10 or 20 mg/kg DM of Cu from Cu sulfate indicating that Cu source is not responsible for these differences (Engle and Spears, 2000b). Further, back fat was decreased in steers receiving either 20 or 40 mg Cu/kg DM from either Cu sulfate, Cu citrate, Cu proteinate, or tribasic Cu chloride in comparison to control steers receiving a basal diet with 10.2 mg Cu/kg DM (Engle et al., 2000).

In some cases, marbling scores tend to be lesser with Cu supplementation. Steers supplemented with 20 mg Cu/kg DM as Availa Cu had lower marbling scores than cattle supplemented with 10 mg Cu/kg DM from the same Cu source (Johnson and Engle, 2003). However, more commonly it has been reported that Cu supplementation does not impact marbling scores in either a negative or positive fashion (Engle et al., 2000; Engle and Spears, 2000b; Engle and Spears, 2001). In addition, KPH has been shown to increase in steers supplemented with 20 mg Cu/kg DM from Availa Cu, having greater percent KPH in comparison to control cattle and those supplemented either 10 or 20 mg Cu/kg DM as Cu sulfate (Johnson and Engle, 2003). Although this difference in KPH is not always noted, as cattle supplemented up to 40 mg Cu/kg DM from multiple sources did not differ in KPH (Engle et al., 2000; Engle and Spears, 2000b; Engle and Spears, 2001). These observances of fat deposition lead to the conclusion that Cu supplementation can depress back fat formation and may have an impact on fat deposition considering varied effects in marbling scores and KPH, but these differences may be cattle dependent and more work is needed to fully understand the role of Cu in fat deposition.

Understanding the effects of Cu supplementation on these fat deposition parameters may be further understood through the role of previously discussed PDE. As a regulator of intracellular cAMP, PDE influences the signaling of lipolysis through hormone sensitive lipase (Shakur et al., 2001). Hormone sensitive lipase initiates lipolysis, the breakdown of triacylglycerol into glycerol and non-esterified fatty acids, through PKA mediation (Yeaman, 1990; Krishnamurthy et al., 2014). Further, Cu has been detected to bind to PDE, thereby inhibiting its ability to degrade cAMP (Krishnamoorthy et al., 2016). Although these effects of Cu were first detected in vitro and only confirmed in mice models, it appears that the decrease in back fat measurements of Cu supplemented cattle observed here may be a result of lipolysis regulation through a Cu dependent mechanism such as Cu inhibited PDE.

Additionally, the supplementation of these Cu sources at 20 or 40 mg/kg DM resulted in decreased HCW in comparison to control steers (Engle et al., 2000). In contrast supplementation of Availa Cu at 10 or 20 mg Cu/kg DM resulted in greater HCW than controls (basal diet, 6.1 mg Cu/kg DM) with cattle receiving 20 mg Cu/kg DM from Availa Cu having greater HCW than steers from the 10 mg Cu/kg DM from Availa Cu treatment (Johnson and Engle, 2003). Further, these opposing HCW results are contradicted by several studies that have noted no effect on HCW from Cu supplementation when fed at 10, 20, or 40 mg Cu/kg DM as Cu sulfate (Engle and Spears, 2000b; Engle and Spears, 2001).

Cattle supplemented with Cu have had varied REA and DP responses and these parameters seem to commonly be unaffected by Cu supplementation (Engle et al., 2000; Engle and Spears, 2000b; Engle and Spears, 2001). However, the supplementation of

Availa Cu at 10 mg Cu/kg DM resulted in greater REA and DP in comparison to the same concentration of Cu sulfate (Johnson and Engle, 2003). Further, DP was improved to a greater extent when supplemented with 20 mg Cu/kg DM as Availa Cu in comparison to the lesser concentration as well as 20 mg Cu/kg DM through Cu sulfate (Johnson and Engle, 2003). Overall, these results indicate that Cu supplementation can improve the leanness of the carcass through decreased back fat measurements in a concentration dependent manner (Engle et al., 2000; Engle and Spears, 2000b; Johnson and Engle, 2003). Further, carcass characteristics discussed here have shown varied results and indicate that study specific parameters and environmental factors may have influenced these findings. Therefore, additional studies are needed to better understand the benefits of Cu supplementation on carcass characteristics, particularly in modern feedlot cattle utilizing cattle with high growth potential and growth promoting technologies.

Copper Interactions with Growth Promoting Technologies

As eluded to previously, BA, hormonal implants and Cu supplementation can impact either performance or carcass characteristics of cattle when examined on their own. Work directed at the possible interactions between bodily requirements for Cu and growth promoting technologies has been scant. It appears that Cu may be linked to cAMP regulation though PDE (Krishnamoorthy et al., 2016). In isoproterenol stimulated adipocytes Cu supplementation led to greater cAMP concentrations confirmed through increased phosphorylated hormone sensitive lipase and perilipin detected in Western blots of Cu treated cells (Krishnamoorthy et al., 2016). Further, this increase in cAMP is a result of PDE inhibition through an interaction with Cu (Krishnamoorthy et al., 2016).

The regulation of PDE by Cu appears to be a reversible bond seen specifically in the variant PDE3B in this study (Krishnamoorthy et al., 2016). As PDE3B contributes to a large proportion of adipocyte PDE activity this regulation by Cu could have a large impact on lipolytic activity (Beavo, 1995). The ability to inhibit PDE activity during a BA response results in greater production of secondary messenger cAMP and therefore greater lipolytic rates that would allow for the leaner carcasses expected in cattle fed a BA. To better understand the details of this Cu and PDE relationship, more research is warranted both in vitro and in vivo.

In addition to the connection between Cu and the BA pathway the role of Cu in growth is evident as the Cu dependent enzyme LOX catalyzes the crosslinking of collagen and elastin within the ECM of cells (Zhang et al., 2015). This crosslinking leads to the structural integrity of the cell thus supporting growth through both structural and functional proteins in the ECM that are involved in tissue development (Gentili and Cancedda, 2009). As described previously LOX activity has been shown to decrease in ligament tissue when treated with E_2 (Lee et al., 2015). This interplay between E_2 treatment and LOX activity provides evidence for a hormonal effect on growth in relation to Cu status. Further, E_2 appears to impact circulating Cp (Sunderman et al., 1971). Daily injections of E_2 in mice resulted in elevated serum Cp that began to plateau after 28 days of E_2 treatment (Sunderman et al., 1971). This increase in circulating Cp suggests that the Cu requirement of tissues treated with E_2 is greater as Cp delivers Cu to target tissues. It is unclear why E_2 would decrease LOX activity while increasing circulating Cp. With the understanding that hormonal implants enhance growth, one would expect E_2 treatments to enhance LOX activity as increased growth would presumably require a greater need for

LOX activity to provide cellular structure. Further, the increase in circulating Cp suggests a greater need for Cu in tissues. As LOX activity is decreased this need for Cu must be used by another source. It is unclear what other enzyme or mechanism is utilizing this upregulated Cu (from Cp) when tissue is treated with E_2.

Beyond the basic interactions between Cu and growth promoting technologies it appears that minerals can impact the performance of cattle receiving growth promoting technologies such as implants and beta agonists. Limited research in cattle on potential relationships between growth promoting technologies and minerals is available. Therefore, these results do not provide conclusive evidence and represent the need for further research in this area. The supplementation of Zn has been shown to accommodate the growth response of implanted cattle. The ADG of steers implanted with 20 mg estradiol benzoate and 200 mg progesterone (Synovex-S) was improved by 7.6 and 17.4% in control steers fed a basal diet containing 84 mg Zn/kg DM and steers fed 200 mg Zn/kg DM from Zn sulfate, respectively, in comparison to nonimplanted steers (Huerta et al., 2002). Implanted heifers of the control and Zn sulfate treatments experienced similar increases in ADG over nonimplanted heifers, 11 and 17% improvements, respectively (Huerta et al., 2002). Further the supplementation of 200 mg Zn/kg DM of Zn-methionine to implanted steers resulted in no differences in gain when compared to nonimplanted steers (Huerta et al., 2002). In contrast, nonimplanted heifers fed the Zn-methionine treatment had 26% greater ADG than implanted heifers of the same treatment (Huerta et al., 2002). An additional study looked at industry versus national recommendations of trace minerals on the performance of cattle receiving a high potency implant program (Niedermayer et al., 2018). This study indicated that during the

first 56 days on a medium potency implant (80 mg TBA and 16 mg E_2) implanted steers fed the national and industry mineral recommendations had improved ADG over their non-trace mineral supplemented counterparts (Niedermayer et al., 2018). However, the following terminal implant (200 mg TBA and 20 mg E_2) revealed no interactions with trace minerals and the implant for the remainder of the study (Niedermayer et al., 2018). This improved growth at the beginning of the trial suggests that proper mineral nutrition may be most critical during this time of high growth as previously discussed with the decline of satellite cell proliferation with age. In addition, minerals have been observed to interact with BA induced growth. Supplementing RAC treated steers (300 $mg \cdot day^{-1} \cdot steer^{-1}$) with 60 mg Zn/kg DM from a Zn amino acid complex plus 60 mg Zn/kg DM from Zn sulfate resulted in a 1.3% increase in HCW in comparison to RAC treated steers not receiving supplemental Zn amino acid complex (Genther-Schroeder et al., 2016).

The augmentation of performance in cattle utilizing growth promoting technologies with mineral supplementation strategies is a new area of research. These performance results suggest that the mineral requirements of cattle utilizing growth promoting technologies are increased to allow for a more optimal growth response as increased supplementation of minerals such as Zn have been shown to improve performance. The limited data within this area of study also indicate that more work is needed to fully understand the ideal supplementation of individual minerals to achieve optimal growth with the use of growth promoting technologies.

Literature Cited

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 cattle1. J. Anim. Sci. 85:3090–3098. doi:10.2527/jas.2007-0263.

Aisen, P., C. Enns, and M. Wessling-Resnick. 2001. Chemistry and biology of eukaryotic iron metabolism. Int. J. Biochem. Cell Biol. 33:940–959. doi:10.1016/S1357-2725(01)00063-2.

Aliaga, M. E., C. López-Alarcón, R. Bridi, and H. Speisky. 2016. Redox-implications associated with the formation of complexes between copper ions and reduced or oxidized glutathione. J. Inorg. Biochem. 154:78–88. doi:10.1016/J.JINORGBIO.2015.08.005.

Allen, J. D., and J. M. Gawthornet. 1987. Involvement of the solid phase of rumen digesta in the interaction between copper, molybdenum and sulphur in sheep. Br. J. Nutr. 58:265. doi:10.1079/BJN19870094.

Aller, S. G., and V. M. Unger. 2006. Projection structure of the human copper transporter CTR1 at 6-Å resolution reveals a compact trimer with a novel channel-like architecture. PNAS. 103:3627–3632. doi:10.1073/PNAS.0509929103.

Alvarez, H. M., Y. Xue, C. D. Robinson, M. A. Canalizo-Hernández, R. G. Marvin, R. A. Kelly, A. Mondragón, J. E. Penner-Hahn, and T. V O'Halloran. 2010. Tetrathiomolybdate inhibits copper trafficking proteins through metal cluster formation. Science. 327:331–4. doi:10.1126/science.1179907.

Alvarez, R., C. Sette, D. Yang, R. M. Eglen, R. Wilhelm, E. R. Shelton, and M. Conti. 1995. Activation and selective inhibition of a cyclic AMP-specific phosphodiesterase, PDE-4D3. Mol. Pharmacol. 48:616–622. doi:0026-89X/95/040616-07$3.00/0.

Antonio, J., J. D. Wilson, and F. W. George. 1999. Effects of castration and androgen treatment on androgen-receptor levels in rat skeletal muscles. J. Appl. Physiol. 87:2016–2019. doi:10.1152/jappl.1999.87.6.2016.

Arredondo, M., P. Muñ Oz, C. V Mura, and M. T. Nú Ñ Ez. 2003. DMT1, a physiologically relevant apical Cu 1+ transporter of intestinal cells. Am J Physiol Cell Physiol. 284:C1525–C1530. doi:10.1152/ajpcell.00480.2002. DMT1,.

Arthington, J. 2003. Copper Antagonists in Cattle Nutrition. In: 14th Annual Florida Ruminant Nutrition Symposium. University of Florida, Gainesville, FL. p. 48–57.

Askwith, C., D. Eide, A. Van Ho, P. S. Bernard, L. Li, S. Davis-Kaplan, D. M. Sipe, and J. Kaplan '. 1994. The FET3 Gene of S. cerevisiae Encodes a Multicopper Oxidase Required for Ferrous Iron Uptake. Cell. 76:403–410. doi:10.1016/0092-8674(94)90346-8.

Baillie, G. S., A. Sood, I. McPhee, I. Gall, S. J. Perry, R. J. Lefkowitz, and M. D. Houslay. 2003. beta-Arrestin-mediated PDE4 cAMP phosphodiesterase recruitment regulates beta-adrenoceptor switching from Gs to Gi. Proc. Natl. Acad. Sci. U. S. A. 100:940–5. doi:10.1073/pnas.262787199.

Bartle, S. J., R. L. Preston, R. E. Brown, and R. J. Grant. 1992. Trenbolone acetate/estradiol combinations in feedlot steers: dose-response and implant carrier effects. J. Anim. Sci. 70:1326–1332. doi:10.2527/1992.7051326x.

Beavo, J. A. 1995. Cyclic nucleotide phosphodiesterases: functional implications of multiple isoforms. Physiol. Rev. 75:725–48. doi:10.1152/physrev.1995.75.4.725.

Beavo, J. A., P. J. Bechtel, and E. G. Krebs. 1974. Activation of protein kinase by physiological concentrations of cyclic AMP. Proc. Natl. Acad. Sci. U. S. A. 71:3580–3. doi:10.1073/PNAS.71.9.3580.

Beitzel, F., P. Gregorevic, J. G. Ryall, D. R. Plant, M. N. Sillence, and G. S. Lynch. 2004. β 2 - Adrenoceptor agonist fenoterol enhances functional repair of regenerating rat skeletal muscle after injury. J. Appl. Physiol. 96:1385–1392. doi:10.1152/japplphysiol.01081.2003.

Benovic, J. L., R. H. Strasser, M. G. Caron, and R. J. Lefkowitz. 1986. Beta-Adrenergic receptor kinase: Identification of a novel protein kinase that phosphorylates the agonist-occupied form of the receptor. PNAS. 83:2797–2801. doi:10.1073/pnas.83.9.2797.

Bento, I., C. Peixoto, V. N. Zaitsev, P. F. Lindley, and IUCr. 2007. Ceruloplasmin revisited: structural and functional roles of various metal cation-binding sites. Acta Crystallogr. Sect. D Biol. Crystallogr. 63:240–248. doi:10.1107/S090744490604947X.

Birch, J. 2015. 2015 Nebraska Feedyard Labor Cost Benchmarks and Historical Trends. Lincoln, NE.

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 steers1. J. Anim. Sci. 94:5382–5392. doi:10.2527/jas.2016-0807.

Bologa, C. G., C. M. Revankar, S. M. Young, B. S. Edwards, J. B. Arterburn, A. S. Kiselyov, M. A. Parker, S. E. Tkachenko, N. P. Savchuck, L. A. Sklar, T. I. Oprea, and E. R. Prossnitz. 2006. Virtual and biomolecular screening converge on a selective agonist for GPR30. Nat. Chem. Biol. 2:207–212. doi:10.1038/nchembio775.

Brewer, G. J. 2000. Recognition, Diagnosis, and Management of Wilson's Disease (44460). Proc Soc Exp Bio Med. 223:39–46. doi:10.1177/153537020022300105.

Calabreses, L., M. Carbonarog, and G. Muscill. 1983. Presence of Coupled Trinuclear Copper Cluster in Mammalian Ceruloplasmin Is Essential for Efficient Electron Transfer to Oxygen. J. Biol. Chem. 264:6183–6187.

Van Campen, D. R. 1967. Zinc interference with copper absorption in rats. J. Nutr. 91:473–476. doi:doi.org/10.1093/jn/91.4.473.

Carroll, M. C., C. E. Outten, J. B. Proescher, L. Rosenfeld, W. H. Watson, L. J. Whitson, P. J. Hart, L. T. Jensen, and V. Cizewski Culotta. 2006. The effects of glutaredoxin and copper activation pathways on the disulfide and stability of Cu,Zn superoxide dismutase. J. Biol. Chem. 281:28648–56. doi:10.1074/jbc.M600138200.

Carruthers, M. E., C. B. Hobbs, and R. L. Warren. 1966. Raised serum copper and caeruloplasmin levels in subjects taking oral contraceptives. J. clin. Path. 19:498–500.

Casareno, R. L., D. Waggoner, and J. D. Gitlin. 1998. The copper chaperone CCS directly interacts with copper/zinc superoxide dismutase. J. Biol. Chem. 273:23625–8. doi:10.1074/JBC.273.37.23625.

Chen, M., H.-Z. Feng, D. Gupta, J. Kelleher, K. E. Dickerson, J. Wang, D. Hunt, W. Jou, O. Gavrilova, J.-P. Jin, and L. S. Weinstein. 2009. $G_s\alpha$ deficiency in skeletal muscle leads to reduced muscle mass, fiber-type switching, and glucose intolerance without insulin resistance or deficiency. Am. J. Physiol. Physiol. 296:C930–C940. doi:10.1152/ajpcell.00443.2008.

Chen, Y., J. D. Zajac, and H. E. MacLean. 2005. Androgen regulation of satellite cell function. J. Endocrinol. 186:21–31. doi:10.1677/joe.1.05976.

Cherukuri, S., R. Potla, J. Sarkar, S. Nurko, Z. L. Harris, and P. L. Fox. 2005. Unexpected role of ceruloplasmin in intestinal iron absorption. Cell Metab. 2:309–319. doi:10.1016/J.CMET.2005.10.003.

Claypool, D. W., F. W. Adams, H. W. Pendell, N. A. Hartmann, and J. F. Bone. 1975. Relationship between the Level of Copper in the Blood Plasma and Liver of Cattle. J. Anim. Sci. 41:911–914. doi:10.2527/jas1975.413911x.

Cong, M., D. E. Goll, and P. B. Antin. 1998. cAMP responsiveness of the bovine calpastatin gene promoter. Biochim. Biophys. Acta - Gene Struct. Expr. 1443:186–192. doi:10.1016/S0167-4781(98)00203-6.

Conti, M., and C. Jin. 1999. The Molecular Biology of Cyclic Nucleotide Phosphodiesterases. In: Progress in Nucleic Acid Research and Molecular Biology. Academic Press. p. 1–38.

Cooksey, C. J., P. J. Garratt, E. J. Land, S. Pavel, C. A. Ramsden, P. A. Riley, and N. P. Smit. 1997. Evidence of the indirect formation of the catecholic intermediate substrate responsible for the autoactivation kinetics of tyrosinase. J. Biol. Chem. 272:26226–35. doi:10.1074/JBC.272.42.26226.

Cray, C., J. Zaias, and N. H. Altman. 2009. Acute Phase Response in Animals: A Review. Comp. Med. 59:517–526.

Csiszar, K. 2001. Lysyl Oxidases: A Novel Multifunctional Amine Oxidase Family. Prog Nucleic Acid Res Mol Biol. 70:1–32.

Denko, C.-X. W. 1979. Protective Role of Ceruloplasmin in Inflammation. Agents Actions. 9:333–336. doi:10.1007/BF01970657.

Deponte, M. 2013. Glutathione catalysis and the reaction mechanisms of glutathione-dependent enzymes. Biochim. Biophys. Acta - Gen. Subj. 1830:3217–3266. doi:10.1016/J.BBAGEN.2012.09.018.

Deshpande, D. A., B. S. Theriot, R. B. Penn, J. K. L. Walker, and ; J K L W Box. 2008. β-Arrestins specifically constrain β2-adrenergic receptor signaling and function in airway smooth muscle. FASEB J. 22:2134–2141. doi:10.1096/fj.07-102459.

Devree, B. T., J. P. Mahoney, G. A. Vélez-Ruiz, S. G. F. Rasmussen, A. J. Kuszak, E. Edwald, J.-J. Fung, A. Manglik, M. Masureel, Y. Du, R. A. Matt, E. Pardon, J. Steyaert, B. K. Kobilka, and R. K. Sunahara. 2016. Allosteric coupling from G protein to the agonist binding pocket in GPCRs HHS Public Access. Nature. 535:182–186. doi:10.1038/nature18324.

Dias, R. S., S. López, Y. R. Montanholi, B. Smith, L. S. Haas, S. P. Miller, and J. France. 2013. A meta-analysis of the effects of dietary copper, molybdenum, and sulfur on plasma and liver copper, weight gain, and feed conversion in growing-finishing cattle1. J. Anim. Sci. 91:5714–5723. doi:10.2527/jas.2013-6195.

Dick, A. T., and L. B. Bull. 1945. Some Preliminary Observations on the Effect of Molybdenum on Copper Metabolism in Herbivorous Animals. Aust. Vet. J. 21:70–72. doi:10.1111/j.1751-0813.1945.tb04462.x.

Dobretsov, G. E., T. A. Borschevskaya, V. A. Petrov, and Y. A. Vladimirov. 1977. The increase of phospholipid bilayer rigidity after lipid peroxidation. FEBS Lett. 84:125–128. doi:10.1016/0014-5793(77)81071-5.

Dodson, M. V., and R. E. Allen. 1987. Interaction of multiplication stimulating activity/rat insulin-like growth factor II with skeletal muscle satellite cells during aging. Mech. Ageing Dev. 39:121–128. doi:10.1016/0047-6374(87)90003-0.

Doumit, M. E., D. R. Cook, and R. A. Merkel. 1996. Testosterone up-regulates androgen receptors and decreases differentiation of porcine myogenic satellite cells in vitro. Endocrinology. 137:1385–1394. doi:10.1210/en.137.4.1385.

Duckett, S. K., F. N. Owens, and J. G. Andrae. 1997. Effects of Implants on Performance and Carcass Traits of Feedlot Steers and Heifers. In: OSU Implant Symposium. Stillwater, OK. p. 63–82.

Duckett, S. K., and S. L. Pratt. 2014. Meat Science and Muscle Biology Symposium—Anabolic implants and meat quality. J. Anim. Sci. 92:3–9. doi:10.2527/jas.2013-7088.

Eisenstein, R. S. 2000. Discovery of the Ceruloplasmin Homologue Hephaestin: New Insight into the Copper/Iron Connection. Nutr. Rev. 58:22–26. doi:10.1111/j.1753-4887.2000.tb01821.x.

Eisses, J. F., and J. H. Kaplan. 2002. Molecular Characterization of hCTR1, the Human Copper Uptake Protein. J. Biol. Chem. 277:29162–29171. doi:10.1074/jbc.M203652200.

El-Shewy, H. M., F. L. Kelly, L. Barki-Harrington, and L. M. Luttrell. 2004. Ectodomain Shedding-Dependent Transactivation of Epidermal Growth Factor Receptors in Response to Insulin-Like Growth Factor Type I. Mol. Endocrinol. 18:2727–2739. doi:10.1210/me.2004-0174.

Elchuri, S., T. D. Oberley, W. Qi, R. S. Eisenstein, L. Jackson Roberts, H. Van Remmen, C. J. Epstein, and T.-T. Huang. 2005. CuZnSOD deficiency leads to persistent and widespread oxidative damage and hepatocarcinogenesis later in life. Oncogene. 24:367–380. doi:10.1038/sj.onc.1208207.

Engle, T. E., and J. W. Spears. 2000a. Effects of dietary copper concentration and source on performance and copper status of growing and finishing steers. J. Anim. Sci. 78:2446–2451. doi:10.2527/2000.7892446x.

Engle, T. E., and J. W. Spears. 2000b. Dietary copper effects on lipid metabolism, performance, and ruminal fermentation in finishing steers. J. Anim. Sci. 78:2452–2458. doi:10.2527/2000.7892452x.

Engle, T. E., and J. W. Spears. 2001. Performance, carcass characteristics, and lipid metabolism in growing and finishing Simmental steers fed varying concentrations of copper 1,2. J. Anim. Sci. 79:2920–2925. doi:10.2527/2001.79112920x.

Engle, T. E., J. W. Spears, T. A. Armstrong, C. L. Wright, and J. Odle. 2000. Effects of dietary copper source and concentration on carcass characteristics and lipid and cholesterol metabolism in growing and finishing steers. J. Anim. Sci. 78:1053–1059. doi:10.2527/2000.7841053x.

Evans, G. W., N. F. Cornatzer, and W. E. Cornatzer. 1970. Mechanism for hormone-induced alterations in serum ceruloplasmin. Am. J. Physiol. 218.

Fan, Y., R. K. Menon, P. Cohen, D. Hwang, T. Clemens, D. J. DiGirolamo, J. J. Kopchick, D. Le Roith, M. Trucco, and M. A. Sperling. 2009. Liver-specific deletion of the growth hormone receptor reveals essential role of growth hormone signaling in hepatic lipid metabolism. J. Biol. Chem. 284:19937–44. doi:10.1074/jbc.M109.014308.

Filardo, E. J., J. A. Quinn, K. I. Bland, and A. R. Frackelton. 2000. Estrogen-Induced Activation of Erk-1 and Erk-2 Requires the G Protein-Coupled Receptor Homolog, GPR30, and Occurs via Trans -Activation of the Epidermal Growth Factor Receptor through Release of HB-EGF. Mol. Endocrinol. 14:1649–1660. doi:10.1210/mend.14.10.0532.

Filardo, E. J., and P. Thomas. 2012. Minireview: G Protein-Coupled Estrogen Receptor-1, GPER-1: Its Mechanism of Action and Role in Female Reproductive Cancer, Renal and Vascular Physiology. Endocrinology. 153:2953–2962. doi:10.1210/en.2012-1061.

Finney, J., H.-J. Moon, T. Ronnebaum, M. Lantz, and M. Mure. 2014. Human copper-dependent amine oxidases. Arch. Biochem. Biophys. 546:19–32. doi:10.1016/J.ABB.2013.12.022.

Fischer, P. W. F., A. Giroux, and M. R. L'Abbe. 1981. The effect of dietary zinc on intestinal copper absorption. Am. J. Clin. Nutr. 34:1670–1675. doi:10.1093/ajcn/34.9.1670.

Fitzpatrick, T. B., and A. Kuikita. 1956. A histochmical autoradiographic method for demonstration of tyrosinase in human melanocytes, nevi and malignant melanoma. J. Invest. Derm. 26:173–183. doi:10.1038/jid.1956.27.

Florini, J. R., D. Z. Ewton, and S. A. Coolican. 1996. Growth Hormone and the Insulin-Like Growth Factor System in Myogenesis. Endocr. Rev. 17:481–517. doi:10.1210/edrv-17-5-481.

Francis, S. H., I. V Turko, and J. D. Corbin. 2000. Cyclic Nucleotide Phosphodiesterases: Relating Structure and Function. In: Progress in Nucleic Acid Research and Molecular Biology. Vol. 65. Academic Press. p. 1–52.

Freedman, J. H., M. R. Ciriolo, and J. Peisach. 1989. The Role of Glutathione in Copper Metabolism and Toxicity. J. Biol. Chem. 264:5598–5605.

Freedman, L. P. 1992. Anatomy of the steroid receptor zinc finger region. Endocr. Rev. 13:129–145. doi:0163-769X/92/1302-0129$03.00/0.

Freestone, D., D. Denoyer, M. Jakab, M. L. Ackland, M. A. Cater, and A. Michalczyk. 2016. Ceruloplasmin is regulated by copper and lactational hormones in PMC42-LA mammary epithelial cell culture models. Metallomics. 8:941–950. doi:10.1039/c6mt00086j.

Frey, R. S., B. J. Johnson, M. R. Hathaway, M. E. White, and W. R. Dayton. 1995. Growth Factor Responsiveness of Primary Satellite Cell Cultures from Steers Implanted with Trenbolone Acetate and Estradiol-17β. Basic Appl Myol. 5:71–79.

Fry, R. S., J. W. Spears, K. E. Lloyd, A. T. O'Nan, and M. S. Ashwell. 2013. Effect of dietary copper and breed on gene products involved in copper acquisition, distribution, and use in Angus and Simmental cows and fetuses1,2. J. Anim. Sci. 91:861–871. doi:10.2527/jas.2011-3888.

Gacheru, S. N., P. C. Trackman, M. A. Shah, C. Y. O'Gara, P. Spacciapoli, F. T. Greenaway, and H. M. Kagan. 1990. Structural and catalytic properties of copper in lysyl oxidase. J Biol Chem. 265:19022–19027.

Gaetke, L. M., and C. K. Chow. 2003. Copper toxicity, oxidative stress, and antioxidant nutrients. Toxicology. 189:147–163. doi:10.1016/S0300-483X(03)00159-8.

Genther-Schroeder, O. N., M. E. Branine, and S. L. Hansen. 2016. The influence of supplemental Zn-amino acid complex and ractopamine hydrochloride feeding duration on growth performance and carcass characteristics of finishing beef cattle. J. Anim. Sci. 94:4338. doi:10.2527/jas.2015-0159.

Gentili, C., and R. Cancedda. 2009. Cartilage and Bone Extracellular Matrix. Curr. Pharm. Des. 15:1334–1348. doi:10.2174/138161209787846739.

Gilman, A. G. 1984. Perspectives Guanine Nucleotide-binding Regulatory Proteins and Dual Control of Adenylate Cyclase. J. Clin. Invest. 73:1–4. doi:0021-9738/84/01/0001/04.

Giurgea, N., M. I. Constantinescu, R. Stanciu, S. Suciu, and A. Muresan. 2005. Ceruloplasmin - acute-phase reactant or endogenous antioxidant? The case of cardiovascular disease. Med. Sci. Monit. 11:RA48-51.

Glerum, D. M., A. Shtanko, and A. Tzagoloff. 1996a. Characterization of COX17, a yeast gene involved in copper metabolism and assembly of cytochrome oxidase. J. Biol. Chem. 271:14504–9. doi:10.1074/JBC.271.24.14504.

Glerum, D. M., A. Shtanko, and A. Tzagoloff. 1996b. SCO1 and SCO2 act as high copy suppressors of a mitochondrial copper recruitment defect in Saccharomyces cerevisiae. J. Biol. Chem. 271:20531–5. doi:10.1074/JBC.271.34.20531.

Goldstein, I. M., B. Kaplan, H. S. Edelson, and G. Weissmann. 1979. Ceruloplasmin a Scavenger of Superoxide Anion Radicals. J. Biol. Chem. 254:4040–4045.

Gonzalez, J. M., S. E. Johnson, T. A. Thrift, J. D. Savell, S. E. Ouellette, and D. D. Johnson. 2009. Effect of ractopamine-hydrochloride on the fiber type distribution and shelf-life of six muscles of steers. J. Anim. Sci. 87:1764–1771. doi:10.2527/jas.2008-1469.

Gooneratnet, S. R., H. W. Symonds, J. V Bailey, and D. A. Christensenl. 1994. Effects of dietary copper, molybdenum and sulfur on biliary copper and zinc excretion in Simmental and Angus cattle. Can. J. Anim. Sci. 315–325. doi:10.4141/cjas94-043.

Greggio, E., E. Bergantino, D. Carter, R. Ahmad, G.-E. Costin, V. J. Hearing, J. Clarimon, A. Singleton, J. Eerola, O. Hellstrom, P. J. Tienari, D. W. Miller, A. Beilina, L. Bubacco, and M. R. Cookson. 2005. Tyrosinase exacerbates dopamine toxicity but is not genetically associated with Parkinson's disease. J. Neurochem. 93:246–256. doi:10.1111/j.1471-4159.2005.03019.x.

Gruber, S. L., J. D. Tatum, T. E. Engle, M. A. Mitchell, S. B. Laudert, A. L. Schroeder, and W. J. Platter. 2007. Effects of ractopamine supplementation on growth performance and carcass characteristics of feedlot steers differing in biological type. J. Anim. Sci. 85:1809–1815. doi:10.2527/jas.2006-634.

Guiroy, P. J., L. O. Tedeschi, D. G. Fox, and J. P. Hutcheson. 2002. The effects of implant strategy on finished body weight of beef cattle. J. Anim. Sci. 80:1791–1800. doi:10.2527/2002.8071791x.

Gurevich, V. V, and J. L. Benovics. 1993. Visual Arrestin Interaction with Rhodopsin Sequential Multisite Binding Ensures Strict Selectivity Toward Light-Activated Phohphorylated Rhodopsin. J. Biol. Chem. 268:11628–11638.

Haemmerle, G., R. Zimmermann, M. Hayn, C. Theussl, G. Waeg, E. Wagner, W. Sattler, T. M. Magint, E. F. Wagner, and R. Zechner. 2002. Hormone-sensitive Lipase Deficiency in Mice Causes Diglyceride Accumulation in Adipose Tissue, Muscle, and Testis. J. Biol. Chem. 277:4806–4815. doi:10.1074/jbc.M110355200.

Hales, K. E., S. D. Shackelford, J. E. Wells, D. A. King, N. A. Pyatt, H. C. Freetly, and T. L. Wheeler. 2016. Effects of dietary protein concentration and ractopamine hydrochloride on performance and carcass characteristics of finishing beef steers1. J. Anim. Sci. 94:2097–2102. doi:10.2527/jas.2015-0225.

Hamza, A., and C.-G. Zhan. 2009. Determination of the Structure of Human Phosphodiesterase-2 in a Bound State and Its Binding with Inhibitors by Molecular Modeling, Docking, and Dynamics Simulation. J. Phys. Chem. B. 113:2896–2908. doi:10.1021/jp8082612.

Hamza, I., M. Schaefer, L. W. Klomp, and J. D. Gitlin. 1999. Interaction of the copper chaperone HAH1 with the Wilson disease protein is essential for copper homeostasis. Proc. Natl. Acad. Sci. U. S. A. 96:13363–8. doi:10.1073/PNAS.96.23.13363.

Handa, N., E. Mizohata, S. Kishishita, M. Toyama, S. Morita, T. Uchikubo-Kamo, R. Akasaka, K. Omori, J. Kotera, T. Terada, M. Shirouzu, and S. Yokoyama. 2008. Crystal structure of the GAF-B domain from human phosphodiesterase 10A complexed with its ligand, cAMP. J. Biol. Chem. 283:19657–64. doi:10.1074/jbc.M800595200.

Hanna, P. M., and R. P. Mason. 1992. Direct evidence for inhibition of free radical formation from Cu(I) and hydrogen peroxide by glutathione and other potential ligands using the EPR spin-trapping technique. Arch. Biochem. Biophys. 295:205–213. doi:10.1016/0003-9861(92)90507-S.

Hart, E. B., H. Steenbock, J. Waddell, and C. A. Elvehjem. 1928. Iron in Nutrition VII. Copper as a supplement to iron for hemoglobin building in the rat. J. Biol. Chem.. 77:797–833.

Hartman, S. J., O. N. Genther-Schroeder, and S. L. Hansen. 2018. Comparison of trace mineral repletion strategies in feedlot steers to overcome diets containing high concentrations of sulfur and molybdenum. J. Anim. Sci. 96:2504–2515. doi:10.1093/jas/sky088.

Hasegawa, T., and Takafumi. 2010. Tyrosinase-Expressing Neuronal Cell Line as in Vitro Model of Parkinson's Disease. Int. J. Mol. Sci. 11:1082–1089. doi:10.3390/ijms11031082.

Hasegawa, T., A. Treis, N. Patenge, F. C. Fiesel, W. Springer, and P. J. Kahle. 2008. Parkin protects against tyrosinase-mediated dopamine neurotoxicity by suppressing stress-activated protein kinase pathways. J. Neurochem. 105:1700–1715. doi:10.1111/j.1471-4159.2008.05277.x.

Hausdorff, W., M. Caron, and R. Lefkowitz. 1990. Turning off the signal: desensitization of B-adrenergic receptor function. The FASEB. 4:2881–2889. doi:10.1096/fasebj.4.11.2165947.

Hausdorff, W. P., M. Bouvier, B. F. O'Dowd, G. P. Irons, M. G. Caron, and R. J. Lefkowitz. 1989. Phosphorylation Sites on Two Domains of the Beta2-Adrenergic Receptor Are Involved in Distinct Pathways of Receptor Desensitization. J. Biol. Chem. 264:12657–12665.

Heinlein, C. A., and C. Chang. 2002. The Roles of Androgen Receptors and Androgen-Binding Proteins in Nongenomic Androgen Actions. Mol. Endocrinol. 16:2181–2187. doi:10.1210/me.2002-0070.

Hellman, N. E., and J. D. Gitlin. 2002. Ceruloplasmin Metabolism and Function. Annu. Rev. Nutr. 22:439–58. doi:10.1146/annurev.nutr.22.012502.114457.

Hirata, F., W. J. Strittmatter, and J. Axelrod. 1979. β-Adrenergic receptor agonists increase phospholipid methylation, membrane fluidity, and β-adrenergic receptor-adenylate cyclase coupling.

Hohenester, E., T. Sasaki, and R. Timpl. 1999. Crystal structure of a scavenger receptor cysteine-rich domain sheds light on an ancient superfamily. Nat. Struct. Biol. 6:228–232. doi:10.1038/6669.

Horng, Y.-C., P. A. Cobine, A. B. Maxfield, H. S. Carr, and D. R. Winge. 2004. Specific copper transfer from the Cox17 metallochaperone to both Sco1 and Cox11 in the assembly of yeast cytochrome C oxidase. J. Biol. Chem. 279:35334–40. doi:10.1074/jbc.M404747200.

Houslay, M. D., G. S. Baillie, and D. H. Maurice. 2007. cAMP-Specific Phosphodiesterase-4 Enzymes in the Cardiovascular System: A Molecular Toolbox for Generating Compartmentalized cAMP Signaling. Circ. Res. 100:950–966. doi:10.1161/01.RES.0000261934.56938.38.

Huerta, M., R. . Kincaid, J. . Cronrath, J. Busboom, A. . Johnson, and C. . Swenson. 2002. Interaction of dietary zinc and growth implants on weight gain, carcass traits and zinc in tissues of growing beef steers and heifers. Anim. Feed Sci. Technol. 95:15–32. doi:10.1016/S0377-8401(01)00334-0.

Hung, I. H., R. L. Casareno, G. Labesse, F. S. Mathews, and J. D. Gitlin. 1998. HAH1 is a copper-binding protein with distinct amino acid residues mediating copper homeostasis and antioxidant defense. J. Biol. Chem. 273:1749–54. doi:10.1074/JBC.273.3.1749.

Hunt, D. W., D. M. Henricks, G. C. Skelley, and L. W. Grimes. 1991. Use of trenbolone acetate and estradiol in intact and castrate male cattle: effects on growth, serum hormones, and carcass characteristics. J. Anim. Sci. 69:2452. doi:10.2527/1991.6962452x.

Ismaya, W. T., H. J. Rozeboom, A. Weijn, J. J. Mes, F. Fusetti, H. J. Wichers, and B. W. Dijkstra. 2011. Crystal Structure of Agaricus bisporus Mushroom Tyrosinase: Identity of the Tetramer Subunits and Interaction with Tropolone. Biochemistry. 50:5477–5486. doi:10.1021/bi200395t.

Jamieson, S., and R. Allcroft. 1949. Copper Pine of Calves. Br. J. Nutr. 4:16–31. doi:10.1079/BJN19500005.

Jensen, L. T., and V. C. Culotta. 2005. Activation of CuZn superoxide dismutases from Caenorhabditis elegans does not require the copper chaperone CCS. J. Biol. Chem. 280:41373–9. doi:10.1074/jbc.M509142200.

Jiang, L., M. D. Garrick, L. M. Garrick, L. Zhao, and J. F. Collins. 2013. Divalent metal transporter 1 (Dmt1) Mediates Copper Transport in the Duodenum of Iron-Deficient Rats and When Overexpressed in Iron-Deprived HEK-293 Cells. J. Nutr. 143:1927–1933. doi:10.3945/jn.113.181867.

Johnson, B. J., P. T. Anderson, J. C. Meiske, and W. R. Dayton. 1996. Effect of a combined trenbolone acetate and estradiol implant on feedlot performance, carcass characteristics, and carcass composition of feedlot steers. J. Anim. Sci. 74:363. doi:10.2527/1996.742363x.

Johnson, B. J., N. Halstead, M. E. White, M. R. Hathaway, A. DiCostanzo, and W. R. Dayton. 1998a. Activation state of muscle satellite cells isolated from steers implanted with a combined trenbolone acetate and estradiol implant. J. Anim. Sci. 76:2779. doi:10.2527/1998.76112779x.

Johnson, B. J., S. B. Smith, and K. Y. Chung. 2014. Historical Overview of the Effect of β-Adrenergic Agonists on Beef Cattle Production. Asian-Australasian J. Anim. Sci. 27:757–66. doi:10.5713/ajas.2012.12524.

Johnson, B. J., M. E. White, M. R. Hathaway, C. J. Christians, and W. R. Dayton. 1998b. Effect of a combined trenbolone acetate and estradiol implant on steady-state IGF-I mRNA concentrations in the liver of wethers and the longissimus muscle of steers. J. Anim. Sci. 76:491. doi:10.2527/1998.762491x.

Johnson, L. R., and T. E. Engle. 2003. The Effects of Copper Source and Concentration on Lipid Metabolism in Growing and Finishing Angus Steers. Asian-Aust. J. Anim. Sci. 16:1131–1136.

Jourdan-Le Saux, C., A. Tomsche, A. Ujfalusi, L. Jia, and K. Csiszar. 2001. Central Nervous System, Uterus, Heart, and Leukocyte Expression of the LOXL3 Gene, Encoding a Novel Lysyl Oxidase-Like Protein. Genomics. 74:211–218. doi:10.1006/geno.2001.6545.

Kalamajski, S., D. Bihan, A. Bonna, K. Rubin, and R. W. Farndale. 2016. Fibromodulin Interacts with Collagen Cross-linking Sites and Activates Lysyl Oxidase. J. Biol. Chem. 291:7951–60. doi:10.1074/jbc.M115.693408.

Kamanga-Sollo, E., M. S. Pampusch, G. Xi, M. E. White, M. R. Hathaway, and W. R. Dayton. 2004. IGF-I mRNA levels in bovine satellite cell cultures: Effects of fusion and anabolic steroid treatment. J. Cell. Physiol. 201:181–189. doi:10.1002/jcp.20000.

Kamanga-Sollo, E., K. J. Thornton, M. E. White, and W. R. Dayton. 2014. Role of G protein-coupled estrogen receptor-1, matrix metalloproteinases 2 and 9, and heparin binding epidermal growth factor-like growth factor in estradiol-17β-stimulated bovine satellite cell proliferation. Domest. Anim. Endocrinol. 49:20–26. doi:10.1016/J.DOMANIEND.2014.04.004.

Kamanga-Sollo, E., M. E. White, K. Y. Chung, B. J. Johnson, and W. R. Dayton. 2008a. Potential role of G-protein-coupled receptor 30 (GPR30) in estradiol-17β-stimulated IGF-I mRNA expression in bovine satellite cell cultures. Domest. Anim. Endocrinol. 35:254–262. doi:10.1016/J.DOMANIEND.2008.06.001.

Kamanga-Sollo, E., M. E. White, M. R. Hathaway, K. Y. Chung, B. J. Johnson, and W. R. Dayton. 2008b. Roles of IGF-I and the estrogen, androgen and IGF-I receptors in estradiol-17β- and trenbolone acetate-stimulated proliferation of cultured bovine satellite cells. Domest. Anim. Endocrinol. 35:88–97. doi:10.1016/J.DOMANIEND.2008.02.003.

Kamanga-Sollo, E., M. E. White, W. J. Weber, and W. R. Dayton. 2013. Role of estrogen receptor-α (ESR1) and the type 1 insulin-like growth factor receptor (IGFR1) in estradiol-stimulated proliferation of cultured bovine satellite cells. Domest. Anim. Endocrinol. 44:36–45. doi:10.1016/J.DOMANIEND.2012.08.002.

Keller, G.-A., T. G. Warner, K. S. Steimert, and R. A. Hallewellt. 1991. Cu,Zn superoxide dismutase is a peroxisomal enzyme in human fibroblasts and hepatoma cells. Proc. Nati. Acad. Sci. USA. 88:7381–7385. doi:10.1073/pnas.88.16.7381.

Kim, B. E., T. Nevitt, and D. J. Thiele. 2008. Mechanisms for copper acquisition, distribution and regulation. Nat. Chem. Biol. 4:176–185. doi:10.1038/nchembio.72.

Kincaid, R. L. 2000. Assessment of trace mineral status of ruminants: A review. J. Anim. Sci. 77:1–10. doi:10.2527/jas2000.77E-Suppl1x.

Kline, W. O., F. J. Panaro, H. Yang, and S. C. Bodine. 2007. Rapamycin inhibits the growth and muscle-sparing effects of clenbuterol. J. Appl. Physiol. 102:740–747. doi:10.1152/japplphysiol.00873.2006.

Klomp, L. W., S. J. Lin, D. S. Yuan, R. D. Klausner, V. C. Culotta, and J. D. Gitlin. 1997. Identification and functional expression of HAH1, a novel human gene involved in copper homeostasis. J. Biol. Chem. 272:9221–6. doi:10.1074/JBC.272.14.9221.

Krishnamoorthy, L., J. A. Cotruvo, J. Chan, H. Kaluarachchi, A. Muchenditsi, V. S. Pendyala, S. Jia, A. T. Aron, C. M. Ackerman, M. N. Vander Wal, T. Guan, L. P. Smaga, S. L. Farhi, E. J. New, S. Lutsenko, and C. J. Chang. 2016. Copper regulates cyclic-AMP-dependent lipolysis. Nat. Chem. Biol. 12:586–592. doi:10.1038/nchembio.2098.

Krishnamurthy, S., B. S. Moorthy, L. Xin Xiang, L. Xin Shan, K. Bharatham, N. K. Tulsian, I. Mihalek, and G. S. Anand. 2014. Active Site Coupling in PDE:PKA Complexes Promotes Resetting of Mammalian cAMP Signaling. Biophys. J. 107:1426–1440. doi:10.1016/J.BPJ.2014.07.050.

Krueger, K. M., Y. Daaka, J. A. Pitcher, and R. J. Lefkowitz. 1997. The role of sequestration in G protein-coupled receptor resensitization. Regulation of beta2-adrenergic receptor dephosphorylation by vesicular acidification. J. Biol. Chem. 272:5–8. doi:10.1074/JBC.272.1.5.

Kugelman, T. P., and E. J. Van Scott. 1961. Tyrosinase activity in melanocytes of human albinos. J. Invest. Dermatol. 37:73–76. doi:10.1038/jid.1961.84.

Kumari, P., A. Srivastava, R. Banerjee, E. Ghosh, P. Gupta, R. Ranjan, X. Chen, B. Gupta, C. Gupta, D. Jaiman, and A. K. Shukla. 2016. Functional competence of a partially engaged GPCR–β-arrestin complex. Nat. Commun. 7:1–16. doi:10.1038/ncomms13416.

Laporte, S. A., R. H. Oakley, J. A. Holt, L. S. Barak, and M. G. Caron. 2000. The interaction of beta-arrestin with the AP-2 adaptor is required for the clustering of beta 2-adrenergic receptor into clathrin-coated pits. J. Biol. Chem. 275:23120–6. doi:10.1074/jbc.M002581200.

Layne, M. D., and S. R. Farmer. 1999. Tumor Necrosis Factor-α and Basic Fibroblast Growth Factor Differentially Inhibit the Insulin-like Growth Factor-I Induced Expression of Myogenin in C2C12 Myoblasts. Exp. Cell Res. 249:177–187. doi:10.1006/EXCR.1999.4465.

Lean, I. J., J. M. Thompson, and F. R. Dunshea. 2014. A Meta-Analysis of Zilpaterol and Ractopamine Effects on Feedlot Performance, Carcass Traits and Shear Strength of Meat in Cattle. PLoS One. 9:1–28. doi:10.1371/journal.pone.0115904.

Lee, C. A., A. Lee-Barthel, L. Marquino, N. Sandoval, G. R. Marcotte, and K. Baar. 2015. Estrogen inhibits lysyl oxidase and decreases mechanical function in engineered ligaments. J Appl Physiol. 118:1250–1257. doi:10.1152/japplphysiol.00823.2014.

Lee, J., M. Marjorette, O. Peñ, Y. Nose, and D. J. Thiele. 2002a. Biochemical Characterization of the Human Copper Transporter Ctr1*. J. Biol. Chem. 277:4380–4387. doi:10.1074/jbc.M104728200.

Lee, J., M. J. Petris, and D. J. Thiele. 2002b. Characterization of Mouse Embryonic Cells Deficient in the Ctr1 High Affinity Copper Transporter. J. Biol. Chem. 277:40253–40259. doi:10.1074/jbc.M208002200.

Lee, J., J. R. Prohaska, S. L. Dagenais, T. W. Glover, and D. J. Thiele. 2000. Isolation of a murine copper transporter gene, tissue specific expression and functional complementation of a yeast copper transport mutant. Gene. 254:87–96. doi:10.1016/S0378-1119(00)00287-0.

Lee, J., J. R. Prohaska, and D. J. Thiele. 2001. Essential role for mammalian copper transporter Ctr1 in copper homeostasis and embryonic development. Proc. Natl. Acad. Sci. U. S. A. 98:6842–7. doi:10.1073/pnas.111058698.

Lefkowitz, R. J. 1998. G protein-coupled receptors. III. New roles for receptor kinases and beta-arrestins in receptor signaling and desensitization. J. Biol. Chem. 273:18677–80. doi:10.1074/JBC.273.30.18677.

Lefkowitz, R. J., and S. K. Shenoy. 2005. Transduction of Receptor Signals by beta-arrestins. Science (80-.). 308:512–517. doi:10.1126/science.1074683.

Lerner, A. B., T. B. Fitzpatrick, E. Calkinsj, and W. H. Summerson. 1950. Mammalian Tyrosinase: The Relationship of Copper to Enzymatic Activity. J. Biol. Chem. 187:793–802.

Linder, M. C. 2016. Ceruloplasmin and other copper binding components of blood plasma and their functions: an update. Metallomics. 8:887–905. doi:10.1039/c6mt00103c.

Linder, M. C., and M. Hazegh-Azam. 1996. Copper biochemistry and molecular biology. Am J Clin N. 63:7975–7981. doi:10.1093/ajcn/63.5.797.

Liu, C., P. Zhu, W. Wang, W. Li, Q. Shu, Z.-J. Chen, L. Myatt, and K. Sun. 2016. Inhibition of lysyl oxidase by prostaglandin E2 via EP2/EP4 receptors in human amnion fibroblasts: Implications for parturition. Mol. Cell. Endocrinol. 424:118–127. doi:10.1016/J.MCE.2016.01.022.

Liu, M., E. J. Cohen, G. J. Brewer, and P. R. Laibson. 2002. Kayser-Fleischer ring as the presenting sign of Wilson disease. Am. J. Ophthalmol. 133:832–834. doi:10.1016/S0002-9394(02)01408-3.

Lupu, F., J. D. Terwilliger, K. Lee, G. V. Segre, and A. Efstratiadis. 2001. Roles of Growth Hormone and Insulin-like Growth Factor 1 in Mouse Postnatal Growth. Dev. Biol. 229:141–162. doi:10.1006/DBIO.2000.9975.

Lutsenko, S. 2010. Human copper homeostasis: a network of interconnected pathways This review comes from a themed issue on Bioinorganic chemistry Edited. Curr. Opin. Chem. Biol. 14:211–217. doi:10.1016/j.cbpa.2010.01.003.

Luza, S. C., and H. C. Speisky. 1996. Liver copper storage and transport during development: implications for cytotoxicity. Am. J. Clin. Nutr. 63:812S–820S. doi:10.1093/ajcn/63.5.812.

Ma, L., and G. Pei. 2007. β-arrestin signaling and regulation of transcription. J. Cell Sci. 120:213–218. doi:10.1242/jcs.03338.

Manglik, A., T. H. Kim, M. Masureel, C. Altenbach, Z. Yang, D. Hilger, M. T. Lerch, T. S. Kobilka, F. S. Thian, W. L. Hubbell, R. S. Prosser, and B. K. Kobilka. 2015. Structural Insights into the Dynamic Process of β2-Adrenergic Receptor Signaling. Cell. 161:1101–1111. doi:10.1016/J.CELL.2015.04.043.

Maryon, E. B., S. A. Molloy, and J. H. Kaplan. 2013. Cellular glutathione plays a key role in copper uptake mediated by human copper transporter 1. Am. J. Physiol. Physiol. 304:C768–C779. doi:10.1152/ajpcell.00417.2012.

Matoba, Y., T. Kumagai, A. Yamamoto, H. Yoshitsu, and M. Sugiyama. 2006. Crystallographic evidence that the dinuclear copper center of tyrosinase is flexible during catalysis. J. Biol. Chem. 281:8981–90. doi:10.1074/jbc.M509785200.

Maurice, D. H., H. Ke, F. Ahmad, Y. Wang, J. Chung, and V. C. Manganiello. 2014. Advances in targeting cyclic nucleotide phosphodiesterases. Nat Rev Drug Discov. 13:290–314. doi:10.1038/nrd4228.

Mersmann, H. J. 1998. Overview of the effects of beta-adrenergic receptor agonists on animal growth including mechanisms of action. J. Anim. Sci. 76:160–172. doi:10.2527/1998.761160x.

Mersmann, H. J. 2002. Beta-Adrenergic receptor modulation of adipocyte metabolism and growth. J. Anim. Sci. 80:E24–E29.

Messerschmidt, A., and R. Huber. 1990. The blue oxidases, ascorbate oxidase, laccase and ceruloplasmin Modelling and structural relationships. Eur. J. Biochem. 187:341–352. doi:10.1111/j.1432-1033.1990.tb15311.x.

Middleton, R. B., and M. C. Linder. 1993. Synthesis and Turnover of Ceruloplasmin in Rats Treated with 17β-Estradiol. Arch. Biochem. Biophys. 302:362–368. doi:10.1006/ABBI.1993.1224.

Mills, C. F. 1987. Biochemical and Physiological Indicators of Mineral Status in Animals: Copper, Cobalt and Zinc. J. Anim. Sci. 65:1702–1711. doi:10.2527/jas1987.6561702x.

Mills, S. E. 2002. Biological basis of the ractopamine response. J. Anim. Sci. 80:E28–E32. doi:10.2527/ANIMALSCI2002.80E-SUPPL_2E28X.

Milne, D. B. 1998. Copper intake and assessment of copper status. Am J Clin Nutr. 67:1041S–5S. doi:10.1093/ajcn/67.5.1041S.

Montarras, D., A. L'honoré, and M. Buckingham. 2013. Lying low but ready for action: the quiescent muscle satellite cell. FEBS J. 280:4036–4050. doi:10.1111/febs.12372.

Montgomery, T. H., P. F. Dew, and M. S. Brown. 2001. Optimizing carcass value and the use of anabolic implants in beef cattle. J. Anim. Sci. 79:E296–E306. doi:10.2527/jas2001.79E-SupplE296x.

Montoliu, L., K. Grønskov, A.-H. Wei, M. Martínez-García, A. Fernández, B. Arveiler, F. Morice-Picard, S. Riazuddin, T. Suzuki, Z. M. Ahmed, T. Rosenberg, and W. Li. 2014. Increasing the complexity: new genes and new types of albinism. Pigment Cell Melanoma Res. 27:11–18. doi:10.1111/pcmr.12167.

Moon, H.-J., J. Finney, T. Ronnebaum, and M. Mure. 2014. Human lysyl oxidase-like 2. Bioorg. Chem. 57:231–241. doi:10.1016/J.BIOORG.2014.07.003.

Morita, H., S.-I. Ikeda, K. Yamamoto, S. Morita, K. Yoshida, S. Nomoto, M. Kato, and N. Yanagisawa. 1995. Hereditary ceruloplasmin deficiency with hemosiderosis: A clinicopathological study of a japanese family. Ann. Neurol. 37:646–656. doi:10.1002/ana.410370515.

Mortimer, R. G., D. A. Dargatz, and L. R. Corah. 1999. Forage analyses from cow-calf herds in 23 states. Fort Collins, CO.

Moss, F. P., and C. P. Leblond. 1971. Satellite cells as the source of nuclei in muscles of growing rats. Anat. Rec. 170:421–435. doi:10.1002/ar.1091700405.

Muhammad, I. F., Y. Borné, B. Hedblad, P. M. Nilsson, M. Persson, and G. Engström. 2016. Acute-phase proteins and incidence of diabetes: a population-based cohort study. Acta Diabetol. 53:981–989. doi:10.1007/s00592-016-0903-8.

Nagalakshmi, N., and M. N. V Prasad. 2001. Responses of glutathione cycle enzymes and glutathione metabolism to copper stress in Scenedesmus bijugatus. Plant Sci. 160:291–299. doi:10.1016/S0168-9452(00)00392-7.

Najafi, P., I. Zulkifli, A. F. Soleimani, and Y. M. Goh. 2016. Acute phase proteins response to feed deprivation in broiler chickens. Poult. Sci. 95:760–763. doi:10.3382/ps/pew001.

NASEM. 2016. Nutrient Requirements of Beef Cattle. 8th ed. National Academy Press, Washington, DC.

Navegantes, L. C. C., N. M. Z. Resano, R. H. Migliorini, and Í. C. Kettelhut. 2000. Role of adrenoceptors and cAMP on the catecholamine-induced inhibition of proteolysis in rat skeletal muscle. Am. J. Physiol. Metab. 279:E663–E668. doi:10.1152/ajpendo.2000.279.3.E663.

Naylor, J. M., and S. L. Ralston. 1991. Large Animal Clinical Nutrition.

Niedermayer, E. K., O. N. Genther-Schroeder, D. D. Loy, and S. L. Hansen. 2018. Effect of varying trace mineral supplementation of steers with or without hormone implants on growth and carcass characteristics. J. Anim. Sci. 96:1159–1170. doi:10.1093/jas/skx063.

Nose, Y., B.-E. Kim, and D. J. Thiele. 2006. Ctr1 drives intestinal copper absorption and is essential for growth, iron metabolism, and neonatal cardiac function. Cell Metab. 4:235–244. doi:10.1016/j.cmet.2006.08.009.

Oakley, R. H., S. A. Laporte, J. A. Holt, L. S. Barak, M. G. Caron, and §. 1999. Association of beta-arrestin with G protein-coupled receptors during clathrin-mediated endocytosis dictates the profile of receptor resensitization. J. Biol. Chem. 274:32248–57. doi:10.1074/JBC.274.45.32248.

Oetting, W. S. 2000. The Tyrosinase Gene and Oculocutaneous Albinism Type 1 (OCA1): A Model for Understanding the Molecular Biology of Melanin Formation. Pigment Cell Res. 13:320–325. doi:10.1034/j.1600-0749.2000.130503.x.

Ogórek, M., M. Lenartowicz, R. Starzyński, A. Jończy, R. Staroń, A. Doniec, W. Krzeptowski, A. Bednarz, O. Pierzchała, P. Lipiński, Z. Rajfur, Z. Baster, P. Gibas-Tybur, and P. Grzmil. 2017. Atp7a and Atp7b regulate copper homeostasis in developing male germ cells in mice. Metallomics. 9:1288–1303. doi:10.1039/C7MT00134G.

Ohrvik, H., and D. J. Thiele. 2014. How copper traverses cellular membranes through the mammalian copper transporter 1 , Ctr1. 1314:32–41. doi:10.1111/nyas.12371.

Oldfield, R. N., · Kathryn, A. Johnston, J. Limones, C. Ghilarducci, and K. M. Lopez. 2018. Identification of Histidine 303 as the Catalytic Base of Lysyl Oxidase via Site-Directed Mutagenesis. Protein J. 37:47–57. doi:10.1007/s10930-017-9749-3.

Omori, K., and J. Kotera. 2007. Overview of PDEs and Their Regulation. Circ. Res. 100:309–327. doi:10.1161/01.RES.0000256354.95791.f1.

Osaki, S., D. A. Johnson, and E. Frieden. 1966. The Possible Significance of the Ferrous Oxidase Activity of Ceruloplasmin in Normal Human Serum. J. Biol. CHEMSTRY. 241:2746–2751.

Pampusch, M. S., M. E. White, M. R. Hathaway, T. J. Baxa, K. Y. Chung, S. L. Parr, B. J. Johnson, W. J. Weber, and W. R. Dayton. 2008. Effects of implants of trenbolone acetate, estradiol, or both, on muscle insulin-like growth factor-I, insulin-like growth factor-I receptor, estrogen receptor-α, and androgen receptor messenger ribonucleic acid levels in feedlot steers. J. Anim. Sci. 86:3418–3423. doi:10.2527/jas.2008-1085.

Park, J. H., P. Scheerer, K. P. Hofmann, H.-W. Choe, and O. P. Ernst. 2008. Crystal structure of the ligand-free G-protein-coupled receptor opsin. Nature. 454:183–187. doi:10.1038/nature07063.

Perry, S. J., G. S. Baillie, T. A. Kohout, I. McPhee, M. M. Magiera, K. L. Ang, W. E. Miller, A. J. McLean, M. Conti, M. D. Houslay, and R. J. Lefkowitz. 2002. Targeting of Cyclic AMP Degradation to β2-Adrenergic Receptors by β-Arrestins. Science (80-.). 298:834–836. doi:10.1126/science.1074683.

Perry, T. C., D. G. Fox, and D. H. Beermann. 1991. Effect of an implant of trenbolone acetate and estradiol on growth, feed efficiency, and carcass composition of Holstein and beef steers1. J. Anim. Sci. 69:4696–4702. doi:10.2527/1991.69124696x.

Petris, M. J., J. F. Mercer, J. G. Culvenor, P. Lockhart, P. A. Gleeson, and J. Camakaris. 1996. Ligand-regulated transport of the Menkes copper P-type ATPase efflux pump from the Golgi apparatus to the plasma membrane: a novel mechanism of regulated trafficking. EMBO J. 15:6084–6095. doi:10.1002/J.1460-2075.1996.TB00997.X.

Pippig, S., S. Andexinger, and M. J. Lohse. 1995. Sequestration and recycling of beta 2-adrenergic receptors permit receptor resensitization. Mol. Pharmacol. 47:666–676.

Polishchuk, E. V, M. Concilli, S. Iacobacci, G. Chesi, N. Pastore, P. Piccolo, S. Paladino, D. Baldantoni, S. C. D. Van Ijzendoorn, J. Chan, C. J. Chang, A. Amoresano, F. Pane, P. Pucci, A. Tarallo, G. Parenti, N. Brunetti-Pierri, C. Settembre, A. Ballabio, and R. S. Polishchuk. 2014. Wilson Disease Protein ATP7B Utilizes Lysosomal Exocytosis to Maintain Copper Homeostasis. Dev. Cell. 29:686–700. doi:10.1016/j.devcel.2014.04.033.

Pompella, A., A. Visvikis, A. Paolicchi, V. De Tata, and A. F. Casini. 2003. The changing faces of glutathione, a cellular protagonist. Biochem. Pharmacol. 66:1499–1503. doi:10.1016/S0006-2952(03)00504-5.

Poujois, A., J.-M. Trocello, N. Djebrani-Oussedik, J. Poupon, C. Collet, N. Girardot-Tinant, R. Sobesky, D. Hab Es, D. Debray, C. Vanlemmens, F. Fluch Ere I, F. Ory-Magne, J. Labreuche, C. Preda, and F. Woimant. 2017. Exchangeable copper: a reflection of the neurological severity in Wilson's disease. Eur. J. Neurol. 24:154–160. doi:10.1111/ene.13171.

Price, J., A. M. Will, G. Paschaleris, and J. K. Chesters. 1987. Identification of thiomolybdates in digesta and plasma from sheep after administration of Mo-labelled compounds into the rumen. Br. J. Nutr. 58:127–138. doi:10.1079/BJN19870076.

Pufahl, R. A., K. L. Singer, S.-J. Peariso, S.-J. Lin, P. J. Schmidt, C. J. Fahrni, V. C. Culotta, J. E. Penner-Hahn, and T. V. O'Halloran. 1997. Metal Ion Chaperone Function of the Soluble Cu(I) Receptor Atx1. Science (80-.). 278:853–856. doi:10.1126/science.7716541.

Puig, S., J. Lee, M. Lau, and D. J. Thiele. 2002. Biochemical and Genetic Analyses of Yeast and Human High Affinity Copper Transporters Suggest a Conserved Mechanism for Copper Uptake. J. Biol. Chem. 277:26021–26030. doi:10.1074/jbc.M202547200.

Rasenick, M. M., M. Watanabeg, M. B. Lazarevic, S. Hatta, and H. E. Hammfl. 1994. Synthetic Peptides as Probes for G Protein Function. Carboxyl-terminal G Alpha s Peptides Mimic Gs and Evoke High Affinity Agonist Binding to Beta-adrenergic Receptors. J. Biol. Chem. 269:21519–21525.

Reeds, R. J., and S. U. S. A. N. M. Hay. 1986. Stimulation of muscle growth by clenbuterol: lack of effect on muscle protein biosynthesis. Br. J. Nutr. 56:249–258. doi:10.1079/BJN19860104.

Reiter, E., and R. J. Lefkowitz. 2006. GRKs and β-arrestins: roles in receptor silencing, trafficking and signaling. Trends Endocrinol. Metab. 17:159–165. doi:10.1016/J.TEM.2006.03.008.

Robison, G. A., R. W. Butcher, and E. W. Sutherland. 1968. Cyclic AMP. Annu. Rev. Biochem. 37:149–174. doi:10.1146/annurev.bi.37.070168.001053.

Roeser, H. P., G. R. Lee, S. Nacht, and G. E. Cartwright. 1970. The role of ceruloplasmin in iron metabolism. J. Clin. Invest. 49:2408–17. doi:10.1172/JCI106460.

Rosenbaum, D. M., S. G. F. Rasmussen, and B. K. Kobilka. 2009. The structure and function of G-protein-coupled receptors. Nature. 459:356–363. doi:10.1038/nature08144.

Rosenzweig, A. C., D. L. Huffman, M. Y. Hou, A. K. Wernimont, R. A. Pufahl, and T. V O 'halloran. 1999. Crystal structure of the Atx1 metallochaperone protein at 1.02 Å resolution. Structure. 7:605–617. doi:10.1016/S0969-2126(99)80082-3.

Rucker, R. B., T. Kosonen, M. S. Clegg, A. E. Mitchell, B. R. Rucker, J. Y. Uriu-Hare, and C. L. Keen. 1998. Copper, lysyl oxidase, and extracellular matrix protein cross-linking. Am J Clin Nutr. 67:996S–1002S. doi:10.1093/ajcn/67.5.996S.

Samber, J. A., J. D. Tatum, M. I. Wray, W. T. Nichols, J. B. Morgan, and G. C. Smith. 1996. Implant Program Effects on Performance and Carcass Quality of Steer Calves Finished for 212 Days. J. Anim. Sci. 74:1470–1476. doi:10.2527/1996.7471470x.

Samuelson, K. L., M. E. Hubbert, M. L. Galyean, and C. A. Löest. 2016. Nutritional recommendations of feedlot consulting nutritionists: The 2015 New Mexico State and Texas Tech University survey. J. Anim. Sci. 94:2648. doi:10.2527/jas.2016-0282.

Samygina, V. R., A. V Sokolov, G. Bourenkov, T. R. Schneider, V. A. Anashkin, A. S. O. Kozlov, N. N. Kolmakov, and V. B. Vasilyev. 2017. Rat ceruloplasmin: a new labile copper binding site and zinc/copper mosaic. Metallomics. 9:1828–1838. doi:10.1039/c7mt00157f.

Sato, M., and J. D. Gitlin. 1991. Mechanisms of Copper Incorporation During the Biosynthesis of Human Ceruloplasmin. J. Biol. Chem. 266:5128–5134.

Schmidt, P. J., C. Kunst, and V. C. Culotta. 2000. Copper activation of superoxide dismutase 1 (SOD1) in vivo. Role for protein-protein interactions with the copper chaperone for SOD1. J. Biol. Chem. 275:33771–6. doi:10.1074/jbc.M006254200.

Schmidt, P. J., T. D. Rae, R. A. Pufahl, T. Hamma, J. Strain, T. V O 'halloran, and V. C. Culotta. 1999. Multiple Protein Domains Contribute to the Action of the Copper Chaperone for Superoxide Dismutase. J. Biol. Chem. 274:23719–23725.

Schwartz, T. W., T. M. Frimurer, B. Holst, M. M. Rosenkilde, and C. E. Elling. 2006. Molecular Mechanism of 7TM Receptor Activation-A Global Toggle Switch Model. Annu. Rev. Pharmacol. Toxicol. 46:481–519. doi:10.1146/annurev.pharmtox.46.120604.141218.

Sette, C., and M. Conti. 1996. Phosphorylation and activation of a cAMP-specific phosphodiesterase by the cAMP-dependent protein kinase. Involvement of serine 54 in the enzyme activation. J. Biol. Chem. 271:16526–34. doi:10.1074/JBC.271.28.16526.

Shakur, Y., L. Stenson Holst, T. Rahn Landstrom, M. Movsesian, E. Degerman, and V. Manganiello. 2001. Regulation and Function of the Cyclic Nucleotide Phosphodiesterase (PDE3) Gene Family. In: Progress in Nucleic Acid Research and Molecular Biology. Academic Press. p. 241–277.

Shenoy, S. K., and R. J. Lefkowitz. 2003. Trafficking patterns of beta-arrestin and G protein-coupled receptors determined by the kinetics of beta-arrestin deubiquitination. J. Biol. Chem. 278:14498–506. doi:10.1074/jbc.M209626200.

Shenoy, S. K., and R. J. Lefkowitz. 2011. β-arrestin-mediated receptor trafficking and signal transduction. Trends Pharmacol. Sci. 32:521–533. doi:10.1016/J.TIPS.2011.05.002.

Shenoy, S. K., P. H. McDonald, T. A. Kohout, and R. J. Lefkowitz. 2001. Regulation of receptor fate by ubiquitination of activated beta 2-adrenergic receptor and beta-arrestin. Science. 294:1307–13. doi:10.1126/science.1063866.

Shi, Y., M. V. Ivannikov, M. E. Walsh, Y. Liu, Y. Zhang, C. A. Jaramillo, G. T. Macleod, and H. Van Remmen. 2014. The Lack of CuZnSOD Leads to Impaired Neurotransmitter Release, Neuromuscular Junction Destabilization and Reduced Muscle Strength in Mice. PLoS One. 9:1–11. doi:10.1371/journal.pone.0100834.

Shukla, A. K., G. H. Westfield, K. Xiao, R. I. Reis, L.-Y. Huang, P. Tripathi-Shukla, J. Qian, S. Li, A. Blanc, A. N. Oleskie, A. M. Dosey, M. Su, C.-R. Liang, L.-L. Gu, J.-M. Shan, X. Chen, R. Hanna, M. Choi, X. J. Yao, B. U. Klink, A. W. Kahsai, S. S. Sidhu, S. Koide, P. A. Penczek, A. A. Kossiakoff, V. L. Woods Jr, B. K. Kobilka, G. Skiniotis, and R. J. Lefkowitz. 2014. Visualization of arrestin recruitment by a G-protein-coupled receptor. Nature. 512:218–222. doi:10.1038/nature13430.

Smith-Mungo, L. I., and H. M. Kagan. 1998. Lysyl Oxidase: Properties, Regulation and Multiple Functions in Biology. Matrix Biol. 16:387–398. doi:10.1016/S0945-053X(98)90012-9.

Smith, K. R., S. K. Duckett, M. J. Azain, R. N. Sonon, and T. D. Pringle. 2007. The effect of anabolic implants on intramuscular lipid deposition in finished beef cattle. J. Anim. Sci. 85:430. doi:10.2527/jas.2006-280.

Sneddon, A. A., M. I. Delday, J. Steven, and C. A. Maltin. 2001. Elevated IGF-II mRNA and phosphorylation of 4E-BP1 and p70 ^{S6k} in muscle showing clenbuterol-induced anabolism. Am. J. Physiol. Metab. 281:E676–E682. doi:10.1152/ajpendo.2001.281.4.E676.

Sprang, S. R. 1997. G protein mechanisms: insights from structural analysis. Annu. Rev. Biochem. 66:639–78. doi:10.1146/annurev.biochem.66.1.639.

Steele-Perkins, G., J. Turner, J. C. Edman, J. Hari, S. B. Pierce, C. Stover, W. J. Rutters, and R. A. Rotho. 1988. Expression and Characterization of a Functional Human Insulin-like Growth Factor I Receptor. J. Biol. Chem. 263:1148–11492.

Strader, C. D., I. S. Sigal, and R. A. Dixon. 1989. Structural basis of beta-adrenergic receptor function. FASEB J. 3:1825–1832. doi:10.1096/fasebj.3.7.2541037.

Strasser, R. H., J. L. Benovic, M. G. Caron, and R. J. Lefkowitz. 1986. Beta-agonist- and prostaglandin E1-induced translocation of the beta-adrenergic receptor kinase: evidence that the kinase may act on multiple adenylate cyclase-coupled receptors. Proc. Natl. Acad. Sci. U. S. A. 83:6362–6. doi:10.1073/PNAS.83.17.6362.

Strosberg, D., and F. Pietri-Reuxel. 1996. Function and regulation of the β3-adrenoceptor Pharmacological properties. TiPS. 17:373–381. doi:10.1016/S0165-6147(96)80011-3.

Sunderman, W. F., S. Nomoto, I. G. Gillies, and P. J. Goldblatt. 1971. Effect of Estrogen Administration upon Ceruloplasmin and Copper Concentrations in Rat Serum. Toxicol. Appl. Pharmacol. 20:588–598.

Suttle, N. F. 1991. The Interactions Between Copper, Molybdenum, and Sulphur in Ruminant Nutrition. Annu. Rev. Nutr. 11:121–140. doi:10.1146/annurev.nu.11.070191.001005.

Suttle, N. F. 2010. Mineral Nutrition of Livestock. 4th ed. CABI Publishing, Wallingford, UK.

Swatland, H. J. 1977. Accumulation of Myofiber Nuclei in Pigs with Normal and Arrested Development. J. Anim. Sci. 44:759–764. doi:10.2527/jas1977.445759x.

Tainer, J. A., E. D. Getzoff, J. S. Richardson, and D. C. Richardson. 1983. Structure and mechanism of copper, zinc superoxide dismutase. Nature. 306:284–287. doi:10.1038/306284a0.

Tang, C., and J. P. Klinman. 2001. The Catalytic Function of Bovine Lysyl Oxidase in the Absence of Copper. J. Biol. Chem. 276:30575–30578. doi:10.1074/jbc.C100138200.

Tang, W., and A. G. Gilman. 1992. Adenylyl Cyclases. Cell. 70:869–872. doi:10.1016/0092-8674(92)90236-6.

Terada, K., Y. Kawarada, N. Miura, O. Yasui, K. Koyama, and T. Sugiyama. 1995. Copper incorporation into ceruloplasmin in rat livers. Biochim. Biophys. Acta - Mol. Basis Dis. 1270:58–62. doi:10.1016/0925-4439(94)00072-X.

Thompson, S. H., L. K. Boxhorn, W. Kong, and R. E. Allen. 1989. Trenbolone alters the responsiveness of skeletal muscle satellite cells to fibroblast growth factor and insulin-like growth factor I. Endocrinology. 124:2110–2117. doi:10.1210/endo-124-5-2110.

Thornton, K. J., E. Kamanga-Sollo, M. E. White, and W. R. Dayton. 2016. Active G protein–coupled receptors (GPCR), matrix metalloproteinases 2/9 (MMP2/9), heparin-binding epidermal growth factor (hbEGF), epidermal growth factor receptor (EGFR), erbB2, and insulin-like growth factor 1 receptor (IGF-1R) are necessary for trenbo. J. Anim. Sci. 94:2332–2343. doi:10.2527/jas.2015-0178.

Thornton, K. J., E. Kamange-Sollo, M. E. White, and W. R. Dayton. 2015. Role of G protein–coupled receptors (GPCR), matrix metalloproteinases 2 and 9 (MMP2 and MMP9), heparin-binding epidermal growth factor–like growth factor (hbEGF), epidermal growth factor receptor (EGFR), erbB2, and insulin-like growth factor 1 receptor (I. J. Anim. Sci. 93:4291–4301. doi:10.2527/jas.2015-9191.

Trenkle, A., D. L. DeWitt, and D. G. Topel. 1978. Influence of Age, Nutrition and Genotype on Carcass Traits and Cellular Development of the M. Longissimus of Cattle. J. Anim. Sci. 46:1597–1603. doi:10.2527/jas1978.4661597x.

Tsukihara, T., H. Aoyama, E. Yamashita, T. Tomizaki, H. Yamaguchi, K. Shinzawa-Itoh, R. Nakashima, R. Yaono, and S. Yoshikawa. 1996. The Whole Structure of the 13-Subunit Oxidized Cytochrome c Oxidase at 2.8 Å. Science (80-.). 272:1136–1144.

Vashchenko, G., and R. MacGillivray. 2013. Multi-Copper Oxidases and Human Iron Metabolism. Nutrients. 5:2289–2313. doi:10.3390/nu5072289.

Velloso, C. P. 2008. Regulation of muscle mass by growth hormone and IGF-I. Br. J. Pharmacol. 154:557–568. doi:10.1038/bjp.2008.171.

Verhoeckx, K. C. M., R. P. Doorrnbos, J. Greef, R. F. Witkamp, and R. J. T. Rodenburg. 2005. Inhibitory effects of the beta2-adrenergic receptor agonist zilpaterol on the LPS-induced production of TNF-alpha in vitro and in vivo. J. Vet. Pharmacol. Ther. 28:531–537. doi:10.1111/j.1365-2885.2005.00691.x.

Vulpe, C. D., Y.-M. Kuo, T. L. Murphy, L. Cowley, C. Askwith, N. Libina, J. Gitschier, and G. J. Anderson. 1999. Hephaestin, a ceruloplasmin homologue implicated in intestinal iron transport,is defective in the sla mouse. Nat. Genet. 21:195–199. doi:10.1038/5979.

Walker, D. K., E. C. Titgemeyer, J. S. Drouillard, E. R. Loe, B. E. Depenbusch, and A. S. Webb. 2006. Effects of ractopamine and protein source on growth performance and carcass characteristics of feedlot heifers1. J. Anim. Sci. 84:2795–2800. doi:10.2527/jas.2005-614.

Wang, H., M.-S. Peng, Y. Chen, J. Geng, H. Robinson, M. D. Houslay, J. Cai, and H. Ke. 2007. Structures of the four subfamilies of phosphodiesterase-4 provide insight into the selectivity of their inhibitors. Biochem. J. 408:193–201. doi:10.1042/BJ20070970.

Wang, S. X., M. Mure, K. F. Medzihradszky, A. L. Burlingame, D. E. Brown, D. M. Dooley, A. J. Smith, H. M. Kagan, and J. P. Klinman. 1996. A Crosslinked Cofactor in Lysyl Oxidase: Redox Function for Amino Acid Side Chains. Science (80-.). 273:1078–1084. doi:10.1126/science.273.5278.1078.

Ward, J. D., and J. W. Spears. 1997. Long-term effects of consumption of low-copper diets with or without supplemental molybdenum on copper status, performance, and carcass characteristics of cattle. J. Anim. Sci. 75:3057. doi:10.2527/1997.75113057x.

Ward, J. D., J. W. Spears, and E. B. Kegley. 1993. Effect of copper level and source (copper lysine vs copper sulfate) on copper status, performance, and immune response in growing steers fed diets with or without supplemental molybdenum and sulfur. J. Anim. Sci. 71:2748–2755. doi:10.2527/1993.71102748x.

Warne, T., R. Moukhametzianov, J. G. Baker, R. Nehmé, P. C. Edwards, A. G. W. Leslie, G. F. X. Schertler, and C. G. Tate. 2011. The structural basis for agonist and partial agonist action on a β 1-adrenergic receptor. Nature. 469:241–244. doi:10.1038/nature09746.

Winterholler, S. J., G. L. Parsons, C. D. Reinhardt, J. P. Hutcheson, W. T. Nichols, D. A. Yates, R. S. Swingle, and B. J. Johnson. 2007. Response to ractopamine-hydrogen chloride is similar in yearling steers across days on feed. J. Anim. Sci. 85:413–419. doi:10.2527/jas.2006-555.

Wong, P. C., D. Waggoner, J. R. Subramaniam, L. Tessarollo, T. B. Bartnikas, V. C. Culotta, D. L. Price, J. Rothstein, and J. D. Gitlin. 2000. Copper chaperone for superoxide dismutase is essential to activate mammalian Cu/Zn superoxide dismutase. PNAS. 97:2886–2891. doi:10.1073/pnas.040461197.

Wu, Y., W. Zhao, J. Zhao, J. Pan, Q. Wu, Y. Zhang, W. A. Bauman, and C. P. Cardozo. 2007. Identification of Androgen Response Elements in the Insulin-Like Growth Factor I Upstream Promoter. Endocrinology. 148:2984–2993. doi:10.1210/en.2006-1653.

Xiao, Z., and A. G. Wedd. 2002. A C-terminal domain of the membrane copper pump Ctr1 exchanges copper(I) with the copper chaperone Atx1. Chem. Commun. 588–589. doi:10.1039/b111180a.

Xu, R. X., A. M. Hassell, D. Vanderwall, M. H. Lambert, W. D. Holmes, M. A. Luther, W. J. Rocque, M. V Milburn, Y. Zhao, H. Ke, and R. T. Nolte. 2000. Atomic structure of PDE4: insights into phosphodiesterase mechanism and specificity. Science. 288:1822–5. doi:10.1126/SCIENCE.288.5472.1822.

Yeaman, S. J. 1990. Hormone-sensitive lipase-a multipurpose enzyme in lipid metabolism. Biochim. Biophys. Acta. 1052:128–132.

Zaitseva, I., V. Zaitsev, G. Card, K. Moshkov, B. Bax, A. Ralph, P. Lindley, I. Zaitseva, V. Zaitsev, G. Card, A. Ralph, P. Lindley, B. Bax, and K. Moshkov. 1996. The X-ray structure of human serum ceruloplasmin at 3.1 Å: nature of the copper centres. JBIC. 1:15–23. doi:10.1007/s007750050018.

Zanardi, A., A. Conti, M. Cremonesi, P. D'Adamo, E. Gilberti, P. Apostoli, C. V. Cannistraci, A. Piperno, S. David, and M. Alessio. 2018. Ceruloplasmin replacement therapy ameliorates neurological symptoms in a preclinical model of aceruloplasminemia. EMBO Mol. Med. 10:91–106. doi:10.15252/emmm.201708361.

Zhang, J., L. S. Barak, P. H. Anborgh, S. A. Laporte, M. G. Caron, and S. S. Ferguson. 1999. Cellular trafficking of G protein-coupled receptor/beta-arrestin endocytic complexes. J. Biol. Chem. 274:10999–1006. doi:10.1074/JBC.274.16.10999.

Zhang, J., S. S. Ferguson, L. S. Barak, L. Ménard, and M. G. Caron. 1996. Dynamin and beta-arrestin reveal distinct mechanisms for G protein-coupled receptor internalization. J. Biol. Chem. 271:18302–5. doi:10.1074/JBC.271.31.18302.

Zhang, J., R. Yang, Z. Liu, C. Hou, W. Zong, A. Zhang, X. Sun, and J. Gao. 2015. Loss of lysyl oxidase-like 3 causes cleft palate and spinal deformity in mice. Hum. Mol. Genet. 24:6174–6185. doi:10.1093/hmg/ddv333.

Zhang, P., E. V. Smith-Nguyen, M. M. Keshwani, M. S. Deal, A. P. Kornev, and S. S. Taylor. 2012. PKA type IIalpha holoenzyme reveals a combinatorial strategy for isoform diversity. Science (80-.). 335:712–716. doi:10.1126/science.1146447.

Zhang, X., Q. Wang, J. Wu, J. Wang, Y. Shi, M. Liu, F. M. Hughson, M. Mure, and L. Tong. 2018. Crystal structure of human lysyl oxidase-like 2 (hLOXL2) in a precursor state. PNAS. 115:3828–3833. doi:10.1073/pnas.1720859115.

Zhou, B., and J. Gitschier. 1997. hCTR1: A human gene for copper uptake identified by complementation in yeast. Genetics. 94:7481–7486. doi:10.1073/pnas.94.14.7481.

Zischka, H., and J. Lichtmannegger. 2014. Pathological mitochondrial copper overload in livers of Wilson's disease patients and related animal models. Ann. N. Y. Acad. Sci. 1315:6–15. doi:10.1111/nyas.12347.

CHAPTER 3. EFFECTS OF LIVER COPPER CONCENTRATION AND DIETARY COPPER CONCENTRATION ON RACTOPAMINE HYDROCHLORIDE PERFORMANCE AND CARCASS CHARACTERISTICS OF FINISHING STEERS

A paper to be submitted to the Journal of Animal Science

E.M. Messsersmith,* M. E. Branine,† O. N. Genther-Schroeder,* S. L. Hansen*

*Department of Animal Science, Iowa State University, Ames, IA 50011 USA

†Zinpro corporation, Eden Prairie, MN, 55344 USA

Abstract

To determine the effect of copper (**Cu**) status on beta agonist (**BA**) induced growth, 96 Angus-cross steers (470 ± 35 kg) were assigned to a 3 × 2 factorial. Factors included Cu-supplementation (mg Cu/kg DM) at: 0 (**LOW**), 10 (**MED**), or 20 (**HIGH**) from Availa-Cu (Zinpro; Eden Prairie, MN) and the BA ractopamine hydrochloride from Optaflexx (Elanco, Greenfield, IN) at 0 (**NoRAC**) or 300 mg·steer^{-1}·d^{-1} (**RAC**) for the final 28-d of the 88-d trial. Steers were blocked by liver Cu, developed in a prior trial to distinct statuses of 6, 14, and 53 mg/kg DM (SEM 2.0) for LOW, MED, and HIGH, respectively, stratified by BW to pens of 6, and fed via GrowSafe bunks. Steer was the experimental unit (n = 12 or 18 per treatment) and initial BW from the prior trial was used as a covariate in performance analyses. Consecutive day BW were collected on d -1, 0, 60, 61, 87, and 88 with intermediate BW on d 28, 40, and 75. Blood samples were collected on d -1, 60, 66, 67, 75, and 87 and liver biopsies collected on d -23, 53, and during harvest. Contrast statements were constructed to determine linear and quadratic

effects of Cu status within BA treatment. Day 53 liver Cu quadratically increased to 14, 166, and 266 mg/kg DM ($P \leq 0.0001$; SEM 8.7). Pre-BA growth was not affected by Cu-supplementation ($P \geq 0.51$). Linear and quadratic effects of Cu within BA treatment were tested in BA period. Copper-supplementation of RAC steers quadratically increased average daily gain ($P = 0.002$) and tended to increase dry matter intake, feed efficiency, and ribeye area ($P \leq 0.1$) driven by greatest performance in MED steers. No other carcass characteristics were affected by Cu-supplementation regardless of BA treatment ($P \geq 0.16$). Regardless of Cu, feeding BA tended to increase hot carcass weight ($P = 0.08$; 412 and 404 kg, for RAC and NoRAC respectively, SEM 3.5). Harvest liver Cu concentrations quadratically increased with dietary Cu-supplementation regardless of BA treatment ($P < 0.0001$). Serum non-esterified fatty acids tended to exhibit a BA × Day interaction ($P = 0.08$) in which NEFA decreased after 14 days of BA treatment for both RAC and NoRAC steer, however RAC steers had lesser serum NEFA concentrations. Feed efficiency during BA period linearly decreased as Cu-supplementation increased in NoRAC steers ($P = 0.04$). Increasing dietary Cu effectively increased Cu status, however RAC steers receiving 10 mg Cu/kg DM performed best during the BA period. These data suggest beta-agonist response is influenced by Cu status of steers.

Key words: beef steers, copper, ractopamine hydrochloride

Introduction

Improved animal performance through the utilization of beta agonists (**BA**) is attributed to the repartitioning of energy from fat deposition to muscle accretion (Mersmann, 1998). Nearly 85% of cattle represented in a survey of consulting nutritionists and feedlot managers are fed a BA (Birch, 2015; Samuelson et al., 2016).

The use of BA is associated with increased live body weight (**BW**), hot carcass weight (**HCW**), and ribeye area (**REA**); as well as improved feed efficiency while decreasing marbling score (Lean et al., 2014). These characteristics are a result of the BA pathway in which intracellular messenger cyclic AMP (**cAMP**) mediates the activation of hormone sensitive lipase for the initiation of lipolysis (Mersmann, 1998). Lipolysis is impeded by phosphodiesterases (**PDE**) regulating BA activity through cAMP degradation (Conti et al., 2003). To accommodate BA induced growth, cattle may require additional nutritional resources such as copper (**Cu**). Growth is affected through Cu-dependent lysyl oxidase which is responsible for the crosslinking of collagen within the extracellular matrix and thus structural integrity of cells (Rucker et al., 1998). Additionally, recent evidence suggests Cu may affect lipolysis as tissue Cu concentrations and lipolytic activity of adipocytes in mice concurrently decreased (Krishnamoorthy et al., 2016). Furthermore, cellular models reveal that reversible binding of Cu to PDE may dampen the activity of this known BA inhibitor (Krishnamoorthy et al., 2016). Taken together, these studies suggest that Cu status may positively impact the performance of cattle fed BA through restricted PDE degradation of the cAMP signal. The objective of this study was to determine the effects of initial Cu status on performance and carcass characteristics of steers consuming or not consuming a BA. It was hypothesized that as Cu status improved the BA response would be consequently enhanced.

Materials and Methods

All procedures and protocol in this study were approved by the Iowa State University Institutional Animal Care and Use Committee (log number 5-17-8522-B).

Animals and experimental design

Ninety-six single source Angus-cross steers (470 ± 35 kg) were utilized in a 3 × 2 factorial design experiment. These steers were utilized in a previous trial in which three distinct Cu statuses were induced through the dietary inclusion of Cu antagonists S and Mo. Steers were blocked by initial liver Cu concentration into three dietary treatments to maintain liver Cu concentration throughout the trial. Prior to initiation of the study three steers were removed for health concerns. Dietary treatments included LOW, MED, and HIGH receiving 0, 10, or 20 mg of Cu/ kg DM from Availa-Cu (Zinpro, Eden Prairie, Mn), respectively. These treatments represent no supplemental Cu, national recommendations and industry consultant reported supplementation for LOW, MED, and HIGH treatments, respectively (NASEM, 2016; Samuelson et al., 2016). Within Cu treatment steers were stratified by BW and randomly assigned to pens of 6 with GrowSafe bunks (GrowSafe Systems Ltd., Airdrie, AB, Canada) and automatic waterers.

Consecutive day BW were taken at the beginning the Pre-BA period (d -1 and 0), the BA period (d 61 and 62), and at the end of the experiment (d 87 and 88). Cattle were implanted with a Component TE-IS (Elanco Animal Health, Greenfield, IN) on day 0 followed by a Component TE-S (Elanco Animal Health, Greenfield, IN) on day 40. On day 62 half of the pens from each dietary Cu treatment were randomly assigned to receive ractopamine hydrochloride (Optaflexx; Elanco Animal Health, Greenfield, IN) at 300 mg·steer^{-1}·day^{-1}. Cattle were shipped to a commercial abattoir (Iowa Premium Beef, Tama, IA) on day 88 and were harvested on day 89. Following a 48-hour chill carcass data were collected including HCW, REA, BF, KPH, marbling score, and YG. Additionally, DP was calculated by dividing the shrunk live final BW by the HCW and

multiplying by 100. A 4 % pencil shrink was applied to all BW before ADG and G:F were calculated.

Feed and tissue sampling and analysis

Cattle were fed a common dry rolled corn-based diet supplemented with NASEM (2016) recommendations for all minerals except Cu (**Table 1**). During the Pre-BA period an error in DM was recognized for modified distillers grains solubles and it was found most appropriate to correct upon the start of the BA period. Thus, steers received the Pre-BA diet from d 0-60, and the BA diet from d 61-87. The diet was delivered at approximately 0800 h daily and bunks were managed to allow for ad libitum feed intake. Total mixed rations (**TMR**) were sampled weekly and dried in a forced air oven at 70˚C for 48 hours to determine dry matter (**DM**). Utilizing the weekly DM from TMR samples, individual steer DMI was determined from as-fed intakes. Feed efficiency (G:F) was calculated for both the Pre-BA and BA periods using total weight gained in that period divided by the total amount of feed consumed on a DM basis during that period. Dried diet samples were ground through a 2-mm screen (Retsch Zm100 grinder; Glen Mills Inc., Clifton, NJ) and samples were composited monthly in accordance with the Pre-BA and BA periods. Liver biopsies were conducted on three randomly sampled steers from each pen on day -23 and 53 in accordance with the method described by Engle and Spears (2000) while final liver samples were collected at harvest. The same three steers were utilized for liver samples throughout the experiment. Jugular blood samples were collected from all animals on days 0, 60, 66, 67, 75, and 87. Composited TMR samples and liver samples were acid digested with trace mineral grade nitric acid (Fisher Scientific, Fair Lawn, NJ) as described by Richter et al. (2012). Liver, plasma, and TMR

samples were analyzed for Cu and Zn using inductively coupled plasma optical emission spectrometry (Optima 7000 DV, Perkin Elmer, Waltham, MA) as described by (Pogge et al., 2014). Further, the S content of TMR samples were calculated from inclusion of feedstuffs in the diet. Blood samples were collected in vacutainer tubes with no additives for serum, trace mineral grade EDTA, and EDTA for other analysis (Becton Dickenson, Rutherford, NJ). Tubes were spun in a centrifuge at 1200 x g for 10 minutes for plasma and at 1200 x g for 20 minutes for serum samples. Serum and trace mineral plasma was stored at -20˚C prior to analysis. Serum NEFA was analyzed using a commercial kit (Wako Pure Chemical Industries Ltd., Chuo-Ku Osaka, Japan). Blood analysis was completed with inter-assay and intra-assay CV values less than or equal to 10.

Statistical analysis

Data from the pre-BA period were analyzed as a randomized block design using the Mixed procedure of SAS 9.4 (SAS Inst. Inc., Cary, NC). Steer was experimental unit (n = 24 for LOW or 36 per MED and HIGH treatment) with a model including the fixed effect of Cu. Contrast statements to test for a linear or quadratic effect of Cu treatment were utilized for analysis of data during this period. Data from the BA period, d 61-88, were analyzed as a 3 × 2 factorial using the Mixed procedure of SAS 9.4 using the fixed effects of Cu, BA, and Cu × BA. Contrast statements were formed to test for both linear and quadratic effects of Cu treatment within cattle that did not receive BA (L-Cu, Q-Cu) and within cattle that did receive BA (L-CuRAC, Q-CuRAC). Analysis of serum NEFA data was conducted as repeated measures with day of sampling as the repeated effect. The experimental unit was steer, except for liver samples (n = 6 for LOW treatments or 9 for all other treatments). Initial liver Cu from the prior trial (d -119) was utilized as a

covariate in liver Cu analysis. Liver trace mineral data were log transformed to fit a normal distribution based on Shapiro-Wilk test for normality. In addition, Cook's D was used to evaluate data for outliers. Data from two steers (one from HIGH and one from MED-RAC) were removed from analysis due to poor performance unrelated to treatment. Prior to the start of the trial 3 steers were removed due to poor performance or health related issues (n = 12 per LOW treatment, 16 for MED-RAC, 17 for MED-NoRAC, 18 for HIGH-RAC, and 16 for HIGH-NoRAC). Data reported are least square means with standard error of the mean. Statistical significance was determined at $P \leq 0.05$, and a statistical tendency was determined at $0.05 < P \leq 0.10$.

Results

Pre-beta agonist period performance and copper status

Throughout the Pre-BA period, no differences in performance were observed for d 0 BW, d 62 BW, DMI, ADG, or feed efficiency (**Table 2**; $P \geq 0.15$). As expected initial liver Cu concentrations of steers linearly increased with greater Cu supplementation ($P = 0.01$). By the end of the Pre-BA (d 61) period liver Cu concentrations quadratically increased with greater concentrations of dietary Cu supplemented ($P = 0.01$).

Beta agonist period and overall growth performance

Steer BA period and overall growth performance data are shown in **Table 3**. During the BA period DMI tended ($P = 0.10$) to quadratically increase due to Cu supplementation within RAC, with MED steers having greater DMI than their counterparts. Moreover, RAC supplemented steers quadratically improved ADG with increasing dietary Cu, again driven by MED-fed steers ($P = 0.01$), resulting in a tendency

for a quadratic increase in G:F due to Cu supplementation of RAC treated steers (P = 0.08). Within NoRAC steers, Cu supplementation did not affect ADG (P = 0.43) and linearly decreased G:F (P = 0.04). Final live BW of steers on d 87, as well as carcass adjusted final BW and overall DMI, ADG, and G:F were not affected by Cu supplementation within BA treatment ($P \geq 0.19$).

Carcass characteristics

Final liver Cu concentrations and steer carcass data are shown in **Table 4**. Following harvest liver Cu concentration of steers was quadratically increased due to greater Cu supplementation regardless of BA treatment (P = 0.01). Further, HCW, DP, marbling, back fat, KPH, and yield grade were unaffected by Cu supplementation within BA strategy ($P \geq 0.31$). However, REA of RAC treated steers tended (P = 0.08) to quadratically increase with MED steers having the largest REA, while no impact of Cu supplementation within NoRAC steers on REA was noted ($P \geq 0.38$).

Blood analysis

There was a tendency for NEFA to display a BA × Day interaction (P = 0.08; **Figure 1**) in which NEFA decreased for both NoRAC and RAC steers on day 14 of BA supplementation in comparison the beginning and end of BA period (d 61 and 87, respectively) although RAC steers had lesser NEFA concentrations throughout trial than NoRAC steers.

Discussion

Beta agonists are widely used throughout the feedlot industry because of the gain in efficiency and lean muscle accretion this technology provides (Birch, 2015; Samuelson

et al., 2016). Beta agonists such as ractopamine hydrochloride or zilpaterol hydrochloride improve live final BW and HCW between 8 and 15 kg (Lean et al., 2014). Previous work has indicated the supplementation of Zn to cattle receiving a BA improves performance suggesting a role for trace minerals in the BA response (Genther-Schroeder et al., 2016a). In contrast to Zn, liver stores of Cu are well accepted as status indexes allowing for clear evaluation of the effect of status through liver Cu concentrations. Therefore, this study was designed to assess the effect of liver Cu concentrations on BA-induced growth and carcass characteristics.

Interestingly, the three Cu treatments at the initiation of the trial represented steers that were deficient or within the marginal range of Cu status based on liver Cu concentrations (Kincaid, 2000). Although initially deficient, the rapid increase in liver Cu concentration by d 53 in MED and HIGH steers may have been enhanced by the transition to a high concentrate diet from an 85% corn silage diet. The change in diet likely resulted in a drop in ruminal pH, allowing for greater solubility of Cu in the rumen (NASEM, 2016). In addition to this ruminal pH shift, steers were previously fed a diet with the potent Cu antagonists S and Mo. With the removal of supplemental S and Mo, MED and HIGH cattle were able to rapidly improve liver Cu concentrations. The stark increases of both MED and HIGH steers in liver Cu concentrations during the 53-day period were in contrast to the minimal improvement in liver Cu concentrations of LOW steers. The continued improvement in liver Cu concentration of MED and HIGH steers and inability of LOW steers to improve deficient liver Cu concentrations through the entirety of the trial suggest that 6.0 mg Cu/kg DM analyzed in the common diet was not enough to meet bodily requirements for Cu and improve Cu concentration in the liver to

an adequate range. Though S was not supplemented in the present study, the inclusion of coproducts such as modified distillers grains with solubles resulted in a calculated dietary S concentration of 0.33% which was likely sufficiently high to interfere with Cu absorption, resulting in the inability of LOW steers to improve liver Cu concentrations to the same degree as MED and HIGH steers.

The three distinct Cu statuses observed in the Cu treatments allow differences in performance and carcass characteristics to be attributed to Cu status based on liver Cu concentrations. During the BA feeding period ADG, DMI, G:F, and REA were greatest in MED-RAC steers while NoRAC steers experienced a numerical increase in DMI and subsequently a linear decrease in feed efficiency with increasing Cu supplementation. Both MED-RAC (227 mg Cu/kg DM) and HIGH-RAC (324 mg Cu/kg DM) treatments regardless of BA treatment had adequate liver Cu concentrations (125-600 mg Cu/kg DM) during the BA period (Kincaid, 2000). The clear improvement in performance of MED-RAC steers over HIGH-RAC during the BA feeding period may suggest that liver Cu concentrations of ~250 mg Cu/kg DM are more favorable than those in excess of 300 mg Cu/kg DM. These differences in performance indicate liver Cu adequacy and dietary supplementation of Cu should be further classified to reflect optimal growth, with or without use of growth promoting technologies. Data in the present study imply that the NASEM (2016) recommendation for Cu supplementation is beneficial to BA-induced growth in comparison to no supplementation or Cu supplementation at current industry reported averages. Additional work is needed to refine optimal liver Cu concentrations and whether more or less than 10 mg Cu/kg DM is optimal to support BA induced growth.

The influence of dietary Cu content on growth of steers during the BA period may be associated with the physiological role of Cu downstream of BA receptor activation. Recent cell culture and rodent research has demonstrated that Cu is positively associated with lipolytic activity through the reversible regulation of PDE in adipocytes (Krishnamoorthy et al., 2016). Previously, Zn was indicated to also reversibly bind to PDE as a regulatory mechanism, and was shown to decrease gene expression of PDE (von Bülow et al., 2005). Through both of these studies cAMP was altered due to mineral concentrations of media (von Bülow et al., 2005; Krishnamoorthy et al., 2016). Further, increasing supplemental Zn in cattle has been shown to linearly increase plasma cAMP, suggesting the inhibition of PDE through Zn (Genther-Schroeder et al., 2016a). Increased availability of cAMP to activate protein kinase A and subsequently hormone sensitive lipase (Beavo et al., 1974; Mersmann, 1998) would support hydrolysis of triacylglycerides, increasing release of free fatty acids from adipose tissue to be used as energy for the accretion of muscle (Yeaman, 1990). As Cu has been identified as a regulator of PDE, the cAMP pool would be expected to be greater with increasing supplementation of Cu leading to more lipolysis. However, plasma cAMP concentrations were not assessed in the present trial. The improved performance of MED-RAC steers in comparison to LOW-RAC and HIGH-RAC steers does not directly follow the linear trend that is observed in Zn studies. It is unclear why supplementing 20 mg Cu/kg DM was not beneficial to BA performance when 10 mg Cu/kg DM elicited a growth response. It is possible that greater concentrations of Cu may have led to faster desensitization of the beta-adrenergic receptor, but research to support this claim is lacking.

Serum NEFA decreased for both NoRAC and RAC steers on d 66 from d 61 values but were greater on d 87 than 66. Although NoRAC and RAC steers followed similar trends, RAC steers had lesser serum NEFA concentrations than NoRAC steers. The decrease in serum NEFA for RAC steers may have been due to released NEFA from BA-induced lipolysis being utilized as energy by growing tissues rather than accumulating in the serum. However, the difference in serum NEFA for both NoRAC and RAC steers between days may have been due to the diet change that occurred at the beginning of the BA period. The inhibition of PDE by Cu described earlier should support increased lipolysis and thus it was expected that serum NEFA would be greater in RAC-fed steers as dietary Cu increased; however, Cu appeared to have no impact on serum NEFA. The role of Cu in potentially stimulating lipolysis may be the underlying reason why previous Cu studies have seen a decrease in back fat with increasing Cu supplementation (Engle et al., 2000a; Engle and Spears, 2000; Engle and Spears, 2001; Johnson and Engle, 2003). However, neither Cu or BA impacted back fat measures in the present trial. Additionally, the effect of Cu on lipolysis is clouded by the numerical trend for marbling to increase with increasing Cu supplementation as inhibition of PDE would suggest greater lipolytic rates. However, studies have shown a decrease in back fat in steers while not affecting marbling suggesting back fat is more directly targeted by lipolysis than intramuscular fat deposits (Engle et al., 2000b; Engle et al., 2000a; Engle and Spears, 2000). Indeed, Pothoven et al. (1975) demonstrated that fatty acid synthesis and thus lipolytic rate vary among tissues suggesting that back fat and marbling may not be affected in a similar manner.

In the present trial LOW steers performed well even though liver Cu concentrations are quite different from Cu supplemented steers. The comparable growth rates of LOW steers to MED and HIGH counterparts may in part have been due to the diet shift at the beginning of the BA period. Through the shift to more corn by-products in the diet, metabolizable protein in the diet during the BA period provided 47% greater metabolizable protein than the steers required. Although adequate, the Pre-BA period provided 21% more metabolizable protein than the requirement of the steers based upon observed BW, DMI, and ADG for each respective period. It is possible that increasing metabolizable protein during the BA period resulted in greater growth rates than may be typically expected late in the finishing period for steers. The consistently excellent growth observed in LOW steers, despite deficient Cu status as determined by liver Cu concentrations, suggests that late stage finishing cattle likely have a lesser Cu requirement than is currently recommended (NASEM, 2016).

Zinc has been more extensively studied than Cu with the supplementation of BA. Increasing supplemental Zn appears to be beneficial during BA supplementation as final BW, ADG, and G:F linearly increased (Genther-Schroeder et al., 2016a). Additional work led to similar results in which supplementation of Zn led to improved HCW, final BW, and carcass adjusted ADG of steers treated with BA (Genther-Schroeder et al., 2016b). Interestingly, in both of these studies steers not supplemented with BA did not exhibit improved performance due to Zn supplementation (Genther-Schroeder et al., 2016b; Genther-Schroeder et al., 2016a). Unlike the linear performance response elicited through Zn supplementation, the response to Cu supplementation appears to have an optimal dietary Cu concentration most near NASEM (2016) recommendations, in which

more or less dietary Cu may limit BA-induced performance. Additionally, the supplementation of trace minerals such as Cu and Zn to cattle not receiving BA appears to have little to no response on performance in these studies. In finishing pigs the supplementation of Cu or Zn at 125 and 150 mg/kg DM, respectively, led to improved feed conversions during BA supplementation in comparison to control pigs; however, combining Cu and Zn did not result in an additive effect or improve efficiency from control pigs (Feldpausch et al., 2015). Because the metabolism of Cu and Zn are interrelated more work is needed to assess the effects of altering one or both of these critical trace minerals in the diets of livestock receiving BA.

Overall, steers receiving BA experience a differential response in performance due to increasing supplemental Cu in comparison to steers within the NoRAC treatment. This suggests that Cu influences the pathway in which BA elicit a growth response, potentially through the inhibition of PDE. Furthermore, these data suggest that NASEM (2016) recommendations for Cu are adequate for BA induced growth while greater or lesser concentrations of Cu may cause adverse impacts on cattle performance when utilizing a BA. To fully understand the mechanism in which Cu influences BA growth and distinguish between Cu status and dietary Cu more work is warranted.

Literature Cited

Beavo, J. A., P. J. Bechtel, and E. G. Krebs. 1974. Activation of protein kinase by physiological concentrations of cyclic AMP. Proc. Natl. Acad. Sci. U. S. A. 71:3580–3. doi:10.1073/PNAS.71.9.3580.

Birch, J. 2015. 2015 Nebraska Feedyard Labor Cost Benchmarks and Historical Trends. Lincoln, NE.

von Bülow, V., L. Rink, and H. Haase. 2005. Zinc-Mediated Inhibition of Cyclic Nucleotide Phosphodiesterase Activity and Expression Suppresses TNF-α and IL-1β Production in Monocytes by Elevation of Guanosine 3′,5′-Cyclic Monophosphate. J. Immunol. 175.

Engle, T. E., and J. W. Spears. 2000. Dietary copper effects on lipid metabolism, performance, and ruminal fermentation in finishing steers. J. Anim. Sci. 78:2452–2458. doi:10.2527/2000.7892452x.

Engle, T. E., and J. W. Spears. 2001. Performance, carcass characteristics, and lipid metabolism in growing and finishing Simmental steers fed varying concentrations of copper. J. Anim. Sci. 79:2920–2925. doi:10.2527/2001.79112920x.

Engle, T. E., J. W. Spears, T. A. Armstrong, C. L. Wright, and J. Odle. 2000a. Effects of dietary copper source and concentration on carcass characteristics and lipid and cholesterol metabolism in growing and finishing steers. J. Anim. Sci. 78:1053–1059. doi:10.2527/2000.7841053x.

Engle, T. E., J. W. Spears, L. Xi, and F. W. Edens. 2000b. Dietary copper effects on lipid metabolism and circulating catecholamine concentrations in finishing steers. J. Anim. Sci. 78:2737. doi:10.2527/2000.78102737x.

Feldpausch, J. A., R. G. Amachawadi, H. M. Scott, and M. D. Tokach. 2015. Effects of Added Copper and Zinc on Growth Performance and Carcass Characteristics of Finishing Pigs Fed Diets with or without Ractopamine HCl. Kansas Agric. Exp. Stn. Res. Reports. 1:1–12. doi:10.4148/2378-5977.1134.

Genther-Schroeder, O. N., M. E. Branine, and S. L. Hansen. 2016a. The effects of increasing supplementation of zinc-amino acid complex on growth performance, carcass characteristics, and inflammatory response of beef cattle fed ractopamine hydrochloride. J. Anim. Sci. 94:3389–3398. doi:10.2527/jas.2015-0209.

Genther-Schroeder, O. N., M. E. Branine, and S. L. Hansen. 2016b. The influence of supplemental Zn-amino acid complex and ractopamine hydrochloride feeding duration on growth performance and carcass characteristics of finishing beef cattle. J. Anim. Sci. 94:4338–4345. doi:10.2527/jas.2015-0159.

Johnson, L. R., and T. E. Engle. 2003. The effects of copper source and concentration on lipid metabolism in growing and finishing Angus steers. Asian-Australasian J. Anim. Sci.

Kincaid, R. L. 2000. Assessment of trace mineral status of ruminants: A review. J. Anim. Sci. 77:1–10. doi:10.2527/jas2000.77E-Suppl1x.

Krishnamoorthy, L., J. A. Cotruvo, J. Chan, H. Kaluarachchi, A. Muchenditsi, V. S. Pendyala, S. Jia, A. T. Aron, C. M. Ackerman, M. N. Vander Wal, T. Guan, L. P. Smaga, S. L. Farhi, E. J. New, S. Lutsenko, and C. J. Chang. 2016. Copper regulates cyclic-AMP-dependent lipolysis. Nat. Chem. Biol. 12:586–592. doi:10.1038/nchembio.2098.

Lean, I. J., J. M. Thompson, and F. R. Dunshea. 2014. A Meta-Analysis of Zilpaterol and Ractopamine Effects on Feedlot Performance, Carcass Traits and Shear Strength of Meat in Cattle. PLoS One. 9:1–28. doi:10.1371/journal.pone.0115904.

Mersmann, H. J. 1998. Overview of the effects of beta-adrenergic receptor agonists on animal growth including mechanisms of action. J. Anim. Sci. 76:160–172. doi:10.2527/1998.761160x.

NASEM. 2016. Nutrient Requirements of Beef Cattle. 8th ed. National Academy Press, Washington, DC.

Pogge, D. J., M. E. Drewnoski, and S. L. Hansen. 2014. High dietary sulfur decreases the retention of copper, manganese, and zinc in steers. J. Anim. Sci. 92:2182–2191. doi:10.2527/jas.2013-7481.

Pothoven, M. A., D. C. Beitz, and J. H. Thornton. 1975. Lipogenesis and lipolysis in adipose tissue of ad libitum and restricted-fed beef cattle during growth. J. Anim. Sci. 40:957–962.

Richter, E. L., M. E. Drewnoski, and S. L. Hansen. 2012. Effects of increased dietary sulfur on beef steer mineral status, performance, and meat fatty acid composition. J. Anim. Sci. 90:3945–3953. doi:10.2527/jas.2011-4512.

Samuelson, K. L., M. E. Hubbert, M. L. Galyean, and C. A. Löest. 2016. Nutritional recommendations of feedlot consulting nutritionists: The 2015 New Mexico State and Texas Tech University survey. J. Anim. Sci. 94:2648. doi:10.2527/jas.2016-0282.

Yeaman, S. J. 1990. Hormone-sensitive lipase-a multipurpose enzyme in lipid metabolism. Biochim. Biophys. Acta. 1052:128–132.

Table 1. Ingredient and composition analysis of un-supplemented diet in pre-beta agonist and beta agonist period

Ingredient	Pre-BA Period % of diet DM	BA Period % of diet DM
Dry rolled corn	68.9	62.0
MDGS[1]	16.7	25.0
Bromegrass hay	8.9	8.0
DDGS[2]	3.33	3.05
Limestone	1.7	1.5
Salt	0.31	0.31
Vitamin & mineral premix[3]	0.13	0.13
Rumensin	0.0135	0.0135
Analyzed composition, %		
Crude protein[4]	12.62	15.31
NDF[4]	20.14	19.35
Ether extract[4]	4.03	4.69
Cu, mg/kg DM[5]	6.0	6.0
Zn, mg/kg DM[6]	64.4	72.0
Calculated composition, %		
Sulfur	0.33	0.33

[1]Modified distillers grains with solubles.

[2]Dried distillers grains with solubles.

[3]Premix provided 2,200 IU vitamin A and 25 IU vitamin E/kg diet and NASEM (2016) recommendations for Co, Mn, Se, Zn, and I from inorganic sources in addition to 0, 10, or 20 mg Cu/kg DM from Availa Cu (Zinpro Corporation, Eden Prairie, MN).

[4]Analysis were completed by Dairyland Laboratories (Arcadia, WI).

[5]Analyzed Cu values represent LOW dietary treatment total, MED and HIGH dietary treatments analyzed at 16 and 25 mg Cu/kg DM, respectively. Trace mineral treatments included: LOW (no supplemental trace mineral), MED (2016 recommendation of 10 mg Cu/kg DM), and HIGH (feedlot consultant recommendations of 20 mg Cu/kg DM; Samuelson et al. (2016)). Supplementation of Cu was delivered as Availa Cu (Zinpro Corporation, Eden Prairie, MN) and all other minerals were supplemented at national recommendations utilizing inorganic sources. Values analyzed by inductively couples plasma optical emission spectrometry (ICP Optima 7000 DV, Perkin Elmer, Waltham, MA).

[6]Analyzed Zn values represent LOW dietary treatment for both Pre-BA and BA period measured by inductively coupled plasma optical emission spectrometry (ICP Optima 7000 DV, Perkin Elmer, Waltham, MA).

Table 2. Effect of supplemental copper on copper status and performance during pre-beta agonist period

	Copper[4]				Contrasts[5]	
Item[1,2,3]	Low	Med	High	SEM	Lin	Quad
steers (n)	24	36	36			
d -23 liver Cu, mg/kg DM	6	14	53	2.0	0.01	0.06
d 53 liver Cu, mg/kg DM	14	166	266	8.7	0.01	0.01
d 0 BW, kg	466	470	473	5.1	0.33	0.91
d 62 BW, kg	579	586	588	6.9	0.32	0.79
DMI, kg/d	12.1	12.3	12.6	0.23	0.15	0.78
ADG, kg	1.8	1.9	1.9	0.05	0.57	0.72
G:F	0.151	0.152	0.148	0.0034	0.61	0.51

[1]Pre-BA period encompasses days 0 through 61 of trial.
[2]A 4% pencil shrink was applied to all live BW measures including ADG.
[3]Performance data were analyzed with prior trial start weight as covariate.
[4]Trace mineral treatments included LOW (no supplemental trace mineral), MED (2016 recommendation of 10 mg Cu/kg DM), and HIGH (feedlot consultant recommendations of 20 mg Cu/kg DM; Samuelson et al. (2016)). Supplementation of Cu was delivered as Availa Cu (Zinpro Corporation, Eden Prairie, MN) and all other minerals were supplemented at national recommendations utilizing inorganic sources.
[5]Contrasts analyzed data to determine either a linear or quadratic relationship among dietary Cu treatments

Table 3. Performance of steers fed varying copper concentrations and ractopamine hydrochloride during beta agonist period

Beta agonist[1]	NoRAC			RAC				Contrasts[2]			
Copper[3]	Low	Med	High	Low	Med	High	SEM	A	B	C	D
Item[4]											
steers (n)	12	18	18	12	18	18					
BA period[5]											
d 87 BW, kg	640	645	642	645	661	662	10.2	0.34	0.62	0.28	0.80
DMI, kg/d	11.7	12.1	12.5	11.8	12.7	12.2	0.38	0.12	0.97	0.45	0.10
ADG, kg	2.39	2.24	2.28	2.33	2.75	2.47	0.1	0.43	0.39	0.27	0.01
G:F	0.206	0.186	0.183	0.198	0.216	0.205	0.0079	0.04	0.34	0.51	0.08
Carcass adjusted[6,7]											
d 88 BW, kg	643	643	641	647	661	661	10.7	0.92	0.92	0.31	0.58
Overall DMI, kg/d	11.9	12.1	12.4	12.0	12.5	12.4	0.33	0.19	0.96	0.32	0.38
Overall ADG, kg	2.0	2.0	2.0	2.1	2.1	2.1	0.07	0.87	0.78	0.89	0.56
Overall G:F	0.164	0.164	0.159	0.171	0.168	0.164	0.0005	0.46	0.65	0.28	0.90

[1]Ractopamine hydrochloride (RAC) treatment was initiated 28 days prior to harvest at an inclusion of 300 mg·steer^{-1}·day^{-1}.

[2]Contrasts utilized to analyze this period of data test linear and quadratic relationships in Cu supplementation when RAC was not supplemented (A and B, respectively) and when RAC was supplemented (C and D, respectively).

[3]Dietary Cu treatments consisted of LOW (no supplemental trace mineral), MED (2016 recommendation of 10 mg Cu/kg DM), and HIGH (feedlot consultant recommendations of 20 mg Cu/kg DM; Samuelson et al. (2016)). Supplementation of Cu was delivered as Availa Cu (Zinpro Corporation, Eden Prairie, MN) and all other minerals were supplemented at national recommendations utilizing inorganic sources

[4]A 4% pencil shrink was applied to all live BW measures including G:F calculations.

[5]Evaluation of performance data during beta agonist period, d 61-88

[6]Carcass adjusted overall performance utilized final BW calculated by dividing HCW by average dressing percent (62.8%).
[7]Performance data analyzed with prior trial starting weight as covariate.

Table 4. The effect of copper supplementation and ractopamine hydrochloride treatment on carcass characteristics of beef finishing steers

Beta agonist[1]	NoRAC			RAC				Contrasts[2]			
Copper[3]	Low	Med	High	Low	Med	High	SEM	A	B	C	D
Item											
steers (n)	12	18	18	12	18	18					
Carcass characteristics[4]											
d 89 liver Cu, mg/kg DM	18	232	289	33	227	324	18.6	0.01	0.01	0.01	0.01
HCW, kg[5]	404	404	403	406	415	415	6.7	0.92	0.91	0.31	0.58
Dressing percent, %	63.1	62.7	62.8	63.0	62.8	62.7	0.34	0.53	0.51	0.59	0.79
Ribeye area, cm^2	93.4	91.1	90.9	92.6	98.1	95.5	2.13	0.38	0.65	0.29	0.08
Marbling[6]	427	463	472	422	458	461	23.4	0.16	0.59	0.21	0.52
Back fat, cm	1.46	1.46	1.37	1.52	1.45	1.41	0.136	0.61	0.71	0.53	0.87
KPH, %[7]	2.3	2.6	2.5	2.4	2.4	2.5	0.16	0.48	0.23	0.64	0.87
Yield grade	3.15	3.33	3.21	3.30	3.02	3.14	0.194	0.82	0.46	0.52	0.36

[1]Ractopamine hydrochloride (RAC) treatment was initiated 28 days prior to harvest at an inclusion of 300 $mg \cdot steer^{-1} \cdot day^{-1}$.
[2]Contrasts for linear and quadratic relationships in dietary Cu treatment within cattle not receiving ractopamine hydrochloride (A and B, respectively) and cattle receiving ractopamine hydrochloride (C and D, respectively) were utilized for analysis.
[3]Dietary Cu treatments consisted of LOW (no supplemental trace mineral), MED (2016 recommendation of 10 mg Cu/kg DM), and HIGH (feedlot consultant recommendations of 20 mg Cu/kg DM; Samuelson et al. (2016)). Supplementation of Cu was delivered as Availa Cu (Zinpro Corporation, Eden Prairie, MN) and all other minerals were supplemented at national recommendations utilizing inorganic sources
[4]Initial weights from prior trial were used as covariate for carcass data analysis.
[5]Hot carcass weight

[6]Marbling scores: slight=300, small=400, modest=500, moderate=600, slightly abundant=700, moderately abundant=800.
[7]Kidney pelvic heart fat

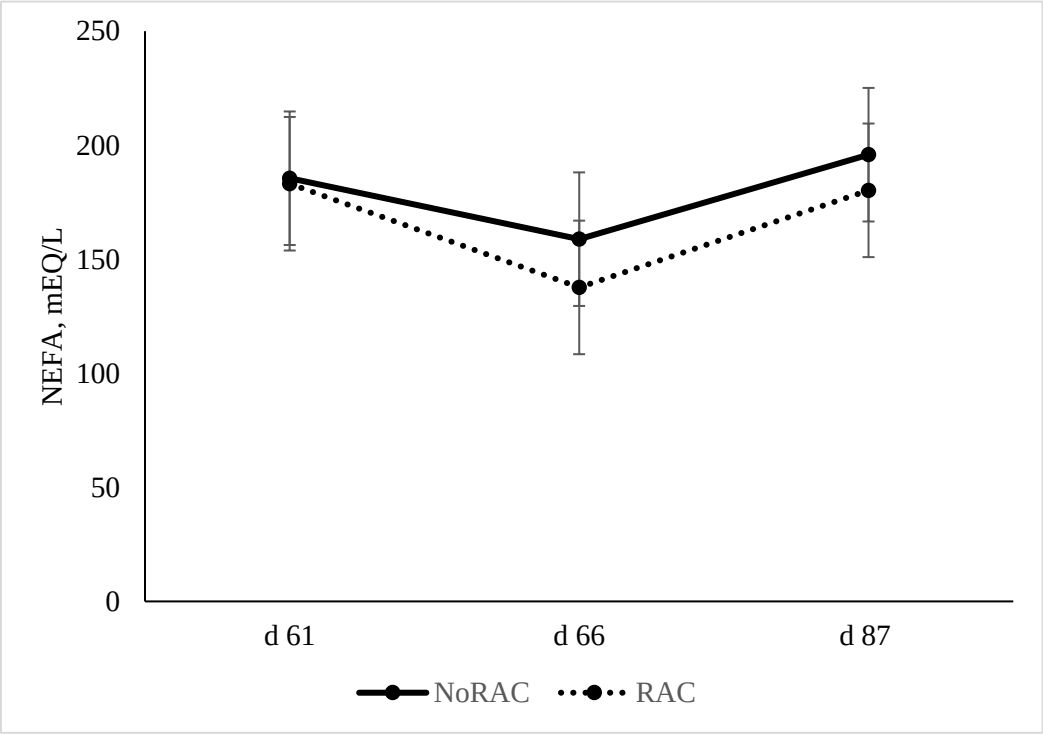

Figure 1. The effect of ractopamine hydrochloride (RAC) on serum NEFA concentrations during BA period. A tendency for a BA × Day interaction was observed (P = 0.08). Cattle were supplemented with 0, 10, or 20 mg Cu/kg DM for LOW, MED, and HIGH dietary Cu treatments and either 0 or 300 mg·steer^{-1}·day^{-}1 of ractopamine hydrochloride (RAC) for 28 days prior to harvest.

CHAPTER 4. EFFECT OF TWO DIETARY COPPER TREATMENTS ON IMPLANT PERFORMANCE RESPONSE AND CARCASS CHARACTERISTICS OF FINISHING BEEF STEERS

A paper to be submitted to the Professional Animal Scientist

E.M. Messersmith, D.D. Loy, and S.L. Hansen

Department of Animal Science, Iowa State University, Ames, IA 50011 USA

Abstract

Sixty Angus-cross steers (477 ± 32 kg) were assigned equally to a 2 × 2 factorial to determine the effect of dietary Cu on implant elicited growth. Factors included Cu supplementation at 0 (**CON**) or 20 mg Cu/kg DM (**IND**) from $CuSO_4$ and implant (**IMP**) administered on d 0 as no implant (**NoIMP**) or Component TE-200 (**TE-200**; 20 mg estradiol + 200 mg TBA; Elanco Animal Health, Greenfield, IN). Steers were blocked to dietary treatments by initial liver Cu concentrations; 97 and 188 mg Cu/kg DM (P = 0.001) for CON and IND treatments, respectively, and stratified by BW to pens (6 steers/pen) for a 74 d study. Liver and blood samples were taken prior to start of trial, 14 days after implant, and before harvest. Steer was the experimental unit (n = 15 per treatment) and initial BW and liver Cu concentrations were used as covariates in analysis of performance and liver Cu concentrations, respectively. TE-200 increased BW, HCW, and ribeye area ($P \leq 0.01$). There tended to be an IMP × Cu interaction (P = 0.13) in which CON elicited a greater live ADG response to implant than IND. Liver Zn concentrations tended to decrease (P = 0.07) while liver Mn was lesser (P = 0.001) on d 14 due to implant. Plasma Cu and ceruloplasmin concentrations were greater due to IND

treatment within TE-200, but lesser due to IND within NoIMP (IMP × Cu; $P \leq 0.13$). These results suggest dietary Cu concentrations may impact growth response in implanted steers and implants may alter trace mineral metabolism.

Key words: beef cattle, copper, implant, trace minerals

Introduction

Implants are commonly used in the beef industry; 70% of cattle represented in a survey of consulting nutritionists are implanted with a two implant protocol (Samuelson et al., 2016). Furthermore, use of combination implants allows for greater feed efficiency and increased growth rate in comparison to single hormone implants and nonimplanted cattle (Bartle et al., 1992). Additionally, the use of two combination implants can result in a 7.5 and 9% increase in HCW and ribeye area (**REA**), respectively, in comparison to nonimplanted cattle (Duckett and Pratt, 2014). This increased growth suggests cattle may have a greater requirement for trace minerals such as copper. The role of Cu in growth is exerted in part through the Cu dependent enzyme lysyl oxidase, which is responsible for crosslinking of collagen and elastin in the extracellular matrix (Rucker et al., 1998). Lambs implanted with 12 mg zeranol, a synthetic estrogen derivative, tended to have lesser fecal excretion and greater absorption of Cu than nonimplanted lambs (Hufstedler and Greene, 1995). Further, steers initially implanted with a combination implant containing 16 mg estradiol (**E_2**) and 80 mg trenbolone acetate (**TBA**) and re-implanted with a combination implant containing 20 mg E_2 and 200 mg TBA had decreased liver Cu concentrations 14 days after reimplantation relative to non-implanted steers (Niedermayer et al., 2018). These data suggest that hormone implants may impact Cu metabolism of ruminants. Little work has been done to determine mineral requirements

of cattle utilizing growth promoting technologies such as implants. Therefore, the objective of this study was to determine the effects of Cu supplementation on performance of steers receiving a potent hormone implant or no implant. It was hypothesized that steers supplemented with Cu would exhibit a greater growth response to implantation compared to steers not supplemented with Cu and Cu supplementation would have little effect on growth of nonimplanted steers.

Materials and Methods

All procedures and protocols in this study were approved by the Iowa State University Institutional Animal Care and Use Committee (log number 2-18-8701-B).

Animals and experimental design

Sixty Angus-cross steers (477 ± 32 kg) were housed in partially-covered pens with concrete bunks and automatic waterers. Steers utilized in the present trial were previously enrolled in another trial, in which the Cu antagonists S and Mo were fed to develop distinct Cu statuses. At the start of the present study steers were blocked by initial liver Cu status and stratified to pens (6 steers per pen; n = 10 pens total) by BW. Pens were randomly assigned to dietary treatments. Cattle were fed a common dry rolled corn based TMR supplemented with NASEM (2016) recommendations of all minerals except for Cu (**Table 1**). The diet was delivered at approximately 0800 h daily and bunks were managed to be slick at feed calls approximately 30 minutes prior to feed delivery.

Experimental treatments were arranged as a 2 x 2 factorial, with 2 dietary Cu treatments: 1) control (**CON**) common TMR with no supplemental Cu, and 2) industry Cu supplementation (**IND**) common TMR supplemented with 20 mg Cu/kg DM from

$CuSO_4$. To complete the factorial two implant strategies (**IMP**) were utilized: 1) no implantation (**NoIMP**) or implanted with a Component TE-200 (**TE-200**; 20 mg E_2 + 200 mg TBA; Elanco Animal Health, Greenfield, IN). Implant treatments were distributed equally among pens (3 steers per treatment per pen).The industry treatment was chosen to be representative of industry consultant recommendations for Cu (Samuelson et al., 2016) in contrast to NASEM (2016) recommendations of 10 mg Cu/kg DM. The common diet analyzed to contain 4.5 mg Cu/kg DM and the IND diet analyzed to contain 21.6 mg Cu/kg DM. The implant utilized was Component TE-200 (20 mg E_2 + 200 mg TBA) given on day 0 of the present trial. All steers were previously implanted with a Component TE-IS (16 mg E_2 + 80 mg TBA; Elanco Animal Health, Greenfield, IN) 107 days prior to the initiation of the present trial.

Sample collection and analysis

Body weight was recorded on consecutive days to start (d -1, 0) and end (d 73 and 74) the trial, and interim weights were recorded on d 13, 28, and 55. For calculations of BW and ADG a 4% pencil shrink was applied to all live BW. Liver biopsies were conducted on d -23, 14, and 62 on 4 steers per pen (2 implanted and 2 nonimplanted) following the method described by Engle and Spears (2000) and blood samples were collected on d 0, 14, and 73 from the same steers (n = 40 total, 10 per treatment). Cattle were shipped to a commercial abattoir (Iowa Premium Beef, Tama, IA) on d 74, harvested on d 75 and HCW were recorded. Carcasses were chilled for 48-hours and carcass data were collected including REA, back fat (**BF**), KPH, marbling score, and YG. Dressing percent was calculated as HCW divided by shrunk final BW and multiplied by 100.

For each dietary treatment TMR were sampled weekly and dried in a forced air oven at 70˚C for 48-hour to calculate DM content of the diet. Dry matter content of weekly TMR were applied to as fed feed disappearance for each pen to determine pen DMI. Dried diet samples were ground through a 2-mm screen (Retsch Zm100 grinder; Glen Mills Inc., Clifton, NJ) and samples were composited by month. Composited TMR samples and liver samples were acid digested with trace mineral grade nitric acid (Fisher Scientific, Fair Lawn, NJ) as previously described by Richter et al. (2012). Plasma, liver, and TMR samples were analyzed for mineral content utilizing inductively coupled plasma optical emission spectrometry (ICP Optima 7000 DV, Perkin Elmer, Waltham, MA). Blood samples were collected in vacutainer tubes with no additives for serum and trace mineral grade EDTA for plasma collection (Becton Dickenson, Rutherford, NJ). Blood was centrifuged at 1200 × *g* for 10 mins for plasma and 1200 × *g* for 20 mins for serum samples. Plasma samples were analyzed for ceruloplasmin concentration described by Demetriou et al. (1974).

Statistical analysis

Data were analyzed as a 2 × 2 factorial using the Mixed procedure of SAS 9.4 (SAS Inst. Inc., Cary, NC) with the fixed effects of Cu, Implant, and Cu × Implant. The experimental unit was steer (n = 15 per treatment for growth and carcass data; n = 10 per treatment for liver and blood data). Initial liver Cu (day -109) and BW (day -107) from the previous trial were used as covariates in liver Cu and performance analysis, respectively. Blood parameters were analyzed as repeated measures, and the compound symmetry covariant structure was selected based on the lowest corrected Akaike's information criterion. In addition, plasma Cu, Zn, and ceruloplasmin data were log

transformed to normalize the data, and back transformed means and standard errors are reported. Cook's D was used to evaluate data for outliers, and two data points for the IND-TE-200 treatment were removed from plasma Cu and Zn analysis. Data reported are least square means with standard error of the means. Statistical significance was determined at $P \leq 0.05$, with tendencies defined as $0.05 < P \leq 0.13$.

Results and Discussion

The wide use of combination implants in the feedlot industry presents an opportunity to improve cattle performance through innovative strategies such as Cu supplementation which may support implant induced growth. While feedlot consultants report supplementing Cu at two times (Samuelson et al., 2016) the NASEM (2016) recommendation, the effect of this range of Cu supplementation on performance of implanted cattle has not previously been examined. In the present 74-d finishing trial, live final BW of TE-200 steers was greater than NoIMP steers by an average of 24 kg ($P \leq 0.001$; **Table 2**). Because TE-200 and NoIMP steers were housed in pens together, individual animal DMI data are not available in the present trial. However, overall DMI between CON (11.6 kg/d) and IND (11.8 kg/d) did not differ ($P = 0.51$). A tendency for an IMP × Cu interaction was detected for overall live ADG ($P = 0.13$). Unexpectedly, CON steers displayed a 24.7% increase in overall ADG when implanted, while IND supplemented steers had a 15.3% ADG increase due to implant. This improvement due to implant for IND steers is in agreement to Smith et al. (2007) where steers implanted with a combination implant (28 mg estradiol benzoate + 200 mg TBA), had a 16% increase in ADG compared to non-implanted controls. These data contrast the 33% increase in ADG due to implant observed in steers with a similar terminal implant program to present trial

(20 mg E_2 + 200 mg TBA; Niedermayer et al., 2018). However, without an intermediate Cu treatment it is unclear if CON steers had better than expected performance or if IND steers underperformed in response to implant. Although an implant response was observed in both CON and IND steers, the degree of response implies that growth elicited by this combination implant was influenced by Cu status of the steer.

There were no IMP × Cu interactions for HCW, REA, BF, KPH, YG, or DP ($P \geq 0.21$; **Table 2**). Additionally, carcass characteristics were unaffected by Cu treatment ($P \geq 0.16$) and KPH, YG, and DP were unaffected by implant treatment ($P \geq 0.30$). Previous work has suggested that Cu supplementation can increase leanness of the carcass through decreased BF thickness (Engle et al., 2000; Engle and Spears, 2000; Johnson and Engle, 2003); however, this response to Cu supplementation was not observed in this study. Rather, BF tended to increase due to implant treatment ($P = 0.12$). Implant also increased HCW and REA of steers ($P \leq 0.01$). Additionally, a tendency for an IMP × Cu interaction ($P = 0.12$) was observed influenced by the lesser marbling score of CON-TE-200 steers while IND-TE-200 marbling remained no different than NoIMP steers. A decrease in marbling score due to implants has been observed by others (Bartle et al., 1992), however, controlling days on terminal implant (74 d in present study) has been suggested as a way to manage impact of implants on carcass quality.

Considering the rapid growth that ensues in response to implantation, dietary mineral inclusions may be of great importance to accommodate this growth. In the current study, no IMP × Cu interactions were observed for liver mineral concentrations. Although liver Cu concentrations of steers in CON and IND treatments measured on d 14 or 62 remained quite distinct ($P = 0.001$; **Table 3**) both treatments maintained adequate

liver Cu status (Kincaid, 2000). Reference ranges for liver Cu as suggested by Kincaid (2000) are: deficient (< 33 mg Cu/ kg DM), marginal (33 to 125 mg Cu/kg DM), and adequate (125 to 600 mg Cu/kg DM). The combined average liver Cu status of 97 mg Cu/kg DM prior to the start of the trial for CON steers is beyond the state of deficiency and would be recognized as physiologically normal although in the top range of the marginal status. Additionally, during deficiency plasma Cu would be expected to fall below 0.50 μg/mL (Kincaid, 2000). Subsequently, all steers in this study would be considered adequate based on plasma Cu concentrations as values did not fall below 0.87 μg/mL. Further, steers fed the IND treatment had on average 91 and 250 mg/kg DM greater liver Cu concentrations than CON steers at the beginning and end of the trial, respectively. That steers with these differences in liver Cu had differential ADG in response to a combination implant suggests the adequate liver Cu status range requires further classification to define a more optimal liver Cu status for growth.

Interestingly, mineral status of the steers measured through liver and plasma concentrations (**Table 3**) suggest that implant may affect mineral metabolism in cattle. However, 14 days after implantation, when hormone payout from implant would be expected to be greatest, liver Cu concentrations tended to be greater in implanted cattle ($P = 0.13$). In contrast, liver Cu was decreased 14 days after implantation (20 mg E_2 + 200 mg TBA) in a previous trial supplementing various trace mineral concentrations to steers (Niedermayer et al., 2018). These differences may be due to the timing of implant between studies. Niedermayer et al. (2018) measured Cu status after steers had been on dietary mineral treatments for 70 days while the present trial collected liver samples after only 14 days of Cu supplementation. However, steers were assigned to dietary Cu

treatments in the present trial based on previous Cu nutrition, thus liver Cu concentrations were already distinct at the start of the trial. This blocking of cattle by liver Cu into dietary treatments led to consistently greater liver Cu in IND steers than CON steers through the entirety of the trial ($P = 0.001$). The present trial contrasts work in rats in which estrogenic treatment has been shown to decrease liver Cu concentrations, suggesting the use of hormonal implants may elicit a similar response (Evans et al., 1970). Additional work observed an increase in Cu retention in sheep implanted with 12 mg zeranol further implying that hormonal implants affect Cu metabolism (Hufstedler and Greene, 1995).

In addition to the change in liver Cu status, a decrease in liver Mn on d 14 after implant ($P = 0.001$) is consistent with previous implant work in steers suggesting Mn metabolism is influenced by combination implants (Niedermayer et al., 2018). Liver Mn tended to remain lesser ($P = 0.08$) in implanted steers and was lesser in CON steers compared to IND steers ($P = 0.002$) near the end of the present trial (d 62). Additionally, an observed tendency for lesser fecal excretion and greater absorption of Mn in lambs implanted with 12 mg zeranol is congruent with these data (Hufstedler and Greene, 1995). Liver Mn is generally tightly controlled as Mn homeostasis in the liver is well maintained with minimal changes beyond the constant influx of Mn from the plasma and removal to the bile (Kincaid, 2000). It is unclear why Cu treatment impacted liver Mn; however, decreased liver Mn due to implant suggests the implant response may require more Mn to account for added growth or there is less need for Mn in the liver during times of rapid growth. The later of these hypotheses may be due to the role of Mn in the urea cycle through Mn dependent arginase (Watts, 1990). During more rapid growth

circulating amino acids may be utilized by muscle cells, resulting in less ammonia needing to be detoxified in the urea cycle. However, the role of Mn in growth is largely thought to be in support of glycosyltransferase and proteoglycan roles in skeletal development (Leach, 1986). Use of estrogenic implants in beef cattle has been demonstrated to increase skeletal growth in late stage finishing cattle (Loy et al., 1988). Manganese is physiologically important in carbohydrate metabolism, contributing to the production of energy to support rapid growth (Scrutton et al., 1966; Failla, 1986; Watts, 1990) and thyroid function is dependent upon Mn for the production of thyroxine (Pfeiffer, 1975). It is important to note that alterations in liver Mn concentration alone due to implant do not indicate if or how Mn is being used or if it is being excreted. Therefore, more work to understand the physiological relevance of this decreased liver Mn due to implant is needed.

Liver Zn also tended to be lessened by implant on day 14 ($P = 0.07$) but was unaffected by implant on d 62 ($P = 0.69$) suggesting a greater demand for Zn at the time of expected maximum hormone payout. However, liver Zn was not affected by Cu treatment ($P \geq 0.16$). Implant has been shown by others to affect liver Zn concentrations. Fed no supplemental Zn, and 59 days after receiving a combination implant (20 mg estradiol benzoate + 200 mg progesterone), implanted steers had lesser liver Zn concentrations than nonimplanted steers (Huerta et al., 2002). In the same study steers fed no supplemental Zn (basal diet contained 84 mg Zn/kg DM) or supplemented with 200 mg Zn/kg DM from Zn sulfate experienced a 7.6 and 17.4% increase in weight gain, respectively, due to implant (Huerta et al., 2002). Using a different growth promoting technology, Genther-Schroeder et al. (2016) reported decreased liver Zn concentrations in

steers after 26 d of ractopamine hydrochloride supplementation, even when steers were supplemented with dietary Zn. Plasma Zn concentrations (**Figure 1**) across the present trial were lesser on d 13 and 73 due to implant (IMP × Day; $P = 0.001$). No IMP × Cu × Day ($P = 0.22$) or IMP × Cu ($P = 0.42$) interactions were observed in the plasma data, but Cu tended to decrease plasma Zn ($P = 0.13$). These changes in tissue Zn concentrations may indicate a demand for Zn in support of increased skeletal and muscle growth of implanted steers. Dietary Zn has been shown to increase N retention suggesting Zn has an important role in accommodating rapid muscle growth (Carmichael et al., 2018) and more work is needed to refine dietary Zn requirements of cattle receiving hormone implants.

While increasing supplemental Zn may support improved growth response to growth promoting technologies (Huerta et al., 2002; Genther-Schroeder et al., 2016), data from the present trial suggest Cu concentrations commonly supplemented in the industry negatively impact implant response. The differential growth response observed in CON and IND steers to implant treatment indicates a physiological difference due to low dietary Cu supplementation. However, the reasoning behind this response remains unclear. The importance of Cu in supporting growth is through collagen crosslinking via lysyl oxidase (Rucker et al., 1998). It is likely that the activity of this enzyme alone does not explain the differences in growth response exhibited by IND steers. Cattle supplemented with 10 or 20 mg Cu/kg DM from Availa Cu (Cu- amino acid complex) had greater HCW than un-supplemented controls suggesting Cu positively impacts growth (Johnson and Engle, 2003). In contrast, increasing dietary Cu concentration up to 40 mg Cu/kg DM has previously not yielded improved performance (Ward et al., 1993;

Engle and Spears, 2000; Engle and Spears, 2001). Cattle response to Cu supplementation is likely a function of rate of growth, cattle genetics, and presence of basal amounts of Cu or Cu antagonists in the diet.

There tended ($P = 0.13$) to be an interaction between IMP and Cu for plasma Cu concentrations and was an interaction ($P = 0.05$) for plasma ceruloplasmin concentrations. For both variables, concentrations were greater in IND within TE-200, but lesser in IND within NoIMP. Previous work in rats has shown an increase in circulating ceruloplasmin concentrations due to estrogen treatment (Sunderman et al., 1971). These data suggest the estrogen dose from the Component TE-200 implant (20 mg E_2 + 200 mg TBA) may have resulted in the spike of plasma ceruloplasmin in IND supplemented steers. The increase in plasma Cu in accordance with ceruloplasmin was expected as ceruloplasmin functions as a carrier protein for Cu (Percival and Harris, 1990).

Due to the potency of the implant utilized in this study (20 mg E_2 + 200 mg TBA), physiological effects of these hormones on trace minerals may be expected, though this is poorly understood in cattle. In human liver cancer cells incubated with different concentrations of Cu in the media, absorption of Cu in cells incubated with E_2 was increased and remained greater following the removal of E_2 (Arredondo et al., 2010). While Cu transport proteins DMT1 and CTR1 are typically increased or decreased in response to cellular Cu availability, incubation with 17-β E_2 ablated this regulatory effect of Cu in human liver cancer cells (Arredondo et al., 2010). These findings suggest that in the presence of estrogen, cellular Cu machinery may be altered to maintain increased cellular Cu concentrations, perhaps to support increased growth. However, further work

is needed to understand the mechanisms by which estrogen may affect the Cu requirement in ruminants.

Implications

Overall, these results indicate that growth performance is differentially affected by dietary Cu concentrations in response to administration of a hormonal implant. It also appears there is a relationship between implants and the trace minerals Zn and Mn, as lesser liver Zn and Mn concentrations were observed after implantation. Further, the IMP × Cu interactions observed in both plasma Cu and ceruloplasmin concentrations indicate Cu metabolism is affected by hormone implants, potentially in relation to the known effects of estrogen on components of Cu biology. The extent to which Cu, Zn, and Mn are involved in the biological implant response remains largely unknown; however, these data suggest minerals may have a role in the growth response elicited from hormonal implant technology. It appears the industry average supplementation of nearly two times NASEM (2016) recommendations for Cu may hinder the implant growth response. Through better understanding the trace mineral requirements of hormone implanted cattle, the cattle feeding industry can move toward more strategic supplementation of trace minerals to improve efficiency and performance of cattle and thus profitability for cattle producers.

Literature Cited

Arredondo, M., H. Núñez, G. López, F. Pizarro, M. Ayala, M. Araya, M. Ayala, and M. Araya. 2010. Influence of Estrogens on Copper Indicators: In Vivo and In Vitro Studies. Biol Trace Elem Res. 134:252–264. doi:10.1007/s12011-009-8475-x.

Bartle, S. J., R. L. Preston, R. E. Brown, and R. J. Grant. 1992. Trenbolone acetate/estradiol combinations in feedlot steers: dose-response and implant carrier effects2. J. Anim. Sci. 70:1326–1332. doi:10.2527/1992.7051326x.

Carmichael, R. N., O. N. Genther-Schroeder, C. P. Blank, E. L. Deters, S. J. Hartman, E. K. Niedermayer, and S. L. Hansen. 2018. The influence of supplemental zinc and ractopamine hydrochloride on trace mineral and nitrogen retention of beef steers 1. J. Anim. Sci. 96:2939–2948. doi:10.1093/jas/sky177.

Duckett, S. K., and S. L. Pratt. 2014. Meat Science and Muscle Biology Symposium—Anabolic implants and meat quality. J. Anim. Sci. 92:3–9. doi:10.2527/jas.2013-7088.

Engle, T. E., and J. W. Spears. 2000. Dietary copper effects on lipid metabolism, performance, and ruminal fermentation in finishing steers. J. Anim. Sci. 78:2452–2458. doi:10.2527/2000.7892452x.

Engle, T. E., and J. W. Spears. 2001. Performance, carcass characteristics, and lipid metabolism in growing and finishing Simmental steers fed varying concentrations of copper. J. Anim. Sci. 79:2920–2925. doi:10.2527/2001.79112920x.

Engle, T. E., J. W. Spears, T. A. Armstrong, C. L. Wright, and J. Odle. 2000. Effects of dietary copper source and concentration on carcass characteristics and lipid and cholesterol metabolism in growing and finishing steers. J. Anim. Sci. 78:1053–1059. doi:10.2527/2000.7841053x.

Evans, G. W., N. F. Cornatzer, and W. E. Cornatzer. 1970. Mechanism for hormone-induced alterations in serum ceruloplasmin. Am. J. Physiol. 218.

Failla, M. L. 1986. Hormonal Regulation of Manganese Metabolism. In: V. L. Schramm and F. C. Wedler, editors. Manganese in Metabolism and Enzyme Function. Academic PRess, Orlando, Florida. p. 93–105.

Genther-Schroeder, O. N., M. E. Branine, and S. L. Hansen. 2016. The effects of increasing supplementation of zinc-amino acid complex on growth performance, carcass characteristics, and inflammatory response of beef cattle fed ractopamine hydrochloride. J. Anim. Sci. 94:3389–3398. doi:10.2527/jas.2015-0209.

Huerta, M., R. . Kincaid, J. . Cronrath, J. Busboom, A. . Johnson, and C. . Swenson. 2002. Interaction of dietary zinc and growth implants on weight gain, carcass traits and zinc in tissues of growing beef steers and heifers. Anim. Feed Sci. Technol. 95:15–32. doi:10.1016/S0377-8401(01)00334-0.

Hufstedler, G. D., and L. W. Greene. 1995. Mineral and nitrogen balance in lambs implanted with zeranol. J. Anim. Sci. 73:3785. doi:10.2527/1995.73123785x.

Johnson, L. R., and T. E. Engle. 2003. The Effects of Copper Source and Concentration on Lipid Metabolism in Growing and Finishing Angus Steers. Asian-Aust. J. Anim. Sci. 16:1131–1136.

Kincaid, R. L. 2000. Assessment of trace mineral status of ruminants: A review. J. Anim. Sci. 77:1–10. doi:10.2527/jas2000.77E-Suppl1x.

Leach, R. M. J. 1986. Mn(II) and glycosyltransferases essential for skeletal development. In: Manganese in Metabolism and Enzyme Function. p. 81–91.

Loy, D. D., H. W. Harpster, and E. H. Cash. 1988. Rate, Composition and Efficiency of Growth in Feedlot Steers Reimplanted with Growth Stimulants. J. Anim. Sci. 66:2668. doi:10.2527/jas1988.66102668x.

NASEM. 2016. Nutrient Requirements of Beef Cattle. 8th ed. National Academy Press, Washington, DC.

Niedermayer, E. K., O. N. Genther-Schroeder, D. D. Loy, and S. L. Hansen. 2018. Effect of varying trace mineral supplementation of steers with or without hormone implants on growth and carcass characteristics. J. Anim. Sci. 96:1159–1170. doi:10.1093/jas/skx063.

Percival, S. S., and E. D. Harris. 1990. Copper transport from ceruloplasmin: characterization of the cellular uptake mechanism. Am. J. Physiol. Physiol. 258:C040-C146. doi:https://doi.org/10.1152/ajpcell.1990.258.1.C140.

Pfeiffer, C. C. 1975. Mental and Elemental Nutrients. Keats Publishing, Inc., New Canaan, Connecticut.

Rucker, R. B., T. Kosonen, M. S. Clegg, A. E. Mitchell, B. R. Rucker, J. Y. Uriu-Hare, and C. L. Keen. 1998. Copper, lysyl oxidase, and extracellular matrix protein cross-linking. Am J Clin Nutr. 67:996S–1002S. doi:10.1093/ajcn/67.5.996S.

Samuelson, K. L., M. E. Hubbert, M. L. Galyean, and C. A. Löest. 2016. Nutritional recommendations of feedlot consulting nutritionists: The 2015 New Mexico State and Texas Tech University survey. J. Anim. Sci. 94:2648. doi:10.2527/jas.2016-0282.

Scrutton, M. C., M. F. Utter, and A. S. Mildvans. 1966. Pyruvate Carboxylase VI. The presence of tightly bound manganese. J. Biol. Chem. 241:3480–3487.

Smith, K. R., S. K. Duckett, M. J. Azain, R. N. Sonon, and T. D. Pringle. 2007. The effect of anabolic implants on intramuscular lipid deposition in finished beef cattle. J. Anim. Sci. 85:430. doi:10.2527/jas.2006-280.

Sunderman, W. F., S. Nomoto, I. G. Gillies, and P. J. Goldblatt. 1971. Effect of Estrogen Administration upon Ceruloplasmin and Copper Concentrations in Rat Serum. Toxicol. Appl. Pharmacol. 20:588–598.

Ward, J. D., J. W. Spears, and E. B. Kegley. 1993. Effect of copper level and source (copper lysine vs copper sulfate) on copper status, performance, and immune response in growing steers fed diets with or without supplemental molybdenum and sulfur. J. Anim. Sci. 71:2748–2755. doi:10.2527/1993.71102748x.

Watts, D. L. 1990. The Nutritional Relationships of Manganese. J. Orthomol. Med. 5:219–222.

Table 1. Ingredient and composition analysis of control diet

Ingredient	% of diet DM
Dry rolled corn	62.0
MDGS[1]	25.0
Bromegrass hay	8.0
DDGS[2]	3.05
Limestone	1.5
Salt	0.31
Vitamin & mineral premix[3]	0.13
Rumensin	0.0135
Analyzed composition	
CP[4]	15.3
NDF[4]	18.0
Ether extract[4]	5.8
Sulfur[4]	0.28
Copper, mg/kg DM[5]	4.5
Zinc, mg/kg DM[6]	61

[1]Modified distillers grains with solubles.
[2]Dried distillers grains with solubles.
[3]Premix provided 2,200 IU vitamin A and 25 IU vitamin E/kg diet DM and NASEM (2016) recommendations for Co, Mn, Se, Zn, and I from inorganic sources. CON premix contained no additional Cu, while IND premix provided 20 mg Cu/kg diet DM from Cu sulfate.
[4]Analysis of CON TMR by Dairyland Laboratories (Arcadia, WI).
[5]Analyzed Cu represents CON dietary treatment total with no supplemental Cu. IND dietary treatment was analyzed to contain 21.6 mg Cu/kg DM by inductively coupled plasma optical emission spectrometry (ICP Optima 7000 DV, Perkin Elmer, Waltham, MA).
[6]Analyzed Zn represents CON dietary treatment with NASEM (2016) recommendations for Zn supplemented. IND treatment analyzed to contain 58 mg Zn/kg DM by inductively coupled plasma optical emission spectrometry (ICP Optima 7000 DV, Perkin Elmer, Waltham, MA).

Table 2. Effect of implant strategy[1] and copper supplementation[2] on growth and carcass characteristics

	NoIMP		TE-200			*P*-value		
	CON	IND	CON	IND	SEM	IMP[3]	Cu	IMP × Cu
steers (n)	15	15	15	15				
Live performance[4 5]								
d 0 BW, kg	475	480	479	475	5.6	-	0.90	-
d 73 BW, kg	595	605	628	620	7.1	0.001	0.91	0.19
Overall ADG, kg	1.62	1.70	2.02	1.96	0.045	0.001	0.87	0.13
CA performance[4 5 6]								
d 74 BW, kg	596	608	627	618	7.9	0.01	0.87	0.21
Overall ADG, kg	1.62	1.69	1.98	1.91	0.052	0.001	0.94	0.18
Carcass characteristics[4 5]								
HCW, kg	383	390	402	397	5.0	0.01	0.87	0.21
Ribeye area, cm^2	84.3	86.0	89.7	89.9	1.56	0.004	0.56	0.63
Back fat, cm	1.72	1.75	1.94	1.88	0.112	0.12	0.86	0.69
KPH	2.4	2.5	2.5	2.6	0.06	0.30	0.16	0.62
Marbling[7]	534	511	459	510	23.6	0.11	0.55	0.12
YG	3.71	3.72	3.83	3.73	0.144	0.67	0.77	0.70
DP, %	64.3	64.4	64.0	64.0	0.32	0.30	0.88	0.95

[1]Implant treatment (IMP) consisted of either a Component TE-200 implant (TE-200; 20 mg estradiol + 200 mg TBA; Elanco Animal Health, Greenfield, IN) on day 0 of the trial or no hormone implant (NoIMP).

[2]The CON treatment received no supplemental Cu whereas the IND treatment received 20 mg Cu/kg DM from $CuSO_4$ per feedlot consultant recommendations (Samuelson et al., 2016).

[3]IMP represents all steers in the TE-200 treatment for implant regardless of Cu treatment.

[4]A 4% pencil shrink was applied to all live BW prior to analysis.

[5]Beginning weight from prior trial was used as covariate in analysis.

[6]Carcass adjusted (CA) overall performance was determined with the average DP of the group (64.20%).
[7]Marbling scores: slight=300, small=400, modest=500, moderate=600, slightly abundant=700, moderately abundant=800.

Table 3. Effect of implant strategy[1] and copper supplementation[2] on liver and plasma mineral parameters

	NoIMP		TE-200			*P*-value		
	CON	IND	CON	IND	SEM	IMP[3]	Cu	IMP × Cu
steer (n)	15	15	15	15				
Liver Cu, mg/kg DM[4]								
d -23 (initial)	92	197	101	178	3.6	-	0.001	-
d 14	117	202	138	207	4.0	0.13	0.001	0.27
d 62	95	344	89	339	4.4	0.62	0.001	0.75
Liver Zn, mg/kg DM								
d 14	126	131	119	121	2.0	0.07	0.37	0.77
d 62	114	117	111	117	1.5	0.69	0.16	0.76
Liver Mn, mg/kg DM								
d 14	9.6	9.7	8.2	8.6	0.13	0.001	0.43	0.55
d 62	8.9	9.7	8.1	9.5	0.13	0.08	0.002	0.30
Plasma[5]								
Cu, mg/L	0.93	0.89	0.87	0.93	0.015	0.72	0.71	0.13
Ceruloplasmin, mg/dL	23.8	21.6	22.3	24.3	0.62	0.91	0.63	0.05

[1]Cattle within the implant treatment received Component TE-200 (TE-200; 20 mg estradiol + 200 mg TBA; Elanco Animal Health, Greenfield, IN) on day 0 of the trial. Non-implanted (NoIMP) cattle did not receive an implant.

[2]Cu supplementation treatments included CON with no supplemental Cu and IND providing 20 mg/kg DM of supplemental Cu from Cu sulfate in the diet as recommended by industry consultants per Samuelson et al. (2016).

[3]IMP represents all steers in the TE-200 implant treatment regardless of Cu supplementation treatment.

[3]Liver Cu from the beginning trial (d -23) was used as covariate in analysis.

[4]Data analyzed as repeated measures, no interactions with day ($P \leq 0.15$).

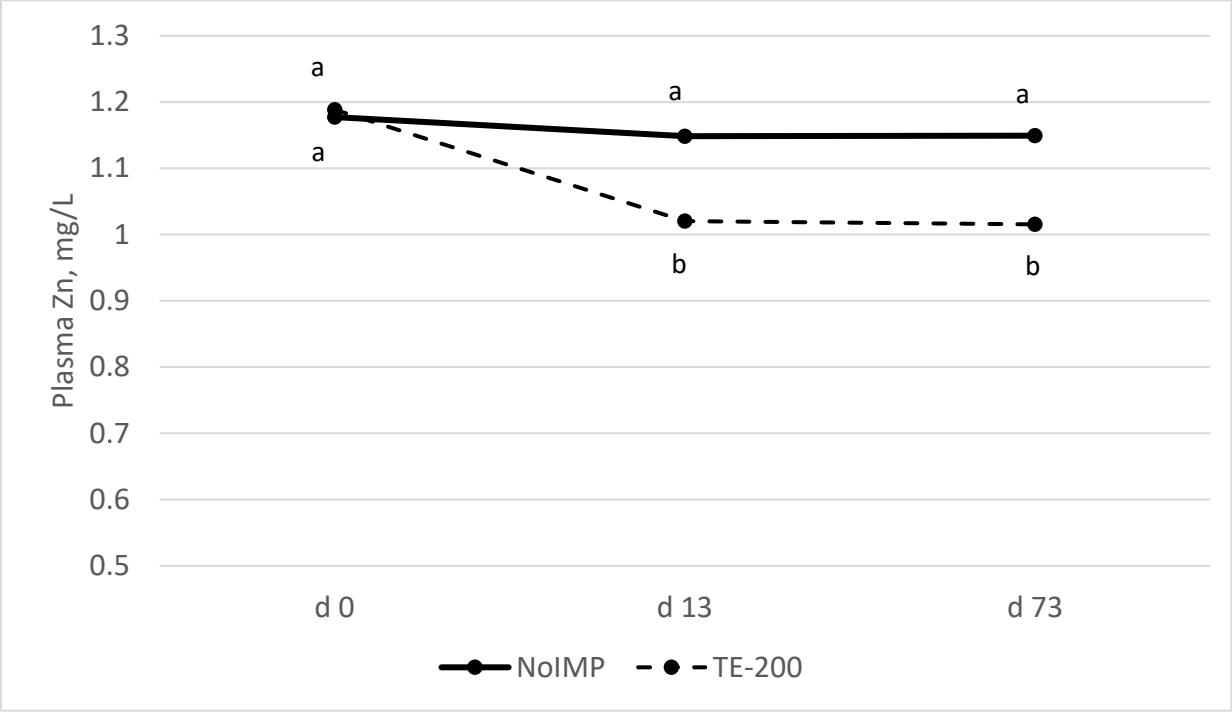

Figure 1. Effect of implant (TE-200) or no implant (NoIMP) and day on plasma Zn concentrations (IMP × Day $P = 0.001$; SEM = 0.075). Cattle receiving the TE-200 treatment were implanted with Component TE-200 (20 mg estradiol + 200 mg TBA; Elanco Animal Health, Greenfield, IN) on day 0 of trial. Unlike superscripts indicate means differ ($P \leq 0.05$).

CHAPTER 5. GENERAL CONCLUSIONS

Growth promoting technologies such as beta agonists and steroid hormone implants are extensively used within the feedlot industry. The increased growth rates observed through the use of these technologies may suggest a greater demand for nutrients such as Cu, which supports development of extracellular matrix through collagen remodeling via lysyl oxidase (Rucker et al., 1998). There is evidence from the basic literature that Cu may impact the biological pathways downstream of both beta agonists and steroid hormones. Additionally, feedlot nutritionists often report supplementing Cu well in excess of the national recommendations for Cu (Samuelson et al., 2016). Therefore, these research studies were designed to gain an understanding of the impact of supplemental Cu concentrations and animal Cu status on growth and carcass characteristics of steers receiving growth promoting technologies.

This research stemmed from the limited data available on effects of trace mineral supplementation on performance of cattle receiving either a beta agonist or hormone implant. It was hypothesized that cattle growth would linearly increase as Cu supplementation increased in diets of cattle receiving the beta agonist ractopamine hydrochloride. This hypothesis is partially based upon the recent revelation that both Zn and Cu reversibly inhibit the enzymatic activity of phosphodiesterase (PDE) and thus influence the availability of cyclic AMP (cAMP) to subsequently stimulate lipolysis downstream of beta adrenergic receptor activation (von Bulow et al., 2005; Krishnamoorthy et al., 2016). Increases in plasma cAMP in steers supplemented with increasing concentrations of Zn, and increasing growth of steers receiving both Zn and ractopamine hydrochloride support these findings (Genther-Schroeder et al., 2016).

Supplementation of Cu was also found to differentially affect performance of steers receiving or not receiving a beta agonist. However, in contrast to the linear improvements noted during increasing Zn supplementation, steers utilized in the present research exhibited maximal growth at NASEM (2016) rates of supplementation (10 mg Cu/kg DM) during the beta agonist feeding period. In addition, the NASEM (2016) rate of Cu supplementation tended to improve ribeye area of steers utilizing beta agonists, but further carcass characteristics were unaffected due to the varying Cu supplementation treatments. This is in opposition to our hypothesis of continually improved growth due to increasing Cu supplementation during the beta agonist feeding period. These performance differences may have been driven by the liver Cu concentrations of steers. Although liver Cu concentrations of steers in treatments supplemented with Cu were within the adequate range, differential growth responses suggest that more work is needed to refine liver Cu concentrations that may best support cattle growth. It is unclear why feedlot consultants often supplement 20 mg Cu/kg DM; however, data from this work suggest this rate of Cu supplemented in the industry is not optimal for beta agonist induced growth. Furthermore, it appears that Cu and Zn have differential impacts on beta agonist induced growth performance in cattle.

In addition to the beta agonist pathway, tissue Cu appears to be affected by hormone implants. Sheep implanted with 12 mg zeranol, an estrogen derivative, tended to have greater Cu retention than nonimplanted sheep (Hufstedler and Greene, 1995). Through the use of a combination implant in steers a decrease in liver Cu status of implanted steers during the period of greatest hormone payout was observed (Niedermayer et al., 2018). Therefore, Cu was targeted to further determine its potential

role in implant growth response. Through this implant study, Cu treatment again resulted in a differential performance due to growth promoting technology. The greater degree of increase in growth for cattle receiving no supplemental Cu in comparison to cattle supplemented with an industry average suggests the industry may be supplementing more Cu than needed, potentially depressing implanted cattle performance. Alternatively, non-Cu supplemented steers may have responded better than expected to the combination implant, potentially because of the relationship between estrogen and bodily Cu metabolism. Further, implant treatment tended to increase liver Cu, decrease Mn and tended to decrease Zn during the period of greatest hormone payout. These findings suggest hormone implants are affecting tissue mineral concentrations and more work is needed to refine the potential implications on cattle performance these changes may be having. Additionally, future work should be undertaken utilizing multiple concentrations of dietary Cu fed to cattle receiving hormone implants to better understand the relationship between these two management factors.

Through both of these studies it is clear Cu supplementation and status are impactful on the performance of steers utilizing growth promoting technologies. Additionally, industry average supplementation of Cu may be adversely affecting performance of cattle utilizing these two technologies. To better understand the role of Cu in these growth responses the initial Cu status of the animal must be taken into consideration as well as dietary constraints such as antagonists. Additional work is warranted to further refine the optimal liver Cu status as well as rate of Cu supplementation to support growth induced by these promotants.