**The effects of strategic zinc supplementation on steroidal implant-induced growth of finishing beef cattle**

by

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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 dissertation. The Graduate College will ensure this dissertation is globally accessible and will not permit alterations after a degree is conferred.

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## NOMENCLATURE

| | |
|---|---|
| ADG | Average daily gain |
| AKT | Protein kinase B |
| AR | Androgen receptor |
| cAMP | Cyclic adenosine monophosphate |
| DM | Dry matter |
| DMI | Dry matter intake |
| DNA | Deoxyribonucleic acid |
| E2 | Estrogen |
| EGFR | Epidermal growth factor receptor |
| ER | Estrogen receptor |
| ERK-1/-2 | Extracellular-signal-regulated kinase 1 and 2 |
| FOXO | Forkhead box O |
| GH | Growth hormone |
| GHRH | Growth hormone-releasing hormone |
| GPER-1 | G protein-coupled estrogen receptor-1 |
| hbEGF | Heparin-binding epidermal growth factor-like growth factor |
| HCW | Hot carcass weight |
| IGF-1 | Insulin like growth factor-1 |
| IGF-1R | Insulin like growth factor-1 receptor |
| IGFBP | Insulin like growth factor binding protein |
| JNK | c-Jun N-terminal kinase |

| | |
|---|---|
| KPH | Kidney, pelvic, and heart fat |
| MAPK | Mitogen-activated protein kinase |
| MMP2/9 | Matrix metalloproteinases 2 and 9 |
| mTOR | Mammalian target of rapamycin |
| PI3K | Phosphoinositide 3-kinase |
| REA | Ribeye area |
| RNA | ribonucleic acid |
| TBA | Trenbolone acetate |

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## ABSTRACT

Extensive use of steroidal implants (NAHMS, 2013; Samuelson et al., 2016) has resulted in increased efficiency of growth and profitability in the beef feedlot industry (Duckett and Pratt, 2014). Steroidal implants increase Zn absorption and retention in lambs (Hufstedler and Greene, 1995) and lessen plasma Zn concentrations compared to non-implanted beef steers (Messersmith, 2018). These data indicate steroidal implants affect Zn metabolism in ruminants, potentially to support the rapid growth occurring in implanted animals. Therefore, strategic supplementation of Zn may optimize growth performance of cattle administered steroidal implants. The research studies discussed in this dissertation were designed to 1) determine the effects of increasing dietary Zn supplementation on the growth performance, carcass characteristics, and expression of genes related to steroidal implant function and Zn metabolism of non-implanted and implanted beef steers, 2) examine how increasing supplemental Zn concentrations influence performance, carcass characteristics, and markers of energy and protein metabolism in beef steers, 3) assess the effects of supplemental Zn on performance, carcass characteristics, and liver trace mineral concentrations of beef heifers administered an extended-release implant or a two-implant strategy, and 4) evaluate the effects of Zn source on the performance, carcass characteristics, and plasma and tissue trace mineral concentrations within non-implanted and implanted beef steers. Through our first research objective, increasing supplemental Zn, as $ZnSO_4$, up to 5 times the NASEM (2016) recommendation linearly increased steer performance and gene expression of steroidal implant signaling proteins in the muscle during peak hormonal payout of a high potency implant but did not influence these parameters in non-implanted steers. During this period implanted steers had 6% lesser plasma Zn concentrations than non-implanted steers, suggesting steroidal implant-induced growth influences Zn metabolism to accommodate

high growth rates. Within our second research objective, increasing Zn supplementation, as $ZnSO_4$, up to 6 times the NASEM (2016) recommendation linearly increased growth during peak hormonal payout of the steroidal implant, but had minimal effects on beta agonist period ADG. Similarly, liver Mn concentrations were correlated with liver arginase activity and ADG directly following implant administration, but not during beta agonist supplementation. These data indicate Zn's effects on growth, in combination with steroidal implants, influence protein degradation. Through our third research objective, beef heifers supplemented consultant-recommended concentrations of Zn (100 mg Zn/kg dry matter from $ZnSO_4$; Samuelson et al., 2016) were 7 kg heavier than heifers supplemented NASEM (2016; 30 mg Zn/kg dry matter from $ZnSO_4$) recommendations of Zn during peak hormonal payout of the extended-release implant and two-implant strategies. Although no differences in final performance were noted between implant strategies, interim period ADG of heifers corresponded to peak hormonal payout of each implant. These data suggest greater supplementation of Zn is beneficial to the growth of heifers administered either an extended-release implant or two-implant strategy and that these implant strategies are equally effective. Finally, our fourth research objective revealed implanted steers were 45 kg heavier than non-implanted steers at the end of the trial. However, an interaction between implant and Zn source (100% $ZnSO_4$ or 70% $ZnSO_4$ + 30% basic ZnCl or Zn glycinate) was observed in final body weight (**BW**) where steers supplemented 100% $ZnSO_4$ tended to be heavier than those supplemented a basic ZnCl blend, but not heavier than steers receiving the Zn glycinate blend. No differences in final BW due to Zn source were observed within non-implanted steers. These data suggest steroidal implants influence Zn metabolism resulting in BW differences between Zn sources within implanted steers, but not non-implanted steers. Together, these studies increased our understanding of the Zn requirements of implanted

vs. non-implanted cattle. These data suggest feeding greater than NASEM (2016) recommendations for Zn is beneficial to implant-induced growth, likely due to Zn's roles in protein synthesis (Oberleas and Prasad, 1969; Duncan and Dreosti, 1976) and the observed effects on the gene expression of implant signaling machinery. However, future work is warranted to determine strategic Zn supplementation to optimize implant-induced growth in beef feedlot cattle.

# CHAPTER 1. GENERAL INTRODUCTION

Steroidal implants have been utilized in the U.S. beef industry for over 60 years (R.L. Preston, 1999) to improve cattle average daily gain (**ADG**) by 16 to 20% and increase the profitability of beef production (Duckett and Pratt, 2014). Nearly 84% of beef cattle in the U.S. are administered a steroidal implant upon arrival to the feedlot, with 71% of cattle implanted at a second processing date (NAHMS, 2013). Recently, Messersmith (2018) observed a potent steroidal implant increased ADG by 29% in the first 14 d after implant administration, and concurrently observed an 11% decrease in plasma Zn concentrations of implanted steers compared to non-implanted steers. These data suggest steroidal implant-induced growth increases the body's demand for Zn. In agreement with this assessment, Hufstedler and Greene (1995) found absorption and retention of Zn were increased in lambs implanted with zeranol. Furthermore, Carmichael et al. (2018) observed a positive correlation between Zn and N retention in finishing beef steers. With roles in deoxyribonucleic acid (**DNA**; Duncan and Dreosti, 1976) and protein synthesis (Wegener and Romano, 1963; Oberleas and Prasad, 1969), Zn is vital to growth processes. Therefore, strategic supplementation of Zn in cattle utilizing steroidal implants may be critical for optimizing cattle growth response. Throughout the research examined in this dissertation, different Zn supplementation strategies were tested to assess the effects of increasing supplemental Zn or Zn source on the performance of cattle administered different steroidal implant strategies. Specifically, two studies focus on differences between non-implanted and implanted cattle supplemented Zn. The remainder of the studies examined the effects of Zn in cattle administered the same steroidal implant or a re-implant program vs. an extended-release implant.

## Dissertation Organization

Following the General Introduction, Chapter II will provide a literature review examining the biological importance of Zn, how steroidal implants function, and the interactions between Zn and steroidal implants in cattle. The research chapters presented next have been published in *The Journal of Animal Science* or will be submitted for publication in *The Journal of Animal Science* or *Translational Animal Science*. Chapter III will examine the effects of increasing supplemental Zn within non-implanted and implanted beef steers on growth performance, carcass characteristics, plasma and liver Zn concentrations, and the expression of genes related to steroidal implant growth and Zn metabolism in muscle. This work is followed in Chapter IV by examining the effects of increasing supplemental Zn on growth performance, carcass characteristics, plasma and liver trace mineral concentrations, and markers of protein and energy metabolism in beef steers administered a steroidal implant. Chapter V investigates the dietary supplementation of two concentrations of Zn to heifers implanted with an aggressive re-implant program or an extended-release implant. In comparison, Chapter VI focuses on the effects of Zn source on the growth performance, carcass characteristics, and plasma and tissue trace mineral concentrations of non-implanted and implanted beef steers. To conclude, Chapter VII will cover the conclusions gathered from the research detailed herein and provide insights into future research directions in this field.

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# CHAPTER 2. LITERATURE REVIEW

## Zinc

Arguably the most utilized trace mineral in the body, Zn, is found in over 2000 transcription factors and various proteins (Beyersmann and Haase, 2001; Cousins et al., 2006a). Specifically, Zn is required for the enzymatic activity involved in deoxyribonucleic acid (**DNA**) synthesis (Duncan and Dreosti, 1976), leading to increased protein synthesis and growth. This growth response is especially observed in Zn deficient animals (Todd et al., 1933; Miller and Miller, 1962; Ott et al., 1964), though Zn supplementation is important regardless of Zn status. This section reviews symptoms of Zn deficiency and toxicity, Zn requirements for cattle, differences in Zn sources, and how these factors influence Zn absorption and the Zn status of the animal. Furthermore, this section will end by describing the role of Zn in protein synthesis and how Zn supplementation can improve growth performance and carcass characteristics in beef cattle.

### Zinc Deficiency

Zinc's role in protein metabolism introduced in the previous section reveals the significance of adequate Zn nutrition, especially in growing animals. Todd et al. (1933) deduced Zn was vital to growth processes in rats. By feeding a diet nearly devoid of Zn (1.6 mg Zn/kg dry matter [**DM**] basis) vs. 50 mg Zn/kg DM from ZnO, vast growth differences were observed within 5 weeks of Zn supplementation in male rats (Todd et al., 1933). Besides inferior weight gain, Zn deficient rats also incurred hair loss noted as coinciding with poor growth rates which could be reversed with the inclusion of Zn in the diet (Todd et al., 1933). Although the detriment of Zn deficiency was clear in species such as the rat, Zn supplementation was thought

unnecessary in many livestock species due to the perceived adequate Zn content of feedstuffs (Miller, 1970).

However, a trademark symptom of Zn deficiency, parakeratosis, has been observed in several livestock species (Stevenson and Earle, 1956; Legg and Sears, 1960; Ott et al., 1964). Parakeratosis is the thickening and hardening of the skin and can result in lesions of the skin. Interestingly, cattle researchers noted incidence of parakeratosis was seasonal and linked to poor forage quality (Legg and Sears, 1960). By administering Zn orally (2 g/wk) or through an injection (1 g/week) as $ZnSO_4$, symptoms of parakeratosis were resolved and new hair growth was observed within 1 week of treatment (Legg and Sears, 1960). The experimental induction of Zn deficiency in male Holstein calves by feeding a purified diet resulted in parakeratosis of the skin and the rumen and interestingly, decreased testicular weight compared to calves fed the purified diet and supplemented 40 mg Zn/kg DM from ZnO (Miller and Miller, 1962). This result was likely related to the later identified role for Zn in spermatogenesis (Underwood and Somers, 1969). Adequate growth rates in calves supplemented 40 mg Zn/kg DM indicated their Zn requirement was being met (Miller and Miller, 1962). At 15 weeks of age, Zn deficient calves were supplemented 260 mg Zn/kg DM from ZnO for 5 weeks, resulting in increased dry matter intake and a 9.1 kg weight gain within 1 week of supplementation (Miller and Miller, 1962). In the swine industry, Zn deficiency and the resulting parakeratosis has been linked to high dietary Ca, an antagonist of Zn absorption, and inadequate supplementation of Zn (Luecke et al., 1956; Stevenson and Earle, 1956). By adjusting dietary Ca and Zn concentrations, symptoms of Zn deficiency such as parakeratosis in swine were resolved (Luecke et al., 1956).

Although parakeratosis is an easily recognized symptom of Zn deficiency, several underlying symptoms do persist. Most commonly, a severe Zn deficiency is associated with a

loss of appetite and decreased growth (Suttle, 2010). Ott et al. (1964) observed Zn deficient lambs exhibited both loss of appetite and poor growth rates in addition to skin lesions. Lambs recovered within 5 weeks of supplementing 100 mg Zn/kg (Ott et al., 1964). At 15 weeks of age, Zn deficient Holstein calves were supplemented 260 mg Zn/kg DM from ZnO for 5 weeks resulting in increased dry matter intake (**DMI**) and a 9.1 kg weight gain within 1 week of supplementation (Miller and Miller, 1962). These data indicate Zn deficiency is detrimental to the health and performance of livestock and that the inclusion of Zn to deficient animals can rapidly remedy deficiency symptoms. Under current livestock production settings, severe Zn deficiencies are unlikely as most producers supplement Zn. However, in the over 50 years since most of this work was conducted, livestock production has greatly changed. Improved genetics and technologies have largely enhanced growth in cattle specifically. Therefore, understanding Zn deficiency symptoms may be important to optimizing performance in modern cattle production systems.

**Zinc Toxicity**

Inducing Zn toxicity is much less likely among livestock species than developing a deficiency. However, high concentrations of Zn in the diet of many species have culminated in Zn toxicity. No adverse effects have been observed from supplementing weanling pigs 1000 mg Zn/kg DM from either $ZnSO_4$ (Lewis et al., 1957) or Zn carbonate (Brink et al., 1959). Though decreased performance and feed intake, as well as increased mortality were observed with the supplementation of 2000, 4000, and 8000 mg Zn/kg DM from Zn carbonate to pigs (Brink et al., 1959). In beef cattle supplemented 100, 500, 900, 1300, 1700, or 2100 mg Zn/kg DM to the diet from ZnO a linear decrease in performance of calves was detected when 900 mg Zn/kg DM or greater was fed (Ott et al., 1966). However, no differences in performance were observed between 100 and 500 mg Zn/kg DM (Ott et al., 1966), suggesting the maximum tolerable limit

for Zn is between 500 and 900 mg Zn/kg DM. Similarly, Jenkins and Hidiroglou (1991) observed supplementing 500 mg Zn/kg DM for five weeks from ZnO was tolerable for Holstein calves, but 700 mg Zn/kg DM and greater resulted in hindered weight gain and feed intake.

Finding the upper tolerable limit of Zn supplementation in livestock species is complicated by various dietary factors. Non-ruminant species struggle with the antagonistic relationship between Zn and phytate (Oberleas et al., 1962; O'Dell et al., 1964). However, ruminants are not as susceptible to this issue due to the breakdown to phytate in the rumen by microbial phytase (Spears, 2003). This is evident in lambs supplemented phytic acid, resulting in no adverse impacts on tissue Zn concentrations (Ott et al., 1964). However, evidence of limited phytate degradation in the rumen has been observed in high milk producing dairy cows, a dilution effect in the rumen allowing for phytate to escape the rumen and proceed as a Zn antagonist in the digestive tract (Humer and Zebeli, 2015). This dilution effect may be applied to feedlot diets containing large percentages of corn, resulting in the escape of phytate from the rumen due to more phytate than the ruminal phytase is capable of breaking down.

Furthermore, the literature suggests ruminants combat many other mineral antagonists such as Ca, Cd, Cu, and Fe influence Zn absorption (Lönnerdal, 2000; Suttle, 2010; NASEM, 2016) and therefore would impact the upper tolerable limit of Zn in the diet. Using a vascularly perfused rat-intestine system Oestreicher and Cousins (1985) found increased Cu concentrations led to an accumulation of Zn in the mucosal cells and less Zn transferred out of the cell to the portal blood supply. Additionally, competition for absorption in the small intestine by Divalent Metal (Ion) Transporter 1 may account for further interactions between these minerals (Garrick et al., 2006). This body of literature indicates dietary components aside from Zn should also be

evaluated when assessing Zn supplementation strategies to prevent both Zn deficiency and toxicity.

**Zinc Requirements in Cattle**

The current recommendation for Zn in cattle is 30 mg Zn/kg DM (NASEM, 2016). At this concentration cattle should be well below risk for toxicity and above the range for deficiency as previously discussed. This recommendation is supported by the classical Zn literature. In a series of experiments conducted by Perry et al. (1968) beef cattle supplemented Zn experienced improvements in daily weight gain when supplemented 100, 185, or 346 mg Zn/kg DM from ZnO compared to cattle receiving the basal diet (24-29 mg Zn/kg DM). However, improvements were not additive with increasing Zn and an additional two studies found either no difference in performance (75, 165, or 186 mg Zn/kg DM) or a slight decrease in performance (132 mg Zn/kg DM) when supplementing ZnO to basal diets containing 18-28 mg Zn/kg DM (Perry et al., 1968). Additionally, no differences in performance due to dietary Zn concentrations between 24 and 32 mg Zn/kg DM in beef cattle were observed by others (Pringle et al., 1973; Pond and Oltjen, 1988). Therefore, it appears the recommended 30 mg Zn/kg DM is sufficient for adequate growth in accordance with this classical literature. However, daily gains were much lower than modern cattle (1.27 to 1.50 kg/d; Dahlke, 2021) with average daily gains ranging from 0.83 to 1.34 kg/d (Perry et al., 1968; Pringle et al., 1973).

This begs the question: do modern feedlot cattle require additional Zn to optimize growth? A survey of consulting nutritionists indicates Zn is commonly supplemented at over 3 times (100 mg Zn/kg DM) the published recommendation of 30 mg Zn/kg DM (Samuelson et al., 2016a). It can be argued that modern cattle have a vastly different genetic potential compared to counterparts utilized in much of the seminal Zn work. Additionally, the advent of growth technologies such as anabolic implants and beta agonists have greatly improved growth (Duckett

and Pratt, 2014; Lean et al., 2014). Together, these changes in modern cattle may have increased the Zn requirement of these animals to reach their full genetic potential for growth. Although the published recommendation remains at 30 mg Zn/kg DM for cattle, the high inclusion of Zn throughout the feedlot industry suggests Zn requirements may be higher. Further research in this area is warranted to reflect the Zn demands of modern cattle utilizing growth promoting technologies.

**Zinc Source**

A variety of Zn sources are available for cattle, including inorganic and organic sources. There are three classifications of organic trace minerals: complexes, chelates, and proteinates (Spears, 1996). Several sources exist within the inorganic classification, including $ZnSO_4$, ZnO, and the hydroxy source, basic ZnCl. Differences between each of these sources are assessed by determining the bioavailability or ability of Zn to be absorbed and utilized of each source compared to another. Zinc sulfate is considered the gold standard for bioavailability, with other sources deemed either more or less bioavailable. While cattle Zn requirements can be met through any source of Zn, some may be advantageous over others given dietary antagonists such as Ca, Cd, Cu, or Fe (Lönnerdal, 2000; Suttle, 2010; NASEM, 2016) mentioned previously. Additionally, Zn sources may differ in absorption due to the mineral's chemical structure influencing its solubility throughout the digestive tract (Cao et al., 2000a). Furthermore, the chemical structure of Zn sources may affect post-absorption metabolism, though this is not well understood.

Although performance responses are often not observed due to different Zn sources (Greene et al., 1988; Malcolm-Callis et al., 2000; Nunnery et al., 2007a), differences in bioavailability of Zn sources have been (Spears, 1989; Wedekind et al., 1992; Cao et al., 2000a; Sridhar et al., 2015b). When lambs were orally dosed 300 mg Zn from either ZnO or Zn

methionine, the percent difference in plasma Zn concentrations from pre-dosing concentrations was greater for Zn methionine (20.9%) than ZnO (12.5%) at 12 h post-dosing proving greater bioavailability of Zn methionine than ZnO in this study (Spears, 1989). Interestingly, while plasma Zn of ZnO supplemented lambs returned to pre-dosing concentrations by 12 h, plasma Zn concentrations remained 11.8% greater than pre-dosing concentrations for Zn methionine-fed lambs at 24 h post-dosing of Zn treatments (Spears, 1989). This sustained increase in plasma Zn is likely a function of how much greater plasma Zn concentrations were for Zn methionine-dosed lambs than those dosed with ZnO. In line with these data, Wedekind et al. (1992) found Zn methionine was more bioavailable than $ZnSO_4$ while $ZnSO_4$ was more bioavailable than ZnO in chicks. These data coincide with Spears' (1989) findings indicating the organic source, Zn methionine, is more available than inorganic sources ZnO and $ZnSO_4$. Similarly, Sridhar et al. (2015) found broiler chicks supplemented 30 mg Zn/kg DM from Zn glycinate had greater Zn retention in the kidney and pancreas than chicks supplemented 40 mg Zn/kg DM from $ZnSO_4$. This improvement in bioavailability over $ZnSO_4$ has also been observed in cattle and rats (Spears et al., 2004; Schlegel and Windisch, 2006).

Considered an inorganic source due to its structure, the hydroxy source, basic Zn chloride, has its advantages. Tests in water, 0.4% HCl, 2% citric acid, and neutral ammonium citrate revealed basic ZnCl is insoluble in water while completely soluble in these other substances compared to $ZnSO_4$, which was completely soluble in all tested substances (Cao et al., 2000a). Therefore basic ZnCl would be insoluble in the rumen, avoiding potential antagonists present in this environment such as other minerals or undigested fiber (Lönnerdal, 2000; Suttle, 2010; NASEM, 2016; VanValin et al., 2018), unlike $ZnSO_4$. Perhaps these solubility characteristics led to the greater apparent absorption and retention of Zn in beef steers fed 25 mg

Zn/kg DM from basic ZnCl vs. $ZnSO_4$ (Shaeffer et al., 2017). However, studies conducted in chickens indicated similar bioavailability coefficients between basic ZnCl and $ZnSO_4$ (Cao et al., 2000a; Batal et al., 2001). Due to the limited research available, Zn glycinate has not been directly compared to basic ZnCl. However, the greater bioavailability of Zn glycinate vs. $ZnSO_4$ (Spears et al., 2004; Schlegel and Windisch, 2006; Sridhar et al., 2015b) implies Zn glycinate may be more bioavailable than basic ZnCl, as well.

In some instances bioavailability of organic sources has not been different from inorganic counterparts. In both chicks and lambs fed increasing concentrations of Zn through $ZnSO_4$, Zn proteinate, or Zn methionine, no differences in bioavailability were observed except for a single Zn proteinate source being more bioavailable than $ZnSO_4$ (Cao et al., 2000b). However, chicks were fed upwards of 600 mg Zn/kg DM and lambs upwards of 2,100 mg Zn/kg DM (Cao et al., 2000b). Therefore, differences in bioavailability may be limited when dietary Zn concentrations are so great. For instance, no differences in liver Zn concentrations between Zn sources were observed in cattle fed a basal diet containing 200 mg Zn/kg DM and supplemented 360 mg Zn/d (~250 mg Zn/kg DM) as Zn methionine, $ZnSO_4$, or ZnO (Rojas et al., 1996). While these studies show bioavailability is not always greater for organic Zn sources, the vast majority of studies reported herein largely suggest bioavailability is favorable for organic sources over inorganic. Though the concentration of Zn present in the basal diet may influence the bioavailability of Zn, regardless of source. Furthermore, structural differences within inorganic sources may further influence the bioavailability and practical application of these sources in feedlot diets.

**Zinc Absorption and Status**

Although Zn supplementation is greater across the beef industry (Samuelson et al., 2016a) than NASEM (2016) requirements, absorption of Zn can greatly influence the effects of dietary Zn in cattle. Absorption and movement of Zn throughout the body is reliant upon the ZIP

and ZnT family of transporters to move Zn into and out of the cytoplasm, respectively (Cousins et al., 2006a). Zinc is absorbed in the jejunum of the small intestine mostly through the apical transporter ZIP4 (Dufner-Beattie et al., 2003). Furthermore, the basolateral transporter ZnT1 is responsible for the passage of Zn from the enterocyte into the portal blood (Mcmahon and Cousins, 1998) where, bound by albumin, it will be transported to the liver to be stored by the protein metallothionein (Cousins, 1985). Dietary supplementation of Zn has been shown to regulate both ZIP4 and ZnT1 expression (Mcmahon and Cousins, 1998; Dufner-Beattie et al., 2003). Therefore, these transporters provide a mechanism for maintaining Zn homeostasis and determine the efficiency of Zn absorption. However, high concentrations of dietary Zn compensate for the down regulation of ZIP and ZnT transporters (Lönnerdal, 2000). Sandström et al. (1980) found the fractional absorption of Zn supplemented to humans through radiolabeled Zn in water was decreased with increasing concentrations. Specifically, supplementation of 40 vs. 200 µmol of Zn resulted in absorption rates of 73 or 46%, respectively, though total Zn absorbed increased three-fold for the higher dose. Evidence of dietary Zn concentration altering absorption has also been found in cattle. Steers (309 kg) fed 36 vs. 156 mg dietary Zn/kg DM had greater apparent absorption, though steers fed the greater concentrations of Zn retained over two times as much Zn as steers fed 36 mg Zn/kg DM (Carmichael et al., 2019b). However, no differences in Zn apparent absorption or retention were observed with similar Zn treatments in heavier steers (485 kg; Carmichael et al., 2018) indicating Zn absorption may be influenced by stage of growth, though additional research in this area is needed to verify this assumption. These data alongside the basic Zn transporter literature, suggest cattle receiving greater concentrations of dietary Zn would have greater total Zn retention than cattle receiving 30 mg Zn/kg DM, irrespective of transporter down regulation.

Yet, Zn absorption may be influenced by additional factors that alter whole body use of Zn, such as growth. Skeletal muscle and bone comprise a large percentage of Zn storage. However, classified as slow Zn metabolizers, release of Zn from muscle and bone is less likely (Gumpper and Ma, 2019). Seeing that 90% of Zn in the body is thought to reside in tissues with slow Zn metabolism (Gumpper and Ma, 2019), labile Zn stores are limited. Therefore, our interpretation of Zn status based on measurable tissue Zn stores may be unreliable, at best. As such, severe Zn deficiency in calves results in only a small decline in Zn concentrations for some tissues such as liver, bone, and pancreas but no changes in muscle and brain (Miller, 1970). However, in situations of Zn deficiency, plasma Zn concentrations appear to reflect Zn status more accurately. In calves fed a diet nearly void of Zn, plasma Zn concentrations dropped, feed intake was depressed, and growth was halted (Mills et al., 1967). Furthermore, removal of supplemental Zn to a subset of calves from this same experiment resulted in a sharp decrease in body weight within 2 weeks of Zn removal, even though plasma Zn concentrations started as adequate (Mills et al., 1967). These data suggest even cattle starting with adequate plasma Zn concentrations (0.8-1.4 mg/L; Kincaid, 2000) can experience detrimental effects of a severely Zn deficient diet. Miller (1970) suggests cattle lack a mechanism to quickly mobilize Zn stores, leading to these quick decreases in circulating Zn concentrations. Feeding sub-optimal concentrations of Zn could also result in depletion of Zn status and performance, though effects would be much more difficult to recognize (Miller, 1970). In modern cattle feeding, severe Zn deficiencies are unlikely due to ample Zn supplementation (Samuelson et al., 2016a), unless antagonists (Ca, Cd, Cu, or Fe) are readily present in the diet (Lönnerdal, 2000; Suttle, 2010; NASEM, 2016). Therefore, circulating Zn concentrations for cattle across the industry are likely adequate but management strategies altering growth rates such as steroidal implants may result

in short term drops in circulating Zn concentrations due to increased Zn utilization in protein synthesis to accommodate increased growth. This marked increase in Zn demand could result in depleted circulating Zn concentrations as other Zn stores (bone, muscle) are not as labile. Therefore, steroidal implants could influence Zn absorption as low plasma Zn concentrations would eventually trigger greater uptake of Zn by the enterocytes to support the increased Zn demand.

**Zinc's Role in Protein Synthesis**

Zinc has been implicated by a variety of studies as essential for growth. Oberleas and Prasad (1969) discovered growth rates in rats were more limited by dietary Zn supplementation than dietary protein concentrations. This decline in growth rate due to inadequate dietary Zn agrees with the depression of growth in Zn deficient rats (Todd et al., 1933), lambs (Ott et al., 1964), and cattle (Miller and Miller, 1962). As such, Zn's role in growth is linked to DNA and subsequently protein synthesis. Duncan and Dreosti (1976) observed thymidine kinase activity was depressed in the liver of Zn deficient rats. However, the exposure of liver tissue to 0.1 µM Zn restored activity to that of liver from control rats (Duncan and Dreosti, 1976). Interestingly, the ratio of ribonucleic acid (**RNA**) and DNA and total collagen and non-collagenous proteins decreased in Zn deficient rats' connective tissue (Fernandez-Madrid et al., 1973). Considering the role of thymidine kinase in incorporating thymidine into DNA and the decreased protein content in connective tissue due to Zn deficiency, these data support the importance of Zn in DNA and protein synthesis.

It appears the function of Zn in DNA and protein synthesis is conserved across organisms. Fungi cultured with 5 mg Zn/kg from $ZnSO_4$ experienced greater RNA synthesis within the first 3.5 h of culture resulting in 2.7 times more RNA synthesis than non-supplemented controls (Wegener and Romano, 1963). Similarly, Wegener and Romano (1963)

observed overall protein synthesis was 1.6 times greater in Zn supplemented fungi cultures than non-supplemented fungi in the first 3.5 h. Using [$^3$H]thymidine incorporation as a labeled DNA precursor, Williams and Chesters (1970) found rats fed a Zn deficient diet for 1 to 5 d had a linearly decrease in [$^3$H]thymidine incorporation in the liver, kidney, and spleen. Interestingly, this decrease in DNA synthesis was observed before the common Zn deficiency symptoms of poor growth and appetite (Williams and Chesters, 1970). These data indicate a Zn deficiency can have immediate effects on growth even if not overt. Using a similar marker, Dorup and Clausen (1991) found rats fed a Zn deficient diet (27 µmol/kg) for 15 d had a 57-64% decrease in [$^3$H]leucine incorporation in skeletal and heart muscle when compared to pair-fed control rats. Although Zn deficiency often results in appetite depression, pair-fed control rats had similar [$^3$H]leucine incorporation compared to rats fed ad libitum (Dorup and Clausen, 1991). Therefore protein synthesis appears to be a function of Zn availability rather than appetite depression.

While it is apparent Zn influences DNA and protein synthesis, Zn also regulates the mechanism by which DNA and protein synthesis are initiated. Jung et al. (2009) determined Zn-induced upregulation of cell cycle regulatory proteins and DNA synthesis were stimulated through phosphoinositide 3-kinase (**PI3K**)/protein kinase B (**AKT**), mitogen-activated protein kinase (**MAPK**), and mammalian target of rapamycin (**mTOR**) pathways. Exposure of mouse embryonic stem cells to 40 µM Zn from ZnCl resulted in a 45.5% increase in the incorporation of [$^3$H]thymidine and a 54% increase in cell proliferation at 24 h after Zn exposure compared to control cells not exposed to Zn (Jung et al., 2009). Furthermore, 40 µM Zn from ZnCl increased AKT, p44/42 MAPKs, c-Jun N-terminal kinase (**JNK**), mTOR, p70S6K, and 4E-BPI phosphorylation (Jung et al., 2009); all proteins upstream up protein synthesis. Treatment of embryonic stem cells to inhibitors of these signaling proteins resulted in decreased

[$^{3}$H]thymidine incorporation and the abundance of cell cycle regulatory proteins such as Cyclin D1, Cyclin E, CDK 2, and CDK 4 (Jung et al., 2009).

Considering the extreme growth occurring during embryonic development, Gao et al. (2014) set out to determine the effects of maternal Zn supplementation on the growth and protein synthesis of chicks born to hens supplemented 0, 50, or 300 mg Zn/kg DM from $ZnSO_4$ for 6 weeks. Examination of eggs revealed the Zn content of the yolk was increased in accordance with maternal Zn supplementation (Gao et al., 2014). By 14 d post-hatch, chicks born to hens supplemented 50 or 300 mg Zn/kg DM had greater phosphorylation of AKT and forkhead box O (**FoxO**) in pectoralis major muscle compared to chicks born to control hens (Gao et al., 2014). Furthermore, by d 35, phosphorylation of mTOR increased in accordance with increasing Zn supplementation, and phosphorylated FoxO numerically increased similarly (Gao et al., 2014). Interestingly, the content of AKT, FoxO, and mTOR in the muscle were not different between treatments (Gao et al., 2014). These data suggest the effects of Zn supplementation are elicited through phosphorylation rather than influencing the amount of these signaling proteins. Additionally, Gao et al. (2014) revealed the importance of maternal Zn nutrition and the beneficial effects observed in offspring.

Although Zn is involved in DNA and protein synthesis, the data gathered here suggest more work is warranted to build upon our understanding of Zn's role in DNA and protein synthesis. Jung et al. (2009) and Gao et al. (2014) both demonstrate the effects of Zn supplementation on the phosphorylation of proteins within protein synthesis pathways. It is likely Zn influences the phosphorylation of several signaling proteins yet to be measured. Furthermore, the timing and concentration of Zn supplementation on a cellular and whole animal

level are necessary to better understand when Zn should be supplemented and how much is needed to support DNA and protein synthesis.

**Zinc Effects on Growth Performance and Carcass Characteristics**

Cattle Zn requirements in the U.S. have been set at 30 mg Zn/kg DM (NASEM, 2016) to prevent Zn deficiency in cattle and provide adequate Zn nutrition for growth. However, evidence exists that Zn supplementation in excess of 30 mg Zn/kg DM can benefit performance. In yearling beef steers, supplementation of 124, 185, or 346 mg Zn/kg DM from ZnO resulted in improved gains over steers fed a control diet containing only 24-29 mg Zn/kg DM (Perry et al., 1968). Furthermore, supplementing cow-calf pairs 900 mg Zn/d while on pasture resulted in a 0.04 kg/d advantage in Zn supplemented calves, though no weight differences were observed in supplemented cows (Mayland et al., 1980). Interestingly, consistent Zn supplementation may be critical to cattle performance. Ott et al. (1965) found that supplementing 11-week-old Holstein steer calves with 100 mg Zn/kg DM from ZnO for 10 weeks improved average daily gain (**ADG**) by 0.34 kg/d. Removal of Zn supplementation and return to the basal diet containing only 3 mg Zn/kg DM resulted in the development of Zn deficiency in calves within 3-4 weeks (Ott et al., 1965).

Similarly, (Miller and Miller, 1962) observed Zn deficiencies in young calves within three weeks of removing supplemental Zn from the diet. These data indicate body Zn stores are likely transient and require consistent supplementation rather than bulk doses to meet cattle requirements and maintain growth. The suggestion that cattle have a daily requirement for Zn indicates absorption is a limiting factor of Zn status. This idea is supported by the quick depletion of Zn status of animals fed diets devoid of Zn discussed previously in the Zn absorption and status section (Mills et al., 1967). In contrast to the transient nature of Zn, both Cu and Se are readily stored in the liver of ruminants for future use (Suttle, 2010). Therefore, Zn

supplementation strategies and Zn absorption require further investigation in modern beef cattle to optimize growth performance.

It has been well documented that Zn is an essential nutrient in growth (Oberleas and Prasad, 1969; Ninh et al., 1996). In humans, Díaz-Gómez et al. (2003) reported supplementation of 10 mg Zn/L of formula to pre-term infants resulted in greater weight gain than pre-term infants supplemented only 5 mg Zn/L. Therefore it is logical that increased Zn supplementation may be critical during times of high growth common in food animal production. Lambs supplemented 65 mg Zn/kg DM from either Zn methionine or ZnO had greater ADG than lambs fed a basal diet containing 15 mg Zn/kg DM (Rodríguez-Maya et al., 2019). Similarly, Spears (1989) and Spears and Kegley (2002) found heifers and steers, respectively, supplemented 25 mg Zn/kg DM from various sources resulted in greater ADG and improved feed efficiency during the growing period even though basal diets contained between 23.8 and 33 mg Zn/kg DM. These Zn responses diminished throughout the finishing period (Spears, 1989; J. W. Spears and Kegley, 2002), likely in accordance with lesser growth rates of cattle towards the end of the finishing period. Others have observed no performance differences in cattle supplemented increased concentrations of Zn during the finishing period (Greene et al., 1988; Spears, 1989; Malcolm-Callis et al., 2000; Nunnery et al., 2007a).

The performance responses observed during the growing period indicate Zn may be more important during periods of high growth rates. Growth promoting technologies such as anabolic implants and beta agonists provide a boost in growth performance of cattle throughout the finishing period or near the end of the finishing period (Duckett and Pratt, 2014; Lean et al., 2014) that may influence growth responses to Zn supplementation in feedlot cattle. This growth

response to Zn has been documented in steers fed a beta agonist. However, the anabolic implant response to Zn supplementation has been less frequently examined.

Supplementing up to 90 mg Zn/kg DM from a Zn amino-acid complex on top of a diet with 88 mg Zn/kg DM has been shown to induce a linear performance response to Zn supplementation only in beta agonist-fed steers during the last 28-d of the 114-d study (Genther-Schroeder et al., 2016a). Furthermore, supplementation of 120 or 160 mg Zn/kg DM from blended organic and inorganic Zn sources improved the performance of steers receiving a beta agonist only (Genther-Schroeder et al., 2016b; Wellmann et al., 2020). Interestingly, these performance responses to Zn supplementation were only observed during the beta agonist period and only to cattle receiving a beta agonist (Genther-Schroeder et al., 2016a; Wellmann et al., 2020) further suggesting Zn supports beta agonist-induced rapid growth. The relationship between Zn supplementation and periods of rapid growth, as induced by growth promoting technologies, will be further detailed later in this literature review.

In addition to an improved performance response, Zn supplementation has been observed to influence carcass characteristics. Supplementation of 120 mg Zn/kg DM from $ZnSO_4$ and Zn amino acid complex (50:50) to steers fed a beta agonist resulted in increased hot carcass weight (**HCW**; Genther-Schroeder et al., 2016b). However, HCW is not always in line with live performance responses. Genther-Schroeder et al. (2016a) observed a linear response to increasing Zn supplementation in final body weight during beta agonist supplementation, but HCW was not affected by Zn supplementation. Furthermore, the influence of Zn on yield grade and quality grade has been observed (Greene et al., 1988; J. W. Spears and Kegley, 2002). Supplementing 360 mg Zn/d (~30 mg Zn/kg DM) from Zn methionine resulted in steers having slightly better USDA quality grade and marbling scores than steers supplemented with the same

concentration of Zn from ZnO (Greene et al., 1988). As detailed previously, an organic source such as Zn methionine is more bioavailable than inorganic ZnO suggesting increased Zn availability is beneficial to marbling and quality grade when the basal diet Zn is marginal. Furthermore, Spears and Kegley (2002) observed steers supplemented only 25 mg Zn/kg DM from both inorganic (Zn oxide) and organic (Zn proteinate) sources had greater quality grade and marbling scores than steers fed the un-supplemented control diet (26 mg Zn/kg DM). Compared to control steers, these steers also had increased YG and backfat thickness (J. W. Spears and Kegley, 2002). These data indicate Zn influences adipose depots, though more work is needed to fully understand this relationship and how Zn concentration and source affect adipose tissue. Perhaps this association is via the regulation of phosphodiesterase and insulin, both of which have been linked to Zn (von Bulow et al., 2005; Ohashi et al., 2015).

Overall, growth and carcass characteristic responses to Zn supplementation have been variable. However, the data presented in this literature review indicate a potential to improve cattle growth rates and efficiency through strategic Zn supplementation tailored to meet the growth demands of modern cattle. Additional work is warranted in this field to unravel why Zn responses in cattle are inconsistent, determine how different Zn sources influence performance responses, and assess the impact of total dietary Zn on cattle performance and carcass characteristics.

## Steroidal Implants

Capturing the growth potential of cattle is critical to improving cattle efficiency and maximizing beef production in the United States. Steroidal implants have been a vital part of a profitable beef industry for the past 60 years (R L Preston, 1999), resulting in a $218 return on investment using 2013 prices when cattle were administered a combination two-implant program (Duckett and Pratt, 2014). Though cattle market prices waver yearly, implants routinely improve

cattle gains by 16 to 20% (Bartle et al., 1992; Johnson et al., 1996a; Duckett and Pratt, 2014). Furthermore, steroidal implants contribute to the economic and environmental sustainability of the beef industry (Capper and Hayes, 2012). Using a model to account for the metabolism and nutrient requirement of cattle, Capper and Hayes (2012) found the removal of steroidal implants along with other growth technologies would increase the amount of land, feedstuffs, and water needed for beef production. Ultimately, the removal of growth technologies such as steroidal implants would greatly increase the number of cattle needed to produce the same amount of beef with growth technologies (Capper and Hayes, 2012). Therefore, steroidal implants are a critical component of a sustainable beef industry.

**Implant Classifications and Performance**

Steroidal implants can be classified by hormone: estrogenic (estrogen [**$E_2$**] or estradiol benzoate), androgenic (testosterone propionate or trenbolone acetate [**TBA**]), or a combination implant with both estrogenic and androgenic components (Smith and Johnson, 2020). Combination implants have proven more effective than estrogenic or androgenic only implants, and androgenic implants are more effective than estrogenic implants at improving cattle performance (Cleale et al., 2013; Reichhardt et al., 2021). Heifers administered implant treatments consisting of 14 mg estradiol benzoate + 100 mg TBA, 14 mg estradiol benzoate, or 100 mg TBA had greater ADG and feed efficiency than sham implanted control heifers (Cleale et al., 2013). Similarly, Reichhardt et al. (2021) found steers administered 24 mg $E_2$ + 120 mg TBA or 200 mg TBA had greater ADG throughout the 129-d study than non-implanted controls, but steers administered 25.7 mg $E_2$ did not have gains that differed from control steers. Additionally, as expected, the combination implant resulted in greater gains than the TBA-only implant (Reichhardt et al., 2021). Although the hormone content of the implant utilized was different, Cleale et al. (2013) found steer performance was also greatest for the combination

implant followed by TBA and $E_2$ only implants. Although trial ADG did not differ between $E_2$ only and non-implanted steers, this may have been due to the type of implant utilized in this study. Reichhardt et al. (2021) used a slow-release estrogen-only implant (Compudose; Elanco Animal Health, Greenfield, IN) with a 200-d duration, thus a greater performance response may have been elicited if the study were longer than 129 d. Bartle et al. (1992) administered an $E_2$ implant to steers resulting in a 7% increase in ADG and a 3% improvement in feed efficiency over 140-160 d compared to non-implanted steers. However, it was noted the study duration was longer than the expected hormonal payout period of the $E_2$ implant utilized. These data indicate the importance of both estrogenic and androgenic hormones to induce growth in cattle. Androgenic hormones appear to have a greater influence on performance than estrogenic hormones.

In addition to hormone type, hormone content or potency of implant greatly influences the performance of cattle. As previously discussed, combination steroidal implants induce more significant growth responses than single hormone implants. Increasing hormone content of implants from 4 mg $E_2$ + 20 mg TBA to 28 mg $E_2$ + 140 mg TBA resulted in a linear improvement in steer performance (Bartle et al., 1992). Bartle et al. (1992) observed that steers administered 28 mg $E_2$ + 140 mg TBA elicited an 18% improvement in ADG and 10% improvement in feed efficiency compared to non-implanted steers. Utilizing a slightly lesser dose of hormone has also been proven just as effective at improving growth rates. Johnson et al. (1996) administered a combination implant containing 24 mg $E_2$ + 120 mg TBA to steers resulting in a 15.6% improvement in ADG throughout the 143-d study. Increasing the potency of implants beyond hormone doses tested in these previously discussed studies has been shown to improve performance benefits further. Smith et al. (2007) found implanting heifers with 28 mg

estradiol benzoate + 200 mg TBA resulted in 36% greater ADG than non-implanted heifers. A meta-analysis of implant studies reported in technical bulletins, University reports, and scientific journals by Reinhardt and Wagner (2014) found combination implants containing 200 mg TBA resulted in greater performance responses than combination implants with 120 mg TBA. Therefore, increased hormone content, specifically TBA, appears to have the greatest influence on a combination implant's potential to improve growth rates.

**Extended-release Implants**

Timing of steroidal implant use is imperative to maximizing cattle growth responses. Historically implants have been administered multiple times throughout production, including upon arrival to the feedlot and once more later in the feeding period (NAHMS, 2013; Samuelson et al., 2016). The advent of the extended-release implant in 2007 (FDA, 2007) provided a new tool for managing steroidal implant administration in the feedlot. Specifically licensed for steers, Revalor-XS (Merck, Madison, NJ) provides 40 mg $E_2$ + 200 mg TBA across 10 pellets containing 4 mg $E_2$ + 20 mg TBA each (FDA, 2007). A similar product was later licensed for heifers; Revalor-XH (Merck) provides 20 mg $E_2$ + 200 mg TBA evenly distributed across 10 pellets (FDA, 2017). These extended-release implants utilize coated pellets to allow for the immediate release of hormone from the uncoated pellets upon implant administration, while coated pellets begin to release hormone around d 70 in accordance with explant data from a similarly coated implant (FDA, 2017b). In contrast, Synovex ONE FEEDLOT (Zoetis, Parsippany, NJ) is an extended-release implant for steers and heifers containing 28 mg estradiol benzoate + 200 mg TBA distributed across 8 coated pellets (FDA, 2014). The coating on Synovex ONE FEEDLOT leads to the slow release of hormone across 200 d (FDA, 2014). Together these different extended-release implant strategies provide options for producers to circumvent the labor and cattle stress associated with re-processing cattle for a terminal implant.

Considering extended-release implants replace the practice of re-implanting cattle, studies have been conducted to determine if performance differs between an extended-release and a two-implant strategy utilizing equivalent hormone content. Parr et al. (2011) found steers implanted with the extended-release implant Revalor-XS (40 mg $E_2$ + 200 mg TBA; Merck) had greater carcass-adjusted final BW and ADG compared to steers implanted with the equivalent Revalor-IS (16 mg $E_2$ + 80 mg TBA; Merck) on d 0 and Revalor-S (24 mg $E_2$ + 120 mg TBA; Merck) on d 90-103 of a 194-200 d trial. This improvement in growth performance may be attributed to not removing steers administered Revalor-XS from pens at re-implanting time. However, three other studies conducted by Parr et al. (2011) and one conducted by Nichols et al. (2014) found no performance differences between steers utilizing the same implant strategies. Cleale et al. (2012) observed steers administered the extended-release implant Synovex ONE FEEDLOT (28 mg estradiol benzoate + 200 mg TBA; Zoetis) had 15.4% greater ADG over the 198-d study in comparison to Synovex PLUS (28 mg estradiol benzoate + 200 mg TBA; Zoetis) implanted steers. These differences in performance responses due to extended-release implant strategy are likely attributed to the proprietary coatings utilized in the implants. Synovex ONE FEEDLOT utilizes a coating that results in the slow release of hormone from all pellets (FDA, 2014). In contrast, only 6 of the 10 pellets in Revalor-XS and Revalor-XH are coated (FDA, 2007; FDA, 2017a), resulting in hormone release from these coated pellets 70-d post-implant administration (FDA, 2017b).

Interestingly, no differences in final BW were observed in heifers administered the extended-release implant Revalor-XH (20 mg $E_2$ + 200 mg TBA; Merck) vs. a Revalor-200 (20 mg $E_2$ + 200 mg TBA; Merck) on d 0 and 100 (Ohnoutka, 2018). Similarly, Crawford et al. (2018) found implanting heifers with a Revalor-IH (8 mg $E_2$ + 80 mg TBA; Merck) on d 0

followed by a Revalor-200 on d 90 resulted in a tendency for greater performance in heifers compared to heifers administered a Revalor-XH. It is unclear why administering a greater hormone dose in these two implant strategies did not always result in greater performance over the extended-release implant. However, these data indicate utilizing an extended-release implant does not forfeit performance compared to some higher dose implant strategies.

**Peak Hormonal Payout**

Although steroidal implants effectively release steroidal hormones for several months per manufacturer and regulatory studies (FDA, 2014; FDA, 2017), research has shown steroidal implants have a peak hormonal payout period (Johnson et al., 1996a). Johnson et al. (1996) observed serum concentrations of trenbolone acetate and estradiol in steers were greatly increased by d 2 after the administration of a moderate potency combination implant (Revalor-S; 120 mg trenbolone acetate + 24 mg estradiol; Merck). Interestingly, circulating trenbolone acetate concentrations were substantially less by d 40 and gradually decreased throughout the 143-d study (Johnson et al., 1996a). Meanwhile, Johnson et al. (1996) observed that circulating estradiol concentrations remained elevated throughout the study following implant administration. These data indicate that the peak hormonal payout of steroidal implants, especially trenbolone acetate, occurs within the first 40 d following implant administration.

The greatest ADG in implanted steers was observed during the first 40 d following implant administration (Johnson et al., 1996a), coinciding with the peak hormonal payout period of the steroidal implant. This growth response has since been associated with a decrease in liver Mn concentrations within 14 d of implant administration in several studies (Messersmith, 2018; Niedermayer et al., 2018; Reichhardt et al., 2021). Interestingly, the majority of Mn in the liver is associated with arginase (Rosebrough et al., 1987), the terminal enzyme of the urea cycle (J. S. Bond et al., 1983; Watts, 1990). It is well established that steroidal implants decrease protein

degradation in cattle (Galbraith, 1980; Parr et al., 2014; Harris et al., 2020). Therefore the decrease in liver Mn concentrations due to implant administration is likely the result of a lesser need for arginase to operate the urea cycle within the liver. However, research is needed to directly link the observed decreases in liver Mn concentrations to arginase and determine if other growth processes during peak hormonal payout of the steroidal implant are responsible for changes in liver Mn concentrations.

**Carcass Characteristics**

In addition to the growth response elicited through steroidal implant administration, carcass characteristics can also be influenced. Hot carcass weight and ribeye area (**REA**) are most commonly benefited from steroidal implants (Perry et al., 1991; Bartle et al., 1992; Johnson et al., 1996a; Smith et al., 2007; Reichhardt et al., 2021). Angus steers administered 28 mg $E_2$ + 140 mg TBA had a 20 kg advantage in HCW compared to non-implanted steers (Perry et al., 1991). Likewise, Johnson et al. (1996) and Reichhardt et al. (2021) observed a 26 kg increase in HCW for steers implanted with 24 mg $E_2$ + 120 mg TBA. Interestingly implanting steers and heifers with 27 mg estradiol benzoate + 200 mg TBA resulted in a 21 kg increase in HCW of steers compared to non-implanted steers (Smith et al., 2007). However, HCW only tended to be greater for implanted heifers (Smith et al., 2007). The lesser HCW response in heifers compared to steers may be due to differences in growth potential, with steers having a greater potential for growth (Owens et al., 1995). However, the observed differences may also be attributed to animal variation.

In agreement with increased HCW due to implant administration, Niedermayer et al. (2018) observed steers implanted with 16 mg $E_2$ + 80 mg TBA on d 0 and 20 mg $E_2$ + 200 mg TBA on d 56 had larger REA than non-implanted steers. These results agree with several studies indicating steroidal implants increase REA (Smith et al., 2007; Cleale et al., 2013; Reichhardt et

al., 2021). A serial slaughter study in steers implanted with 24 mg $E_2$ + 120 mg TBA increased REA over non-implanted steers by d 115 when subsets of steers were slaughtered on d 40, 115, and 143 post-implant administration (Johnson et al., 1996a). Furthermore, increasing hormone content of combination implants has been observed to increase REA of steers (Bartle et al., 1992). This increase in hormone content also resulted in a linear decrease in marbling scores (Bartle et al., 1992). Likewise, Johnson et al. (1996) observed implanted steers had lesser percent kidney, pelvic, and heart fat (**KPH**) by 143-d post-implant administration.

Indeed, these data indicate steroidal implants affect fat stores. However, the depression in marbling scores and decreased KPH are likely attributed to implant timing. A study using Holstein steers found the closer to harvest an implant is administrated (24 mg $E_2$ + 120 mg TBA), the greater the effect on marbling depression (Scheffler et al., 2003). However, the timing of implant from this study was either 291, 179, or 67 d out from harvest (Scheffler et al., 2003). Feeding implanted cattle for a longer period of time can help alleviate differences in fat deposition and avoid depression in marbling (Johnson et al., 1996a; Guiroy et al., 2002). Therefore, steroidal implants likely depress marbling scores by shifting the growth curve of cattle, meaning fat deposition is delayed compared to non-implanted cattle with the same d on feed. Ultimately, steroidal implants can be beneficial to carcass characteristics if proper strategies are implemented. Timing of implant administration is one of the biggest factors influencing carcass characteristics, and work must be continued to simplify this information for cattle producers to make educated and timely decisions for their implant strategies.

**Mechanism of Action**

Cattle are born with a finite number of muscle fibers (Mills, 2002), indicating growth must occur through hypertrophy. This growth is accomplished through nuclei accumulation in the multi-nucleated muscle fibers (Moss and Leblond, 1971; Thompson et al., 1989). Satellite

cells reside in a quiescent state between the basal lamina and sarcolemma of the muscle fiber and are responsible for this contribution of nuclei to growing muscle fibers (Moss and Leblond, 1971; Chen et al., 2005). Satellite cells become myoblasts or myogenic precursor cells upon activation and begin to proliferate and differentiate into myotubules before fusing with existing muscle fibers (Moss and Leblond, 1971; Charge and Rudnicki, 2004). The exact mechanism by which steroidal implants induce growth through satellite cells is uncertain. However, it is clear that both estrogen and androgen hormones influence this growth response through genomic and non-genomic mechanisms of action detailed below.

**Genomic**

Nuclear hormone receptors represent the classic mechanism by which steroid hormones influence growth. This mechanism regulates gene transcription and is a relatively slow process compared to nongenomic hormone receptors that will be discussed later. Steroidal implants in cattle production predominantly release $E_2$ and androgen hormones into circulation (Johnson et al., 1996a). There are two $E_2$ nuclear hormone receptors (**ER**) that have been discovered, including ERα and ERβ (Heldring et al., 2007), while only one androgen nuclear hormone receptor (**AR**) has been named (Davey and Grossmann, 2016). Steroid hormone receptors are type I nuclear receptors within the nuclear receptor superfamily (Mangelsdorf et al., 1995; Yen, 2015). Nuclear receptors are composed of a carboxy-terminal ligand-binding domain, a central DNA binding domain, and an amino-terminal activation domain (Mangelsdorf et al., 1995; Evans and Mangelsdorf, 2014; Yen, 2015). The DNA binding domain is the most conserved across nuclear receptors. It is made up of two Zn fingers that mediate the formation of high-affinity bonds between the nuclear receptor and the target gene (Yen, 2015).

These type I nuclear receptors are located within the cell's cytoplasm, where they are bound to heat shock proteins 70 and 90 that act as chaperones until a hormone ligand binds

(Smith, 1993; Mangelsdorf et al., 1995; Yen, 2015). This intracellular location is possible due to the fat-soluble nature of steroid hormones allowing for their passage through the lipid bilayer of the cell (Mangelsdorf et al., 1995). A chain of responses occurs once the hormone binds to the ligand-binding domain. First, the receptor undergoes a conformational change that results in the shedding of the heat shock proteins and dimerization. These changes are followed by the translocation of the receptor into the nucleus to bind to the corresponding DNA sequence or hormone response element of the target gene (Freedman, 1992; Chen et al., 2005; Yen, 2015). These hormone response elements are located within the promoter region of the gene of interest and result in the regulation of gene transcription (Yen, 2015). Therefore, steroid nuclear receptors can be considered transcription factors.

Specifically, evidence of these receptors acting as transcription factors for genes relevant to the steroidal implant mechanism has been observed in cell culture. Wu et al. (2007) found the treatment of human liver and prostate cells with testosterone in cell culture resulted in the upregulation of insulin like growth factor-1 (**IGF-1**) gene expression due to the recruitment of the AR to androgen response elements located within the promoter region of the IGF-1 gene. The use of an AR inhibitor, flutamide, indicates the importance of AR to this IGF-1 response. Treatment of bovine satellite cells with flutamide repressed the ability of the trenbolone acetate (10 nM) to increase IGF-1 mRNA expression (Kamanga-Sollo et al., 2008). These data are congruent with Wu et al. (2007), indicating that the IGF-1 response was dependent upon a functional AR. Treatment of bovine satellite cells with 10 nM of estrogen or trenbolone acetate resulted in 2.6 and 1.5 times greater expression of IGF-1 mRNA, respectively, compared to controls (Kamanga-Sollo et al., 2004). However, the increased expression of IGF-1 caused by $E_2$ is not through an $E_2$ nuclear receptor as no estrogen response elements are found on the IGF-1

gene (Umayahara et al., 1994). Instead, $E_2$ appears to work through a nongenomic $E_2$ receptor, G protein-coupled estrogen receptor-1 (**GPER-1**), to induce local IGF-1 production (Kamanga-Sollo et al., 2008). This nongenomic mechanism will be discussed later.

Although an ER is not responsible for local IGF-1 production, an $E_2$ response element has been located on the growth hormone-releasing hormone (**GHRH**) gene in the hypothalamus (Lalmansingh and Uht, 2008). These data agree with Preston's (1975) hypothesis that $E_2$ stimulates the release of GHRH from the hypothalamus, thus triggering the release of growth hormone (**GH**) from the anterior pituitary. In turn, GH stimulates the production of IGF-1 in the liver and locally in the muscle (Velloso, 2008). Insulin-like growth factor-1 is a key inducer of growth, resulting in increased protein synthesis and decreased protein degradation (Duan et al., 2010; Yoshida and Delafontaine, 2020). Therefore, it is not surprising that beef steers implanted with a combination implant (Revalor-S; 24 mg $E_2$ + 120 mg TBA; Merck Animal Health, Madison, NJ) had greater circulating IGF-1 within six days of implant administration (Johnson et al., 1998a) and 68% greater IGF-1 mRNA gene expression in the muscle than non-implanted steers forty days after implant administration (Johnson et al., 1998b). These data showcase how steroid hormone nuclear receptors elicit their growth response in implanted cattle. However, genomic and nongenomic receptors work together to induce the steroid hormone growth response we observe in cattle.

**Non-genomic**

While it is clear nuclear hormone receptors aid in the genomic growth response elicited through steroidal implants (Preston, 1975; Kamanga-Sollo et al., 2008), additional types of hormone receptors have been discovered. By definition, non-genomic mechanisms more rapidly induce growth than genomic mechanisms due to the use of secondary messenger systems that are insensitive to gene transcription and translation inhibitors (Heinlein and Chang, 2002). Most

non-genomic responses of $E_2$ and androgen hormones are relayed through G protein-coupled receptors (Heinlein and Chang, 2002; Kamanga-Sollo et al., 2008; Filardo and Thomas, 2012). This mechanism will be the main focus of this section.

As previously discussed, $E_2$ and androgens lead to increased circulating IGF-1 and IGF-1 mRNA in the muscle (Johnson et al., 1998b; Johnson et al., 1998a; Kamanga-Sollo et al., 2004; Kamanga-Sollo et al., 2008). Although nuclear hormone receptors influence this response, the sustained IGF-1 response in the presence of ER and AR inhibitors indicates another mechanism is in use, as showcased by Kamanga-Sollo et al. (2008). By treating bovine satellite cells with 10 nM ICI, an inhibitor of ER, Kamanga-Sollo et al. (2008) found $E_2$ induced IGF-1 expression was not suppressed in bovine satellite cells. Furthermore, treatment of 100 nM ICI increased IGF-1 mRNA expression (Kamanga-Sollo et al., 2008). These data point to GPER-1 as the additional mechanism for $E_2$ induced IGF-1 upregulation of gene expression. However, IGF-1 mRNA expression was increased when bovine satellite cells were treated with 100 nM of G1, a GPER-1 agonist (Kamanga-Sollo et al., 2008). The authors believe these data indicate IGF-1 expression is mediated through other genomic and non-genomic mechanisms, though the exact mechanism is unclear. In contrast to the inhibition of $E_2$ receptors, treatment of bovine satellite cells with flutamide, an AR inhibitor, resulted in the complete suppression of androgen-induced IGF-1 gene expression (Kamanga-Sollo et al., 2008). This response indicates that androgen regulation of IGF-1 gene expression is likely mediated through AR, not a non-genomic mechanism. However, inhibition of matrix metalloproteinases 2 and 9 (**MMP2/9**), a vital component of the non-genomic mechanism, leads to the suppression of IGF-1 receptor (**IGF-1R**) induced proliferation in bovine satellite cells (Thornton et al., 2015). Additionally, TBA-stimulated bovine satellite cell proliferation was impeded due to the use of a G protein-coupled receptor

inhibitor (Thornton et al., 2015). Therefore, G protein-coupled receptors are vital to cell proliferation and the steroidal implant growth response for $E_2$ and androgen hormones.

Following hormone activation of these G protein-coupled receptors, a cascade of responses ensues, increasing the production of intracellular secondary messengers such as cyclic adenosine monophosphate (**cAMP**; Filardo and Thomas, 2005). These secondary messengers result in the activation of the signaling proteins protein kinase A, protein kinase C, and MAPK (Heinlein and Chang, 2002). Among the activated proteins, MMP2/9 cleave heparin-binding epidermal growth factor-like growth factor (**hbEGF**) from the cell membrane (Dempsey et al., 1997; Dethlefsen et al., 1998) resulting in the binding of cleaved hbEGF to epidermal growth factor receptor (**EGFR**) and the activation of downstream pathways that induce cell proliferation (Prenzel et al., 1999; Filardo et al., 2000; Thornton et al., 2015). This mechanism of action appears conserved across $E_2$ and androgen steroid hormones. In breast cancer cells specifically transfected with GPER-1, the inhibition of hbEGF activity with CRM-197 resulted in a decrease in EGFR, extracellular-signal-regulated kinase 1 and 2 (**ERK-1/-2**), and MAPK activity when treated with $E_2$ (Filardo et al., 2000). Furthermore, Thornton et al. (2015) found the inhibition of MMP2/9 in TBA treated bovine satellite cells impeded cell proliferation mediated through EGFR. These data indicate ERK-1/-2 and MAPK activation and their subsequent response on cell proliferation are mediated through EGFR and are dependent upon MMP2/9 cleavage of hbEGF (Filardo et al., 2000; Thornton et al., 2015). Although the exact mechanism by which steroidal implants elicit their growth response still needs refinement, these data suggest non-genomic signaling through G protein-coupled receptors elicits a rapid response to steroidal implants, induced growth.

## Interactions Between Growth Processes and Zinc

Throughout this literature review Zn (Ott et al., 1965; Perry et al., 1968; Mayland et al., 1980) and steroidal implants (Bartle et al., 1992; Johnson et al., 1996a; Duckett and Pratt, 2014) have both been implicated in supporting growth. Interestingly, many of the mechanisms by which Zn influences growth overlap with mechanisms identified within the steroidal implant growth pathway (Ninh et al., 1996; Wu et al., 2004; Hamza et al., 2012; Pisano et al., 2017). This section will detail the interactions between Zn and proteins within the steroidal implant pathway including IGF-1 and hormone receptors. Furthermore, the effects of Zn supplementation on implant-induced growth and effects of steroidal implants on Zn metabolism will be explored herein.

### Insulin-like Growth Factor-1

Although Zn supplementation has been shown to improve growth rates in rats (Oberleas and Prasad, 1969), links between Zn and growth pathways are emerging. An interaction is likely between Zn and the GH-IGF system, considering its significant role in regulating growth (Underwood et al., 1994). As discussed earlier, steroidal implants increase gene expression in the muscle and circulating IGF-1 concentrations (Johnson et al., 1998b; Johnson et al., 1998a; Kamanga-Sollo et al., 2004; Kamanga-Sollo et al., 2008). Of note, IGF-1 stimulates protein synthesis through the phosphorylation of AKT and the subsequent activation of mTOR and glycogen synthase kinase 3 beta pathways and hinder protein degradation in skeletal muscle (Yoshida and Delafontaine, 2020). Furthermore, IGF-1 hinders protein degradation in skeletal muscle by inhibiting the transcription factor FoxO (Yoshida and Delafontaine, 2020). Due to the many targets of IGF-1, most circulating IGF-1 is bound by an IGF binding protein (**IGFBP**), such as IGFBP-3, that prolongs the half-life of IGF-1 (Allard and Duan, 2018). These binding proteins guard against unwarranted IGF-1 stimulation (Duan and Clemmons, 1998) and indicate

measurements of circulating IGF-1 are likely correlated with circulating IGFBP such as IGFBP3. This correlation will be important in interpretation of the data forthcoming.

Much of the work detailing the effects of Zn on IGF-1 have been in Zn deficient children along with some research conducted with rodent models. Both Cesur et al. (2009) and Hamza et al. (2012) observed serum IGF-1 and IGFBP-3 below the normal reference range in over 94% of Zn deficient children considered short for their age group. However, rats fed very low (< 1 mg Zn/kg) or low concentrations of Zn (7 mg Zn/kg) had no differences in serum IGF-1 or IGFBP-3 compared with control rats (25 mg Zn/kg; Hall et al., 2005). Furthermore, supplementation of Zn increased the height and weight of previously Zn deficient children (Ninh et al., 1996), while Hamza et al. (2012) only observed an improvement in the height of Zn supplemented children. Differences may have been attributed to the difference in the period of Zn supplementation as Ninh et al. (1996) supplemented for 5 mo compared to only 3 mo by Hamza et al. (2012). Interestingly, Hamza et al. (2012) fed nearly 5 times more Zn per day to children than Ninh et al. (1996).

However, circulating concentrations of IGF-1 and/or IGFBP-3 increase with Zn supplementation (Nishi et al., 1989; Nakamura et al., 1993; Ninh et al., 1996; Hamza et al., 2012). Rats fed low concentrations of Zn (7 mg Zn/kg) had greater liver IGF-1 and IGFBP-3 gene expression than rats fed the control diet containing 25 mg Zn/kg (Hall et al., 2005). However, in this study, serum glucose concentrations were significantly greater for rats fed < 1 mg Zn/kg than rats pair-fed the control diet (25 mg Zn/kg; Hall et al., 2005). Indeed, Zn supplementation influences IGF-1 gene expression and likely influences supplemented subjects' downstream growth and energy metabolism. Although these relationships between Zn supplementation and IGF-1 are intriguing, the severely Zn deficient models used in the studies

above are unlikely to be readily observed in beef cattle. However, these data greatly contribute to our understanding of the apparent interactions between Zn and IGF-1 signaling and suggest body Zn concentrations and supplemental Zn influence IGF-1 gene expression and circulating concentrations. Therefore, Zn supplementation may work synergistically with steroidal implants to positively affect growth through IGF-1 signaling.

**Hormone Receptors**

Though Zn has been implicated in many downstream pathways of hormone receptors, some literature suggests these responses start with the receptors themselves. The proposed relationships between Zn and the steroidal implant mechanism are detailed in **Figure 1**. Specifically, GPER-1 is directly influenced by Zn treatment. In human breast cancer cells, Zn was shown to phosphorylate EGFR and IGF-1R leading to the downstream activation of ERK and AKT in a dose-dependent manner (Pisano et al., 2017). These data agree with previous studies that have observed increased phosphorylation of EGFR due to Zn supplementation (Wu et al., 1999; Wu et al., 2002; Samet et al., 2003). Interestingly, Pisano et al. (2017) found the inhibition of GPER-1 halted the Zn-induced phosphorylation of EGFR and IGF-1R as well as the downstream activation of ERK and AKT. Therefore, these data indicate Zn-induced actions were mediated through GPER-1.

However, Zn may have additional functions within this pathway. Treatment of normal human bronchial epithelial cells with Zn resulted in increased shedding of hbEGF from EGFR, resulting in EGFR phosphorylation (Wu et al., 2004). Phosphorylation of specific tyrosine kinase residues is vital to EGFR downstream signaling by promoting binding of the receptor by proteins that lead to cell proliferation and migration, among other aspects of growth (Wells, 1999). Zinc supplementation has been observed to increase phosphorylation at the autophosphorylation sites Tyr1068 and Tyr1173, as well as Tyr845, a known transphosphorylation site (Samet et al.,

2003). Furthermore, testing the mechanism by which hbEGF is released, Wu et al. (2004) utilized an MMP inhibitor and observed a decrease in Zn but not epidermal growth factor-

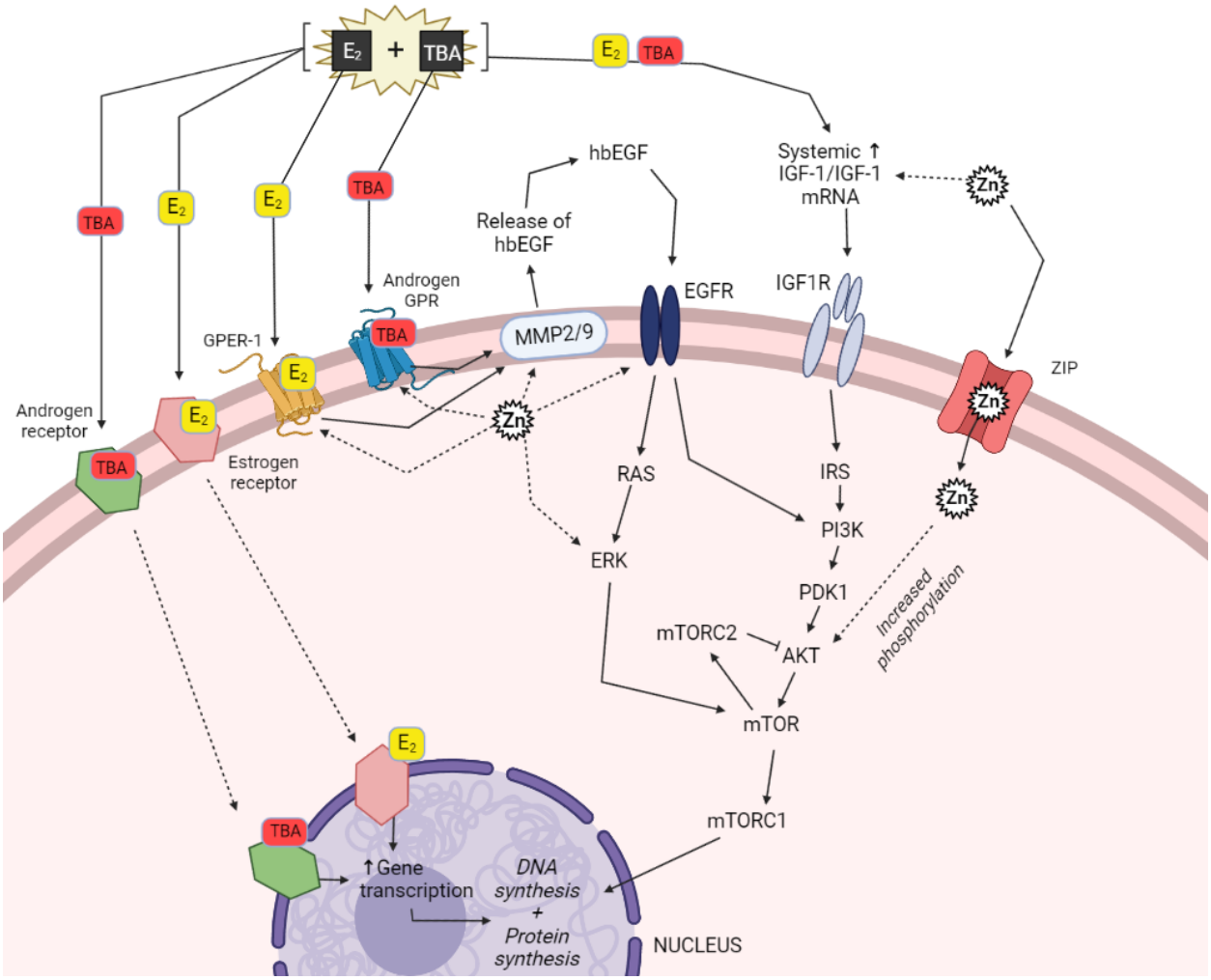


**Figure 1**. The proposed mechanism by which Zn influences the effects of steroidal hormones adapted from (Messersmith et al., 2021).

induced EGFR phosphorylation. Matrix metalloproteinases, Zn-dependent enzymes (Pardo and Selman, 2005; Krane and Inada, 2008), are known for their role in cleaving hbEGF (Dempsey et al., 1997; Dethlefsen et al., 1998). Further investigation implicated MMP3 as the responsible enzyme due to an association between the release of hbEGF and an increase in free MMP3 in normal human bronchial epithelial cells treated with Zn (Wu et al., 2004). However, MMP2/9

have been implicated in this release of hbEGF in muscle (Thornton et al., 2015), kidney (Dempsey et al., 1997), prostate (Dethlefsen et al., 1998), and cancer cells (Prenzel et al., 1999; Filardo et al., 2000). It is likely many isoforms of MMP cleave hbEGF from the cell membrane. Therefore, Zn appears to influence growth in several tissues through MMP cleavage of hbEGF.

Although Zn has not been directly related to androgen receptor function like the previously discussed $E_2$ receptor, a role for Zn in androgen signaling is emerging. Interestingly, the Zn transporter ZIP9 was discovered as a membrane androgen receptor that mediates non-genomic androgen signaling in fish (Berg et al., 2014) and human breast and prostate cancer cells (Thomas et al., 2014). The direct mechanism of action is not clear. However, ZIP9 has been observed to activate G proteins and increase intracellular concentrations of cAMP when cells were treated with testosterone (Berg et al., 2014; Converse and Thomas, 2019). Additionally, testosterone binding appears to activate the Zn transporter function of ZIP9 as intracellular concentrations of Zn were increased in cultured granulosa and theca cells treated with testosterone (Berg et al., 2014). Knocking ZIP9 out provided additional evidence of testosterone's effects on ZIP9 function, as Zn transport was mitigated by the knockout (Berg et al., 2014; Converse and Thomas, 2019). However, Converse and Thomas (2019) found intracellular Zn concentrations and incidence of apoptosis mediated by ZIP9 were dependent upon the stage of growth in granulosa and theca cells. Testosterone treated early-stage follicles exhibited less apoptosis and lesser concentrations of intracellular Zn and cAMP (Converse and Thomas, 2019). In contrast, late-stage follicles had increased intracellular Zn and cAMP concentrations and increased incidence of apoptosis mediated through testosterone binding of ZIP9 (Converse and Thomas, 2019). It appears Zn may play a dynamic role in cellular signaling

dependent upon the stage of growth. However, more work is warranted to fully relate the impacts of ZIP9 as an androgen receptor to muscle growth in cattle utilizing steroidal implants.

**Cattle Growth**

Although updated in 2016 (NASEM, 2016), trace mineral recommendations have changed little over the past 40 years (NRC, 1984). However, supplementation rates for trace minerals across the beef industry are commonly 2 to 3 times the NASEM (2016) recommendations (Samuelson et al., 2016a). Niedermayer et al. (2018) supplemented Zn, Cu, Mn, Se, and Co from inorganic sources at 2 to 3 times NASEM (2016) recommendations resulting in improved gain and hot carcass weight over non-supplemented and NASEM supplemented steers regardless of implant administration. In this study, due to feeding greater concentrations of multiple trace minerals simultaneously, it is difficult to determine the growth performance-enhancing effects of individual trace minerals. However, increasing trace mineral supplementation did improve the growth rates of feedlot cattle. While the impact of Zn on growth performance in this particular study cannot be pinpointed, the role Zn plays in growth, and numerous other physiological and biochemical pathways, make it a suitable candidate for follow-up studies. **Table 1** outlines several studies detailing the effects of steroidal implants on growth performance and Zn metabolism.

Indeed, Zn supplementation influences the steroidal implant-induced performance of cattle. When 200 mg Zn/kg DM from $ZnSO_4$ was fed to heifers and steers steroidal implant administration improved ADG by more than 17% (Huerta et al., 2002). However, Zn source may influence this response as supplementation of 200 mg Zn/kg DM from Zn-methionine resulted in 26% lesser gains for implanted heifers than non-implanted heifers and no differences in ADG between implanted and non-implanted steers (Huerta et al., 2002). Both the steer and heifer studies fed high grain diets typical for finishing phase beef feedlot cattle in the U.S. It is not

**Table 1.** Literature examining steroidal implants and zinc metabolism in cattle adapted from Messersmith et al. (2021).

| Ref. | Sex | Steroidal implant[1] | Potency[2] | Supplemental Zn[3] | Day[4] | $\Delta^5$ ADG, % | $\Delta^5$ Plasma Zn, % | $\Delta^5$ Liver Zn, % |
|---|---|---|---|---|---|---|---|---|
| Huerta et al., 2002 | Steer | 200 mg P + 20 mg EB | Moderate | 0 | 59 | +7.6 | +1.8 | -26.1 |
| | | | | 200[a] | 59 | +17.4 | +3.2 | +30.7 |
| | | | | 200[b] | 59 | -- | -15.0 | +8.5 |
| Huerta et al., 2002 | Heifer | 200 mg TP + 20 mg EB | Moderate | 0 | 50 | +11.0 | +7.0 | -38.2 |
| | | | | 200[a] | 50 | +17.0 | +24.7 | +25.4 |
| | | | | 200[b] | 50 | -26.0 | -2.5 | -40.8 |
| Dorton et al., 2010 | Steer | 200 mg P + 20 mg EB (Initial) | Moderate | 360[d] mg·steer$^{-1}$·d$^{-1}$ | 28 | -- | -4.8 | +12.2 |
| | | 80 mg TBA + 16 mg $E_2$ | Moderate | 360[d] mg·steer$^{-1}$·d$^{-1}$ | 56 | -- | -7.7 | +4.7 |
| Messersmith, 2018 | Steer | 200 mg TBA + 20 mg $E_2$ | High | 30[a] | 13/14 | +29.0 | -11.2 | -6.6 |
| Niedermayer et al., 2018 | Steer | 200 mg TBA + 20 mg $E_2$ | High | 0 | 69 | -- | -- | +10.6 |
| | | | | 30[a] | 69 | -- | -- | +11.0 |
| | | | | 100[a] | 69 | -- | -- | +22.9 |
| Reichhardt et al., 2021 | Steer | 200 mg TBA | High | 55[c] | 2 | -- | -4.3 | -16.2 |
| | | 120 mg TBA + 24 mg $E_2$ | Moderate | 55[c] | 2 | -- | -8.5 | -16.2 |

[1]Steroidal implant hormone concentrations are listed using the following abbreviations: $E_2$: estradiol, EB: estradiol benzoate, P: progesterone, TBA: trenbolone acetate, TP: testosterone propionate. All steroidal implants were given as the terminal implant, unless otherwise noted as initial implant.

[2]Steroidal implant potency is arbitrarily categorized as low, moderate, or high based on hormone combination and dose.

[3]Supplemental Zn is expressed as mg/kg DM, unless otherwise stated. Source of Zn supplemented is represented by superscript: [a] inorganic, [b] organic, [c] blend of inorganic and organic, [d] means of inorganic and organic Zn supplementation were combined due to no source differences.

[4]Represents day relative to administration of the steroidal implant used in each study.

[5]The percent delta (Δ) represents the effect of steroidal implant compared to non-implanted cohorts on growth and Zn metabolism parameters on the noted day post-steroidal implant administration. Changes in plasma Zn concentrations for Huerta et al., 2002 represent changes in serum Zn concentrations.

apparent why the Zn source would result in such drastic differences in implant-induced performance between studies as well as between steers and heifers when diet type and days on feed were similar between studies. Perhaps, too much supplementation of a highly bioavailable Zn source is cause for detrimental performance, though Zn concentrations fed by Huerta et al. (2002) were far from toxic. Follow-up work to answer these questions has not been conducted to the authors' knowledge.

Though few studies have been conducted to understand performance responses to supplemental Zn within implanted and non-implanted cattle, other growth technologies may help link Zn and growth. Genther-Schroeder et al. (2016) observed a positive linear performance response due to increasing Zn supplementation up to 180 mg Zn/kg DM in steers receiving a beta agonist, while no performance response to Zn supplementation was observed within cattle not receiving the beta agonist. These data suggest cattle experiencing high growth rates, such as that induced by beta agonists, may have a greater demand for Zn. Furthermore, the timing of Zn supplementation as it relates to the administration of a growth promoting technology may be vital to capturing a growth response to Zn supplementation. Peak payout of hormone from steroidal implants occurs within 40 d of implant administration (Johnson et al., 1996a), resulting in a period with the greatest growth potential for the implanted animal. Therefore strategic supplementation of increased Zn may be beneficial to growth performance during periods of peak hormonal payout or other times of high growth rates. Furthermore, research is warranted to determine how increasing supplemental Zn prior to a growth event influences Zn's ability to support growth through protein synthesis machinery.

**Zinc Metabolism**

Changes in liver and plasma Zn concentrations provide compelling evidence of modified physiological Zn requirements due to steroidal implants. In lambs implanted with zeranol (12

mg), Zn retention was increased by 60% (Hufstedler and Greene, 1995). A positive correlation between Zn and N retention in feedlot steers (Carmichael et al., 2018) corroborates a connection between growth and Zn requirements.

In recent studies (Table 1) implant administration has been observed to alter Zn requirements as assessed through plasma and liver Zn concentrations (Huerta et al., 2002; Dorton et al., 2010; Messersmith, 2018; Niedermayer et al., 2018; Reichhardt et al., 2021). Messersmith (2018) found implanted steers decreased plasma Zn concentrations by 11.2% on d 13 post-administration of a high potency steroidal implant coinciding with a 29% greater ADG in implanted steers during the first 14 d post-implant. Interestingly, dietary Zn concentrations were well above NASEM (30 mg Zn/kg DM; 2016) recommendations (61 mg Zn/kg DM), indicating the effect on plasma Zn due to implant was minimally influenced by dietary Zn concentrations. Plasma Zn concentrations of implanted steers remained lesser than non-implanted steers through d 73 of the study (Messersmith, 2018).

In contrast, Huerta et al. (2002) observed an increase in serum Zn concentrations throughout the 120-d study in heifers implanted with two moderate potency implants, though Zn supplementation was relatively high. Fifty d following the first implant, serum Zn was relatively unchanged by implant in heifers fed 0 or 200 mg Zn/kg DM from Zn-methionine, but increased nearly 25% in heifers fed 200 mg Zn/kg DM from $ZnSO_4$ (Huerta et al., 2002). However, 14 d post-administration of the second implant, serum Zn concentrations for heifers supplemented 0 or 200 mg Zn/kg DM from Zn-methionine appeared to decrease 6.0 and 9.3% due to implant administration, respectively (Huerta et al., 2002). Yet, these data differ from steers administered a similar steroidal implant and fed the same dietary Zn treatments. In the companion study utilizing steers, Huerta et al. (2002) observed no differences in serum Zn due to implant

administration. Sex differences may have driven these different responses. Utilization of intact heifers, regardless of melengestrol acetate supplementation to suppress estrus, presents many challenges when assessing the effects of steroidal implants. Mainly, endogenous $E_2$ cannot be quantified and accounted for in a controlled study. Therefore, the effects of steroidal implants on Zn metabolism in heifers may not be as reliable as in steers or spayed heifers. Although steers generally have greater growth potential than heifers, the implant-induced growth response observed by Huerta et al. (2002) for steers and heifers was relatively similar. This further complicates why heifers observed an implant-induced decrease in serum Zn concentrations while steers did not.

In addition to circulating Zn concentrations, steroidal implants have been found to influence concentrations of Zn in the liver. As previously mentioned in the Zn section, liver Zn concentrations are more difficult to change than plasma due to the less labile nature of Zn stored in the liver. Steers administered a two-implant strategy utilizing moderate potency implants had greater liver Zn concentrations 59 and 74 d post-implant administration than non-implanted controls when supplemented 200 mg Zn/kg DM from $ZnSO_4$ or Zn-methionine (Huerta et al., 2002). In comparison, steers fed no supplemental Zn had decreased liver Zn concentrations due to implant at the same time points (Huerta et al., 2002). This response may have been driven by the bulk supplementation of Zn increasing Zn storage. However, it is interesting that non-supplemented steers observed an opposite response considering the high concentration of Zn in the basal diet (84 mg Zn/kg DM). Perhaps, high dietary Zn concentrations replenished Zn storage before decreases in liver Zn were detected.

While this has not been studied extensively, work by Niedermayer et al. (2018) observed an increase in liver Zn concentrations 70 d post-administration of a high potency steroidal

implant in steers. An increase in Zn absorption and retention in sheep implanted with a low potency zeranol implant observed by Hufstedler and Greene (1995) suggests that this increase in liver Zn concentrations may result from upregulated Zn absorption due to steroidal implant administration. However, Dorton et al. (2010) observed no liver Zn response 28 and 56 d post-administration of a moderate potency implant at the beginning of the growing and finishing phase, respectively, in steers fed diets above NASEM (2016) Zn recommendations (45 to 51 mg Zn/kg DM). Even though the sheep utilized by Hufstedler and Greene (1995) were only implanted with a low potency implant, it can be argued that zeranol is a high potency implant for sheep due to differences in growth rates between sheep and cattle. Together these data provide preliminary evidence that steroidal implant potency in relation to the growth potential of the animal may influence liver Zn concentrations and absorption.

In conjunction with the plasma Zn response reported earlier, Messersmith (2018) observed a decrease in liver Zn concentrations 14 d post-administration of a high potency implant, though this effect was not evident by d 62. Interestingly, Reichhardt et al. (2021) observed liver Zn concentrations in steers were decreased within 2 d of administration of 3 different moderate potency steroidal implants. These studies suggest the effect of steroidal implants on Zn metabolism is rapid, regardless of implant potency, with differences observed within 2 to 14 d of implant administration.

These data indicate changes in plasma Zn concentrations may be more consistent than liver Zn concentrations due to steroidal implant administration. Although the detected differences in Zn concentrations remained within the wide reference ranges (Kincaid, 2000) for plasma (0.8–1.4 mg/L) and liver (25 to 200 mg/kg DM), the corresponding performance differences suggest these changes are likely physiologically relevant. Interestingly, the observed

changes in plasma Zn concentrations occurred within the aforementioned peak hormonal payout period of the steroidal implant (first 40 d post-implant administration; Johnson et al., 1996a) and were associated with implant-induced improvements in growth (Messersmith, 2018). Similarly, an increased rate of gain in children recovering from malnourishment was correlated with lesser plasma Zn concentrations (Golden and Golden, 1981), indicating circulating Zn is critical to support the growth response and may be an applicable biomarker of growth. Moreover, these Zn responses were more prominent in cattle receiving high potency steroidal implants. Indeed, these data indicate steroidal implants influence Zn metabolism. However, variables such as hormone potency, dietary Zn concentrations, and assessment of liver or plasma Zn concentration timing must be examined to further our understanding of steroidal implants' effects on Zn metabolism and subsequent requirements of high growth rate cattle.

## Conclusions and Future Directions

Strategic supplementation of trace minerals, including Zn, to cattle based upon expected performance and management practices such as steroidal implant use appears to be the future of cattle feeding. This literature review details the multi-faceted interactions between Zn and steroidal implants on growth ranging from effects on hormone receptors to Zn status and reveals many aspects that need further evaluation. There is immense opportunity to capture additional growth in implanted cattle through strategic supplementation of Zn in the diet. Currently, the available literature suggests increased Zn supplementation improves steroidal implant-induced growth. However, it is unclear how much Zn should be supplemented to capitalize on the genetic potential and implant-induced growth rates of cattle.

Furthermore, optimal timing of Zn supplementation is unknown. While increased energy and protein requirements are commonly associated with steroidal implant-induced growth, micronutrients such as trace minerals should also be accounted for during periods of high growth

rates. Therefore, phase feeding of greater concentrations of Zn during peak hormonal payout or other periods of high growth rates may optimize growth. Furthermore, supplementation of all trace minerals at greater concentrations should be evaluated to determine the first limiting trace mineral and build a strategic trace mineral supplementation strategy. In addition to determining the optimal concentration and timing of Zn supplementation for high growth animals, understanding how steroidal implants impact Zn absorption is vital to determining Zn supplementation strategies. This review of the literature indicates the effects of steroidal implants on Zn absorption have only been evaluated in lambs. Replication of this work in cattle of both sexes utilizing varied potency of steroidal implants is necessary to ascertain how different steroidal implants influence Zn and other trace minerals' absorption. Upon understanding the effects of steroidal implants on Zn absorption, Zn source should be further evaluated in implanted cattle to assess the use of more bioavailable Zn sources as a viable Zn supplementation strategy to accommodate implant-induced growth rates. Lastly, roles for Zn in growth processes and signaling appears to be a growing area of discovery. Understanding how Zn influences hormone signaling and protein synthesis is critical to deepening our understanding of the many influential roles of Zn in growth.

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# CHAPTER 3. THE EFFECTS OF INCREASING SUPPLEMENTAL ZINC TO NON-IMPLANTED AND IMPLANTED FEEDLOT BEEF STEERS ON PERFORMANCE, CARCASS CHARACTERISTICS, TRACE MINERAL CONCENTRATIONS, AND MUSCLE GENE EXPRESSION

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## Abstract

The effects of supplemental Zn within implant strategy on performance, carcass characteristics, trace mineral status, and muscle gene expression were tested in a 59-d study using 128 Angus-crossbred steers (492 ± 29 kg) in a 2 × 4 factorial. Implant strategies included no implant (**NoIMP**) or Component TE-200 (**TE200**; Elanco, Greenfield, IN) administered on d 0. Zinc was supplemented at 0, 30, 100, or 150 mg Zn/kg dry matter (**Zn0**, **Zn30**, **Zn100**, **Zn150**, respectively) from $ZnSO_4$. Steers were stratified by body weight (**BW**) to pens (*n* = 5 or 6 steers/pen) equipped with GrowSafe bunks (GrowSafe Systems Ltd., Airdrie, AB, Canada) and assigned to pen-wide treatments (*n* = 15, 16, or 17 steers/treatment). Cattle were weighed on d -1, 0, 18, and 59 with blood collected on d -1, 18, 40, and 59. Muscle samples were collected from the longissimus thoracis on d 11 and liver samples collected on d 55 or 56. Data were analyzed using the Mixed Procedure of SAS via contrast statements testing the linear and quadratic response to Zn supplementation within implant treatment and NoIMP vs. TE200 for performance, carcass, blood, and liver parameters. Specific contrast statements were formed for

the analysis of gene expression in muscle including: Zn0 vs. Zn150 within NoIMP and TE200, NoIMP vs. TE200 (Zn0 and Zn150 only), and the linear effect of supplementing Zn0, Zn100, and Zn150 within TE200. Steer was the experimental unit. Day 18 BW and d 0-18 average daily gain (**ADG**) were linearly increased due to Zn supplementation within TE200 ($P \leq 0.002$) in conjunction with a linear increase from Zn in d 11 muscle epidermal growth factor receptor, matrix metalloproteinase 2, and phosphodiesterase 4B gene expression of TE200 steers ($P \leq 0.05$). Plasma Zn on d 18 and 40 linearly increased with increasing Zn supplementation regardless of implant treatment ($P \leq 0.03$) and was lesser for TE200 than NoIMP steers on d 18 ($P = 0.001$). Day 59 BW and hot carcass weight (**HCW**) were greater for TE200 vs. NoIMP ($P \leq 0.002$) and HCW of implanted steers tended to linearly increase with increasing Zn supplementation ($P = 0.09$). No effects of Zn supplementation were observed in NoIMP for HCW, BW, or ADG ($P \geq 0.17$).Yield grade and 12$^{th}$ rib fat tended to quadratically decrease within NoIMP ($P \leq 0.09$) with Zn100 being most lean. These data indicate increasing supplemental Zn influences steroidal implant signaling machinery while increasing the Zn status and steroidal implant-induced growth of beef feedlot cattle.

Keywords: cattle, steroidal implant, zinc

## Introduction

Steroidal implants have been commonly used in U.S. beef production since the 1950s (R.L. Preston, 1999), resulting in improvements in the average daily gain (**ADG**) of cattle by 16 to 20% (Bartle et al., 1992; Duckett and Pratt, 2014). This growth response is thought to be elicited through genomic and nongenomic modes of action that regulate gene transcription (Yen, 2015) and stimulate growth processes through secondary messengers (Heinlein and Chang, 2002;

Filardo and Thomas, 2012), respectively. Zinc may augment steroidal implant signaling. For example, Zn acts on the nongenomic G protein-coupled estrogen receptor (**GPER1**) to phosphorylate epidermal growth factor receptor (**EGFR**) and insulin-like growth factor-1 receptor (**IGF1R**) in a dose-dependent manner (Pisano et al., 2017), leading to the activation of growth processes. Zinc supports transcription factors, deoxyribonucleic acid, and protein synthesis (Oberleas and Prasad, 1969; Duncan and Dreosti, 1976; Cousins et al., 2006a), all vital components of growth. Steroidal implants appear to influence plasma Zn concentrations as Messersmith (2018) found steers implanted with a high potency implant had lesser plasma Zn concentrations throughout the 73-d finishing period than non-implanted counterparts. Furthermore, the steroidal implant, zeranol, increased Zn absorption and retention in lambs (Hufstedler and Greene, 1995) and the supplementation of 200 mg Zn/kg dry matter (**DM**) basis from $ZnSO_4$ to steers resulted in a greater ADG response to a potent steroidal implant than steers receiving a control diet that exceeded NASEM (2016) Zn recommendations (Huerta et al., 2002). These data suggest administration of steroidal implants may increase Zn requirements of the animal to accommodate increased growth rates and greater supplementation of Zn may promote implant-induced growth. Therefore, the objective of this study was to determine the effects of increasing dietary Zn supplementation within implanted and non-implanted steers on performance, carcass characteristics, liver and plasma Zn concentrations, and the expression of genes associated with growth and Zn metabolism. It was hypothesized that Zn supplementation would be most beneficial to growth and carcass parameters within implanted steers.

## Materials and Methods

### Care and Use of Animals

All procedures and protocols utilized in this study were approved by the Iowa State University Institutional Animal Care and Use Committee (log number: IACUC-20-053).

### Animals and Experimental Design

One hundred and twenty-eight Angus-crossbred steers (492 ± 29 kg) were utilized in a 2 × 4 randomized design to test the effect of Zn supplementation within implant treatment. Implant treatments included no implant (**NoIMP**) or a Component TE-200 (**TE200**; 200 mg trenbolone acetate + 20 mg estradiol; donated by Elanco Animal Health, Greenfield, IN) administered on d 0. Cattle were fed 0, 30, 100, or 150 mg Zn/kg DM (**Zn0**, **Zn30**, **Zn100**, or **Zn150**, respectively) from $ZnSO_4$ starting on d 0. Cattle were stratified by body weight (**BW**) into pens ($n$ = 5 or 6 steers per pen) equipped with GrowSafe (GrowSafe Systems Ltd., Airdrie, AB, Canada) bunks and fed ad libitum. Radio frequency tags on each steer relayed individual steer feed disappearance data from the bunk to GrowSafe software. Cattle were randomly assigned to implant and supplemental Zn treatments within pen ($n$ = 3 pens/treatment). All cattle within a pen received the same implant and supplemental Zn treatment, and individual intake and performance data were collected for each steer. Therefore, steer was the experimental unit. Prior to the start of the experiment all steers received a corn silage based growing diet supplemented with 30 mg Zn/kg DM from $ZnSO_4$. Steers were fed once daily (0800 h) and transitioned to a dry-rolled corn-based diet during the first 14 d of the experiment and remained on the finishing diet through the remainder of the 59-d study (**Table 1**). Cattle were harvested on d 61 at a commercial abattoir (Greater Omaha Beef, Omaha, NE) via industry accepted practices. Trained Greater Omaha Beef personnel collected hot carcass weight (**HCW**) while ribeye area

(**REA**), 12th rib fat (**BF**), marbling, and yield grade (**YG**) were recorded by a camera system following a 48-h chill.

**Sample Collection and Analysis**

Total mixed ration (**TMR**) samples of each treatment were collected weekly to calculate DM by drying in a forced air oven for 48 h at 70ºC. Dried TMR samples were ground through a 2-mm screen (Retsch Zm100 grinder; Glen Mills Inc., Clifton, NJ) and composited by month within each dietary Zn treatment. The composited Zn0 TMR was sent to Dairyland Laboratories (Arcadia, WI) for nutrient analysis (Heiderscheit and Hansen, 2020). Body weights were taken on d -1, 0, 18, 40, 55/56, and 59 ($n$ = 15, 16, or 17 steers/treatment). Blood was collected on d -1, 18, and 40 ($n$ = 8, 9, or 10 steers/treatment) via jugular venipuncture in vacuum capped tubes (Becton Dickerson, Rutherford, NJ) containing trace mineral grade $K_2$EDTA for plasma trace mineral analysis or sodium heparin for plasma urea nitrogen (**PUN**) analysis. Blood samples were stored at -20ºC until analysis was completed. A commercial kit (Teco Diagnostics, Anaheim, CA) was utilized to determine PUN concentrations with an intra-assay and inter-assay CV of 6.33% and 7.15%, respectively.

Muscle biopsies were conducted on 9 randomly selected sampler steers per treatment on d 11 utilizing procedures adapted from Pampusch et al. (2008). At timepoints split across two days, half of the cattle were biopsied on each day. Biopsies were extracted from the longissimus thoracis between the 10th and 13th rib space. Muscle samples were flash frozen in liquid nitrogen and stored at -80ºC prior to analysis.

Muscle samples from 5 of the 8 treatments (NoIMP: Zn0 and Zn150 and TE200: Zn0, Zn100, and Zn150) were analyzed for quantitative gene expression using the 48.48 Dynamic Array Integrated Fluidic Circuit (Fluidigm, San Francisco, CA) as described by Suasnavas et al. (2015). Prior to quantitative gene expression analysis, RNA was isolated from muscle samples

using Trizol Reagent (Life Technologies, Carlsbad, CA, USA) and cDNA was synthesized using random primers and Superscript III Reverse Transcriptase (Invitrogen, Waltham, MA, USA) in accordance with manufacturer's instructions (McGill et al., 2016). Twenty-four genes were targeted for analysis (**Table 2**) and primer sets for each gene were designed and validated by Fluidigm. In brief, a Specific Target Amplification was performed to enrich each sample for target-specific cDNA prior to quantitative gene expression analysis, in accordance with Fluidigm protocol. For STA thermal cycling, each reaction consisted of 1.25 µl of primer mix, 2.5 µl of the TaqMan PreAmp Master Mix (Applied Biosystems, Foster City, CA), and 1.25 µl of cDNA diluted 1:20 in nuclease free water. Enzyme activation took place at 95ºC for 10 min before amplification was conducted for 14 cycles (95ºC for 15 s then 60ºC for 4 min). The Fluidigm IFC chip was run on the Biomark thermocycler/detection module. The average expression of housekeeping genes eukaryotic translation elongation factor 1 alpha 2 (**EEF1A2**) and ribosomal protein S9 (**RPS9**) was utilized as a reference to determine relative gene expression of each parameter using the $2^{-\Delta\Delta Ct}$ method (Livak and Schmittgen, 2001). Relative gene expression was calculated relative to the NoIMP-Zn0 treatment.

Liver biopsies using procedures described by Engle and Spears (2000) were conducted on d 55/56 with half of the steers sampled on each day. Liver samples were stored at -20º C before analysis for trace mineral. Liver and composited TMR samples were acid digested following the procedures of Pogge and Hansen (2013) and Richter et al. (2012), respectively. Trace mineral concentrations for liver, TMR, and plasma were measured via inductively coupled plasma optical emission spectrometry (Optima 7000 DV, Perkin Elmer, Waltham, MA) as described by Pogge and Hansen (2013) and Richter et al. (2012). A standard was utilized on each run to verify instrument accuracy (Trace Elements Serum Control #66816; UTAK Laboratories Inc.,

Valencia, CA; Bovine Liver #1577c; National Institute of Standards and Technology, Gaithersburg, MD).

**Statistical Analysis**

Data were analyzed via the Mixed Procedure of SAS 9.4 (SAS Inst. Inc., Cary, NC) with the fixed effect of treatment. Contrast statements were formed to specifically test for linear and quadratic effects of Zn supplementation within NoIMP and TE200 treatments as well as to test NoIMP vs. TE200, regardless of Zn treatment. Steer served as the experimental unit for all analysis ($n$ = 15, 16, or 17 steers/treatment for performance and carcass parameters or $n$ = 8, 9, or 10 steers/treatment for blood and liver data). Initial BW was utilized as a covariate in all performance and carcass data analysis and initial plasma Zn and PUN concentrations served as covariates for plasma Zn and PUN analysis, respectively. Covariates were excluded from d 0 BW, d -1 plasma Zn, and d-1 PUN analysis. Because not all treatments were included in the assessment of relative gene expression in d 11 muscle samples additional contrast statements were formed. These statements included testing the effect of Zn0 vs. Zn150 within NoIMP and TE200 treatments, NoIMP vs. TE200 (Zn0 and Zn150 only), and the linear effect of Zn0, Zn100, and Zn150 within TE200. Outliers were assessed using the Cook's D statistical test with values above 0.20 removed from analysis. One steer was removed from performance and carcass parameters (NoIMP-Zn30) due to poor performance related to health concerns. Data are reported as the least squares means with the standard error of the mean, and statistical significance was determined as $P \leq 0.05$ with tendencies between $0.06 \leq P \leq 0.10$.

## Results

**Performance**

No differences in BW were observed on d 0 (**Table 3**; $P \geq 0.67$) between Zn treatments. However, d 18 BW, d 0-18 ADG, and d 0-18 feed efficiency (**G:F**) linearly increased as Zn

supplementation increased within TE200 ($P \leq 0.02$) and were greater for implanted steers than non-implanted steers ($P \leq 0.01$). Implanted steers also tended to have greater d 0-18 dry matter intake (**DMI**) than NoIMP ($P = 0.09$). Zinc supplementation tended to quadratically decrease d 0-18 DMI ($P = 0.09$) and quadratically increased d 0-18 G:F ($P = 0.05$) of NoIMP.

By the end of the trial (d 59), no linear or quadratic effects of Zn supplementation were observed for NoIMP or TE200 ($P \geq 0.17$) on live performance. However, TE200 were 8.25 kg heavier than NoIMP on d 59 ($P = 0.002$) and had greater d 0-59 ADG and G:F ($P \leq 0.02$). Although d 0-59 DMI was not influenced by Zn supplementation or implant treatment ($P \geq 0.15$), d 0-59 G:F quadratically increased within non-implanted steers ($P = 0.01$) with NoIMP-Zn100 having the greatest G:F.

Carcass-adjusted final BW, ADG, and G:F were greater for TE200 than NoIMP ($P \leq 0.0001$). A tendency for a linear increase due to Zn supplementation in carcass-adjusted final BW and ADG was observed in implanted steers ($P \leq 0.10$), while carcass-adjusted G:F quadratically increased within NoIMP ($P = 0.04$) with Zn100 having the greatest G:F.

**Carcass Characteristics**

Hot carcass weight tended to linearly increase (**Table 4**; $P = 0.09$), while dressing percentage linearly increased ($P = 0.01$) with increasing Zn supplementation within TE200. Implanted steers had greater HCW, dressing percentage, and REA ($P \leq 0.02$) than NoIMP. Both BF and yield grade tended to quadratically decrease with increasing Zn supplementation within NoIMP ($P \leq 0.09$) with Zn100 having lesser BF and YG than other Zn treatments. However, marbling was not influenced by Zn supplementation or implant treatment ($P \geq 0.17$).

**Trace Mineral and Plasma Urea Nitrogen Concentrations**

As expected, d -1 plasma Zn concentrations were not influenced by Zn or implant treatments (**Table 5**; $P \geq 0.16$). Day 18 and 40 plasma Zn concentrations linearly increased with

increasing Zn supplementation within both NoIMP and TE200 ($P \leq 0.03$). Furthermore, plasma Zn was lesser for implanted steers on d 18 than NoIMP ($P = 0.001$), but no effect of implant was observed for d 40 plasma Zn concentrations ($P = 0.49$).

Liver Zn, Cu, and Fe concentrations measured on d 55 or 56 were not affected by increasing Zn supplementation ($P \geq 0.15$) or implant treatment ($P \geq 0.20$). Liver Mn tended to be lesser for TE200 steers than NoIMP steers ($P = 0.10$) and was not affected by Zn supplementation ($P \geq 0.15$).

A tendency for a linear increase due to Zn supplementation within TE200 steers was observed for d -1 PUN concentrations ($P = 0.07$), though no other polynomial effects of Zn or differences been NoIMP and TE200 were observed for d -1 PUN ($P \geq 0.42$). By d 18, implanted cattle had lesser PUN concentrations than NoIMP ($P = 0.05$). Furthermore, a tendency for a negative quadratic response to Zn supplementation within NoIMP was apparent for d 40 PUN ($P = 0.06$) with Zn30 having the lowest PUN concentrations. At the end of the trial, a linear and quadratic response to Zn supplementation ($P \leq 0.05$) was observed for d 59 PUN concentrations within TE200 with Zn30 having the lowest PUN concentrations, though no differences in PUN concentrations were observed due to implant ($P = 0.16$).

**Gene Expression**

The relative gene expression of steroidal implant hormone receptors and signaling machinery are displayed in **Figure 1**. Neither androgen receptor (**AR**; **Fig. 1A**), estrogen receptor 1 (**ESR1**; **Fig. 1B**), or IGF1R (**Fig. 1C**) were influenced by Zn0 or Zn150 supplementation within NoIMP or TE200 treatments ($P \geq 0.17$), implant treatment ($P \geq 0.14$), or a linear response to 0, 100, or 150 mg Zn/kg DM supplementation within TE200 steers ($P \geq 0.26$). However, EGFR relative gene expression was greater for Zn150 than Zn0 within TE200 (**Fig. 1D**; $P = 0.03$). Furthermore, EGFR gene expression was greatest for implanted cattle ($P =$

0.02) and linearly increased with supplementation of 0, 100, and 150 mg Zn/kg DM within TE200 ($P = 0.05$).

Matrix metalloproteinase 2 (**MMP2**) tended to be greater for Zn150 than Zn0 within TE200 (**Fig. 1E**; $P = 0.10$) and linearly increased ($P = 0.04$) with increasing supplemental Zn at 0, 100, and 150 mg Zn/kg DM within TE200. Similarly, phosphodiesterase 4B (**PDE4B**) relative gene expression tended to be greater for Zn150 than Zn0 within TE200 steers (**Fig. 1F**; $P = 0.10$) but was lesser for implanted steers than NoIMP supplemented Zn0 or Zn150 ($P = 0.02$). However, PDE4B gene expression linearly increased with the supplementation of 0, 100, and 150 mg Zn/kg DM within TE200 ($P = 0.03$). The linear responses observed in both MMP2 and PDE4B gene expression were largely driven by high relative gene expression of Zn100-TE200 steers.

The relative gene expression of select genes involved in growth signaling are found in **Figure 2**. No effects of Zn or IMP were observed through contrast statements formed for gene expression for protein kinase B (**AKT1**; **Fig. 2A**), eukaryotic elongation factor 2 kinase (**EEF2K**; **Fig. 2B**), ribosomal protein S6 kinase B1 (**RPS6KB1**; **Fig. 2C**), mitogen-activated protein kinase 1 (**MAPK1**; **Fig. 2D**), or forkhead box O-3 (**FOXO3**; **Fig 2E**; $P \geq 0.17$). However, some markers of satellite cell development were influenced by implant strategy (**Figure 3**). Both myogenin (**MYOG**; **Fig. 3B**) and myogenic factor 5 (**MYF5**; **Fig. 3C**) were greater for Zn0 and Zn150 implanted steers than NoIMP counterparts ($P \leq 0.04$). No further differences in relative gene expression were observed for markers of satellite cell development ($P \geq 0.11$).

Within TE200, Zn150 tended to have greater relative gene expression of the Zn transporter solute carrier family 30 member 7 (**SLC30A7**) than Zn0 (**Fig. 4A**; $P = 0.07$) and

SLC30A7 gene expression tended to linearly increase with supplementation of 0, 100, and 150 mg Zn/kg DM within TE200 steers ($P = 0.06$). Furthermore, TE200 steers had lesser SLC30A7 relative gene expression than NoIMP ($P = 0.05$). Neither solute carrier family 39 member 7 (**SLC39A7**; **Fig. 4B**) or solute carrier family 39 member 14 (**SLC39A14**; **Fig. 4C**) relative gene expression were influenced by the tested contrast statements ($P \geq 0.11$).

## Discussion

Steroidal implants improve cattle ADG (Bartle et al., 1992; Duckett and Pratt, 2014) with the greatest potential for gain occurring in the first 40 d post-implant during peak hormonal payout of the implant (Johnson et al., 1996a). It was hypothesized Zn concentrations above NASEM (2016) recommendations may be needed to optimize this implant-induced growth because of Zn's role in a multitude of biological pathways supporting growth (Oberleas and Prasad, 1969; Duncan and Dreosti, 1976). Indeed, increasing supplemental Zn linearly increased ADG of implanted cattle within 18 d of implant administration, though no effects of Zn were observed for non-implanted steers at this time. By supplementing Zn, Cu, Mn, Se, and Co at 2 to 3 times NASEM (2016) recommendations, Niedermayer et al. (2018) observed improved gain and HCW over non-supplemented and NASEM (2016) supplemented steers. Due to Zn's roles in DNA and protein synthesis (Oberleas and Prasad, 1969; Duncan and Dreosti, 1976), this growth response is likely supported, at least in part, by increased Zn supplementation. Similar to the present study, others have suggested increasing Zn supplementation to cattle is beneficial to implant-induced growth. Huerta et al. (2002) found steers supplemented 200 mg Zn/kg DM from $ZnSO_4$ had a 9.8% greater growth response to a high potency steroidal implant than steers fed the control diet containing 84 mg Zn/kg DM. Furthermore, heifers implanted with a high potency two-implant strategy or an extended-release implant had greater BW on days corresponding to periods of peak hormonal payout of each implant strategy (Messersmith et al., 2021b).

Biopsies of the longissimus thoracis collected on d 11 during the early period of rapid growth post-implant administration allow for closer examination of growth processes that Zn may be affecting. Upon estrogen stimulation from a steroidal implant, GPER initiates non-genomic signaling through the cleaving action of MMP2 or 9 on heparin-binding epidermal growth factor-like growth factor and the subsequent activation of EGFR to trigger growth processes (Thornton et al., 2015). Pisano et al. (2017) found Zn acts through GPER to activate signaling through IGF1R and EGFR. Although GPER gene expression was undetected in the analysis of d 11 muscle samples, the linear response of EGFR relative gene expression to increasing Zn supplementation within implanted steers is aligned with the results of Pisano et al. (2017) and the linear increase in d 0-18 ADG of the current study. As a component of the non-genomic steroidal implant signaling mechanism, EGFR leads to the activation of downstream proteins such as extracellular-signal-regulated-kinase 1 and 2 (**ERK-1/-2**) that induce cell proliferation and migration (Wells, 1999; Filardo et al., 2000). Both ERK and AKT activation in human breast cancer cells have been observed with increasing Zn exposure (Pisano et al., 2017), indicating the effects of Zn supplementation on growth pathways are observed downstream of cell surface receptors. However, MAPK1 (representative of ERK-2) gene expression and AKT1 gene expression were not influenced by implant or Zn supplementation within implanted steers of the current study. This lack of response may be due to the role of AKT1 and MAPK1 in many growth signaling pathways, thereby diluting a Zn or implant response on these proteins. However, measurements at the protein level may be more reflective of Zn's effects on implant-induced signaling as Zn is associated with increased protein phosphorylation (Wu et al., 1999; Samet et al., 2003; Pisano et al., 2017). Furthermore, the small sampling size in the present study may have also contributed to these results.

Additionally, MMP2 and PDE4B are components of non-genomic implant-induced signaling (Shakur et al., 2001; Thornton et al., 2015). As a Zn-dependent enzyme, MMP2 cleaves hbEGF from the cell membrane to bind EGFR (Thornton et al., 2015), and it is interesting to note the linear response to Zn for MMP2 and EGFR gene expression and the correspondingly increased d 0-18 growth of implanted steers. As a regulator of G protein-coupled receptor signaling, PDE4B degrades intracellular cyclic adenosine monophosphate (**cAMP**) to inhibit downstream non-genomic signaling (Shakur et al., 2001). Gene expression of PDE4B is inhibited by Zn in cell culture (von Bulow et al., 2005). Therefore, it was hypothesized increasing Zn supplementation would decrease the relative gene expression of PDE4B within implanted steers in a dose dependent manner, leading to sustained cAMP signaling and increased growth rates. Indeed, implanted steers had lesser PDE4B gene expression than non-implanted steers. However, a linear increase in PDE4B relative gene expression with increasing Zn supplementation of implanted steers was observed, largely attributed to high Zn100 expression. It is uncertain why PDE4B gene expression would increase with Zn supplementation, though small sampling size may have exaggerated this response.

Interestingly, non-implanted steers supplemented 100 mg Zn/kg DM seemed to perform comparably to implanted steers throughout the trial and had numerically greater HCW than NoIMP counterparts. Therefore, supplementing 100 mg Zn/kg DM to non-implanted cattle may make up for the lost growth potential of not utilizing an implant. Although Zn supplementation did not affect d 59 BW, the early effects of Zn on implant-induced growth performance were apparent in HCW, and Zn linearly increased dressing percentage of implanted steers. While this trial was relatively short, 59 d, it provides valuable insight into the Zn requirements of late-stage beef feedlot cattle. With increasing days on feed, carcass transfer, the transfer of live gain to

HCW gain, increases (Macdonald et al., 2007). Similarly, Genther-Schroeder et al. (2016a) noted an increase in HCW of steers fed a beta agonist and supplemented 120 mg Zn/kg DM at the end of the finishing period. It is interesting to consider that as carcass transfer increases late in the feeding period, Zn may be particularly important to help capture live gain as carcass gain. However, serial slaughter studies are warranted to understand the full effects of supplemental Zn on carcass transfer across the finishing period.

Within the limited literature focused on Zn supplementation to cattle utilizing growth promoting technologies, the live growth response to supplemental Zn is inconsistently transferred to HCW. For example, the 7 kg BW advantage Messersmith et al. (2021) observed in implanted heifers supplemented 100 vs. 30 mg Zn/kg DM 48 d before harvest was not observed in HCW. Furthermore, Huerta et al. (2002) found no differences in HCW due to Zn supplementation of 0 or 200 mg Zn/kg DM in steers and heifers, even though ADG differed throughout the trial. Due to the greatest potential for Zn to influence implant-induced growth occurring within the early peak hormonal payout of the implant, Zn performance responses may be diluted by the end of the trial. Therefore, more statistical power appears necessary to pick up these subtle, yet impactful effects of Zn on HCW.

Plasma Zn concentrations are arguably the most reliable marker of transient Zn status in cattle. As expected, plasma Zn concentrations linearly increased with increasing Zn supplementation regardless of implant treatment on d 18 and 40. Interestingly, Zn100 and Zn150 plasma Zn concentrations were relatively similar and did not exceed 1.44 mg Zn/L. Considering the adequacy range of 0.8-1.4 mg Zn/L for plasma Zn concentrations proposed by Kincaid (2000), cattle Zn status was adequate to high. The negligible differences in plasma Zn concentrations between Zn100 and Zn150 suggest plasma Zn is highly regulated. To maintain

plasma Zn concentrations, the downregulation of intestinal Zn absorption (Sandström et al., 1980; Carmichael et al., 2019a) may combat this influx of dietary Zn. However, Zn absorption was not measured in the present study.

Plasma Zn concentrations were also influenced by steroidal implant administration, being lesser on d 18 in implanted steers. These data agree with the implant-induced decrease in plasma Zn concentrations observed by Messersmith (2018) 13 d post-implant administration. However, the depression in plasma Zn concentrations observed by Messersmith (2018) persisted through d 73, unlike the present study where d 40 plasma Zn was not affected by implant administration. The lesser plasma Zn concentrations of implanted vs. non-implanted steers is likely due to increased Zn demand in the muscle for growth processes (Oberleas and Prasad, 1969; Duncan and Dreosti, 1976) corresponding with the linear increase in d 0-18 performance and gene expression of implant signaling machinery such as EGFR and MMP2 of TE200 steers due to Zn supplementation. Furthermore, this implant-induced growth response appears to influence the Zn transporter SLC30A7 (ZnT7) that is important in uptake of cytosolic Zn by both the Golgi apparatus and sarcoplasmic reticulum (Kirschke and Huang, 2002; Tuncay et al., 2017).

Although Zn was supplemented up to 5 times the NASEM (2016) recommendation, no effects of Zn supplementation or implant administration were observed on d 55/56 liver Zn concentrations. Steroidal implants have been found to decrease liver Zn concentrations within 2 to 14 d of implant administration (Messersmith, 2018; Reichhardt et al., 2021), though implant administration did not affect liver Zn concentrations on d 62 (Messersmith, 2018). These early liver Zn responses likely correspond to the peak hormonal payout of the implant (Johnson et al., 1996a). In contrast, Niedermayer et al. (2018) observed greater liver Zn concentrations in implanted steers than non-implanted steers 69 d post-implant administration. Perhaps, in the

present study, liver samples collected closer to peak hormonal payout of the steroidal implant would have resulted in changes in liver Zn concentration.

In addition to plasma Zn, d 55/56 liver Mn and d 18 PUN concentrations were decreased due to steroidal implant administration. Although implants decrease liver Mn concentrations within 14 d of implant administration (Messersmith, 2018; Niedermayer et al., 2018; Messersmith et al., 2021b; Reichhardt et al., 2021), this effect appears to diminish with increasing days post-implant. However, Messersmith (2018) observed a tendency for liver Mn to remain depressed through d 62, similar to the time point of the current study. Interestingly, this liver Mn response is likely associated with the decrease in d 18 PUN due to implant administration. Steroidal implants decrease muscle degradation leading to downregulation of the urea cycle (Galbraith, 1980). This is consistent with observed decreases in PUN concentrations due to implants (Parr et al., 2014; Harris et al., 2020). Interestingly, the terminal enzyme of the urea cycle, arginase, is Mn dependent (J. S. Bond et al., 1983; Watts, 1990). Furthermore, PUN concentrations were abnormally high at the beginning of the study before substantially dropping by d 18 and the remainder of the study. This is inconsistent with our understanding of PUN, as PUN concentrations generally increase with days on feed (Wellmann et al., 2020). However, post hoc review of cattle intakes revealed low DMI on d -1 which may have contributed to high PUN.

Overall, these data indicate increasing supplemental Zn concentrations are beneficial to steroidal implant-induced growth, especially within the peak hormonal payout of the implant. Zinc appears to support gene expression of implant signaling machinery, such as EGFR and MMP2. The decrease in d 18 plasma Zn concentrations of implanted cattle indicate a greater demand for Zn to sustain implant-induced growth. These data imply implanted cattle have

different Zn demands than non-implanted cattle. Therefore, more work is warranted to further identify the signaling mechanisms by which Zn influences growth and determine the concentration of supplemental Zn to optimize steroidal implant-induced cattle performance.

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**Table 1.** Compositional analysis of non-zinc supplemented diet.

| Ingredient, % of diet DM | Transition 1 d 0-6 | Transition 2 d 7-13 | Finisher d 14-59 |
|---|---|---|---|
| Dry rolled corn | 30 | 45 | 57 |
| Sweet Bran[1] | 25 | 20 | 20 |
| DDGS[2] | 18.06 | 13.06 | 13.06 |
| Bromegrass hay | 5 | 8 | 8 |
| Corn silage | 20 | 12 | - |
| Limestone | 1.5 | 1.5 | 1.5 |
| Salt | 0.31 | 0.31 | 0.31 |
| Vitamin & mineral premix[3] | 0.1159 | 0.1159 | 0.1159 |
| Rumensin | 0.0135 | 0.0135 | 0.0135 |
| Analyzed composition, % | | | |
| Crude protein[4] | 16.6 | 13.1 | 13.9 |
| NDF[4] | 28.0 | 31.2 | 20.9 |
| Ether extract[4] | 5.5 | 5.0 | 4.4 |
| Zn[5], mg/kg DM | 48 | 40 | 42 |
| Cu[5], mg/kg DM | 17 | 13 | 12 |
| Calculated composition[6], Mcal/kg | | | |
| NEm | 1.97 | 1.98 | 2.05 |
| NEg | 1.32 | 1.33 | 1.39 |

[1]Branded wet corn gluten feed (Cargill Corn Milling, Blair, NE).

[2]Dried distillers grains with solubles.

[3]Premix provided 2,200 IU vitamin A and 25 IU vitamin E/kg diet. All diets included NASEM (2016) recommendations for Co, Cu, I, Mn, and Se. Diets were supplemented with Zn at 0, 30, 100, or 150 mg Zn/kg DM. All trace minerals were from inorganic sources.

[4]Analysis of Zn0 TMR was conducted by Dairyland Laboratories (Arcadia, WI).

[5]Trace minerals were analyzed by inductively coupled plasma optical emission spectrometry (ICP Optima 7000 DV, Perkin Elmer, Waltham, MA). Analyses were of Zn0 TMR. Analysis of Zn30, Zn100, and Zn150 were 68, 122, and 148 mg Zn/kg DM, 77, 121, and 107 mg Zn/kg DM, and 48, 101, and 137 mg Zn/kg DM for transition 1, transition 2, and finishing diets, respectively.

[6]Net energy of maintenance (NEm) and gain (NEg) were calculated using ingredient nutrient values from NASEM (2016).

**Table 2.** Forward and reverse primers used for quantitative real-time PCR using Fluidigm technology.

| Gene[1] | Accession number | Strand | Sequence (5'-3') |
|---|---|---|---|
| AKT1 | NM_173986.2 | Forward | CACACGCTCACAGAGAACC |
| | | Reverse | TCGTGTGTCTGGAAGGAGTAC |
| AR | NM_001244127.1 | Forward | ATGCTCTACTTTGCCCCTGAC |
| | | Reverse | TTCGGACACACTGGCTGTAC |
| EEF2K | NM_001192542.1 | Forward | GGAGGTACGAGTCGGATGAA |
| | | Reverse | GGGAGGAGTTGAAGAGATTCCA |
| EGFR | XM_002696890.5 | Forward | GACCTCCATGCTTTTGAGAACC |
| | | Reverse | CCGACAACCGCAAGAGAAAA |
| ESR1 | NM_001001443.1 | Forward | AACTCCTCCTCATCCTCTCTCA |
| | | Reverse | CACCACGTTCTTGCACTTCA |
| FOXO3 | NM_001206083.1 | Forward | GCGTGCCCTACTTCAAGGATA |
| | | Reverse | GTGACAGGTTGTGCCGGATA |
| GPER1[2] | XM_024985174.1 | Forward | GCTTTGGCAACACTTTGGAAAC |
| | | Reverse | AGTTGCACAGGTGGAAGGAA |
| IGF1R | NM_001244612.1 | Forward | ACGGATCCCGTGTTCTTCTAC |
| | | Reverse | AATGGGCAGAGCGATCATCA |
| MAPK1 | NM_175793.2 | Forward | CGCTACACCAATCTCTCGTAC |
| | | Reverse | CGACTCGGACTTTGTTGACA |
| MMP2 | NM_174745.2 | Forward | CCATGATGGAGAGGCTGACA |
| | | Reverse | GCCCGTCTTTGCCATCAAA |
| MMP9[2] | NM_174744.2 | Forward | AGCACGCACGACATCTTTCA |
| | | Reverse | GAACTCACGCGCCAGTAGAA |
| MTOR[2] | XM_002694043.6 | Forward | CCAAACTGCTGTGGCTGAA |
| | | Reverse | ACCGCTAAAGAGCGGGTATAA |
| MYF5 | NM_174116.1 | Forward | CACCAGCCCCACCTCAA |
| | | Reverse | CAGGACAGTAGACGCTGTCA |
| MYOD | NM_001040478.2 | Forward | ACTGCTACGACCGCACTTAC |
| | | Reverse | ACCGCAGCGCTCTTCC |
| MYOG | NM_001111325.1 | Forward | CAGCGCACTGGAGTTTGG |
| | | Reverse | AGGTGAGGGAGTGCAGATTG |
| PAX7 | XM_616352.4 | Forward | AGACCGACTGCTGAAGGAC |
| | | Reverse | TGAGCACTCGGCTAATCGAA |
| PDE4B | NM_001102546.1 | Forward | AGCCCCATGTGTGATAAGCA |
| | | Reverse | CCCACAAGGGATGGACAATGTA |
| RPS6KB1 | NM_205816.1 | Forward | TGACGGAACAGTCACACACA |
| | | Reverse | ACGATTGTGGCCACTTCTCA |
| SLC30A10[2] | NM_001192180.1 | Forward | ATGGTCCCCAAAGGTGTCAA |
| | | Reverse | GCTCCCAGATGTGGACTTCA |
| SLC30A7 | NM_001083760.1 | Forward | CTGGATTGGCAGCGTCTGTTA |
| | | Reverse | AGCCAGAACCTCCGCTCTAA |

Table 2 Continued

| Gene[1] | Accession number | Strand | Sequence (5'-3') |
|---|---|---|---|
| SLC39A14 | NM_001098036.1 | Forward | TTTGCACTGGCTGGAGGAA |
| | | Reverse | CTCTTGGCTCACCTCGTTCA |
| SLC39A7 | NM_001076237.3 | Forward | CATGCTCATGGTCACACACA |
| | | Reverse | TCCTCTGAGCTCTGTTTCTCC |
| EEF1A2 (Housekeeping) | NM_001037464.2 | Forward | CGACAAGAGGACCATCGAGAA |
| | | Reverse | TTGTCCAGTACCCAGGCATAC |
| RPS9 (Housekeeping) | NM_001101152.2 | Forward | GCCTCGACCAAGAGCTGAA |
| | | Reverse | GAATTTGACCCTCCAGACCTCA |

[1]Gene abbreviations: AKT1: protein kinase B, AR: androgen receptor, EEF2K: eukaryotic elongation factor 2 kinase, EGFR: epidermal growth factor receptor, ESR1: estrogen receptor 1, FOXO3: forkhead box O-3, GPER1: G protein-coupled estrogen receptor 1, IGF1R: insulin-like growth factor 1 receptor, MAPK: mitogen-activated protein kinase 1, MMP2: matrix metalloproteinase 2, MMP9: matrix metalloproteinase 9, MTOR: mechanistic target of rapamycin kinase, MYF5: myogenic factor 5, MYOD: myogenic differentiation 1, MYOG: myogenin, PAX7: paired box protein 7, PDE4B: phosphodiesterase 4B, RPS6KB1: ribosomal protein S6 kinase B1, SLC30A10: solute carrier family 30 member 10, SLC30A7: solute carrier family 30 member 7, SLC39A14: solute carrier family 39 member 14, SLC39A7: solute carrier family 39 member 7, EEF1A2: eukaryotic translation elongation factor 1 alpha 2, RPS9: ribosomal protein S9.

[2]GPER1, MMP9, MTOR, and SLC30A10 gene expression were not detected in quantitative real-time PCR using Fluidigm technology (Fluidigm, San Francisco, CA). Therefore, these genes were excluded from analysis.

**Table 3.** Effects of zinc[1] supplementation on performance parameters of non-implanted and implanted[2] beef feedlot steers.

| | NoIMP | | | | TE200 | | | | | Contrasts[3] | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Zn0 | Zn30 | Zn100 | Zn150 | Zn0 | Zn30 | Zn100 | Zn150 | SEM | ZnL-NoIMP | ZnQ-NoIMP | ZnL-TE200 | ZnQ-TE200 | NoIMP vs. TE200 |
| Steer (*n*)[4] | 17 | 15 | 16 | 16 | 15 | 16 | 16 | 16 | | | | | | |
| BW, kg | | | | | | | | | | | | | | |
| d 0 | 490 | 495 | 491 | 492 | 495 | 492 | 492 | 489 | 7.6 | 0.99 | 0.91 | 0.67 | 0.95 | 0.98 |
| d 18 | 528 | 532 | 532 | 531 | 531 | 534 | 534 | 540 | 1.8 | 0.34 | 0.18 | 0.002 | 0.34 | 0.005 |
| d 59 | 595 | 596 | 603 | 596 | 605 | 607 | 608 | 603 | 3.8 | 0.50 | 0.17 | 0.81 | 0.26 | 0.002 |
| d 0-18 | | | | | | | | | | | | | | |
| ADG, kg | 2.01 | 2.24 | 2.22 | 2.18 | 2.17 | 2.31 | 2.32 | 2.65 | 0.101 | 0.34 | 0.18 | 0.002 | 0.34 | 0.005 |
| DMI, kg | 11.4 | 11.2 | 11.2 | 12.1 | 11.5 | 11.4 | 11.7 | 12.0 | 0.24 | 0.11 | 0.09 | 0.09 | 0.48 | 0.52 |
| G:F | 0.177 | 0.192 | 0.200 | 0.181 | 0.188 | 0.203 | 0.199 | 0.222 | 0.0094 | 0.71 | 0.05 | 0.02 | 0.61 | 0.01 |
| d 0-59 | | | | | | | | | | | | | | |
| ADG, kg | 1.75 | 1.76 | 1.88 | 1.76 | 1.91 | 1.95 | 1.97 | 1.88 | 0.064 | 0.50 | 0.17 | 0.81 | 0.26 | 0.002 |
| DMI, kg | 11.1 | 11.0 | 11.0 | 11.6 | 11.5 | 11.2 | 11.5 | 11.5 | 0.24 | 0.20 | 0.15 | 0.69 | 0.72 | 0.19 |
| G:F | 0.157 | 0.161 | 0.172 | 0.153 | 0.166 | 0.175 | 0.172 | 0.165 | 0.0054 | 0.95 | 0.01 | 0.59 | 0.16 | 0.02 |
| Carcass-adjusted[5] | | | | | | | | | | | | | | |
| Final BW | 594 | 593 | 600 | 593 | 604 | 606 | 610 | 613 | 4.0 | 0.81 | 0.35 | 0.10 | 0.99 | <0.0001 |
| ADG | 1.70 | 1.69 | 1.79 | 1.68 | 1.87 | 1.89 | 1.96 | 2.01 | 0.066 | 0.81 | 0.35 | 0.10 | 0.99 | <0.0001 |
| G:F | 0.151 | 0.152 | 0.163 | 0.145 | 0.162 | 0.168 | 0.170 | 0.174 | 0.0053 | 0.74 | 0.04 | 0.12 | 0.86 | <0.0001 |

[1]Cattle were supplemented 0, 30, 100, or 150 mg Zn/kg DM (**Zn0**, **Zn30**, **Zn100**, or **Zn150**, respectively) from $ZnSO_4$.

[2]Implant strategies included no implant (**NoIMP**) or a Component TE-200 (**TE200**; 200 mg trenbolone acetate + 20 mg estradiol; Elanco Animal Health, Greenfield, IN) on d 0.

[3]Contrast statements were formed to test for linear (L) and quadratic (Q) effects of Zn supplementation within non-implanted (**L-NoIMP** and **Q-NoIMP**) or implanted (**L-TE200** or **Q-TE200**) steers. A separate contrast statement was formed to test for differences between non-implanted steers and all implanted steers (**NoIMP vs. TE200**).

[4]Initial body weight (BW) was utilized as a covariate in all performance analysis including body weight (**BW**), average daily gain (**ADG**), dry matter intake (**DMI**), and gain:feed (**G:F**).

[5]Carcass adjusted performance was calculated using the average dressing percentage for all treatments: 62.25%.

**Table 4.** Effects of zinc[1] supplementation within non-implanted and implanted[2] beef steers on carcass characteristics.

| | NoIMP | | | | TE200 | | | | | Contrasts[3] | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Zn0 | Zn30 | Zn100 | Zn150 | Zn0 | Zn30 | Zn100 | Zn150 | SEM | L-NoIMP | Q-NoIMP | L-TE200 | Q-TE200 | NoIMP vs. TE200 |
| Steer (*n*)[4] | 17 | 15 | 16 | 16 | 15 | 16 | 16 | 16 | | | | | | |
| Hot carcass weight, kg | 370 | 369 | 373 | 369 | 376 | 377 | 380 | 381 | 2.5 | 0.80 | 0.39 | 0.09 | 0.98 | < 0.0001 |
| Dress, % | 62.2 | 62.0 | 61.9 | 62.0 | 62.3 | 62.1 | 62.4 | 63.2 | 0.30 | 0.62 | 0.65 | 0.01 | 0.13 | 0.02 |
| Ribeye area, $cm^2$ | 84.4 | 81.7 | 86.5 | 83.1 | 89.4 | 88.8 | 87.9 | 87.6 | 1.96 | 0.76 | 0.56 | 0.48 | 0.91 | 0.001 |
| $12^{th}$ rib fat, cm | 1.61 | 1.67 | 1.43 | 1.69 | 1.50 | 1.44 | 1.52 | 1.56 | 0.086 | 0.87 | 0.09 | 0.43 | 0.67 | 0.12 |
| Marbling[5] | 491 | 470 | 483 | 495 | 472 | 460 | 452 | 472 | 22.0 | 0.70 | 0.52 | 0.96 | 0.43 | 0.17 |
| Yield grade[6] | 3.6 | 3.6 | 3.4 | 3.7 | 3.5 | 3.4 | 3.5 | 3.5 | 0.08 | 0.87 | 0.09 | 0.43 | 0.67 | 0.12 |

[1]Zinc was supplemented to cattle at 0, 30, 100, or 150 mg Zn/kg DM from $ZnSO_4$ (**Zn0**, **Zn30**, **Zn100**, or **Zn150**, respectively).

[2]Implant strategies included no implant (**NoIMP**) or a Component TE-200 (**TE-200**; 200 mg trenbolone acetate + 20 mg estradiol; Elanco Animal Health, Greenfield, IN) on d 0.

[3]Contrast statements were formed to test for linear (L) and quadratic (Q) effects of Zn supplementation within non-implanted (**L-NoIMP** and **Q-NoIMP**) or implanted (**L-TE200** or **Q-TE200**) steers. A separate contrast statement was formed to test for differences between non-implanted steers and all implanted steers (**NoIMP vs. TE200**).

[4]Intial body weight was utilized as a covariate for carcass characteristics.

[5]Marbling scores: slight = 300, small = 400, modest = 500, moderate = 600, slightly abundant = 700, moderately abundant = 800.

[6]Yield grade was assigned by personnel of the commercial abattoir.

**Table 5.** Effects of zinc[1] supplementation within non-implanted and implanted[2] beef steers on trace mineral and plasma urea nitrogen concentrations.

| | NoIMP | | | | TE200 | | | | | Contrasts[3] | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Zn0 | Zn30 | Zn100 | Zn150 | Zn0 | Zn30 | Zn100 | Zn150 | SEM | L-NoIMP | Q-NoIMP | L-TE200 | Q-TE200 | NoIMP vs. TE200 |
| Steer (*n*) | 10 | 9 | 9 | 9 | 8 | 9 | 9 | 9 | | | | | | |
| Plasma Zn[4], mg/L | | | | | | | | | | | | | | |
| d -1 | 1.22 | 1.31 | 1.32 | 1.32 | 1.28 | 1.28 | 1.37 | 1.35 | 0.053 | 0.17 | 0.41 | 0.16 | 0.67 | 0.42 |
| d 18 | 1.31 | 1.34 | 1.43 | 1.44 | 1.25 | 1.21 | 1.33 | 1.39 | 0.037 | 0.003 | 0.53 | 0.001 | 0.36 | 0.001 |
| d 40 | 1.33 | 1.25 | 1.36 | 1.42 | 1.29 | 1.27 | 1.35 | 1.38 | 0.038 | 0.01 | 0.12 | 0.03 | 0.82 | 0.49 |
| Liver[5], mg/kg DM | | | | | | | | | | | | | | |
| Zn | 115 | 113 | 122 | 115 | 118 | 118 | 125 | 127 | 6.1 | 0.69 | 0.59 | 0.20 | 0.97 | 0.20 |
| Mn | 8.7 | 7.9 | 8.6 | 8.6 | 8.2 | 7.9 | 7.6 | 8.4 | 0.46 | 0.65 | 0.55 | 0.82 | 0.15 | 0.10 |
| Cu | 310 | 274 | 277 | 252 | 278 | 268 | 268 | 293 | 29.9 | 0.15 | 0.86 | 0.67 | 0.49 | 0.94 |
| Fe | 181 | 181 | 173 | 184 | 178 | 170 | 171 | 184 | 11.2 | 0.99 | 0.47 | 0.59 | 0.28 | 0.59 |
| PUN[6], mg/dL | | | | | | | | | | | | | | |
| d -1 | 14.0 | 13.6 | 14.4 | 14.9 | 13.6 | 13.8 | 14.4 | 16.2 | 1.08 | 0.42 | 0.78 | 0.07 | 0.50 | 0.73 |
| d 18 | 10.1 | 11.9 | 11.2 | 10.7 | 10.9 | 10.0 | 8.6 | 9.9 | 0.86 | 0.95 | 0.19 | 0.29 | 0.12 | 0.05 |
| d 40 | 11.9 | 9.9 | 11.3 | 12.9 | 11.2 | 10.7 | 10.2 | 10.5 | 0.96 | 0.19 | 0.09 | 0.58 | 0.59 | 0.18 |
| d 59 | 9.8 | 9.1 | 10.5 | 10.3 | 9.5 | 8.1 | 8.9 | 10.8 | 0.61 | 0.20 | 0.97 | 0.05 | 0.01 | 0.16 |

[1]Zinc was supplemented to cattle at 0, 30, 100, or 150 mg Zn/kg DM from $ZnSO_4$ (**Zn0**, **Zn30**, **Zn100**, or **Zn150**, respectively).

[2]Implant strategies included no implant (**NoIMP**) or a Component TE-200 (**TE-200**; 200 mg trenbolone acetate + 20 mg estradiol; Elanco Animal Health, Greenfield, IN) on d 0.

[3]Contrast statements were formed to test for linear (L) and quadratic (Q) effects of Zn supplementation within non-implanted (**L-NoIMP** and **Q-NoIMP**) or implanted (**L-TE200** or **Q-TE200**) steers. A separate contrast statement was formed to test for differences between non-implanted steers and all implanted steers (**NoIMP vs. TE200**).

[4]Plasma Zn from d -1 was utilized as a covariate in d 18 and 40 plasma Zn analysis.

[5]Liver samples were collected on d 55/56 with half of the samples collected on each d.

[6]Plasma urea nitrogen (**PUN**) used d -1 values as a covariate in d 18, 40, and 59 PUN analysis.

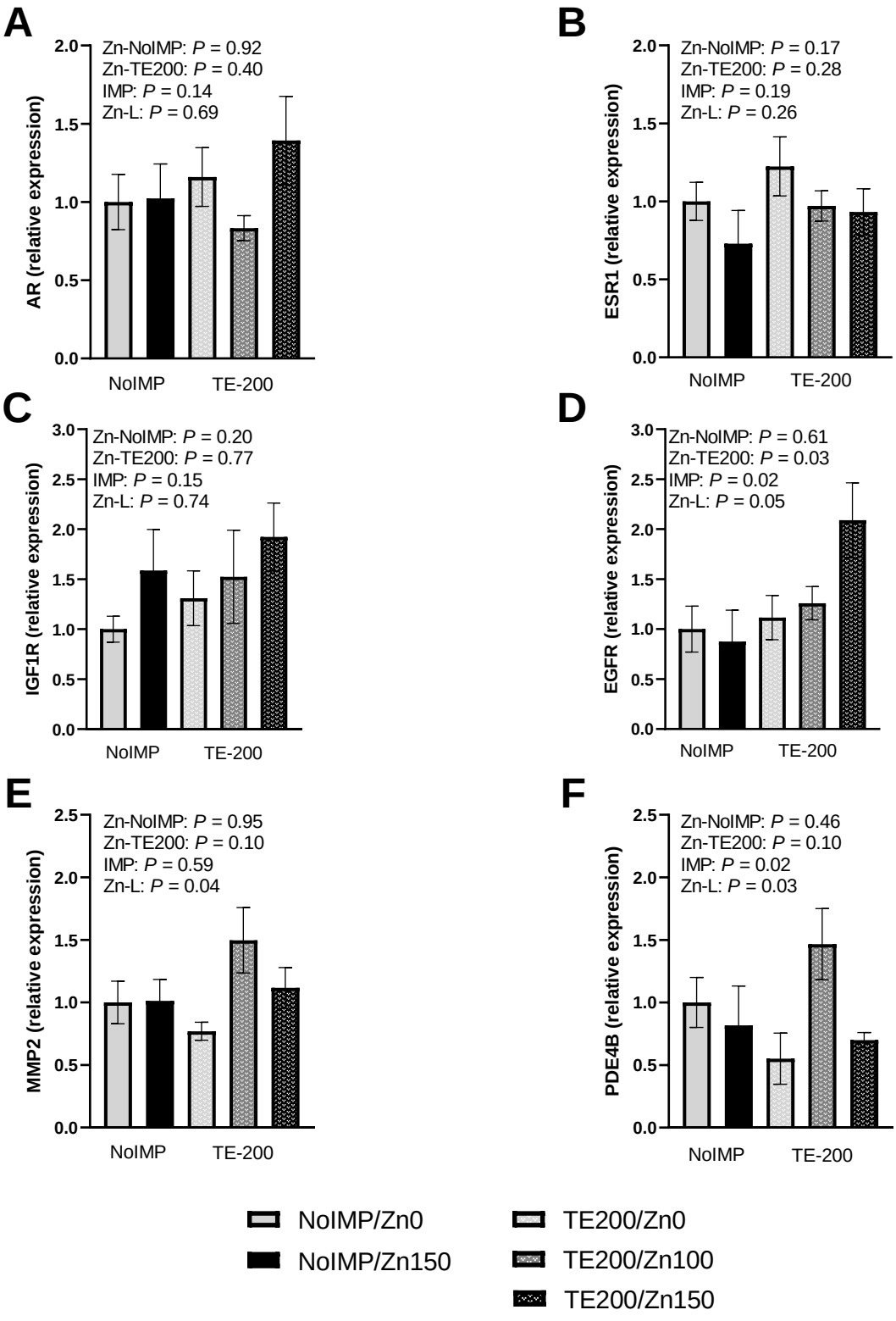


**Figure 1.** Relative gene expression of steroidal implant receptors and growth signaling machinery from muscle of steers receiving no implant (**NoIMP**) or an implant(**TE200**) and fed

0, 100, or 150 mg Zn/kg DM (**Zn0**, **Zn100**, **Zn150**, respectively). Samples were collected from the longissimus thoracis on d 11 and relative gene expression was calculated relative to NoIMP-Zn0 treatment. Contrast statements were formed to test the effect of increasing Zn from Zn0 to Zn150 within NoIMP (**Zn-NoIMP**) and TE200 (**Zn-TE200**), in addition to the main effect of implant (**IMP**; Zn0 and Zn150) and the linear effect of increasing Zn as Zn0, Zn100, and Zn150 within TE200 (**Zn-L**). **A)** Androgen receptor (AR), **B)** estrogen receptor (ESR1), and **C)** insulin-like growth factor-1 receptor (IGF1R) gene expression were not influenced by Zn or implant contrast statements ($P \geq 0.14$). **D)** Epidermal growth factor receptor (EGFR) gene expression was greater for Zn150 than Zn0 steers administered TE200 ($P = 0.03$) and linearly increased with 0, 100, and 150 mg/kg DM of supplemental Zn within TE200 steers ($P = 0.05$). Implanted cattle (Zn0 and Zn150) had greater EGFR gene expression than NoIMP ($P = 0.02$). **E)** Matrix metalloproteinase 2 (MMP2) gene expression tended to be greater for Zn150 than Zn0 within TE200 ($P = 0.10$) and linearly increased with 0, 100, and 150 mg/kg DM of supplemental Zn for TE200 steers ($P = 0.04$). **F)** Phosphodiesterase 4B (PDE4B) gene expression tended to be greater for Zn150 than Zn0 within TE200 ($P = 0.10$) and linearly increased in TE200 steers with 0, 100, and 150 mg/kg DM of supplemental Zn ($P = 0.03$). Implanted cattle had lesser muscle PDE4B gene expression than NoIMP for Zn0 and Zn150 cattle ($P = 0.02$).

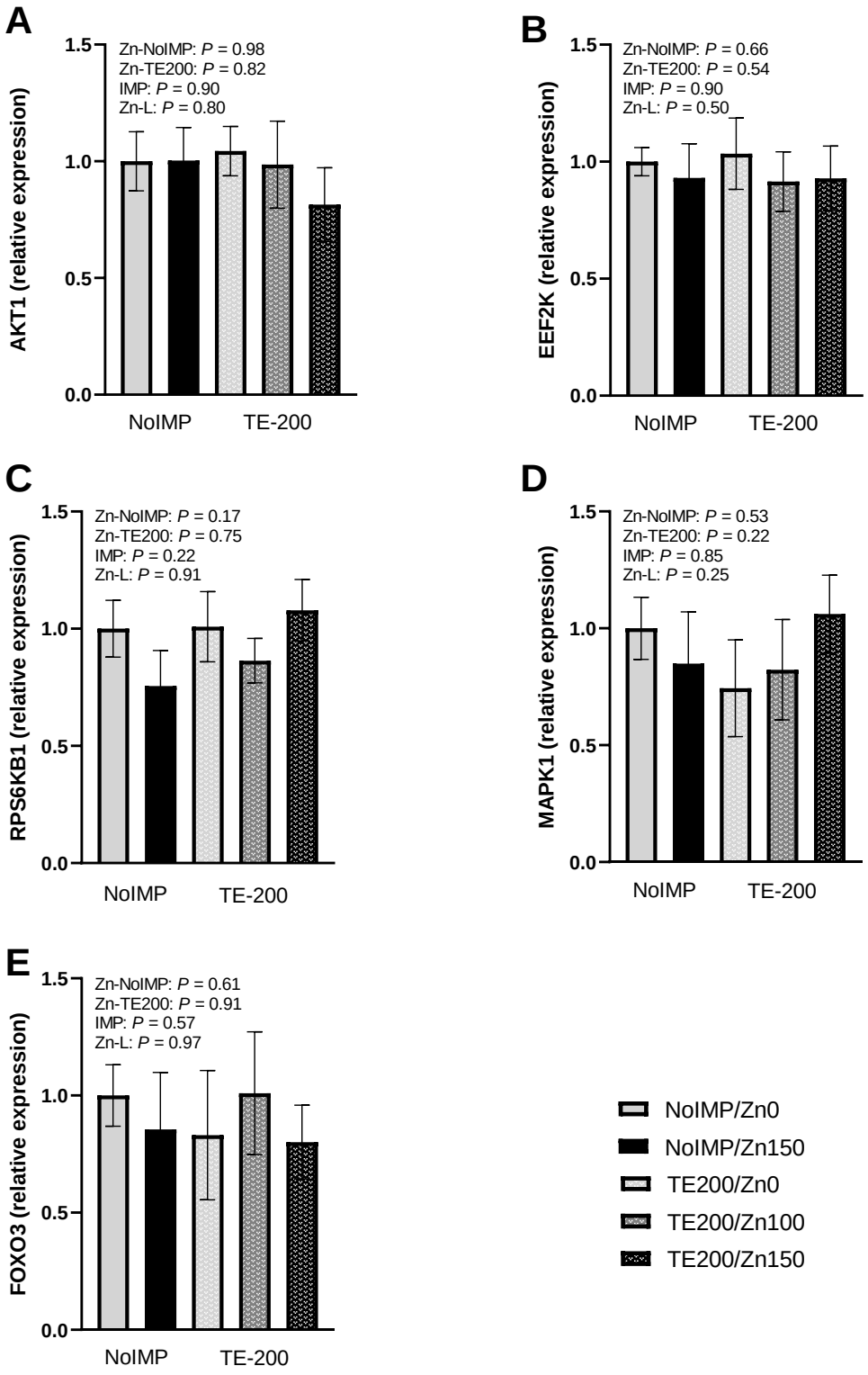


**Figure 2.** Relative gene expression of growth signaling proteins from muscle of steers not implanted (**NoIMP**) or implanted (**TE200**) and supplemented 0, 100, or 150 mg Zn/kg DM

(**Zn0**, **Zn100**, **Zn150**, respectively). Samples were collected on d 11 from the longissimus thoracis. Relative gene expression was calculated relative to NoIMP-Zn0 treatment. Contrast statements were formed to test the effect of increasing Zn from Zn0 to Zn150 within NoIMP (**Zn-NoIMP**) and TE200 (**Zn-TE200**), in addition to the main effect of implant (**IMP**; Zn0 and Zn150) and the linear effect of increasing Zn as Zn0, Zn100, and Zn150 within TE200 (**Zn-L**). **A)** Protein kinase B (AKT1), **B)** eukaryotic elongation factor 2 kinase (EEF2K), **C)** ribosomal protein S6 kinase B1 (RPS6KB1), **D)** mitogen-activate protein kinase 1 (MAPK1), and **E)** forkhead box O-3 (FOXO3) gene expression were not affected by Zn or implant contrast statements ($P \geq 0.17$).

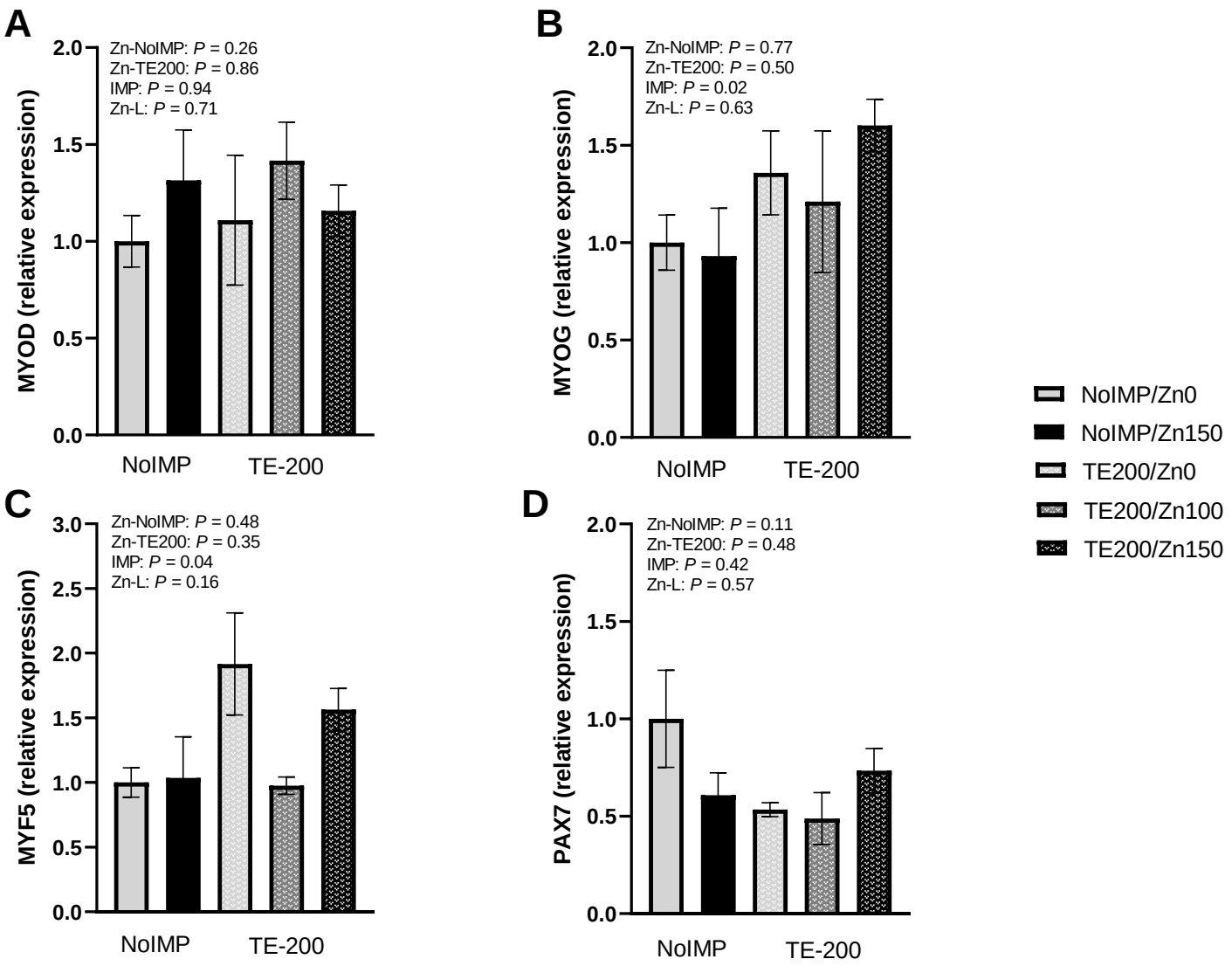


**Figure 3.** The relative gene expression of markers of satellite cell development were analyzed in non-implanted (**NoIMP**) and implanted (**TE200**) steers that were supplemented 0, 100, or 150 mg Zn/kg DM (**Zn0**, **Zn100**, **Zn150**, respectively). Samples from the

longissimus thoracis were collected on d 11 and relative gene expression was calculated relative to NoIMP-Zn0 treatment. Contrast statements were formed to test the effect of increasing Zn from Zn0 to Zn150 within NoIMP (**Zn-NoIMP**) and TE200 (**Zn-TE200**), in addition to the main effect of implant (**IMP**; Zn0 and Zn150) and the linear effect of increasing Zn as Zn0, Zn100, and Zn150 within TE200 (**Zn-L**). **A)** Myogenic differentiation 1 (MYOD) gene expression was not influenced by the tested contrast statements ($P \geq 0.26$). **B)** Myogenin (MYOG) and **C)** myogenic factor 5 (MYF5) gene expression were greater for TE200 than NoIMP for Zn0 and Zn150 treatments ($P \leq 0.04$). **D)** Paired box protein 7 (PAX7) gene expression was not influenced by the tested contrast statements ($P \geq 0.11$

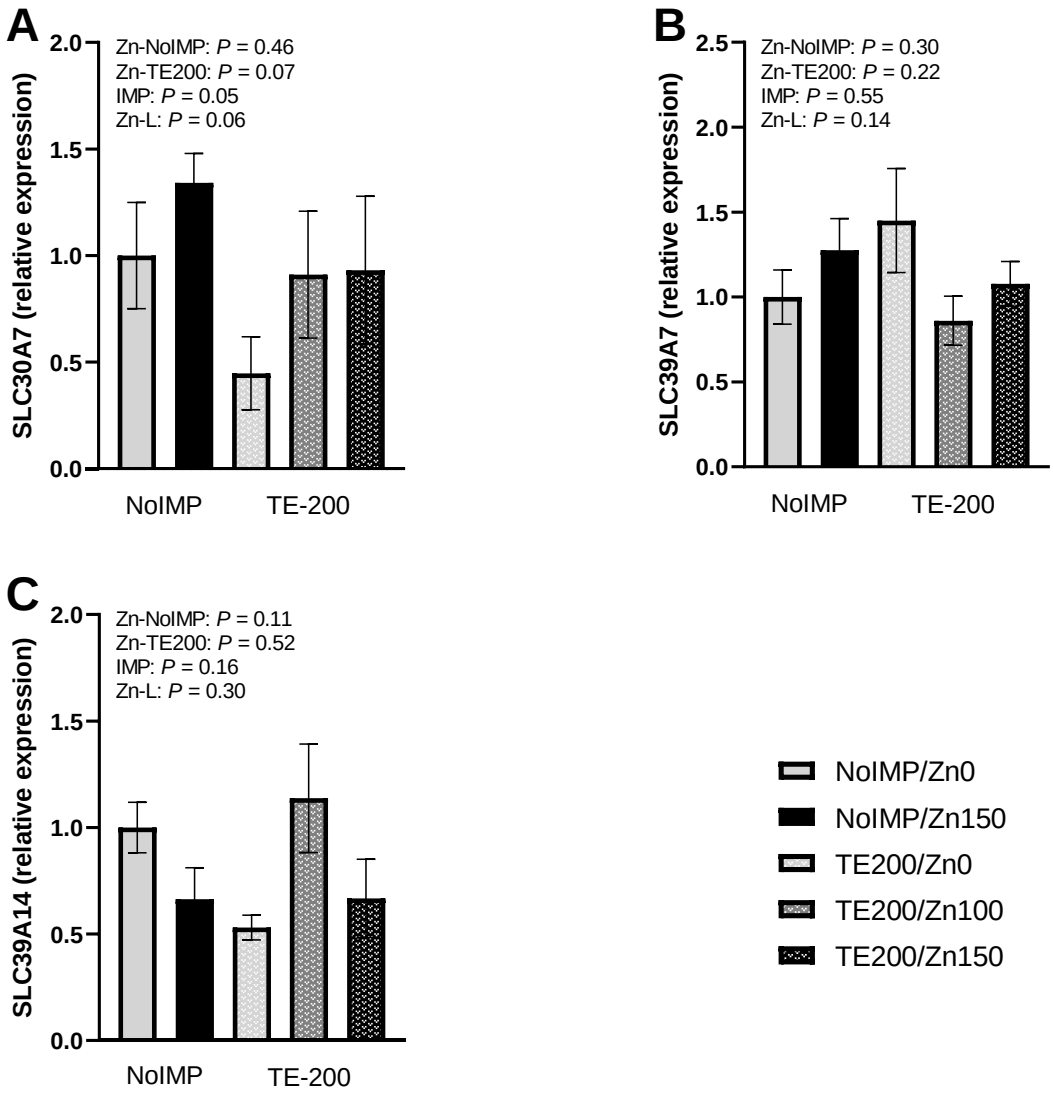


**Figure 4.** Relative gene expression of Zn transporters in muscle of non-implanted (**NoIMP**) and implanted (**TE200**) steers supplemented 0, 100, or 150 mg Zn/kg DM (**Zn0**, **Zn100**, **Zn150**, respectively). Samples were collected on d 11 from the longissimus thoracis and relative gene expression was determined relative to NoIMP-Zn0 treatment. Contrast statements were formed to test the effect of increasing Zn from Zn0 to Zn150 within NoIMP (**Zn-NoIMP**) and TE200 (**Zn-TE200**), in addition to the main effect of implant (**IMP**; Zn0 and Zn150) and the linear effect of increasing Zn as Zn0, Zn100, and Zn150 within TE200 (**Zn-L**). **A)** Solute carrier family 30 member 7 (SLC30A7) gene expression tended to be greater for Zn150 than Zn0 within TE200 steers ($P = 0.07$) and tended to linearly increase with increasing Zn supplementation (Zn0, Zn100, and Zn150) within TE200 ($P = 0.06$). Gene expression of SLC30A7 was lesser for TE200 than NoIMP ($P = 0.05$). **B)** Solute carrier family 39 member 7 (SLC39A7) and **C)** solute carrier family 39 member 14 (SLC39A14) were not affected by the tested contrast statements ($P \geq 0.11$).

# CHAPTER 4. EFFECTS OF INCREASING SUPPLEMENTAL ZINC IN BEEF FEEDLOT STEERS ADMINISTERED A STEROIDAL IMPLANT AND BETA AGONIST

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## Abstract

Ninety-two Angus-crossbred steers (424 ± 28 kg initial body weight) were used in a 98-d study to assess the effects of increasing Zn supplementation on cattle performance, carcass characteristics, liver and plasma trace mineral concentrations, and blood metabolites. All steers were implanted with a Component TE-200 (200 mg trenbolone acetate + 20 mg estradiol; Elanco Animal Health, Greenfield, IN) on d 0 and fed 300 mg·steer$^{-1}$·d$^{-1}$ of ractopamine hydrochloride (Zoetis, Parsippany, NJ) from d 70 to 98. Cattle were fed via GrowSafe bunks (GrowSafe Systems Ltd., Airdrie, AB, Canada), and steer served as the experimental unit (*n* = 22 or 23 steers/treatment). Supplemental Zn was administered through the diet at 0, 100, 150, or 180 mg Zn/kg on a dry matter basis from $ZnSO_4$ (**Zn0**, **Zn100**, **Zn150**, or **Zn180**, respectively). Cattle were weighed on d -1, 0, 9/10, 20, 41, 59, 69, 70, 78/79, 97, and 98. Blood was collected on d 0, 9/10, 69, 78/79, and 97, and liver biopsies on d 9/10 and 78/79 (*n* = 12 steers/treatment). Data were analyzed via Proc Mixed and Proc Corr of SAS. Contrast statements were formed to test the linear, quadratic, and cubic effects of Zn supplementation and test Zn0 vs. Zn

supplementation. Day 10 and 70 body weight (BW) and d 0-10 and 0-70 average daily gain were linearly increased with Zn supplementation ($P \leq 0.05$), and greater for Zn supplemented steers ($P \leq 0.03$). No effects of Zn supplementation were observed on final BW, dressing percentage, ribeye area, 12$^{th}$ rib fat, or marbling ($P \geq 0.11$). Hot carcass weight tended to be 7 kg greater for Zn supplemented steers than Zn0 ($P = 0.07$), and yield grade linearly increased with increasing Zn supplementation ($P = 0.02$). Day 10 liver Mn concentrations tended to quadratically decrease ($P = 0.08$) with increasing Zn supplementation, though d 79 liver Mn concentrations and arginase activity were not influenced by Zn ($P \geq 0.28$). Day 10 liver arginase activity tended to be ($r = 0.27$; $P = 0.07$) and d 10 serum urea nitrogen was correlated with d 10 liver Mn ($r = 0.55$; $P < 0.0001$). Zinc supplementation linearly increased d 10 liver Zn and d 10, 69, 79, and 97 plasma Zn concentrations ($P \leq 0.05$). A cubic effect of Zn was observed on d 79 liver Zn ($P = 0.01$) with lesser liver Zn in Zn0 and Zn150 steers. These data suggest increasing dietary Zn improves growth directly following the administration of a steroidal implant and that steroidal implants and beta agonists differ in their effects on protein metabolism.

Keywords: arginase, beef steers, manganese, protein metabolism, zinc sulfate

## Introduction

Steroidal implants and beta agonists are technologies commonly utilized in the U.S. feedlot industry (NAHMS, 2013; Samuelson et al., 2016). The economic importance of these technologies is demonstrated by the 16 to 20% improvement in average daily gain (**ADG**) of implanted cattle (Duckett and Pratt, 2014) and improvements in hot carcass weight (**HCW**) and ribeye area (**REA**) of cattle fed a beta agonist (Lean et al., 2014). However, just as energy has been shown to be a potentially limiting factor in implant-induced growth of cattle (Guiroy et al.,

2002) so too might other nutrients be preventing optimal growth responses to these technologies. Zinc may benefit the growth of cattle utilizing both steroidal implants and beta agonists due to its function in over 2000 transcription factors (Cousins et al., 2006b) and role in both deoxyribonucleic acid (**DNA**) and protein synthesis (Oberleas and Prasad, 1969; Duncan and Dreosti, 1976). Messersmith and Hansen (2021) reported increasing supplemental Zn up to 150 mg Zn/kg dry matter (**DM**) linearly increased ADG of implanted cattle, but not non-implanted cattle, within 18 d of implant administration. Similarly, Genther-Schroeder et al. (2016) observed a linear improvement in final body weight (**BW**) of beta agonist-fed cattle when increasing Zn supplementation up to 150 mg Zn/kg DM, but no effects of Zn were observed in cattle fed no beta agonist. Growth rates of cattle utilizing these technologies appear to be greatest early after administration (Johnson et al., 1996a; Maxwell et al., 2015), indicating Zn may have the greatest potential to improve growth during this time. These data suggest Zn supplementation above NASEM (2016) recommendations may be crucial to optimizing the growth of cattle utilizing growth promoting technologies. The objective of this study was to examine the effects of increasing Zn supplementation from inorganic Zn on growth and mineral status of feedlot cattle with particular emphasis on the periods of peak growth following the administration of a steroidal implant and the initiation of beta agonist feeding. It was hypothesized cattle growth would linearly increase with increasing Zn supplementation during both periods of high growth.

## Materials and Methods

### Care and Use of Animals

Iowa State University Institutional Animal Care and Use Committee (log number: IACUC-20-182) approved all procedures and protocols utilized in this study.

**Animals and Experimental Design**

Ninety-two Angus-crossbred steers (424 ± 28 kg) were utilized in a randomized design to determine the effect of increasing Zn supplementation on cattle utilizing both anabolic implant and beta agonist growth promoting technologies. Cattle were stratified by BW into pens (*n* = 5 or 6 steers/pen). All cattle were implanted with a high potency implant (Component TE-200; 200 mg trenbolone acetate + 20 mg estradiol; donated by Elanco Animal Health, Greenfield, IN) on d 0 and fed a beta agonist for the last 28-d of the trial starting on d 70 (300 mg·steer$^{-1}$·d$^{-1}$ of ractopamine hydrochloride; donated by Zoetis, Parsippany, NJ). Steers were fed ad libitum a corn-silage based finishing diet (**Table 1**) throughout the 98-d trial. Feed was delivered once daily (0800 h) to GrowSafe bunks (GrowSafe Systems Ltd., Airdrie, AB, Canada). Radio frequency tags on each steer relayed individual steer feed disappearance data from the bunk to GrowSafe software. Therefore, individual intake data was recorded for each animal in each pen. Cattle were fed one of four Zn treatments (n = 22 or 23 steers per treatment): 0, 100, 150, or 180 mg supplemental Zn/kg on a DM basis (**Zn0**, **Zn100**, **Zn150**, or **Zn180**, respectively) from $ZnSO_4$ administered daily through a dried distillers grains plus solubles-based premix in the total mixed ration (**TMR**).

Following the completion of the 98-d study, cattle were harvested at a commercial abattoir (National Beef, Tama, IA) via industry accepted practices. Trained National Beef personnel collected HCW on the day of harvest while REA, 12$^{th}$ rib fat (**BF**), and marbling data were collected following a 48-h chill. Yield grade data represents the abattoir assigned number and was not calculated via the USDA yield grade calculation due to a lack of kidney, pelvic, and heart fat data.

**Sample Collection and Analysis**

Consecutive day BW were taken at the beginning of the trial (d -1 and 0), the start of the beta agonist period (d 69 and 70), and at the end of the trial (d 97 and 98) with interim BW recorded on d 9/10, 20, 41, 59, and 78/79 (*n* = 22 or 23 steers/treatment). Liver biopsies were collected from the same steers on d 9 and 10, and again on d 78 and 79 with 2 of the 4 pens sampled on each day (n = 12 steers/treatment). Cattle from different treatments were sampled relatively equally across these day pairings to accommodate logistics related to biopsy collections. Liver samples were collected via biopsy procedures outlined by Engle and Spears (2000) and stored at 20ºC prior to analysis for trace mineral concentration.

Liver arginase activity was analyzed utilizing procedures adapted from Lin et al. (2017). Briefly, liver samples were homogenized with a phosphate buffered saline, protease, and phosphatase buffer cocktail followed by centrifugation of the sample at 14,000 × *g* for 15 min. The resulting supernatant was diluted with 30x phosphate buffered saline solution. The diluted supernatant (50 µl) was added to test tubes in triplicate and incubated with 220 µl glycine-NaOH buffer (pH 9.6) and 100 µl arginine (68 mM, pH 9.6) at 37ºC for 10 min. Following incubation the reaction was stopped with the addition of 900 µl $H_2SO_4/H_3PO_4/H_2O$ (1:3:7). Next, 40 µl of α-isonitrosopropiophenone (9%) dissolved in ethanol was added before samples were heated at 95ºC for 30 min to develop color. Samples were cooled in the dark for 15 min before being transferred to a 96-well plate and read at 540 nm. Sample urea production was normalized to protein content of the sample analyzed using a commercial Coomassie Bradford Protein Assay kit (Thermo Fisher Scientific, Waltham, MA) and arginase activity was determined by the amount of urea produced per µg protein in 1 min (nM urea/µg protein/min).

Samples of each treatment's TMR were collected weekly and dried in a forced air oven at 70ºC for 48 h to calculate diet dry matter (DM). Dried TMR samples were ground through a 2-

mm screen (Retsch Zm100 grinder; Glen Mills Inc., Clifton, NJ) and composited by month within each treatment. Nutrient analysis of the composited Zn0 TMR was conducted by Dairyland Laboratories (Arcadia, WI) in accordance with procedures outlined by Heiderscheit and Hansen (2020). Blood was collected from the same steers on d 0, 9/10, 69, 78/79, and 97 (*n* = 12 steers/treatment) via jugular venipuncture in vacuum capped tubes (Becton Dickerson, Rutherford, NJ). Tubes contained either trace mineral grade $K_2$EDTA for plasma trace mineral analysis or no additives for serum collection. Blood samples were stored at -20ºC until analysis. Serum urea nitrogen (**SUN**) concentrations were measured at all timepoints from serum samples using a commercial kit (Teco Diagnostics, Anaheim, CA). Intra-assay and inter-assay CV were 6.3 and 5.9%, respectively for SUN analysis. Non-esterified fatty acid (**NEFA**) concentrations were also measured in serum for d 69, 78/79, and 97 using a commercial kit (Wako Pure Chemical Industries Ltd., Chuo-Ku Osaka, Japan). Intra-assay and inter-assay CV for NEFA data were 5.9 and 5.5%, respectively.

Composited TMR and liver samples were acid digested via procedures outlined by Richter et al. (2012) and Pogge and Hansen (2013), respectively. Trace mineral concentrations of composited TMR, liver, and plasma samples were analyzed via inductively coupled plasma optical emission spectrometry (Optima 7000 DV, Perkin Elmer, Waltham, MA) following procedures described by Richter et al. (2012) and Pogge and Hansen (2013). Instrument accuracy was ensured by using a standard on each run (Trace Elements Serum Control #66816; UTAK Laboratories Inc., Valencia, CA; Bovine Liver #1577c; National Institute of Standards and Technology, Gaithersburg, MD).

**Statistical Analysis**

Data were analyzed using the Mixed Procedure of SAS 9.4 (SAS Inst. Inc., Cary, NC). Zinc treatment was utilized as the fixed effect. Contrast statements were formed to test for linear,

quadratic, and cubic effects of Zn supplementation as well as to test for differences between non-Zn supplemented and Zn supplemented treatments. Steer was utilized as the experimental unit for all analysis ($n$ = 22 or 23 steers/treatment for performance parameters or $n$ = 12 steers/treatment for blood and liver data). Initial BW was utilized as a covariate in performance and carcass data analysis while d 0 plasma values were utilized as a covariate for the respective analysis of SUN and plasma trace mineral concentrations. The Correlation Procedure of SAS was utilized to assess correlations between liver arginase activity, SUN concentrations, and liver Mn concentrations. All data are reported as the least squares means with the standard error of the mean. Cook's D statistical test was utilized to test for outliers. Cook's D values above 0.20 were removed from analysis. Two steers were removed from all performance and carcass analysis due to chronic health events ($n$ = 1 each for Zn100 and Zn180). Statistical significance was determined as $P \leq 0.05$ and tendencies between $0.06 \leq P \leq 0.10$.

## Results

### Performance and Carcass Characteristics

Body weights from throughout the trial are included in **Table 2**. A positive linear response to Zn supplementation was observed for d 10 and 70 BW ($P \leq 0.05$) that can be explained by the 3.3 kg advantage in d 10 BW and 9.7 kg advantage in d 70 BW of all Zn supplemented steers vs. non-Zn supplemented steers ($P \leq 0.03$). Furthermore, a tendency for a cubic response to Zn supplementation on d 20 BW was observed ($P = 0.10$) in which Zn0 and Zn150 had lesser BW than Zn100 and Zn180. No other polynomial effects were observed for BW throughout the trial ($P \geq 0.11$). However, d 79 BW tended to be 8.3 kg greater for Zn supplemented steers than non-Zn supplemented steers ($P = 0.09$).

Both d 0-10 and d 0-70 ADG linearly increased with Zn supplementation (**Table 3**; $P \leq 0.05$) and were greater for Zn supplemented steers than Zn0 ($P \leq 0.03$). However, neither dry

matter intake (**DMI**) or feed efficiency (**G:F**) for d 0-70 were influenced by any contrasts ($P \geq 0.15$). Although final BW was not influenced by Zn supplementation ($P \geq 0.15$), a cubic response to Zn supplementation was observed for d 70-98 ADG and G:F ($P \leq 0.02$) driven by lesser ADG and G:F of Zn150 during this beta agonist feeding period. Furthermore, Zn supplementation tended to decrease d 70-98 G:F ($P = 0.08$), although no effects of Zn supplementation were observed on d 70-98 DMI ($P \geq 0.16$). Likewise, overall (d 0-98) G:F tended to cubically respond to Zn supplementation ($P = 0.06$) with Zn100 having the greatest G:F and Zn150 the lowest G:F. However, no effects of Zn supplementation were observed for d 0-98 ADG or DMI ($P \geq 0.16$).

Carcass-adjusted G:F tended to quadratically decrease (**Table 4**; $P = 0.10$) with Zn150 having the lowest G:F. However, Zn supplementation did not affect carcass-adjusted final BW or ADG ($P \geq 0.15$). Hot carcass weight, dressing percentage, REA, BF, and marbling were not affected by polynomial effects of Zn supplementation ($P \geq 0.11$). However, HCW tended to be 7 kg greater for Zn supplemented steers than Zn0 ($P = 0.07$). Interestingly, yield grade linearly increased with increasing Zn supplementation ($P = 0.02$), corresponding to Zn supplemented cattle having greater yield grade than Zn0 steers ($P = 0.04$). This is in accordance with the numerical ($P = 0.11$) linear increase in BF with increasing Zn supplementation.

**Blood Metabolites**

Serum urea nitrogen and NEFA concentrations presented in **Table 5** were analyzed as single timepoints and covariate adjusted with d 0 or d 69 values, respectively. Although dietary treatments had not yet begun, d 0 SUN linearly decreased with increasing Zn concentrations ($P = 0.02$) and were lesser for Zn supplemented steers than non-Zn supplemented steers ($P = 0.01$) prior to the administration of the implant. Zinc supplementation did not influence SUN concentrations ($P \geq 0.47$) 10 d-post administration of the implant but tended to quadratically

increase SUN on d 69 ($P = 0.08$). No further effects of Zn supplementation were observed for d 0, 69, 79 or 97 SUN ($P \geq 0.19$). Representing the percent change in SUN during the first 10 d after implant administration and beta agonist supplementation, respectively, d 0 to 10 percent change in SUN was not affected by Zn supplementation ($P \geq 0.24$). The percent change in SUN from d 69 to 79 linearly ($P = 0.05$) and quadratically ($P = 0.001$) decreased with Zn100 having the greatest decrease in SUN.

Non-esterified fatty acid concentrations were measured beginning on d 69, at the start of the beta agonist feeding period. After 10 d of beta agonist supplementation, d 79 NEFA concentrations linearly decreased ($P = 0.05$) with increasing Zn concentrations and a tendency for a cubic response to Zn supplementation ($P = 0.08$) was observed for d 97 NEFA concentrations with Zn0 and Zn150 having greater NEFA concentrations than Zn100 and Zn180. No effects of Zn supplementation were observed for d 69 NEFA concentrations ($P \geq 0.27$) or d 69 to 79 percent change in NEFA ($P \geq 0.19$).

**Liver Trace Mineral Concentrations and Arginase Activity**

Liver Cu tended to quadratically increase with increasing Zn supplementation on d 10 (**Table 6**; $P = 0.07$) while d 10 liver Mn concentrations tended to quadratically decrease ($P = 0.08$) with increasing Zn supplementation. Liver Zn concentrations linearly increased with increasing Zn supplementation ($P = 0.04$) on d 10. However, no effects of Zn were observed for d 10 liver Fe concentrations ($P \geq 0.20$).

After 10 d of beta agonist supplementation, d 79 liver Cu and Fe concentrations quadratically decreased with increasing Zn supplementation ($P \leq 0.04$) and d 79 liver Cu concentrations were lesser for Zn supplemented steers than Zn0 ($P < 0.01$). Liver Cu concentrations on d 79 also linearly decreased ($P < 0.01$). A linear ($P = 0.02$) and cubic response ($P = 0.01$) for d 79 liver Zn concentrations was observed with Zn0 and Zn150 having lesser liver

Zn concentrations than Zn100 and Zn180. However, grouped together, Zn supplemented steers had greater liver Zn concentrations than Zn0 ($P = 0.03$) on d 79. Liver Mn concentrations on d 79 were not influenced by Zn supplementation ($P \geq 0.28$).

Liver arginase activity measured 10 d post-implant administration (d 10) quadratically decreased ($P = 0.02$) with Zn100 having the lowest arginase activity. However Zn supplementation did not further influence d 10 or d 79 liver arginase activity ($P \geq 0.41$).

**Correlations Between Markers of Growth and Protein Degradation**

Correlations between d 10 or 79 liver arginase activity, liver Mn, SUN, and d 0-10 or d 70-79 ADG are presented in **Table 7**. Day 10 liver arginase activity tended to be positively correlated ($r = 0.27$; $P = 0.07$) with d 10 liver Mn concentrations but not d 10 SUN concentrations ($r = -0.04$; $P = 0.77$) or d 0-10 ADG ($r = -0.01$; $P = 0.95$). However, d 10 liver Mn concentrations were correlated with d 10 SUN concentrations ($r = 0.55$; $P < 0.0001$) and tended to be correlated with d 0-10 ADG ($r = 0.25$; $P = 0.09$). Day 10 SUN were not correlated with d 0-10 ADG ($r = 0.10$; $P = 0.49$).

Following 10 d of beta agonist supplementation, d 79 liver arginase activity was not correlated with either d 79 liver Mn ($r = 0.19$), SUN concentrations ($r = 0.07$), or d 70-79 ADG ($r = -0.15$; $P \geq 0.21$). However d 79 liver Mn concentrations were positively correlated with d 79 SUN concentrations ($r = 0.29$; $P = 0.05$). Neither d 79 liver Mn ($r = 0.003$) or SUN ($r = -0.18$) were correlated with d 70-79 ADG ($P \geq 0.23$).

**Plasma Trace Mineral Concentrations**

Plasma trace mineral concentrations analyzed as single timepoints and covariate adjusted with d 0 values are presented in **Table 8**. Although treatments had not yet been assigned, plasma Zn concentrations quadratically decreased ($P = 0.01$) and were lesser ($P = 0.05$) within Zn supplemented treatments on d 0. Plasma Cu and Fe on d 0 and plasma Cu on d 10 were not

affected by Zn supplementation ($P \geq 0.23$). Ten d post-implant administration (d 10) plasma Fe quadratically increased ($P = 0.05$) with Zn100 and Zn150 having the greatest plasma Fe concentrations. Furthermore, d 10 plasma Zn concentrations linearly increased ($P = 0.02$) with increasing Zn supplementation and Zn supplemented steers had greater plasma Zn concentrations than Zn0 on d 10 ($P = 0.02$).

By the start of the beta agonist period (d 69), plasma Cu concentrations tended ($P = 0.10$) to quadratically decrease with Zn150 having the lowest plasma Cu concentration. Plasma Fe concentrations tended to increase ($P = 0.10$) and plasma Zn concentrations did increase ($P = 0.03$) with increasing Zn supplementation on d 69. Furthermore, d 69 plasma Fe concentrations tended to be greater ($P = 0.06$) and d 69 plasma Zn concentrations were greater ($P = 0.02$) for Zn supplemented steers. During beta agonist supplementation, d 79 and 97 plasma Zn concentrations linearly increased within increasing Zn supplementation ($P \leq 0.05$) and were greater for Zn supplemented steers than Zn0 ($P \leq 0.05$). No effects of Zn supplementation were observed for d 79 or 97 plasma Cu or Fe ($P \geq 0.15$).

## Discussion

Steroidal implants increase ADG of cattle 16-20% (Bartle et al., 1992; Johnson et al., 1996a; Duckett and Pratt, 2014), but it is thought the greatest growth stimulation occurs during the first 40 d after implant administration, corresponding to peak hormonal payout of the implant (Johnson et al., 1996a). Similarly, beta agonists induce rapid growth (Johnson et al., 2014; Lean et al., 2014), albeit across a much shorter timeframe. This growth appears to be greatest early in the beta agonist feeding period (Maxwell et al., 2015; Genther-Schroeder et al., 2018). Because of the need for Zn to support protein synthesis, it was hypothesized that increasing Zn supplementation would be most beneficial during these rapid growth periods occurring shortly after administration of these widely used growth promoting technologies.

Indeed, Zn supplementation linearly increased d 10 BW and d 0-10 ADG, with all three concentrations of supplemental Zn similarly improving implanted cattle growth over un-supplemented controls. Messersmith and Hansen (2021) found supplementing up to 150 mg Zn/kg DM from $ZnSO_4$ linearly improved ADG during the first 18-d after administration of the same high potency implant utilized in this study. In heifers, supplementation of 100 vs. 30 mg Zn/kg DM from $ZnSO_4$ led to a 6 kg advantage in BW 29 d after administering a high potency implant (Messersmith et al., 2021b). Collectively, it appears dietary Zn may be limiting implant-induced growth, and Zn supplementation at greater rates may be necessary to capture the full benefits of this technology, as even basal concentrations of Zn in these studies were well above NASEM (30 mg Zn/kg DM; 2016) recommendations at 42 or 68 mg Zn/kg DM (Messersmith et al., 2021b; Messersmith and Hansen, 2021) with the current study diet analyzing at 39 mg Zn/kg DM.

The current trial's early benefits of Zn supplementation on growth performance of steers persisted until the start of beta agonist supplementation on d 70. Though BW tended to be greater for Zn supplemented steers on d 79, Zn supplementation did not affect d 98 live or carcass-adjusted final BW. While controls lagged early in the trial, once beta agonist supplementation began on d 70, these steers matched or exceeded rates of daily gain of Zn supplemented steers. In combination with the large increase in NEFA concentrations from d 69 to 79 for Zn0 steers this may suggest Zn0 steers were experiencing greater rates of lipolysis in response to beta agonist supplementation. It is tempting to suggest the greater liver Cu concentrations of Zn0 steers on d 79 supported greater lipolytic rates, as Cu status has been shown to influence in vitro lipolysis (Johnson and Engle, 2003; Messersmith et al., 2021a). More work is needed to clarify the potential interaction between tissue Zn and Cu concentrations and the beta agonist growth

response. Genther-Schroeder et al. (2016) found a linear increase in final BW, ADG, and G:F in beta agonist-fed steers when Zn was supplemented up to 150 mg Zn/kg DM total. Likewise, supplementation of 120 or 160 mg Zn/kg DM improved the performance of steers receiving a beta agonist only (Genther-Schroeder et al., 2016b; Wellmann et al., 2020). However, these studies utilized blended organic and inorganic Zn sources (Genther-Schroeder et al., 2016c; Genther-Schroeder et al., 2016b; Wellmann et al., 2020) compared to only $ZnSO_4$ utilized in the current study. It is unclear why Zn source may affect performance response during the beta agonist feeding period. More work is needed to clarify the optimum concentrations and sources of Zn to utilize in feedlot cattle diets during this time.

Hot carcass weight tended to be greater in Zn supplemented steers, though this advantage is most obvious in Zn100 and Zn180 steers. This advantage corresponds to 8 and 6 kg advantages in final BW for Zn100 and Zn180, respectively, over Zn0 and Zn150 steers. Zinc impacts on carcass accretion appear to be subtle but relatively consistent across published works in modern cattle utilizing growth promoting technologies (Genther-Schroeder et al., 2016c; Genther-Schroeder et al., 2016b; Wellmann et al., 2020; Messersmith et al., 2021b; Messersmith and Hansen, 2021). Although $12^{th}$ rib fat was not statistically affected by Zn supplementation, numerically $12^{th}$ rib fat linearly increased with increasing Zn supplementation in agreement with Zn effects observed by Spears and Kegley (2002) and Greene et al. (1988). This numerical trend in $12^{th}$ rib fat is likely why YG linearly increased with increasing Zn supplementation.

Considering all treatments were implanted on d 0 and started a beta agonist on d 70, the well-defined decrease in SUN concentrations observed from d 0 to 10 and d 69 to 79 agree with data from Parr et al. (2014) and Harris et al. (2020) and indicate these technologies result in decreased protein degradation. Although Zn did not influence SUN concentrations during beta

agonist supplementation, the quadratic decrease in the percent change in SUN concentrations from d 69 to 79 suggests supplemental Zn influences protein degradation. However, this response may be directly related to the quadratic tendency of Zn supplementation observed for d 69 SUN instead. Interestingly, this percent change in SUN concentrations from d 69 to 79 was greatest in Zn100 steers. These steers had numerically greater ADG during this period than Zn150 and Zn180, suggesting growth rates did influence protein degradation even though d 79 SUN were not correlated with d 70-79 ADG.

We have previously observed steroidal implants decrease liver Mn concentrations (Messersmith, 2018; Niedermayer et al., 2018; Messersmith et al., 2021b; Reichhardt et al., 2021). Interestingly, cytosolic Mn in the liver is associated with arginase (Rosebrough et al., 1987), the terminal enzyme of the urea cycle (J. S. Bond et al., 1983; Watts, 1990). Given that steroidal implants decrease the demand for the urea cycle (Galbraith, 1980), we suspected the decrease in liver Mn of implanted cattle was associated with decreasing liver arginase. Indeed, we found d 10 liver arginase activity was positively correlated with d 10 liver Mn concentrations and tended to be correlated with d 0-10 ADG. These data support the implant-induced decreases in liver Mn previously observed and suggest changes in protein degradation during the implant response are impacting liver Mn concentrations. It is intriguing to consider liver Mn concentrations in the ruminant may be more directly influenced by N metabolism than previously thought.

In contrast to the first 10 days following implant administration, there was no correlation between d 79 liver arginase activity and d 79 liver Mn concentrations, corresponding to the first 10 d of beta agonist feeding. Perhaps, steroidal implants and beta agonists affect N metabolism differently. Bryant et al. (2010) found steroidal implants improved ADG by 20.7% over non-

implanted heifers during the beta agonist supplementation period while beta agonist supplementation increased ADG by 60.7% over the 28-d beta agonist feeding period. Although beta agonist supplementation induces high growth rates, Ji and Orcutt (1991) indicate beta agonists stimulate protein synthesis more than they decrease protein degradation. These data are consistent with the liver arginase activity and liver Mn response observed during beta agonist supplementation.

By analyzing the liver at time points coordinated with projected peak growth responses to technology, liver trace minerals were expected to shift with growth pressure. Liver Zn has a debatable value as a marker of Zn status (Suttle, 2010); however, the linear response to Zn supplementation on d 10 and 79 suggests high concentrations of supplemental Zn can influence liver Zn stores. Plasma Zn concentrations provide a more robust measurement of Zn that more readily changes with Zn needs. For example, Messersmith (2018) observed implanted steers had lesser plasma Zn 13 and 73 d post-implant administration, suggesting increased demand for Zn to support implant-induced growth. In the current study, plasma Zn concentrations were linearly increased with increasing Zn supplementation on all sampling days. And, similar to Messersmith's (2018) findings, plasma Zn concentrations decreased for all Zn treatments from d 0 to 10; however, this decrease in plasma Zn differs across treatments. Steers receiving no supplemental Zn decreased plasma Zn from d 0 to 10 by 19%, while Zn100, Zn150, and Zn180 dropped by 3, 3, and 9%, respectively. Perhaps the increased growth rates observed in Zn supplemented treatments during this period were due to increased availability of circulating Zn to support protein accretion. If so, these data suggest a threshold for plasma Zn concentrations should be determined to accommodate implant-induced growth.

Interestingly, plasma Zn concentrations increased in all treatments in the first 10 days of beta agonist supplementation even though cattle had been on Zn treatments for 69 d prior to the start of the beta agonist. Both Zn0 and Zn150 increased 9%, while Zn100 and Zn180 increased plasma Zn concentrations by 4.5%. On average, these changes in plasma Zn concentrations were not as strong as the changes observed within the first 10 d after implant administration and were opposite in effect. It is possible plasma Zn concentrations were sufficiently high in all treatments at start of beta agonist feeding on d 69 to prevent any drop in circulating Zn even as growth rates increased across treatments due to ractopamine feeding. More work is warranted to determine how each technology impacts circulating Zn stores and if protein synthesis and degradation rates influence plasma Zn concentrations.

Together these data indicate increasing dietary Zn supplementation well above NASEM (2016) recommendations increased growth, and based on interim performance, this was most dramatic very early after implant administration. Therefore, Zn may be needed to prevent the decline in circulating Zn observed in implanted cattle (Huerta et al., 2002; Messersmith, 2018) and support the demands of growth. It is unclear why steers supplemented 150 mg Zn/kg DM experienced lesser performance than steers supplemented 100 or 180 mg Zn/kg DM throughout the trial. These data suggest 100 mg Zn/kg DM is optimal for improving performance, and no further benefits were noted in treatments receiving more than 100 mg supplemental Zn/kg DM. Furthermore, these data provide a link between growth rates, liver Mn concentrations, and protein degradation, indicating implants influence liver Mn concentrations through regulation of the urea cycle and its terminal enzyme, arginase. These data warrant further research to determine strategic Zn supplementation programs to accommodate the growth of cattle

administered steroidal implants and fed a beta agonist and to understand differences in N metabolism of cattle utilizing these technologies.

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**Table 1.** Diet composition

| Ingredient | % DM basis |
|---|---|
| Dry rolled corn | 45.0 |
| Sweet Bran[1] | 20.0 |
| Corn silage | 15.0 |
| DDGS[2] | 18.06 |
| Limestone | 1.5 |
| Salt | 0.31 |
| Vitamin & mineral premix[3,4] | 0.1165 |
| Rumensin | 0.0135 |
| Analyzed composition | |
| Crude protein[5] | 15.4 |
| Neutral detergent fiber[5] | 19.8 |
| Ether extract[5] | 4.8 |
| Cu, mg/kg DM[6] | 12 |
| Fe, mg/kg DM[6] | 118 |
| Mn, mg/kg DM[6] | 30 |
| Zn, mg/kg DM[6] | 39 |
| Calculated composition[7], Mcal/kg | |
| NEm | 2.05 |
| NEg | 1.39 |

[1]Branded wet corn gluten feed (Cargill Corn Milling, Blair, NE).
[2]Dried distillers grains with solubles.
[3]Premix provided 2,200 IU vitamin A and 25 IU vitamin E/kg diet.
[4]Wtih the exception of Zn, trace minerals were supplemented at NASEM (2016) recommendations for Co, Cu, I, Mn, and Se, from inorganic sources. Diets were supplemented with 0, 100, 150, or 180 mg Zn/kg dry matter (**DM**) from $ZnSO_4$.
[5]Analysis of Zn0 total mixed ration (**TMR**) conducted by Dairyland Laboratories (Arcadia, WI).
[6]Analyzed values for trace minerals represent the Zn0 TMR measured by inductively coupled plasma optical emission spectrometry (ICP Optima 7000 DV, Perkin Elmer, Waltham, MA). Dietary Zn was analyzed as 148, 167, and 205 mg Zn/kg DM for Zn100, Zn150, and Zn180, respectively.
[7]Calculations for net energy of maintenance (**NEm**) and net energy of gain (**NEg**) utilized NASEM (2016) nutrient values of diet ingredients.

**Table 2.** Effect of zinc supplementation on body weights of implanted and beta agonist-fed finishing beef steers

| | Treatments[1] | | | | | Contrasts[2] | | | |
|---|---|---|---|---|---|---|---|---|---|
| | Zn0 | Zn100 | Zn150 | Zn180 | SEM | Lin | Quad | Cub | No Zn vs. Zn |
| Steers (*n*)[3] | 23 | 22 | 23 | 22 | | | | | |
| Weight, kg | | | | | | | | | |
| d 0 (start implant) | 422 | 424 | 425 | 424 | 5.6 | 0.81 | 0.84 | 0.96 | 0.76 |
| d 10 | 440 | 444 | 443 | 444 | 1.3 | 0.03 | 0.33 | 0.61 | 0.02 |
| d 20 | 464 | 468 | 465 | 468 | 1.6 | 0.21 | 0.67 | 0.10 | 0.15 |
| d 41 | 494 | 500 | 494 | 497 | 2.6 | 0.59 | 0.20 | 0.11 | 0.26 |
| d 59 | 522 | 528 | 525 | 526 | 3.7 | 0.48 | 0.43 | 0.65 | 0.32 |
| d 70 (start beta agonist) | 535 | 546 | 543 | 545 | 3.9 | 0.05 | 0.34 | 0.51 | 0.03 |
| d 79 | 555 | 566 | 560 | 564 | 4.2 | 0.18 | 0.32 | 0.26 | 0.09 |
| d 98 | 589 | 599 | 591 | 599 | 5.0 | 0.28 | 0.62 | 0.15 | 0.19 |

[1]Steers were fed 0, 100, 150, or 180 mg Zn/kg DM from $ZnSO_4$ throughout the 98-d trial (**Zn0**, **Zn100**, **Zn150**, and **Zn180**, respectively). All cattle were implanted with a Component TE-200 (TE-200; 200 mg trenbolone acetate + 20 mg estradiol; donated by Elanco Animal Health, Greenfield, IN) on d 0 and fed a beta agonist at 300 $mg \cdot steer^{-1} \cdot d^{-1}$ from d 70 to 98 (ractopamine hydrochloride, donated by Zoetis, Parsippany, NJ).

[2]Contrast statements were formed to test linear (**Lin**), quadratic (**Quad**), and cubic (**Cub**) effects of Zn supplementation and to test for a difference between Zn0 and all other Zn treatments (**No Zn vs. Zn**; Zn100, Zn150, and Zn180).

[3]Initial body weight and pre-trial average daily gain (**ADG**; d -22 through d 0) served as covariates in performance analysis. Initial body weight was not used as a covariate in d 0 body weight.

**Table 3.** Effects of increasing zinc supplementation on average daily gain, dry matter intake, and feed efficiency

| | Treatments[1] | | | | | Contrasts[2] | | | |
|---|---|---|---|---|---|---|---|---|---|
| | Zn0 | Zn100 | Zn150 | Zn180 | SEM | Lin | Quad | Cub | No Zn vs. Zn |
| Steers (*n*)[3] | 23 | 22 | 23 | 22 | | | | | |
| ADG, kg | | | | | | | | | |
| d 0-10 | 1.62 | 2.00 | 1.93 | 1.98 | 0.126 | 0.03 | 0.29 | 0.58 | 0.02 |
| d 0-70 | 1.58 | 1.74 | 1.71 | 1.74 | 0.056 | 0.05 | 0.35 | 0.51 | 0.03 |
| d 70-79 | 2.28 | 2.28 | 1.88 | 2.06 | 0.160 | 0.13 | 0.74 | 0.16 | 0.25 |
| d 70-98 | 1.93 | 1.91 | 1.71 | 1.91 | 0.070 | 0.25 | 0.58 | 0.02 | 0.27 |
| d 0-98 | 1.68 | 1.79 | 1.71 | 1.78 | 0.051 | 0.27 | 0.57 | 0.16 | 0.18 |
| DMI, kg | | | | | | | | | |
| d 0-70 | 10.1 | 10.0 | 10.6 | 10.5 | 0.27 | 0.15 | 0.40 | 0.40 | 0.37 |
| d 70-98 | 10.1 | 9.9 | 10.7 | 10.5 | 0.30 | 0.21 | 0.42 | 0.16 | 0.49 |
| d 0-98 | 10.1 | 10.0 | 10.6 | 10.5 | 0.27 | 0.16 | 0.40 | 0.30 | 0.40 |
| G:F | | | | | | | | | |
| d 0-70 | 0.160 | 0.177 | 0.162 | 0.167 | 0.0065 | 0.56 | 0.16 | 0.19 | 0.23 |
| d 70-98 | 0.192 | 0.195 | 0.160 | 0.177 | 0.0072 | 0.01 | 0.47 | 0.004 | 0.08 |
| d 0-98 | 0.169 | 0.182 | 0.162 | 0.166 | 0.0057 | 0.46 | 0.08 | 0.06 | 0.83 |

[1]Steers were fed 0, 100, 150, or 180 mg Zn/kg DM from $ZnSO_4$ throughout the 98-d trial (**Zn0**, **Zn100**, **Zn150**, and **Zn180**, respectively). All cattle were implanted with a Component TE-200 (TE-200; 200 mg trenbolone acetate + 20 mg estradiol; donated by Elanco Animal Health, Greenfield, IN) on d 0 and fed a beta agonist at 300 $mg \cdot steer^{-1} \cdot d^{-1}$ from d 70 to 98 (ractopamine hydrochloride, donated by Zoetis, Parsippany, NJ).

[2]Contrast statements were formed to test linear (**Lin**), quadratic (**Quad**), and cubic (**Cub**) effects of Zn supplementation and to test for a difference between Zn0 and all other Zn treatments (**No Zn vs. Zn**; Zn100, Zn150, and Zn180).

[3]Initial body weight and pre-trial average daily gain (**ADG**; d -22 through d 0) served as covariates in performance analysis of ADG, dry matter intake (**DMI**), and feed efficiency (**G:F**).

**Table 4.** Effects of increasing zinc supplementation on carcass-adjusted performance and carcass characteristics

| | Treatments[1] | | | | | Contrasts[2] | | | |
|---|---|---|---|---|---|---|---|---|---|
| | Zn0 | Zn100 | Zn150 | Zn180 | SEM | Lin | Quad | Cub | No Zn vs. Zn |
| Steers (*n*)[3] | 23 | 22 | 23 | 22 | | | | | |
| Carcass-adjusted[4] | | | | | | | | | |
| Final weight, kg | 588 | 599 | 591 | 599 | 4.9 | 0.23 | 0.61 | 0.15 | 0.16 |
| ADG, kg | 1.66 | 1.77 | 1.69 | 1.77 | 0.049 | 0.22 | 0.59 | 0.15 | 0.15 |
| G:F | 0.168 | 0.180 | 0.161 | 0.165 | 0.0059 | 0.45 | 0.10 | 0.11 | 0.90 |
| Carcass characteristics | | | | | | | | | |
| Hot carcass weight, kg | 384 | 393 | 389 | 391 | 3.2 | 0.17 | 0.25 | 0.36 | 0.07 |
| Dress, % | 65.3 | 65.6 | 65.8 | 65.2 | 0.33 | 0.79 | 0.29 | 0.41 | 0.53 |
| Ribeye area, $cm^2$ | 88.3 | 90.3 | 87.6 | 88.8 | 1.09 | 0.95 | 0.33 | 0.13 | 0.65 |
| $12^{th}$ rib fat, cm | 1.18 | 1.18 | 1.27 | 1.38 | 0.079 | 0.11 | 0.22 | 0.89 | 0.33 |
| Marbling[5] | 442 | 456 | 452 | 457 | 18.6 | 0.57 | 0.84 | 0.81 | 0.54 |
| Yield grade[6] | 2.1 | 2.3 | 2.5 | 2.6 | 0.16 | 0.02 | 0.74 | 0.86 | 0.04 |

[1]Steers were fed 0, 100, 150, or 180 mg Zn/kg DM from $ZnSO_4$ throughout the 98-d trial (**Zn0**, **Zn100**, **Zn150**, and **Zn180**, respectively). All cattle were implanted with a Component TE-200 (TE-200; 200 mg trenbolone acetate + 20 mg estradiol; donated by Elanco Animal Health, Greenfield, IN) on d 0 and fed a beta agonist at 300 $mg \cdot steer^{-1} \cdot d^{-1}$ from d 70 to 98 (ractopamine hydrochloride, donated by Zoetis, Parsippany, NJ).

[2]Contrast statements were formed to test linear (**Lin**), quadratic (**Quad**), and cubic (**Cub**) effects of Zn supplementation and to test for a difference between Zn0 and all other Zn treatments (**No Zn vs. Zn**; Zn100, Zn150, and Zn180).

[3]Initial body weight and pre-trial average daily gain (**ADG**; d -22 through d 0) served as covariates in carcass-adjusted final weight, ADG, and feed efficiency (**G:F**), as well as carcass characteristics analysis.

[4]Carcass-adjusted data were calculated using treatment dressing percentage averages: 65.30, 65.60, 65.77, and 65.21% for Zn0, Zn100, Zn150, and Zn180, respectively.

[5]Marbling scores: slight = 300, small = 400, modest = 500, moderate = 600.

[6]Yield grade was assigned by the personnel at the commercial abattoir.

**Table 5.** Effects of zinc supplementation on serum urea nitrogen and non-esterified fatty acids.

| | Treatments[1] | | | | | Contrasts[2] | | | |
|---|---|---|---|---|---|---|---|---|---|
| | Zn0 | Zn100 | Zn150 | Zn180 | SEM | Lin | Quad | Cub | No Zn vs. Zn |
| Steers (*n*) | 12 | 12 | 12 | 12 | | | | | |
| SUN[3], mg/dL | | | | | | | | | |
| d 0 | 13.6 | 11.4 | 11.7 | 11.3 | 0.61 | 0.02 | 0.27 | 0.53 | 0.01 |
| d 10 | 9.7 | 9.9 | 10.3 | 9.7 | 0.59 | 0.78 | 0.61 | 0.47 | 0.69 |
| d 69 | 12.2 | 13.3 | 12.4 | 11.5 | 0.70 | 0.61 | 0.08 | 0.99 | 0.82 |
| d 79 | 11.2 | 11.5 | 10.8 | 11.5 | 0.77 | 0.95 | 0.99 | 0.47 | 0.92 |
| d 97 | 11.1 | 13.0 | 11.7 | 12.2 | 0.75 | 0.39 | 0.22 | 0.27 | 0.19 |
| Δ SUN[4], % | | | | | | | | | |
| d 0 to 10 | -21.53 | -12.86 | -11.84 | -17.29 | 5.470 | 0.39 | 0.31 | 0.71 | 0.24 |
| d 69 to 79 | -11.58 | -18.39 | -12.68 | 1.80 | 3.525 | 0.05 | 0.001 | 0.33 | 0.65 |
| NEFA[3], mEq/L | | | | | | | | | |
| d 69 | 137 | 156 | 130 | 122 | 18.0 | 0.55 | 0.27 | 0.66 | 0.96 |
| d 79 | 177 | 166 | 162 | 128 | 13.8 | 0.05 | 0.26 | 0.35 | 0.14 |
| d 97 | 142 | 119 | 141 | 108 | 11.9 | 0.20 | 0.92 | 0.08 | 0.21 |
| Δ NEFA[4], % | | | | | | | | | |
| d 69 to 79 | 31.75 | 17.91 | 42.08 | 7.14 | 17.643 | 0.61 | 0.78 | 0.19 | 0.65 |

[1]Steers were fed 0, 100, 150, or 180 mg Zn/kg DM from $ZnSO_4$ throughout the 98-d trial (**Zn0**, **Zn100**, **Zn150**, and **Zn180**, respectively). All cattle were implanted with a Component TE-200 (200 mg trenbolone acetate + 20 mg estradiol; donated by Elanco Animal Health, Greenfield, IN) on d 0 and fed ractopamine hydrochloride from d 70 to 98 (300 $mg \cdot steer^{-1} \cdot d^{-1}$, donated by Zoetis, Parsippany, NJ).

[2]Contrast statements were formed to test linear (**Lin**), quadratic (**Quad**), and cubic (**Cub**) effects of Zn supplementation and to test for a difference between Zn0 and all other Zn treatments (**No Zn vs. Zn**; Zn100, Zn150, and Zn180).

[3]Serum urea nitrogen (**SUN**) data were analyzed with d 0 values as a covariate, except for d 0 analysis. Non-esterified fatty acid (**NEFA**) data were analyzed with d 69 values as a covariate, expect for d 69 analysis.

[4]Percent change (Δ) was calculated between timepoints for SUN and NEFA data using individual steer data. No covariates were utilized in Δ analysis.

**Table 6.** Effect of increasing zinc supplementation on liver trace mineral concentrations and liver arginase after implant and beta agonist administration.

| | Treatments[1] | | | | | Contrasts[2] | | | |
|---|---|---|---|---|---|---|---|---|---|
| | Zn0 | Zn100 | Zn150 | Zn180 | SEM | Lin | Quad | Cub | No Zn vs. Zn |
| Steers (*n*) | 12 | 12 | 12 | 12 | | | | | |
| Liver[3], mg/kg DM | | | | | | | | | |
| d 10 | | | | | | | | | |
| Cu | 205 | 210 | 204 | 163 | 14.6 | 0.13 | 0.07 | 0.33 | 0.47 |
| Fe | 132 | 139 | 139 | 136 | 4.2 | 0.31 | 0.36 | 0.76 | 0.20 |
| Mn | 7.7 | 6.9 | 7.3 | 7.6 | 0.34 | 0.74 | 0.08 | 0.77 | 0.30 |
| Zn | 108 | 111 | 111 | 119 | 3.0 | 0.04 | 0.31 | 0.26 | 0.13 |
| d 79 | | | | | | | | | |
| Cu | 265 | 248 | 214 | 166 | 14.6 | <0.01 | 0.02 | 0.59 | <0.01 |
| Fe | 170 | 152 | 151 | 187 | 12.3 | 0.75 | 0.04 | 0.26 | 0.62 |
| Mn | 8.1 | 7.7 | 7.7 | 7.4 | 0.46 | 0.28 | 0.95 | 0.76 | 0.33 |
| Zn | 116 | 132 | 120 | 143 | 5.8 | 0.02 | 0.65 | 0.01 | 0.03 |
| Liver arginase activity, nmol/µg/min | | | | | | | | | |
| d 10 | 0.97 | 0.78 | 0.99 | 1.20 | 0.114 | 0.19 | 0.02 | 0.94 | 0.86 |
| d 79 | 0.87 | 0.90 | 0.86 | 1.00 | 0.099 | 0.51 | 0.59 | 0.41 | 0.65 |

[1]Steers were fed 0, 100, 150, or 180 mg Zn/kg DM from $ZnSO_4$ throughout the 98-d trial (**Zn0**, **Zn100**, **Zn150**, and **Zn180**, respectively). implanted with a Component TE-200 (TE-200; 200 mg trenbolone acetate + 20 mg estradiol; donated by Elanco Animal Health, Greenfiel a beta agonist at 300 mg·steer$^{-1}$·d$^{-1}$ from d 70 to 98 (ractopamine hydrochloride, donated by Zoetis, Parsippany, NJ).

[2]Contrast statements were formed to test linear (**Lin**), quadratic (**Quad**), and cubic (**Cub**) effects of Zn supplementation and to test for a d between Zn0 and all other Zn treatments (**No Zn vs. Zn**; Zn100, Zn150, and Zn180).

[3]Liver biopsies were collected on d 9/10 and 78/79, representing 9 or 10 d-post administration of either growth promoting technology.

**Table 7.** Correlations between liver arginase activity, liver manganese, serum urea nitrogen, and average daily gain.

| | Liver Mn[1] | | SUN[1] | | ADG[2] | |
|---|---|---|---|---|---|---|
| | Corr[3] | *P*-value | Corr[3] | *P*-value | Corr[3] | *P*-value |
| d 10 | | | | | | |
| Liver arginase | 0.27 | 0.07 | - 0.04 | 0.77 | - 0.01 | 0.95 |
| Liver Mn | - | - | 0.55 | < 0.0001 | 0.25 | 0.09 |
| SUN | - | - | - | - | 0.10 | 0.49 |
| d 79 | | | | | | |
| Liver arginase | 0.19 | 0.21 | 0.07 | 0.64 | - 0.15 | 0.33 |
| Liver Mn | - | - | 0.29 | 0.05 | 0.003 | 0.98 |
| SUN | - | - | - | - | - 0.18 | 0.23 |

[1]Liver biopsies and serum urea nitrogen (**SUN**) were collected on d 9/10 and 78/79, representing 9 or 10 d-post administration of either growth promoting technology.
[2]Average daily gain (**ADG**) represents the average daily gain of steers from d 0-10 for correlations within d 10 values and d 70-79 for correlations within d 79 values.
[3]Corr: *r*, Pearson's correlation coefficient.

**Table 8.** Effects of increasing zinc supplementation on plasma trace mineral concentrations after implant and beta agonist administration.

| | Treatments[1] | | | | | Contrasts[2] | | | |
|---|---|---|---|---|---|---|---|---|---|
| | Zn0 | Zn100 | Zn150 | Zn180 | SEM | Lin | Quad | Cub | No Zn vs. Zn |
| Steers (*n*) | 12 | 12 | 12 | 12 | | | | | |
| Plasma[3], mg/L | | | | | | | | | |
| d 0 | | | | | | | | | |
| Cu | 0.90 | 0.87 | 0.87 | 0.86 | 0.045 | 0.51 | 0.92 | 0.79 | 0.50 |
| Fe | 2.13 | 1.90 | 2.06 | 1.79 | 0.152 | 0.24 | 0.99 | 0.23 | 0.25 |
| Zn | 1.32 | 1.21 | 1.18 | 1.31 | 0.040 | 0.24 | 0.01 | 0.14 | 0.05 |
| d 10 | | | | | | | | | |
| Cu | 0.98 | 0.96 | 0.99 | 1.01 | 0.032 | 0.65 | 0.38 | 0.97 | 0.96 |
| Fe | 2.04 | 2.23 | 2.23 | 2.05 | 0.081 | 0.50 | 0.05 | 0.47 | 0.17 |
| Zn | 1.07 | 1.17 | 1.15 | 1.19 | 0.034 | 0.02 | 0.54 | 0.33 | 0.02 |
| d 69 | | | | | | | | | |
| Cu | 0.97 | 0.91 | 0.90 | 0.96 | 0.034 | 0.52 | 0.10 | 0.51 | 0.23 |
| Fe | 1.86 | 2.26 | 2.24 | 2.17 | 0.157 | 0.10 | 0.23 | 0.99 | 0.06 |
| Zn | 1.17 | 1.33 | 1.28 | 1.33 | 0.048 | 0.03 | 0.32 | 0.32 | 0.02 |
| d 79 | | | | | | | | | |
| Cu | 1.00 | 0.98 | 0.99 | 1.00 | 0.047 | 0.95 | 0.70 | 0.98 | 0.92 |
| Fe | 1.96 | 2.20 | 2.03 | 1.91 | 0.144 | 0.89 | 0.15 | 0.83 | 0.61 |
| Zn | 1.28 | 1.39 | 1.39 | 1.39 | 0.042 | 0.04 | 0.35 | 0.82 | 0.03 |
| d 97 | | | | | | | | | |
| Cu | 0.97 | 0.95 | 0.96 | 1.01 | 0.031 | 0.52 | 0.24 | 0.68 | 0.89 |
| Fe | 2.17 | 2.18 | 2.26 | 2.20 | 0.111 | 0.69 | 0.94 | 0.66 | 0.72 |
| Zn | 1.20 | 1.31 | 1.29 | 1.35 | 0.047 | 0.05 | 0.71 | 0.39 | 0.05 |

Table 8 Continued

[1]Steers were fed 0, 100, 150, or 180 mg Zn/kg DM from $ZnSO_4$ throughout the 98-d trial (Zn0, Zn100, Zn150, and Zn180, respectively). All cattle were implanted with a Component TE-200 (TE-200; 200 mg trenbolone acetate + 20 mg estradiol; donated by Elanco Animal Health, Greenfield, IN) on d 0 and fed a beta agonist at 300 $mg \cdot steer^{-1} \cdot d^{-1}$ from d 70 to 98 (ractopamine hydrochloride, donated by Zoetis, Parsippany, NJ).

[2]Contrast statements were formed to test linear (**Lin**), quadratic (**Quad**), and cubic (**Cub**) effects of Zn supplementation and to test for a difference between Zn0 and all other Zn treatments (**No Zn vs. Zn**; Zn100, Zn150, and Zn180).

[3]Blood was collected on d 0, 9/10, 69, 78/79, and 97. Trace mineral concentrations from d 0 were utilized as a covariate in analysis of that respective mineral on subsequent days.

# CHAPTER 5. ZINC SUPPLEMENTATION STRATEGIES IN FEEDLOT HEIFERS RECEIVING AN EXTENDED-RELEASE IMPLANT OR AN AGGRESSIVE RE-IMPLANT PROGRAM

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## Abstract

Two-hundred and eight Angus-crossbred heifers (291 ± 23 kg) from four sources were used in a randomized complete block design. The objective of the study was to determine the effects of implant strategy and Zn supplementation on performance, carcass characteristics, muscle fiber diameter, and mineral status of heifers. Heifers were assigned to a 2 × 2 factorial study for 168 d, factors included Zn and implant (**IMP**). Heifers were supplemented Zn (mg/kg dry matter [**DM**]; $ZnSO_4$) at national (30; **NRC**) or industry (100; **IND**) recommendations. Implant strategies (Merck Animal Health, Madison, NJ) included extended-release Revalor-XH on d 0 (**REV-XH**; 20 mg estradiol + 200 mg trenbolone acetate) containing 4 uncoated pellets

and 6 coated pellets or the uncoated implant Revalor-200 on d 0 and again on d 91 (**REV-200**; 20 mg estradiol + 200 mg trenbolone acetate). Heifers were blocked by weight within source to pens of 5 or 6 heifers per pen (9 pens per treatment). A corn silage-based diet was fed during the growing period (d 0-55) followed by transition to a corn-based finishing diet. Weights were taken consecutively on d -1/0, 55/56, and 167/168. Liver and muscle from the longissimus thoracis were collected from one heifer per pen on d -5, 14, 105, and 164. Data were analyzed via Mixed Procedure of SAS. Average daily gain (**ADG**) and liver mineral used Period as the repeated effect. Corresponding to periods of high hormone payout from each implant, d 0-28 and 91-120 ADG were greatest for REV-200, while REV-XH numerically peaked during d 56-91 (IMP × Period; $P = 0.02$). Day 91 IND body weight tended to be heavier ($P = 0.06$) and d 120 body weight was heavier ($P = 0.05$) than NRC heifers. No effect of Zn or IMP on final body weight was observed ($P \geq 0.21$). Muscle fiber cross-sectional diameter on d 164 was greater ($P = 0.05$) in IND than NRC. Liver Mn concentrations decreased by d 14 regardless of implant, though d 105 and 164 concentrations were lesser for REV-200 than REV-XH (IMP × Period; $P = 0.02$). No effects of Zn, IMP, or the interaction were observed for carcass-adjusted gain to feed, d 0-168 DM intake, hot carcass weight, or ribeye area ($P \geq 0.11$). The nominal differences in performance between implant strategies suggests extended-release implants may be an effective implant strategy to replace re-implant programs in heifers, while the improved performance of heifers fed IND vs. NRC during times of peak hormone payout suggests a role for Zn in periods of rapid growth.

Key words: anabolic implant, beef cattle, extended-release, muscle fiber cross-sectional diameter, trace mineral

## Introduction

The use of anabolic steroidal implants in the feedlot is common practice (Samuelson et al., 2016), with approximately 84% of cattle on feed in the U.S. receiving an implant upon arrival, and 71% of cattle receiving an implant at a second processing date (NAHMS, 2013). Continuous anabolic implant exposure through an initial and terminal implant program improves growth performance (Reinhardt, 2007), but additional costs are associated with a second processing date. Revalor-XH, an extended-release implant developed specifically for heifers, provides 200 mg trenbolone acetate + 20 mg estradiol (Merck Animal Health; Madison, NJ) evenly dispersed across ten pellets (FDA [Food and Drug Administration], 2017a). Revalor-XH consists of 4 uncoated and 6 coated pellets (FDA, 2017a). Uncoated pellets provide immediate release of trenbolone acetate and estradiol, while hormone payout from coated pellets begins at approximately d 70 (FDA, 2017b). In a re-implant program, feedlot heifers may receive Revalor-200 (200 mg trenbolone acetate + 20 mg estradiol; Merck Animal Health) as both the initial and terminal implant. This strategy offers more than twice the anabolic hormone dose in comparison to a single Revalor-XH. Although increased hormone potency may be expected to improve cattle growth (Bartle et al., 1992) previous studies have reported minimal differences in final body weight (**BW**) between these strategies (Crawford et al., 2018; Ohnoutka et al., 2018). However, few studies have reported cattle performance during interim periods when comparing extended-release implant and re-implant programs.

Zinc may be essential to support optimal carcass accretion in implanted cattle as lambs implanted with zeranol exhibited greater absorption and retention of Zn (Hufstedler and Greene, 1995). Furthermore, Carmichael et al. (2018) reported a positive correlation between Zn and N retention in late-stage finishing beef steers. Arguably the most utilized trace mineral in the body,

Zn is involved in thousands of proteins (Andreini et al., 2006) and has a vital role in protein synthesis (Wegener and Romano, 1963; Oberleas and Prasad, 1969). In spite of substantial improvements in beef cattle growth due to genetic selection and development of growth promoting technologies, Zn recommendations have remained steady (NRC, 1996; NASEM, 2016), though nutritional consultants report feeding Zn at three times published recommendations (Samuelson et al., 2016a). Niedermayer et al. (2018) found increasing supplemental trace mineral concentrations to industry rather than published recommendations improved hot carcass weight (**HCW**) and feed efficiency in steers. Therefore, the objective of this study was to determine if supplemental Zn (as $ZnSO_4$) at national (30 mg Zn/kg dry matter [**DM**]) or industry (100 mg Zn/kg DM) recommendations would affect performance of heifers given a two-implant (Revalor-200, Revalor-200) or extended-release (Revalor-XH) implant strategy. It was hypothesized that increased supplemental Zn would increase growth rate with greatest effects in heifers receiving a two-implant strategy compared to a single extended-release implant.

## Materials and Methods

### Animal Use and Care

The Iowa State University Institutional Animal Care and Use Committee (log number: IACUC-18-103) approved all procedures and protocols used in this study.

### Animals and Experimental Design

A total of 208 Angus-crossbred heifers (291 ± 23 kg) from 4 sources of origin were used in a 2 × 2 factorial randomized complete block design study examining the effect of Zn supplementation on the performance of beef heifers receiving different implant strategies throughout the 168-d study conducted from July through December 2018. Cattle were processed

before the start of the study to induce luteolysis (Estrumate; Merck Animal Health, Madison, NJ), prevent clostridial diseases (Vision 7; Merck Animal Health), deter respiratory diseases (Vista Once; Merck Animal Health) and defend against parasites (Safe-Guard; Merck Animal Health). Within source, heifers were blocked by weight to pens of 5 or 6 (36 pens total) and fed a corn silage-based growing diet from d 0-56 and then transitioned to a dry rolled corn-based finishing diet for the remainder of the study (**Table 1**). Each block consisted of 4 pens (*n* =1/treatment). Melengestrol acetate (**MGA**), Rumensin, and trace minerals were supplemented to the diet through dried distillers grains with solubles-based premixes. During the first 23 days of the growing diet an ingredient DM error occurred, though differences in MGA, Rumensin, and Zn supplementation were negligible. The error was corrected on d 24 and is reflected in the diet table. Cattle were fed ad-libitum via concrete bunks at approximately 0800 h daily and had access to automatic waterers. Two dietary Zn treatments were fed, including Zn supplementation at national (**NRC**; 30 mg Zn/kg DM; NASEM, 2016) or industry (**IND**; 100 mg Zn/kg DM; Samuelson et al., 2016) recommendations from $ZnSO_4$. Within Zn treatment, heifers received either the extended-release implant Revalor-XH (200 mg trenbolone acetate + 20 mg estradiol; Merck Animal Health) on d 0 or Revalor-200 (200 mg trenbolone acetate + 20 mg estradiol; Merck Animal Health) on d 0 and again on d 91. There were nine pens per full factorial treatment combination. Cattle were shipped to a commercial abattoir (Iowa Premium Beef, Tama, IA; trucking distance: 100 km) in the afternoon of d 168 and were harvested the morning of d 169.

**Sample Collection and Analysis**

All heifers were weighed individually on consecutive days at the beginning of the study (d -1/0), end of the growing period (d 55/56), and end of the study (d 167/168). Intermediate BW

were collected on d 28, 91, and 120. Cattle were harvested on d 169 at a commercial abattoir and carcass characteristics were collected by trained University personnel on the left half of the carcass. No camera data were available. Hot carcass weight data were recorded at harvest and following a 48-h chill, ribeye area (**REA**), back fat (**BF**), kidney, pelvic, heart fat (**KPH**), and marbling score were collected, and yield grade (**YG**) was calculated. Carcass-adjusted final BW was calculated by dividing HCW by treatment averages for dressing percent (63.46, 63.95, 64.14, and 63.90% for NRC/REV-XH, NRC/REV-200, IND/REV-XH, and IND/REV-200, respectively) and gain to feed ratio (**G:F**) was calculated by dividing average daily gain (**ADG**) by dry matter intake (**DMI**).

Liver and muscle biopsies ($n$ = 9/treatment) were conducted on one heifer, randomly selected prior to initiation of the study, from each pen on d -5, 14, 105, and 164 following the method described by Engle and Spears (2000) and adapted procedures from Pampusch et al. (2008), respectively. These sampling d represented initial and final samples as well as 14 d post-implant administration to capture effects of peak hormonal payout of implants on liver and muscle parameters. Muscle samples were collected from the longissimus thoracis between the $10^{th}$ and $13^{th}$ ribs. Rib space and side of biopsy were alternated between timepoints. Liver and composites of total mixed ration (**TMR**) were acid digested with trace mineral grade nitric acid (Fisher Scientific, Fair Lawn, NJ) following the procedures outlined by Richter et al. (2012). Inductively coupled plasma optical emission spectrometry (Optima 7000 DV, Perkin Elmer, Waltham, MA) was used to analyze plasma, liver, and TMR composites for trace mineral concentration as described by Pogge and Hansen (2013) and Richter et al. (2012).

Muscle samples were analyzed for cross-sectional diameter and area at Utah State University. Samples from the longissimus thoracis were mounted with the muscle fibers

perpendicular to the cork, cryosectioned (10 μm thick), and used for histochemical fiber type staining. Serially sectioned samples were stained using a succinate dehydrogenase stain following previously described methods (Pearse, 1968; Picard et al., 1998; Thornton et al., 2012). In brief, samples were incubated for 60 min in a medium consisting of 0.2 M phosphate buffer, 0.17 M sodium succinate, and 1.2 mM nitro blue toluene. Then, samples were washed serially with deionized $H_2O$, 30% acetone, 60% acetone, 90% acetone, 60% acetone, 30% acetone, and deionized water, respectfully. Cover slips were then mounted onto sectioned samples using a mounting medium containing 0.09 M gelatin, 6.7 M glycerol, and 0.12 M phenol. Images of sectioned samples were taken using a Zeiss AXIO Observer.Z1 microscope (Carl Zeiss Meditec, Inc., Dublin, CA). At least 3 different captured images from each sample were taken and used for analysis. Using Image-Pro® Plus (Media Cybernetics, Inc., Rockville, MD), average area and diameter were calculated from at least 20 skeletal muscle fibers of each sample.

Weekly TMR samples were dried in a forced air oven at 70˚C for 48 h for determination of diet DM. Dried TMR were ground through a 2-mm screen (Retsch Zm100 grinder; Glen Mills Inc., Clifton, NJ) and samples were composited for each Zn treatment by month within growing and finishing period. Excess feed in the bunk was weighed and a sub-sample was dried as previously described at the end of the growing and finishing period for calculation of feed disappearance during these periods. Total DMI per pen was calculated utilizing DM from weekly TMR. The DM weight of excess feed was subtracted from each pen, and this value divided by the number of heifers in each pen to determine the average heifer DMI for the pen. After proper weighing and sampling, off condition feed was discarded as necessary throughout the study.

### Statistical Analysis

Performance and carcass data of this randomized complete block design were analyzed as a 2 × 2 factorial using the Mixed procedure of SAS 9.4 (SAS Inst. Inc., Cary, NC). The model included the fixed effects of Zn, implant, the interaction, and block. Initial BW was used as a covariate in the analysis of performance and carcass characteristics. Analysis of ADG and liver trace mineral were conducted as repeated measures with Period or d of sampling as the repeated effect, respectively. Compound symmetry and unstructured covariance matrixes were selected for ADG and trace mineral analysis, respectively, based on the lowest Akaike Information Criterion. The experimental unit was pen ($n$ = 9 per treatment). Cook's D was used to assess outliers. Outliers were discovered for muscle cross-section diameter (d 1: REV-XH-IND [1], d 2: REV-XH-NRC [2] and REV-200-NRC [1], d 3: REV-XH-IND [1]), but no other variables. Six heifers, all from different pens, were removed from the study due to health issues unrelated to experimental treatment (REV-XH-NRC: 1, REV-XH-IND: 2, REV-200-NRC: 1, REV-200-IND: 2). Data for these animals were removed from analysis on the d of dismissal from the study. All data are reported as least square means with the standard error of the mean. Data were determined statistically significant at $P \leq 0.05$, and a statistical tendency at $0.05 < P \leq 0.10$.

## Results

### Performance and Carcass Characteristics

Performance parameters are displayed in **Table 2**. Heifer BW were not impacted by Zn × IMP ($P \geq 0.27$), and Zn treatment did not affect initial, d 28, d 56, or final live BW ($P \geq 0.34$). However, IND heifers tended to be heavier on d 91 ($P = 0.06$) and were heavier on d 120 ($P = 0.05$), with a 7 kg advantage over NRC by d 120. Implant did not affect initial, d 91, or final live

BW ($P \geq 0.21$), but interim BW were affected by IMP, as REV-200 heifers were heavier on d 28 and 56 ($P \leq 0.04$) and tended to be heavier on d 120 ($P = 0.09$).

Dry matter intake was unaffected by Zn × IMP ($P \geq 0.58$) or IMP ($P \geq 0.51$). Dry matter intake during the initial implant period and d 0-168 DMI was not affected by Zn ($P \geq 0.15$); however, IND tended to have greater DMI than NRC during the re-implant period (d 91-168; $P = 0.09$). There was no interaction between Zn and IMP for the initial implant period G:F ($P = 0.94$), nor was there an effect of IMP ($P = 0.44$), while IND improved feed efficiency during this period (d 0-90; $P = 0.04$). However, re-implant period G:F was affected by Zn × IMP ($P = 0.03$), where feed efficiency was greater for REV-200-NRC heifers than any other treatment (d 91-169). No differences in carcass adjusted final BW, overall ADG, or overall G:F due to Zn, IMP, or the interaction were observed ($P \geq 0.11$).

Average daily gain data analyzed as repeated measures across the study indicated an effect of IMP × Period ($P = 0.02$; **Figure 1**). In general, improvements in ADG followed expected peak hormone payout for the two implant strategies, where ADG was greater from d 0-28 and 91-120 for REV-200 vs. REV-XH, while ADG was not different due to IMP during the periods of d 28-56, 56-91 or 120-168. Additionally, no Zn, IMP, Zn × IMP, Zn × Period, or Zn × IMP × Period effects were observed for ADG ($P \geq 0.12$; Period $P = 0.0001$; Period effect data not shown).

Carcass characteristics shown in **Table 3** indicated no Zn, IMP, or Zn × IMP effects for HCW, REA, BF, KPH, marbling, or YG ($P \geq 0.11$). However, dressing percentage tended ($P = 0.08$) to be affected by the interaction of Zn and IMP, where Rev-XH-IND heifers had greater dressing percent than REV-XH-NRC, while REV-200 heifers were intermediate, regardless of Zn treatment.

**Liver and Muscle Parameters**

Treatment means for liver trace mineral concentrations analyzed as repeated measures are shown in **Table 4**. Liver Cu, Fe, and Zn concentrations were not affected by Zn, IMP, Zn × IMP, Zn × Day, IMP × Day, or Zn × IMP × Day ($P \geq 0.12$). However, a Zn × IMP effect ($P = 0.03$) was observed for liver Mn concentrations where, within IND heifers, concentrations were less in REV-200 vs. REV-XH. No difference in liver Mn concentrations between implant strategies within NRC treatment was observed. Liver Mn decreased across the first 14 d regardless of implant strategy, but REV-XH heifers returned to initial concentrations by d 105 while REV-200 heifers maintained lesser liver Mn concentrations from d 14 to the end of the study (**Figure 2**; IMP × Day; $P = 0.02$). No Zn × Day or Zn × IMP × Day effects ($P \geq 0.58$) were observed for liver Mn concentrations while a Day effect was observed for liver Cu, Fe, Mn, and Zn ($P \leq 0.0002$).

Muscle cross-sectional diameter and area measured from biopsy samples removed from the longissimus thoracis at three-time points are shown in **Table 5**. No effects of Zn, IMP, or the interaction were observed on d 14 or 105 ($P \geq 0.12$) on cross-sectional diameter. Near the end of the study, d 164, muscle cross-sectional diameter was greater for IND supplemented heifers than NRC ($P = 0.05$). However, no IMP or Zn × IMP effects were observed for d 164 muscle cross-sectional diameter ($P \geq 0.28$) and no Zn, IMP, or Zn × IMP effects were observed for muscle cross-sectional area on d 14, 105, or 164 ($P \geq 0.11$).

## Discussion

A shift towards heavier cattle carcasses has led to longer days on feed and, subsequently, more potent implant strategies. The present study compared a potent two-implant strategy, REV-200 on d 0 and again on d 91, with the extended-release implant REV-XH administered on d 0.

Considering implant administration induces protein accretion, and Zn is critical in many biological processes that support protein synthesis (Oberleas and Prasad, 1969; Suttle, 2010), the effect of Zn supplemented at national (30 mg/kg DM; NASEM, 2016) or industry (100 mg/kg DM; Samuelson et al., 2016) recommendations was examined.

No difference in final BW between REV-XH and REV-200 heifers was detected in the present study, similar to observations of Ohnoutka et al. (2018) when Revalor-200 was administered on both d 0 and 100 (151, 165, 179, or 193 d on feed). Likewise, when Revalor-XH was compared to a less potent initial implant (Revalor-IH) followed by a Revalor-200 on d 90 (172, 193, or 214 d on feed), nominal differences in performance were observed (Crawford et al., 2018). However, the comparison of Revalor-XS vs. a two-implant strategy of the equivalent hormone potency (Revalor-IS followed by Revalor-S) revealed greater carcass-adjusted final BW and ADG for Revalor-XS steers (Parr et al., 2011). Three additional experiments conducted by Parr et al. (2011) and a study by Nichols et al. (2014) observed no differences in performance using the Revalor-IS/Revalor-S implant program. These studies in combination with the present work indicate little to no difference in performance of cattle receiving an extended-release implant vs. a two-implant strategy of equal or greater hormone potency.

In contrast to many extended-release implant comparison studies (Parr et al., 2011; Nichols et al., 2014; Crawford et al., 2018), BW data from both REV-200 and REV-XH heifers were collected at all interim weigh dates in the present study. The more potent REV-200 treatment exhibited greater ADG during d 0-28 and 91-120, directly following the first and second Revalor-200 implant administration. Furthermore, ADG of REV-XH was numerically greater than REV-200 during d 56-91, presumably due to the coated pellets' hormonal payout

around d 70 as suggested by explant data of a similarly coated implant, Revalor-XR (FDA, 2017b).

Increasing dietary Zn positively affected interim BW during peak implant-induced growth on d 91 (re-implant) and 120. The increase in REV-XH heifer performance between d 56 and 91 corresponds to the release of the second portion of Revalor-XH hormone around d 70. Coinciding with this peak payout of hormone, supplementing IND vs. NRC to REV-XH heifers resulted in 6, 8, and 9 kg advantages on d 91, 120, and 168, respectively. However, in REV-200 heifers, the numerical BW advantage due to IND over NRC is more gradual across the study and peaks at 6 kg on d 120. In alignment with the observed Zn effects in the present study, Huerta et al. (2002) found steers supplemented with 200 mg Zn/kg DM from $ZnSO_4$ had a greater improvement in ADG due to implant than steers fed the basal diet (84 mg Zn/kg DM). Additionally, heifers receiving Revalor-H (140 mg trenbolone acetate + 14 mg estradiol; Merck Animal Health) and supplemented with Zn (75 mg Zn/kg DM) regardless of $ZnSO_4$, Zn-methionine, or Zn-propionate source numerically had 6.0% greater ADG compared to un-supplemented heifers consuming a diet containing 52.5 or 50.5 mg Zn/kg DM (Nunnery et al., 2007b).

Final BW and carcass characteristics in the present study were nearly identical between IND and NRC for REV-200 heifers. However, IND heifers had greater longissimus thoracis muscle fiber diameter than NRC near the end of the study (d 164). Such effects on muscle fiber diameter supports the role of Zn in muscle growth. More work is needed to determine the impact of different Zn supplementation strategies on muscle growth in heifers. Furthermore, DMI was minimally influenced by Zn in the present study, similar to work of others (Greene et al., 1988). Improved performance in IND vs. NRC during the initial implant period resulted in greater G:F

during this time, possibly because this period captured greater growth rates from both the hormone release of coated (d 0) and uncoated (d 70) pellets for REV-XH as well as the potent initial Revalor-200 for REV-200 heifers. In the re-implant period, there was a trend for IND to have greater DMI than NRC heifers. This corresponded to poorer feed efficiency in IND heifers during the terminal implant period, while efficiency was also decreased in REV-XH heifers fed NRC Zn because of poorer gains in this terminal window.

The effect of anabolic implants on trace mineral status of cattle is not well understood. However, the association of Zn with protein synthesis (Oberleas and Prasad, 1969) and Mn with N metabolism as a cofactor for arginase, the terminal enzyme of the urea cycle (Bond et al., 1983; Watts, 1990), suggest trace minerals are pertinent to implant-induced growth. Administering sheep an implant containing the estrogen derivative zeranol resulted in increased Zn absorption and retention as well as a tendency for increased Mn absorption and retention (Hufstedler and Greene, 1995). Furthermore, concurrent increases in Zn and Mn retention when steers were supplemented 120 mg Zn/kg DM (Carmichael et al., 2018) also indicates a relationship between Zn and Mn utilization in cattle experiencing high growth rates.

Previous work in our laboratory has indicated administration of a potent terminal implant decreases liver Mn 14-d post-implant (Messersmith, 2018; Niedermayer et al., 2018), and in the present study both implant strategies decreased liver Mn in the first 14 d of the study. The nominal decrease in liver Mn of REV-XH may be due to the lesser potency of hormone released over the 168-d study compared to the REV-200 treatment. Because there were no non-implanted heifers in this study it is unclear if REV-XH liver Mn would be lesser than non-implanted heifers, though this would be anticipated. Increasing hormone potency would be expected to increase cattle growth response (Bartle et al., 1992) and thus muscle catabolism would be

decreased in implanted cattle (Galbraith, 1980). We hypothesize liver Mn is decreased in more potent implant strategies due to lesser demand for the urea cycle and subsequently the Mn dependent enzyme arginase. Little work has been done to quantify exactly why this change in liver Mn occurs in implanted cattle.

Messersmith (2018) observed a decrease in liver and plasma Zn 14 d following administration of a potent implant (Component TE-200; 200 mg trenbolone acetate + 20 mg estradiol; Elanco Animal Health; Greenfield, IN) compared to non-implanted steers. A lack of available biomarkers of Zn status complicates the search to better understand the complex interactions between trace minerals and anabolic implant-induced growth. Furthermore, no effect of implants or dietary Zn concentration was found on liver Zn or Cu concentrations, in contrast to work by Niedermayer et al. (2018) who reported liver Cu to be decreased 14 d after delivery of a moderate potency implant.

Consistent with previous work (Bondurant et al., 2018; Ohnoutka et al., 2018), minimal differences in HCW, dressing percent, REA, or YG were observed between Revalor-XH and Revalor-200 followed by a second Revalor-200. Ohnoutka et al. (2018) noted a tendency for Revalor-XH heifers to have better USDA marbling scores than those receiving the Revalor-200 strategy while the current study revealed no differences in marbling due to implant. Overall, the lack of differences in carcass characteristics due to implant strategy suggest cattle feeders may select whichever strategy best fits their production system. However, Zn supplementation may drive this decision as within REV-XH, heifers fed IND tended to have greater dressing percent than NRC with no dress differences within REV-200. Zinc treatment did not impact additional carcass characteristics in the current study. Effects of Zn supplementation on carcass characteristics have been mixed, likely due to differences in basal Zn concentrations for non-

supplemented animals. Spears and Kegley (2002) observed an increase in quality grade and marbling while both YG and BF tended to increase due to supplementation of 25 mg Zn/kg DM to a basal diet containing either 33 or 26 mg Zn/kg DM for the growing and finishing periods, respectively. Similarly, Malcolm-Callis et al. (2000) observed a quadratic increase in YG and BF due to supplementation of 20, 100, or 200 mg Zn/kg DM from $ZnSO_4$ (basal diet ~70 mg Zn/kg DM) with steers supplemented 100 mg Zn/kg DM having the greatest BF and YG.

This work suggests overall performance and carcass characteristics were not drastically affected by either Zn or implant treatment. Revalor-XH is a viable alternative to re-implanting heifers without losing potential gains, though this result appears to be somewhat dependent on dietary Zn concentration. Interim performance improvements due to IND supplementation of Zn provides evidence Zn is an important trace mineral during periods of high growth rates such as that occurring during peak hormonal payout. Future work is warranted to determine optimal implant strategies for long-fed cattle and refine Zn requirements of cattle receiving anabolic implants to best capture carcass gains.

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**Table 1.** Diet composition

| % DM basis | Growing[1,2] | Finishing[3] |
|---|---|---|
| Ingredient | | |
| Dry rolled corn | --- | 40.0 |
| Sweet Bran[4] | 40.0 | 20.0 |
| Corn silage | 40.0 | 20.0 |
| DDGS[5] | 18.04 | 18.04 |
| Limestone | 1.5 | 1.5 |
| Salt | 0.31 | 0.31 |
| Vitamin premix[6] | 0.1 | 0.1 |
| Mineral premix[7] | 0.02 | 0.02 |
| Rumensin[8] | 0.0135 | 0.0135 |
| MGA[9] | 0.0134 | 0.0134 |
| Analyzed composition[10] | | |
| Crude protein[10] | 18.44 | 15.45 |
| NDF[10] | 33.02 | 22.46 |
| Ether extract[10] | 4.14 | 4.32 |
| NEg, Mcal/kg[11] | 1.30 | 1.40 |
| Sulfur[10] | 0.31 | 0.25 |
| Cu, mg/kg DM[12] | 15 | 14 |
| Fe, mg/kg DM[12] | 90 | 69 |
| Mn, mg/kg DM[12] | 40 | 38 |
| Zn, mg/kg DM[12,13] | 77 | 68 |

[1]Growing period d 0-55; heifers transitioned to finishing diet over 2 transition diets.

[2]Ingredient dry matter error occurred during the first 23 d of growing period before correction to displayed growing diet. Differences in MGA, Rumensin, and Zn supplementation were negligible.

[3]Finishing period d 84-169.

[4]Branded wet corn gluten feed (Cargill Corn Milling, Blair, NE).

[5]Dried distillers grains with solubles.

[6]Premix provided 2,200 IU vitamin A and 25 IU vitamin E/kg diet.

[7]Minerals were provided at NASEM (2016) recommendations for Co, Mn, Se, Cu, and I from inorganic sources in addition to 30 (**NRC**) or 100 (**IND**) mg Zn/kg DM from $ZnSO_4$.

[8]Active ingredient Monensin (Elanco, Greenfield, IN).

[9]Melengestrol acetate (Zoetis, Florham Park, NJ).

[10]Analysis of total mixed rations by Dairyland Laboratories (Arcadia, WI).

[11]Net energy of gain was calculated using NASEM (2016) values for ingredients.

Table 1 Continued

[12]Analyzed values for trace minerals represent NRC treatment for both growing and finishing periods measured by inductively coupled plasma optical emission spectrometry (ICP Optima 7000 DV, Perkin Elmer, Waltham, MA).

[13]Analyzed Zn concentrations for IND treatment total mixed ration during growing and finishing periods were 149 and 129 mg Zn/kg DM, respectively.

**Table 2.** Effect of zinc[1] supplementation and implant[2] strategy on performance of finishing heifers

| | NRC | | IND | | | *P*-value | | |
|---|---|---|---|---|---|---|---|---|
| | REV-XH | REV-200 | REV-XH | REV-200 | SEM | Zn | IMP | Zn × IMP |
| Pens (n) | 9 | 9 | 9 | 9 | | | | |
| Live performance[3] | | | | | | | | |
| BW, kg | | | | | | | | |
| d 0 (Initial) | 294 | 295 | 294 | 295 | 7.0 | 0.96 | 0.90 | 0.98 |
| d 28 | 337 | 342 | 338 | 343 | 1.7 | 0.34 | 0.004 | 0.98 |
| d 56 | 381 | 385 | 382 | 387 | 2.3 | 0.50 | 0.04 | 0.86 |
| d 91 (Re-implant) | 439 | 442 | 445 | 447 | 2.8 | 0.06 | 0.35 | 0.83 |
| d 120 | 485 | 492 | 493 | 498 | 3.3 | 0.05 | 0.09 | 0.86 |
| d 168 | 561 | 571 | 570 | 570 | 4.6 | 0.37 | 0.21 | 0.27 |
| DMI, kg | | | | | | | | |
| d 0-90 | 9.5 | 9.6 | 9.6 | 9.6 | 0.11 | 0.68 | 0.51 | 0.89 |
| d 91-168 | 11.4 | 11.3 | 11.6 | 11.7 | 0.20 | 0.09 | 0.97 | 0.58 |
| d 0-168 | 10.4 | 10.4 | 10.6 | 10.6 | 0.13 | 0.15 | 0.80 | 0.72 |
| G:F | | | | | | | | |
| d 0-90 | 0.170 | 0.172 | 0.176 | 0.177 | 0.0025 | 0.04 | 0.44 | 0.94 |
| d 91-168[3] | 0.137[b] | 0.147[a] | 0.138[b] | 0.135[b] | 0.0030 | 0.06 | 0.25 | 0.03 |
| CA performance[4,5] | | | | | | | | |
| Final BW, kg | 561 | 572 | 570 | 571 | 5.0 | 0.45 | 0.25 | 0.34 |
| Overall ADG, kg | 1.58 | 1.65 | 1.64 | 1.64 | 0.030 | 0.47 | 0.28 | 0.29 |
| Overall G:F | 0.154 | 0.160 | 0.156 | 0.156 | 0.0021 | 0.71 | 0.18 | 0.11 |

[1]The NRC treatment received 30 mg supplemental Zn/kg DM from $ZnSO_4$ and IND treatment received 100 mg supplemental Zn/kg DM

[2]Implant strategies included either a single Revalor-XH implant (REV-XH; 20 mg estradiol + 200 mg TBA; Merck Animal Health, Madison, NJ) on d 0 or a Revalor-200 implant on d 0 and again on d 91 (REV-200; 20 mg estradiol + 200 mg TBA; Merck Animal

[3]Unlike superscripts indicate differences in treatment means ($P \leq 0.05$).

[4] Day 0 BW was used as a covariate in performance analysis, excluding d 0 BW.

Table 2 Continued

[5]Carcass-adjusted (CA) overall performance was determined with the average dressing percent of treatment groups (63.46, 63.95, 64.14, and 63.90% for NRC/REV-XH, NRC/REV-200, IND/REV-XH, and IND/REV-200, respectively).

**Table 3.** Effect of zinc[1] supplementation and implant[2] strategy on carcass characteristics of finishing beef heifers

| | NRC | | IND | | | *P*-value | | |
|---|---|---|---|---|---|---|---|---|
| | REV-XH | REV-200 | REV-XH | REV-200 | SEM | Zn | IMP | Zn × IMP |
| Pens (n) | 9 | 9 | 9 | 9 | | | | |
| Carcass characteristics[3] | | | | | | | | |
| HCW, kg | 356 | 366 | 365 | 365 | 3.2 | 0.19 | 0.18 | 0.11 |
| Dress[4], % | 63.5$^{y}$ | 64.0$^{xy}$ | 64.1$^{x}$ | 63.9$^{xy}$ | 0.21 | 0.17 | 0.59 | 0.08 |
| REA, sq cm | 79.0 | 82.1 | 81.3 | 81.0 | 1.07 | 0.60 | 0.18 | 0.11 |
| Back fat, cm | 1.81 | 1.94 | 1.94 | 1.89 | 0.073 | 0.60 | 0.54 | 0.24 |
| KPH, % | 2.6 | 2.7 | 2.7 | 2.6 | 0.03 | 0.82 | 0.57 | 0.11 |
| Marbling[5] | 576 | 554 | 571 | 548 | 16.7 | 0.75 | 0.18 | 0.99 |
| YG[6] | 3.87 | 3.94 | 3.97 | 3.93 | 0.110 | 0.68 | 0.91 | 0.64 |

[1]The NRC treatment received 30 mg Zn/kg DM from $ZnSO_4$ and IND treatment received 100 mg Zn/kg DM from $ZnSO_4$.

[2]Implant strategies included either a single Revalor-XH implant (REV-XH; 20 mg estradiol + 200 mg TBA; Merck Animal Health, Madison, NJ) on d 0 or a Revalor-200 on d 0 and again on d 91 (REV-200; 20 mg estradiol + 200 mg TBA; Merck

[3]Day 0 BW was used as covariate in the analysis of carcass characteristics.

[4]Unlike superscripts indicate tendencies for differences in treatment means ($0.05 < P \leq 0.10$).

[5]Marbling scores: slight=300, small=400, modest=500, moderate=600, slightly abundant=700, moderately abundant=800.

[6]Yield grade (YG) was calculated utilizing the USDA yield grade equation.

**Table 4.** Effect of zinc[1] supplementation and implant[2] strategy on liver mineral concentrations of finishing heifers

| | NRC | | IND | | | *P*-value | | |
|---|---|---|---|---|---|---|---|---|
| | REV-XH | REV-200 | REV-XH | REV-200 | SEM | Zn | IMP | Zn × IMP |
| Heifers (n) | 9 | 9 | 9 | 9 | | | | |
| Trace mineral concentrations[3] | | | | | | | | |
| Liver, mg/kg DM[4,5] | | | | | | | | |
| Cu | 350 | 371 | 353 | 321 | 26.8 | 0.28 | 0.85 | 0.24 |
| Fe | 172 | 167 | 167 | 171 | 9.5 | 0.97 | 0.99 | 0.61 |
| Mn[6] | 8.32[a] | 7.94[ab] | 8.71[a] | 7.40[b] | 0.217 | 0.70 | 0.0007 | 0.03 |
| Zn | 113 | 117 | 115 | 121 | 4.1 | 0.47 | 0.13 | 0.81 |

[1]The NRC treatment received 30 mg Zn/kg DM from $ZnSO_4$ and IND treatment received 100 mg Zn/kg DM from $ZnSO_4$.

[2]Implant strategies included either a single Revalor-XH implant (REV-XH; 20 mg estradiol + 200 mg TBA; Merck Animal Health, Madison, NJ) on d 0 or a Revalor-200 on d 0 and again on d 91 (REV-200; 20 mg estradiol + 200 mg TBA; Merck Animal

[3]Data were analyzed using repeated measures of the mixed procedure of SAS and represent overall treatment means.

[4]No Zn × IMP × Day ($P \geq 0.25$) effect was observed. Day ($P \leq 0.0002$); IMP × Day ($P \geq 0.12$) except for Mn (**Figure 2**; $P = 0.02$).

[5]Liver samples were collected on d -5, 14, 105, 164.

[6]Unlike superscripts indicate differences in treatment means ($P \leq 0.05$).

**Table 5.** Effect of zinc[1] supplementation and implant[2] strategy on longissimus thoracis cross-sectional diameter and area from finishing heifers

| | NRC | | IND | | | *P*-value | | |
|---|---|---|---|---|---|---|---|---|
| | REV-XH | REV-200 | REV-XH | REV-200 | SEM | Zn | IMP | Zn × IMP |
| Heifers (n) | 9 | 9 | 9 | 9 | | | | |
| Muscle cross-sectional diameter, μm | | | | | | | | |
| d 14 | 50.0 | 48.7 | 53.9 | 50.4 | 2.05 | 0.12 | 0.19 | 0.54 |
| d 105 | 69.0 | 68.1 | 69.7 | 74.3 | 3.16 | 0.23 | 0.52 | 0.36 |
| d 164 | 61.3 | 57.5 | 69.3 | 64.8 | 4.28 | 0.05 | 0.28 | 0.93 |
| Muscle cross-sectional area, $\mu m^2$ | | | | | | | | |
| d 14 | 2,144 | 2,026 | 2,350 | 2,183 | 175.7 | 0.22 | 0.33 | 0.87 |
| d 105 | 4,016 | 4,000 | 4,157 | 4,765 | 385.6 | 0.20 | 0.40 | 0.40 |
| d 164 | 3,250 | 2,958 | 3,769 | 3,674 | 451.6 | 0.11 | 0.61 | 0.79 |

[1]The NRC treatment received 30 mg Zn/kg DM from $ZnSO_4$ and IND treatment received 100 mg Zn/kg DM from $ZnSO_4$.

[2]Implant strategies included either a single Revalor-XH implant (REV-XH; 20 mg estradiol + 200 mg TBA; Merck Animal Health, Madison, NJ) on d 0 or a Revalor-200 on d 0 and again on d 91 (REV-200; 20 mg estradiol + 200 mg TBA; Merck

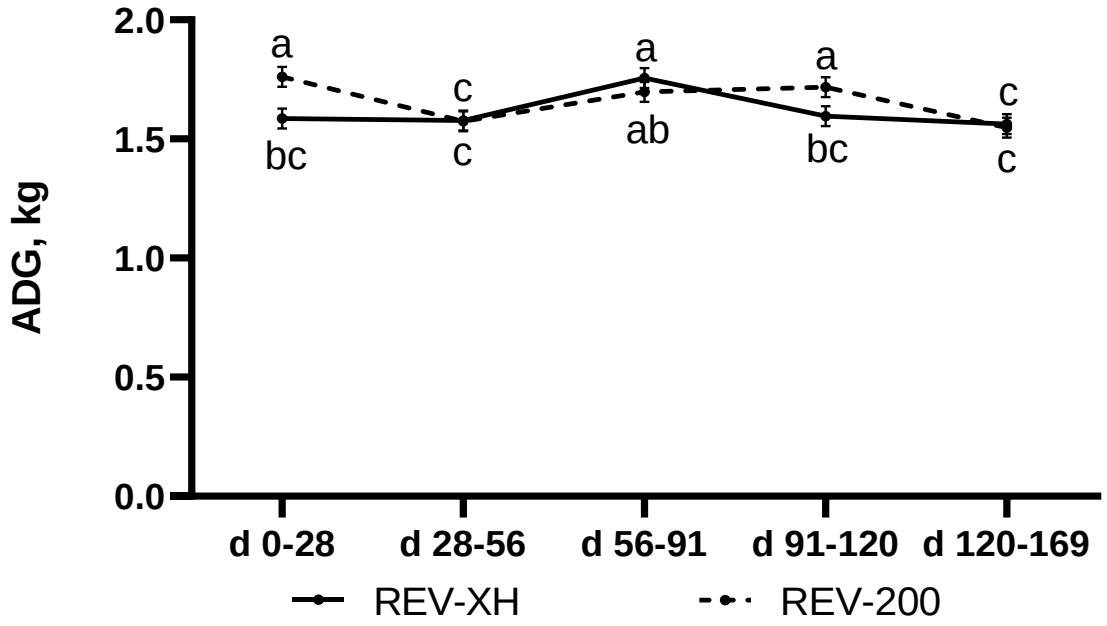


**Figure 1.** The effect of implant strategy and day on ADG throughout the 169-day study (IMP × Period $P = 0.02$). Implant treatments included Revalor-XH (**REV-XH**; 200 mg trenbolone acetate + 20 mg estradiol; Merck Animal Health Madison, NJ) on d 0 or Revalor-200 (**REV-200**; 200 mg trenbolone acetate + 20 mg estradiol; Merck Animal Health) on d 0 and again on d 91. Data were analyzed using repeated measures of the mixed procedure in SAS. Unlike superscripts indicate differences between means across all time points ($P \leq 0.05$).

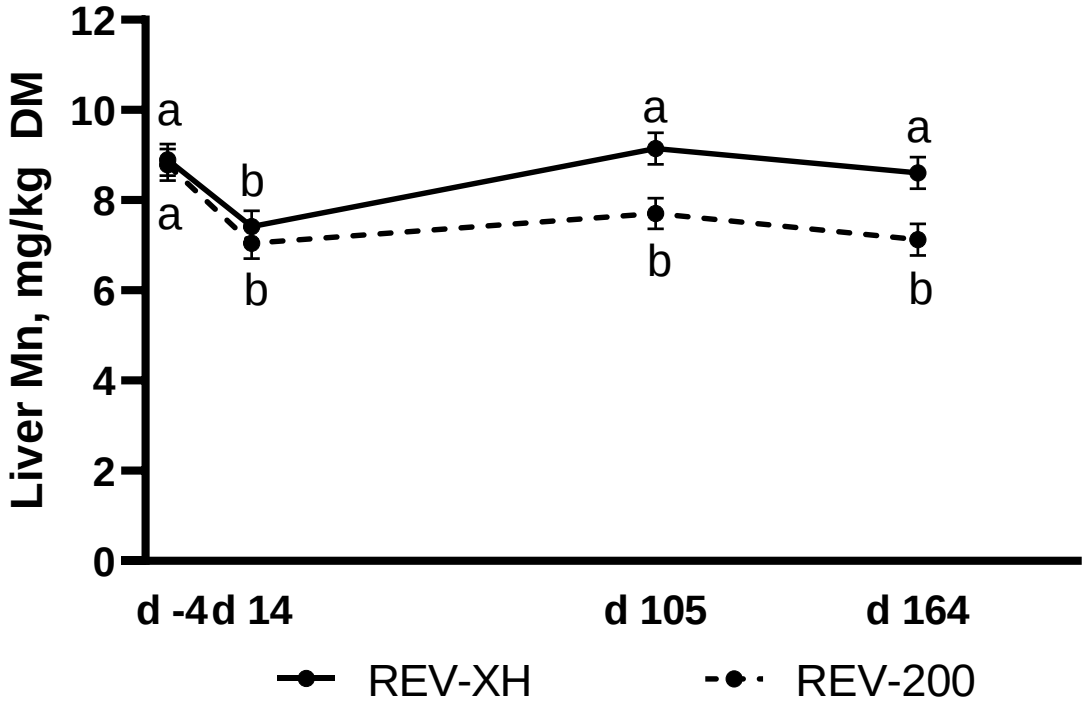


**Figure 2.** Liver manganese response to implant strategy over course of study (IMP × Day $P$ = 0.02). Heifers received either Revalor-XH (REV-XH; 200 mg trenbolone acetate + 20 mg estradiol; Merck Animal Health; Madison, NJ) on d 0 or Revalor-200 on d 0 and again on d 91 (REV-200; 200 mg trenbolone acetate + 20 mg estradiol; Merck Animal Health). Data were analyzed as repeated measures of the mixed procedure of SAS. Unlike superscripts indicate differences between treatment means across all time points ($P \leq 0.05$).

# CHAPTER 6. EFFECT OF ZINC SOURCE AND IMPLANT STRATEGY ON PERFORMANCE, CARCASS CHARACTERISTICS, AND TRACE MINERAL CONCENTRATIONS IN FINISHING FEEDLOT STEERS

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## Abstract

Seventy-two Angus-crossbred steers (411 ± 16 kg) were assigned to a 2 × 3 factorial arrangement of treatments to examine the effects of blended Zn source supplementation on performance, carcass characteristics, and trace mineral parameters of steers administered no implant or a two-implant program. Factors included implant (**IMP**) strategies and Zn supplementation. During the 126-d study steers were either not implanted (**NoIMP**) or implanted (**IS/200**; Elanco, Greenfield, IN) on d 0 (Component TE-IS; 80 mg trenbolone acetate + 16 mg estradiol) and 57 (Component TE-200; 200 mg trenbolone acetate + 20 mg estradiol). All steers were fed 70 mg Zn/kg on a dry matter (**DM**) basis from $ZnSO_4$ + 30 mg Zn/kg DM from either basic ZnCl (Vistore Zn, Phibro Animal Health, Teaneck, NJ), Zn glycinate (Gemstone Zn, Phibro Animal Health), or $ZnSO_4$ (**ZnB**, **ZnG**, or **ZnS**, respectively). Steers were blocked by weight into pens of 6 and fed a dry rolled corn-based diet via GrowSafe bunks (GrowSafe Systems Ltd.; Airdrie, AB, Canada). Data were analyzed using the Mixed Procedure of SAS, with fixed effects of Zn, IMP, and the interaction. Steer was the experimental unit ($n$ = 12

steers/treatment). Liver and muscle collected on d -5, 14, 71, and 120 were analyzed for Zn concentration, and data were analyzed as repeated measures (repeated effect = Day). An IMP × Zn tendency ($P = 0.07$) was observed for d 126 body weight with no effects of Zn within NoIMP while ZnS tended to be heavier than ZnB with ZnG intermediate within IS/200. Carcass-adjusted overall feed efficiency (**G:F**) was greatest for ZnS (Zn; $P = 0.02$). Implanted cattle had greater DM intake, G:F, and carcass-adjusted performance ($P \leq 0.01$). Liver Zn concentrations were greater for IS/200 by d 120 (IMP × Day; $P = 0.02$). Within IS/200, ZnG tended to have greater muscle Zn than ZnS while ZnB was intermediate (Zn × IMP; $P = 0.09$). No Zn or IMP × Zn ($P \geq 0.12$) effects were observed for carcass data. However, IS/200 had greater hot carcass weight, dressing percentage, and ribeye area than NoIMP ($P \leq 0.001$). These data suggest implants improve growth and influence Zn metabolism. Future work should examine Zn sources and supplementation alongside implant strategies.

Key words: anabolic implant, cattle, chelated zinc, growth, hydroxy zinc

## Introduction

Widespread use of anabolic implants in feedlot cattle (NAHMS, 2013) stems from considerable improvements in performance of implanted cattle (Duckett and Pratt, 2014). Furthermore, supplementation of Zn, Cu, Mn, Co, and Se at concentrations 2 to 3 times greater than NASEM (2016) recommendations improved carcass-adjusted average daily gain (**ADG**) by 13.3% compared to non-supplemented steers (Niedermayer et al., 2018). In particular, Zn supports protein synthesis (Oberleas and Prasad, 1969; Suttle, 2010) and N retention (Carmichael et al., 2018), suggesting increased dietary Zn may be needed in growing cattle.

Inorganic $ZnSO_4$ is commonly fed to feedlot cattle, but blends of inorganic and organic trace minerals are also fed (Samuelson et al., 2016). Zinc glycinate, an organic amino acid

chelate, is considered more bioavailable than $ZnSO_4$ in rats, chickens, and cattle (Spears et al., 2004; Schlegel and Windisch, 2006; Sridhar et al., 2015a). Limited research on basic ZnCl, a hydroxy source, suggests similar bioavailability for basic ZnCl as $ZnSO_4$ (Cao et al., 2000; Batal et al., 2001), though Shaeffer et al. (2017) found basic ZnCl to be more bioavailable than $ZnSO_4$ in cattle. To our knowledge, a direct comparison between Zn glycinate and basic ZnCl has not been reported.

Though nutritional Zn requirements can be met with any Zn source, those with greater bioavailability may be optimal during periods of high Zn demand such as implant-induced growth and may effectively decrease the amount of supplemental Zn needed to meet these demands. Messersmith (2018) found implanted steers experiencing 29% greater ADG than non-implanted steers 14 d after implant administration had a 14% decrease in plasma Zn concentrations. This suggests implanted cattle have a demand for Zn to support rapid growth, drawing down circulating concentrations. Therefore, this study's objective was to determine the effects of feeding 100% $ZnSO_4$, or 70% $ZnSO_4$ with 30% of either basic ZnCl or Zn glycinate on performance, carcass characteristics, and trace mineral parameters of feedlot steers not implanted or implanted with a moderately aggressive two implant strategy. It was hypothesized that implanted cattle would better utilize the more available Zn sources towards muscle accretion.

## Materials and Methods

### Care and Use of Animals

All procedures and protocols utilized in this trial were approved by the Iowa State University Institutional Animal Care and Use Committee (log number: IACUC-18-344).

**Animals and Experimental Design**

Seventy-two Angus-cross steers (initial body weight = 411 ± 16 kg) were utilized in a 2 × 3 factorial arrangement of treatments. The effects of implant strategy and Zn source on performance and carcass characteristics in a 126-d study were examined using a randomized complete block design. Steers were housed in partially covered concrete pens (*n* = 6 steers/pen) equipped with automatic waterers and GrowSafe bunks (GrowSafe Systems Ltd.; Airdrie, AB, Canada). Individual steer's radio frequency tags were linked to GrowSafe software to record individual steer feed disappearance. As-fed feed disappearance was corrected for dry matter (**DM**) and used to calculate individual steer dry matter intake (DMI). Implant strategies included either no implant (**NoIMP**) or a Component TE-IS (80 mg trenbolone acetate + 16 mg estradiol; Elanco Animal Health, Greenfield, IN) on d 0 followed by a Component TE-200 (200 mg trenbolone acetate + 20 mg estradiol; Elanco Animal Health**; IS/200)** on d 57. Additionally, all cattle received 70 mg Zn/kg DM from $ZnSO_4$ + 30 mg Zn/kg DM from one of three sources: basic ZnCl (Vistore Zn, Phibro Animal Health, Teaneck, NJ), Zn glycinate (Gemstone Zn, Phibro Animal Health) and $ZnSO_4$ (**ZnB**, **ZnG**, and **ZnS**, respectively). Steers were assigned to block (light or heavy) by body weight (**BW**) and randomly assigned to treatment and pen; all treatments were equally represented within each weight block. On d 127 steers were harvested at a commercial abattoir (National Beef, Tama, IA) via industry standard captive bolt and hot carcass weight (**HCW**) data were obtained. Following a 48-h chill, ribeye area (**REA**), $12^{th}$ rib fat (**BF**), percent kidney, pelvic, and heart fat (**KPH**), and marbling score data were collected by trained University personnel and yield grade (**YG**) was calculated.

**Sample Collection and Analysis**

Steers were fed a dry rolled corn-based diet (**Table 1**) once daily (0800 h) with weekly total mixed ration (**TMR**) samples dried in a forced air oven for 48 h at 70˚C to determine diet DM. A grinder fitted with a 2-mm screen (Retsch Zm100 grinder; Glen Mills Inc., Clifton, NJ) was utilized to grind dried TMR. Ground samples were composited by month for nutrient analysis by Dairyland Laboratories (Arcadia, WI). Body weights were collected approximately every 28 d with consecutive d BW to begin the study (d -1, 0), at re-implant (d 56, 57), and before harvest (d 125, 126). Pre-feeding blood was collected via jugular venipuncture in trace mineral grade EDTA and heparin-containing vacuum capped tubes (Becton Dickenson, Rutherford, NJ) for TM and plasma urea nitrogen (**PUN**) analysis, respectively, on d -1, 14, 56, 71 or 72, and 125 for all steers. Blood was centrifuged at 1,000 × *g* for 20 min to separate plasma for storage at -20˚C until analysis. A Urea Nitrogen Reagent (Colorimetric Method, Teco Diagnostics, Anaheim, CA) was used to determine PUN. Inter-assay and intra-assay CV were 7.33% and 3.02%, respectively. Liver and muscle biopsies were conducted on all steers over two d for each time point (d -5/-4, 14/15, 71/72, and 120/121) with half of the steers biopsied on each d utilizing the methods described by Engle and Spears (2000) for liver and adapted from Pampusch et al. (2008) for muscle. Muscle biopsies were collected from the longissimus thoracis between the $10^{th}$ and $13^{th}$ rib. Rib spacing and side were alternated between each muscle biopsy timepoint. Liver and muscle samples were stored at -20˚C prior to trace mineral analyses. Composited TMR, liver, muscle, and plasma samples were analyzed for trace mineral concentrations via inductively coupled plasma optical emission spectrometry (ICP Optima 7000 DV, Perkin Elmer, Waltham, MA) as described by Pogge and Hansen (2013) and Richter et al. (2012). Each run utilized a standard to ensure instrument accuracy (plasma: Trace Elements

Serum Control #66816; UTAK Laboratories Inc., Valencia, CA; liver, muscle and TMR: Bovine Liver #1577c; National Institute of Standards and Technology, Gaithersburg, MD).

### Statistical Analysis

Data were analyzed as a randomized complete block design with a 2 × 3 factorial arrangement of treatments for performance, blood, tissue, and carcass characteristics using the Mixed Procedure of SAS 9.4 (SAS Inst. Inc., Cary, NC). Fixed effects included implant (**IMP**), Zn, and IMP × Zn. The experimental unit was steer ($n$ = 11 or 12/treatment) and initial BW was used as a covariate in performance and carcass analyses. Plasma trace mineral, PUN, and liver trace mineral data were analyzed as repeated measures using the compound symmetry covariance structure while muscle Zn concentration data utilized repeated measures with the heterogeneous compound symmetry covariance structure determined by the lowest corrected Akaike's information criterion. The repeated effect was Day. Normality was assessed using Shapiro-Wilks test and outliers were evaluated using Cook's D, with no outliers being found. One steer was removed from all analysis due to health concerns unrelated to treatment (NoIMP-ZnG) and another removed from carcass data analysis due to unaccepted hide coloration at the commercial abattoir (IS/200-ZnB). Data are reported as least squares means with the SEM. Statistical significance was determined at $P \leq 0.05$, with tendencies defined as $0.05 < P \leq 0.10$.

## Results and Discussion

Although anabolic implants are extensively used in the feedlot industry (NAHMS, 2013; Samuelson et al., 2016), implanted cattle's micronutrient requirements are poorly understood. Incorporation of trace minerals into the diet at greater than NASEM (2016) recommendations has been found to increase HCW (Niedermayer et al., 2018). However, little research has been conducted to determine if a single trace mineral is responsible for this growth response in cattle.

A multitude of enzymes require Zn for catalytic or structural roles (Suttle, 2010) and Zn is required for protein synthesis (Oberleas and Prasad, 1969). Therefore, Zn is a promising candidate for strategic supplementation aimed to improve growth in high growth potential animals such as those utilizing anabolic implants. Furthermore, Zn source, and ultimately bioavailability, must be considered to determine a strategic trace mineral program targeting optimal performance. Therefore, we hypothesized that steers supplemented a more bioavailable Zn source would have improved performance over steers receiving less bioavailable Zn sources, with greater benefits within implanted steers.

Organic Zn-glycinate has been observed to be more bioavailable than inorganic $ZnSO_4$ in both rats and broiler chickens (Schlegel and Windisch, 2006; Sridhar et al., 2015a). Although a direct comparison between Zn-glycinate and basic ZnCl has not been reported, research in chickens and cattle has indicated basic ZnCl has a similar bioavailability as $ZnSO_4$ (Cao et al., 2000; Batal et al., 2001). While others suggest basic ZnCl is more bioavailable than $ZnSO_4$ in cattle (Shaeffer et al., 2017). Although not definitive, these data suggest Zn-glycinate may be more bioavailable than the hydroxy source, basic ZnCl. However, basic ZnCl may be considered advantageous over $ZnSO_4$ due to its insoluble properties (Cao et al., 2000a; Shaeffer, 2006), protecting this source of Zn from dietary antagonists in the rumen.

Assessment of performance measures (**Table 2**) in the current study revealed no differences in body weights taken on d 28, 57, 84, 112, and 126 due to Zn source ($P \geq 0.22$); nor were IMP × Zn effects observed for d 28, or 57 BW. However, by d 28, IS/200 steers tended to be heavier than NoIMP ($P = 0.08$), leading to a 14 kg advantage in BW for IS/200 steers compared to NoIMP on d 57 ($P = 0.001$). Interestingly, a tendency for an IMP × Zn ($P \leq 0.10$) effect for d 84, 112, and 126 BW was observed, where Zn source did not affect BW of NoIMP

steers, while within IS/200 treatment ZnS tended to have greater BW than ZnB, with ZnG being intermediate. A tendency for an IMP × Zn effect ($P = 0.08$) was observed for d 0-56 ADG in which ZnS was numerically lesser than ZnB and ZnG within NoIMP and ZnB was numerically lesser than ZnG and ZnS. However, a tendency for an IMP × Zn ($P = 0.07$) effect in overall ADG found no differences in overall ADG between Zn sources within NoIMP while ZnS was greater than ZnB and ZnG was intermediate within IS/200. Furthermore, implanted cattle had greater d 57-126 ADG ($P < 0.0001$), though no Zn or IMP × Zn effects ($P \geq 0.12$) were observed for ADG during this period.

Though ADG was influenced by IMP × Zn, no effects of the interaction were observed for d 0-56 DMI, daily Zn intake, or feed efficiency (**G:F**), d 57-126 DMI, daily Zn intake, or G:F, overall DMI or G:F, carcass-adjusted final BW, and carcass-adjusted overall ADG or G:F ($P \geq 0.12$). Implanted cattle had greater d 0-56 G:F, d 57-126 DMI, daily Zn intake, and G:F, overall DMI and G:F, carcass-adjusted final BW, carcass-adjusted overall ADG, and G:F ($P \leq 0.01$). Daily Zn intake, d 0-56 G:F, and carcass-adjusted overall G:F were greater for ZnS than ZnB and ZnG ($P \leq 0.02$). These effects were likely driven by greater ADG throughout most of the trial and a tendency ($P = 0.08$) for lesser DMI during the d 0-56 initial implant period for ZnS. Numerical differences in overall intakes between Zn sources may also be driving the overall ADG IMP × Zn response in which steers that ate more had greater gains. No other effects of IMP or Zn on performance measures were observed ($P \geq 0.13$).

Although performance was hypothesized to improve with greater bioavailability of Zn, neither d 0-56 or d 57-126 ADG were influenced by Zn treatment. The numerical BW advantage of ZnG steers on d 28 may have been exacerbated by a modest response to the initial implant by other Zn treatments and aided by the availability of ZnG in the diet. Broilers fed 30 mg Zn/kg

DM from Zn-glycinate had improved feed efficiency over broilers fed at the requirement (40 mg Zn/kg DM) from $ZnSO_4$ (Sridhar et al., 2014), indicating a more bioavailable source can outperform a less bioavailable source, particularly when supplemented at a low concentration in the diet. In the present study, the benefits of a more bioavailable source may have been outweighed by the large concentration of supplemental Zn, regardless of source. In salmon, low concentrations of Mn-glycinate were more bioavailable than $MnSO_4$, while high concentrations of Mn-glycinate downregulated Mn absorption (Prabhu et al., 2019). Similarly, in the current study, the supplementation of 100 mg Zn/kg DM may have led to the downregulation of Zn absorption. However, the bioavailability of Zn was not directly measured in this study.

As reported by others, differences in animal performance due to dietary Zn supplementation have been variable. Cao et al. (2000) and Gunter et al. (2001) detected no differences in chick and steer performance, respectively, due to Zn source ($ZnSO_4$, basic ZnCl, ZnO, Zn polysaccharide, or Zn amino acid complex). However, Genther-Schroeder et al. (2016) found increasing supplemental Zn (Zn amino acid chelate and $ZnSO_4$ blend supplemented up to 150 mg Zn/kg DM) linearly increased final BW and ADG of steers that received ractopamine hydrochloride. Spears and Kegley (2002) found steers supplemented Zn-proteinate had greater HCW than steers receiving the control diet or ZnO. Although the current study supplemented the same concentration of Zn between each Zn treatment, more bioavailable sources of Zn would be hypothesized to improve cattle performance if Zn had been limiting growth. The high inclusion of supplemental Zn across Zn treatments may have limited the detection of individual Zn source differences in the present study.

It is common in the feedlot industry to supplement blends of trace mineral sources (Samuelson et al., 2016b), therefore we examined varying Zn sources at industry relevant blends

of 70 mg Zn/kg DM from inorganic $ZnSO_4$ and 30 mg Zn/kg DM from the other Zn source (basic ZnCl, Zn glycinate, or $ZnSO_4$). Interestingly, Shaeffer (2006) found basic ZnCl fed steers had poorer performance than $ZnSO_4$ supplemented steers when supplemented at 30 mg Zn/kg DM. However, at higher concentrations, similar to this study (90 mg Zn/kg DM), no differences in performance were observed between these two inorganic sources (Shaeffer, 2006). Perhaps, a greater inclusion of supplemental Zn is adequate to meet the Zn requirements of the animal regardless of source; however, this does not support why basic ZnCl did not perform similarly to $ZnSO_4$ in the current study.

No differences in carcass characteristics were noted in this study (**Table 3**; $P \geq 0.12$). Previous work examining different concentrations of Zn in the diet of heifers has revealed interim performance improvements but no differences in carcass characteristics due to Zn concentrations similar to the present study (100 mg Zn/kg DM from $ZnSO_4$) or national recommendations for cattle (30 mg Zn/kg DM from $ZnSO_4$; Messersmith et al., 2021). However, others have noted positive effects of Zn on carcass characteristics when supplemental Zn was added to a Zn deficient basal diet (Spears and Kegley, 2002). Such differences in carcass characteristics may not have been observed in the current study because all treatments were supplemented 100 mg Zn/kg DM regardless of source. The portion of Zn supplemented from basic ZnCl, or Zn glycinate may not have been enough to impact carcass characteristics regardless of bioavailability differences between sources. However, implanted steers had greater HCW, DP, and REA than NoIMP ($P \leq 0.001$) as expected with anabolic implant use (Duckett and Pratt, 2014). No effects of IMP were observed for BF, KPH, marbling, or yield grade ($P \geq 0.23$).

The evaluation of plasma and tissue trace mineral concentrations of these steers provides a more in-depth understanding of mineral metabolism in implanted cattle. An IMP × Day effect ($P = 0.003$) and a tendency for an Zn × Day effect ($P = 0.10$) were observed for plasma Zn concentrations. Furthermore, a tendency for an IMP × Zn × Day effect (**Figure 1**; $P = 0.06$) was observed for plasma Zn in which differences between the full factorial of treatments began to emerge by d 56. Assessing differences in LSM of treatments revealed plasma Zn of IS/200 steers was less than NoIMP steers on d 71, with a more substantial drop in plasma Zn for IS/200 steers from d 56 to 71 than NoIMP. Decreases in plasma Zn concentrations following a terminal implant have previously been reported within 14 to 18 d of implant administration (Messersmith, 2018; Messersmith and Hansen, 2021) indicating Zn metabolism is influenced during the period of peak hormonal release of implants (first 40 d after implant administration; Johnson et al., 1996). Zinc is heavily involved in growth processes through protein synthesis (Oberleas and Prasad, 1969). Therefore, plasma Zn may be decreasing as tissues take up increased amounts of Zn to support growth in high performing animals. However, it is unclear why plasma Zn for NoIMP-ZnG decreased between d 56 and 71.

Liver Zn, a poor biomarker of Zn status due to the steady nature of tissue Zn concentrations (Suttle, 2010), was not influenced by Zn or Zn × Day ($P \geq 0.62$). However, an IMP × Day effect in liver Zn revealed greater liver Zn concentrations in IS/200 steers by d 120 than NoIMP (**Figure 2A**; $P = 0.02$). This increase in liver Zn concentrations may be linked to greater Zn retention of implanted animals as observed in lambs (Hufstedler and Greene, 1995). Although both immediate and persistent effects of implant administration on Zn absorption and retention have not been examined in cattle. Perhaps, the slight increase in liver Zn concentrations of implanted steers is reflective of greater DMI and subsequently greater Zn intake in IS/200

steers. However, this is in contrast to the decrease in liver Zn concentrations observed by Messersmith (2018) within 14-d of implant administration when 61 mg Zn/kg DM was fed in the diet. Considering dietary Zn in the current study was much higher than this (130 mg Zn/kg DM), feeding high concentrations of Zn in the diet may have prevented a decline in liver Zn concentrations of implanted cattle (**Table 4**).

Zinc effects were observed on liver and plasma Fe concentrations ($P \leq 0.04$) where ZnB increased liver Fe concentrations while decreasing plasma Fe concentrations relative to ZnG. However, ZnS was not different from either Zn treatment in both liver and plasma analyses of Fe. Greater bioavailability of Zn source may have contributed to competition for absorption between Zn and Fe leading to lesser plasma Fe for ZnG but this does not explain the increase in liver Fe observed for ZnB. Additionally, an IMP × Day (**Figure 2B**; $P = 0.01$) effect was observed on plasma Fe in which NoIMP plasma Fe increased from d 15 to 56 before decreasing to concentrations similar to d 15 for the remainder of the trial; whereas IS/200 plasma Fe remained similar during this time point with no further differences in plasma Fe on d 0, 15, 71, or 125. Steers within ZnS had lesser liver Cu within NoIMP, and no differences in liver Cu concentrations due to Zn source were observed within IS/200 (IMP × Zn; $P = 0.02$). No further effects of IMP × Day, Zn × Day, or IMP × Zn × Day were observed for plasma or liver Fe and Cu ($P \geq 0.17$).

Albeit its relatively slow Zn metabolism compared to other Zn stores, skeletal muscle comprises a large percentage of Zn storage in the body making it a potential indicator of Zn nutrition (Gumpper and Ma, 2019). A tendency for an IMP × Zn effect for muscle Zn concentration was observed ($P = 0.09$) in which IS/200-ZnG steers had greater muscle Zn than IS/200-ZnS, NoIMP-ZnB, and NoIMP-ZnG steers while not different from IS/200-ZnB or

NoIMP-ZnS. We hypothesized that muscle Zn concentrations would increase with greater Zn source bioavailability and that implanted cattle would experience additional increases in muscle Zn concentrations due to the demands of Zn in growth processes such as DNA synthesis. However, muscle Zn responses were variable. Additionally, muscle Zn was increased by d 120, though d 15 and 71 muscle Zn concentrations were not different from d -5 (120, 119, 121, and 132 mg Zn/kg DM SEM: 2.0 for d -5, 15, 71, and 120, respectively; $P < 0.0001$). No IMP × Day, Zn × Day, or IMP × Zn × Day effects ($P \geq 0.44$) were observed for muscle Zn concentrations.

Interestingly, liver Mn concentrations of IS/200 steers on d 71 were lesser than NoIMP, but liver Mn concentrations were not affected by implant on any other sampling d (**Figure 3A**; IMP × Day; $P = 0.001$). Various implant studies conducted in our laboratory have revealed that implants decreased liver Mn concentrations by d 14 post-implant (Messersmith, 2018; Niedermayer et al., 2018). Although no differences in liver Mn were observed on d 14 of the present trial, d 71 corresponds to 14 d following the terminal implant matching the implant potency of these previous trials. This liver Mn response may be linked to increased growth from potent implants leading to greater protein synthesis and lesser protein degradation. A decrease in protein degradation would cause lesser need for the urea cycle to detoxify ammonia. Arginase, the urea cycle's terminal enzyme, is a Mn-dependent enzyme (Judith S Bond et al., 1983; Watts, 1990). Therefore, a decrease in protein degradation could indicate a lesser need for arginase, and thus Mn, resulting in the observed decrease in liver Mn concentrations. Considering arginase is thought be a main driver of Mn requirements, implant-induced decreases in liver Mn concentrations may be linked to increased biliary excretion of Mn and lesser reabsorption of Mn from the bile, a primary mechanism in Mn metabolism in cattle (Suttle, 2010). The decrease in liver Mn in the present study pairs with the observed lesser PUN concentrations of implanted vs.

non-implanted steers. Across the trial PUN were lesser for IS/200 on d 15, 56, and 71 while no difference was observed by d 125 (**Figure 3B**; $P = 0.001$). In general, PUN increased in NoIMP until d 56, then held steady for the remainder of the feeding period, while IS/200 increased from d 15 to 56, dropped again after re-implant, and then increased to similar concentrations as NoIMP at the end of the trial. No Zn × Day or IMP × Zn × Day effects were observed for liver Mn or PUN ($P \geq 0.34$). As expected, these data appear inversely related to the ADG response observed consistent with periods of increased growth and lesser protein degradation as reflected by decreased PUN. However, this relationship between liver Mn concentrations and implant use has not been further evaluated. No further effects of IMP, Zn, or the interaction were observed for trace mineral or blood parameters ($P \geq 0.14$).

## Conclusion

Collectively, these data revealed interim performance differences due to feeding 100% $ZnSO_4$, or 70% $ZnSO_4$ with 30% of either basic ZnCl or Zn glycinate within both implanted and non-implanted steers and effects on steer trace mineral concentrations in plasma and liver. Zinc source did not impact carcass characteristics in this study. Further work should investigate titration of lesser concentrations of dietary Zn from highly bioavailable sources to determine source differences in performance of implanted cattle, as the dietary consumption of Zn may have been high enough in all treatments in the present study to mask potential differences due to source.

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**Table 1.** Diet composition

| Ingredient | % of diet DM |
|---|---|
| Dry rolled corn | 57.0 |
| Sweet Bran[1] | 20.0 |
| Bromegrass hay | 8.0 |
| DDGS[2] | 13.03 |
| Limestone | 1.5 |
| Salt | 0.31 |
| Vitamin & mineral premix[3,4,5] | 0.1441 |
| Rumensin | 0.0135 |
| Analyzed composition | |
| Crude protein[6] | 13.9 |
| Neutral detergent fiber[6] | 23.9 |
| Ether extract[6] | 4.2 |
| Cu, mg/kg DM[7] | 13 |
| Fe, mg/kg DM[7] | 134 |
| Mn, mg/kg DM[7] | 37 |
| Zn, mg/kg DM[7] | 130 |

[1]Branded wet corn gluten feed (Cargill Corn Milling, Blair, NE).
[2]Dried distillers grains with solubles.
[3]Premix provided 2,200 IU vitamin A and 25 IU vitamin E/kg diet.
[4]Wtih the exception of Zn, trace minerals were supplemented at NASEM (2016) recommendations for Co, Cu, I, Mn, and Se, from inorganic sources.
[5]All diets included supplemental Zn at 70 mg Zn/kg DM provided as $ZnSO_4$. Additional Zn (30 mg Zn/kg DM) was provided for respective treatments from $ZnSO_4$ (ZnS), basic ZnCl (ZnB; Phibro Animal Health) or Zn glycinate (ZnG; Gemstone Zn; Phibro Animal Health), to achieve a total of 100 mg supplemental Zn/kg DM.
[6]Analysis of ZnS TMR conducted by Dairyland Laboratories (Arcadia, WI).
[7]Analyzed values for trace minerals represent the average of ZnB, ZnG, and ZnS treatments measured by inductively coupled plasma optical emission spectrometry (ICP Optima 7000 DV, Perkin Elmer, Waltham, MA).

**Table 2.** The effects of implant[1] and zinc source[2] on performance measures in finishing feedlot steers.

| Implant treatments | NoIMP | | | IS/200 | | | | *P*-values | | |
|---|---|---|---|---|---|---|---|---|---|---|
| Zinc treatments | ZnB | ZnG | ZnS | ZnB | ZnG | ZnS | SEM | IMP | Zn | IMP × Zn |
| Steer (*n*) | 12 | 11 | 12 | 12 | 12 | 12 | | | | |
| Bodyweight, kg | | | | | | | | | | |
| d 0 (Initial) | 411 | 411 | 409 | 410 | 412 | 412 | 3.1 | 0.70 | 0.96 | 0.80 |
| d 28 | 462 | 461 | 452 | 459 | 469 | 465 | 4.0 | 0.08 | 0.22 | 0.11 |
| d 57 (Terminal) | 508 | 506 | 497 | 511 | 520 | 522 | 5.1 | <0.001 | 0.74 | 0.11 |
| d 84 | $542^{yz}$ | $538^{yz}$ | $536^{z}$ | $555^{xy}$ | $567^{wx}$ | $575^{w}$ | 6.3 | <0.001 | 0.48 | 0.10 |
| d 112 | $586^{z}$ | $579^{z}$ | $581^{z}$ | $611^{y}$ | $622^{xy}$ | $637^{x}$ | 7.3 | <0.001 | 0.30 | 0.10 |
| d 126 | $603^{z}$ | $594^{z}$ | $591^{z}$ | $630^{y}$ | $639^{xy}$ | $654^{x}$ | 7.8 | <0.001 | 0.62 | 0.07 |
| Average daily gain, kg | | | | | | | | | | |
| d 0-56 | $1.70^{yz}$ | $1.65^{yz}$ | $1.55^{z}$ | $1.77^{xy}$ | $1.90^{x}$ | $1.91^{x}$ | 0.069 | <0.001 | 0.75 | 0.08 |
| d 57-126 | 1.38 | 1.28 | 1.37 | 1.72 | 1.72 | 1.91 | 0.070 | <0.001 | 0.12 | 0.32 |
| Dry matter intake, kg | | | | | | | | | | |
| d 0-56 | 10.5 | 10.6 | 9.8 | 10.5 | 10.8 | 10.4 | 0.28 | 0.23 | 0.08 | 0.55 |
| d 57-126 | 10.4 | 10.5 | 10.3 | 10.9 | 11.0 | 11.5 | 0.36 | 0.01 | 0.84 | 0.45 |
| Zn intake, mg/d | | | | | | | | | | |
| d 0-56 | 1367 | 1295 | 1310 | 1367 | 1321 | 1398 | 37.2 | 0.20 | 0.24 | 0.45 |
| d 57-126 | 1361 | 1294 | 1383 | 1419 | 1353 | 1549 | 46.9 | 0.01 | 0.01 | 0.40 |
| G:F[3] | | | | | | | | | | |
| d 0-56 | 0.161 | 0.147 | 0.174 | 0.200 | 0.194 | 0.228 | 0.0093 | <0.001 | 0.01 | 0.70 |
| d 57-126 | 0.132 | 0.121 | 0.133 | 0.160 | 0.156 | 0.169 | 0.0072 | <0.001 | 0.19 | 0.83 |
| Overall performance | | | | | | | | | | |
| ADG[4], kg | $1.49^{z}$ | $1.41^{z}$ | $1.38^{z}$ | $1.69^{y}$ | $1.74^{xy}$ | $1.84^{x}$ | 0.060 | <0.0001 | 0.53 | 0.07 |
| DMI, kg | 10.5 | 10.6 | 10.1 | 10.7 | 10.9 | 11.0 | 0.30 | 0.03 | 0.74 | 0.45 |
| G:F | 0.15 | 0.14 | 0.15 | 0.16 | 0.17 | 0.18 | 0.005 | <0.0001 | 0.13 | 0.36 |
| Carcass-adjusted performance[5] | | | | | | | | | | |
| Final BW, kg | 598 | 590 | 590 | 635 | 637 | 660 | 8.7 | <0.0001 | 0.33 | 0.12 |
| ADG, kg | 1.48 | 1.42 | 1.44 | 1.79 | 1.79 | 1.98 | 0.062 | <0.0001 | 0.21 | 0.12 |
| G:F | 0.14 | 0.13 | 0.14 | 0.17 | 0.16 | 0.18 | 0.005 | <0.0001 | 0.02 | 0.49 |

Table 2 Continued

[1]Implant strategies included no implant (**NoIMP**) or a Component TE-IS (**IS/200**; 80 mg trenbolone acetate + 16 mg estradiol; Elanco Animal Health, Greenfield, IN) on d 0 followed by a Component TE-200 (200 mg trenbolone acetate + 20 mg estradiol; Elanco Animal Health) on d 57.

[2]Cattle received 70 mg Zn/kg DM from ZnSO4 + 30 mg Zn/kg DM from basic ZnCl, Zn glycinate, or $ZnSO_4$ (**ZnB**, **ZnG**, or **ZnS**, respectively). Total Zn supplementation was targeted at 100 mg Zn/kg DM to meet industry consultant recommendations (Samuelson et al., 2016).

[3]Feed efficiency.

[4]Average daily gain was analyzed as repeated measures with the repeated effect of Day. Zn × Day $P = 0.01$; IMP × Day $P = 0.02$; Day $P < 0.0001$; Zn × IMP × Day $P = 0.86$.

[5]Carcass adjusted performance was calculated using the average dressing percent: 64.35%.

**Table 3.** The effects of implant[1] and zinc source[2] on carcass characteristics and carcass adjusted performance in finishing feedlot steers.

| Implant treatments | NoIMP | | | IS/200 | | | | *P*-values | | |
|---|---|---|---|---|---|---|---|---|---|---|
| Zinc treatments | ZnB | ZnG | ZnS | ZnB | ZnG | ZnS | SEM | IMP | Zn | IMP × Zn |
| Steer (*n*) | 12 | 11 | 12 | 11 | 11 | 9 | | | | |
| Hot carcass weight, kg | 385 | 380 | 380 | 408 | 410 | 424 | 5.6 | <0.001 | 0.33 | 0.12 |
| Dress, % | 63.8 | 63.9 | 64.2 | 64.6 | 64.8 | 64.8 | 0.37 | 0.01 | 0.72 | 0.85 |
| Ribeye area, $cm^2$ | 80.0 | 81.2 | 80.2 | 86.8 | 83.4 | 83.9 | 2.23 | 0.01 | 0.76 | 0.51 |
| Rib fat, cm | 1.69 | 1.71 | 1.80 | 1.80 | 1.87 | 1.81 | 0.125 | 0.31 | 0.86 | 0.79 |
| KPH[3], % | 2.6 | 2.6 | 2.6 | 2.6 | 2.6 | 2.7 | 0.09 | 0.63 | 0.77 | 0.96 |
| Marbling[4] | 530 | 511 | 572 | 546 | 517 | 515 | 35.7 | 0.66 | 0.63 | 0.50 |
| Yield grade | 3.94 | 3.85 | 4.00 | 3.90 | 4.17 | 4.21 | 0.181 | 0.23 | 0.53 | 0.55 |

[1]Cattle were either non-implanted (**NoIMP**) or received a Component TE-IS (**IS/200**; 80 mg trenbolone acetate + 16 mg estradiol; Elanco Animal Health, Greenfield, IN) on d 0 followed by a Component TE-200 (200 mg trenbolone acetate + 20 mg estradiol; Elanco Animal Health) on d 57.

[2]Cattle received 70 mg Zn/kg DM from ZnSO4 + 30 mg Zn/kg DM from basic ZnCl, Zn glycinate, or $ZnSO_4$ (**ZnB**, **ZnG**, or **ZnS**, respectively). Total Zn supplementation was targeted at 100 mg Zn/kg DM to meet industry consultant recommendations (Samuelson et al., 2016).

[3]Kidney, pelvic, and heart fat.

[4]Marbling scores: slight = 300, small = 400, modest = 500, moderate = 600, slightly abundant = 700, moderately abundant = 800.

**Table 4.** The effects of implant[1] and zinc source[2] on liver, muscle, and plasma parameters[3].

| Implant treatments | NoIMP | | | IS/200 | | | | *P*-values | | |
|---|---|---|---|---|---|---|---|---|---|---|
| Zinc treatments | ZnB | ZnG | ZnS | ZnB | ZnG | ZnS | SEM | IMP | Zn | IMP × Zn |
| Steer (*n*)[4] | 12 | 11 | 12 | 11 | 11 | 9 | | | | |
| Liver, mg/kg DM[5] | | | | | | | | | | |
| Zn[6] | 103 | 102 | 101 | 105 | 106 | 112 | 2.9 | 0.02 | 0.62 | 0.21 |
| Cu | 303$^{a}$ | 314$^{a}$ | 240$^{b}$ | 298$^{a}$ | 278$^{ab}$ | 301$^{a}$ | 18.3 | 0.62 | 0.19 | 0.02 |
| Mn[6] | 9.0 | 8.5 | 9.1 | 8.5 | 8.3 | 8.8 | 0.30 | 0.19 | 0.19 | 0.86 |
| Fe | 167 | 153 | 156 | 164 | 147 | 155 | 6.3 | 0.53 | 0.04 | 0.92 |
| Muscle, mg/kg DM[5,7] | | | | | | | | | | |
| Zn | 118$^{y}$ | 122$^{y}$ | 123$^{xy}$ | 124$^{xy}$ | 133$^{x}$ | 119$^{y}$ | 3.7 | 0.16 | 0.14 | 0.09 |
| Plasma, mg/L[5] | | | | | | | | | | |
| Cu | 0.99 | 1.05 | 1.02 | 1.00 | 1.07 | 1.03 | 0.046 | 0.76 | 0.34 | 0.99 |
| Fe[6] | 1.92 | 2.24 | 2.13 | 1.97 | 2.25 | 2.15 | 0.107 | 0.79 | 0.02 | 0.98 |
| PUN, mg/dL[5,6] | 8.20 | 9.02 | 9.12 | 7.96 | 7.83 | 7.37 | 0.485 | 0.01 | 0.78 | 0.30 |

[1]Implant strategies included no implant (**NoIMP**) or a Component TE-IS (**IS/200**; 80 mg trenbolone acetate + 16 mg estradiol; Elanco Animal Health, Greenfield, IN) on d 0 followed by a Component TE-200 (200 mg trenbolone acetate + 20 mg estradiol; Elanco Animal Health) on d 57.

[2]Cattle received 70 mg Zn/kg DM from ZnSO4 + 30 mg Zn/kg DM from basic ZnCl, Zn glycinate, or $ZnSO_4$ (**ZnB**, **ZnG**, or **ZnS**, respectively). Total Zn supplementation was targeted at 100 mg Zn/kg DM to meet industry consultant recommendations (Samuelson et al., 2016).

[3]Liver and muscle samples were taken on d -5/-4, 14/15, 71/72, and 120/121 while blood for plasma trace mineral and PUN analysis were collected on d -1, 14, 56, 71/72, and 125.

[4]Unlike superscripts indicate differences between treatment means: a,b,c represent $P \leq 0.05$ and x, y, z represent $0.05 < P \leq 0.10$.

[5]Data were analyzed using repeated measures of the mixed procedure of SAS and represent treatment means. No IMP × Zn × Day or Zn × Day interactions were observed ($P \geq 0.15$) for all measurements except plasma Zn (IMP × Zn × Day; $P = 0.06$; Fig. 5).

[6]An IMP × Day effect was observed ($P \leq 0.01$) for liver Zn (Fig. 2A) and plasma Fe (Fig 2B), liver Mn (Fig 3A), and plasma urea nitrogen (PUN; Fig 3B). No other IMP × Day effects were observed ($P \geq 0.27$).

[7]A Day effect was observed ($P < 0.0001$; Fig. 3).

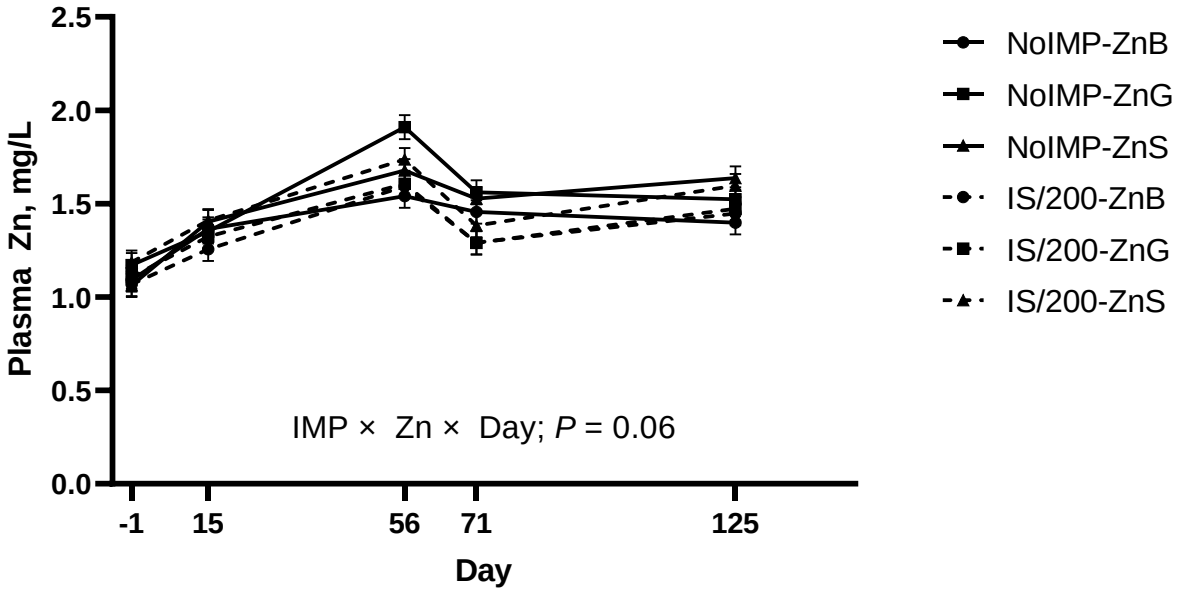


**Figure 1.** Plasma Zn tends to respond to implant and Zn treatments throughout study. Steers received either no hormonal implant (**NoIMP**) or were implanted with Component TE-IS on d 0 followed by Component TE-200 on d 57 (**IS/200**; Elanco) and fed 70 mg Zn/kg DM from $ZnSO_4$ + 30 mg Zn/kg DM from basic ZnCl, Zn glycinate, or $ZnSO_4$ (**ZnB**, **ZnG**, and **ZnS**, respectively). Blood was collected on d -1, 15, 56, 71/72, and 125 for plasma trace mineral analysis. Data were analyzed using repeated measures of the mixed procedure of SAS.

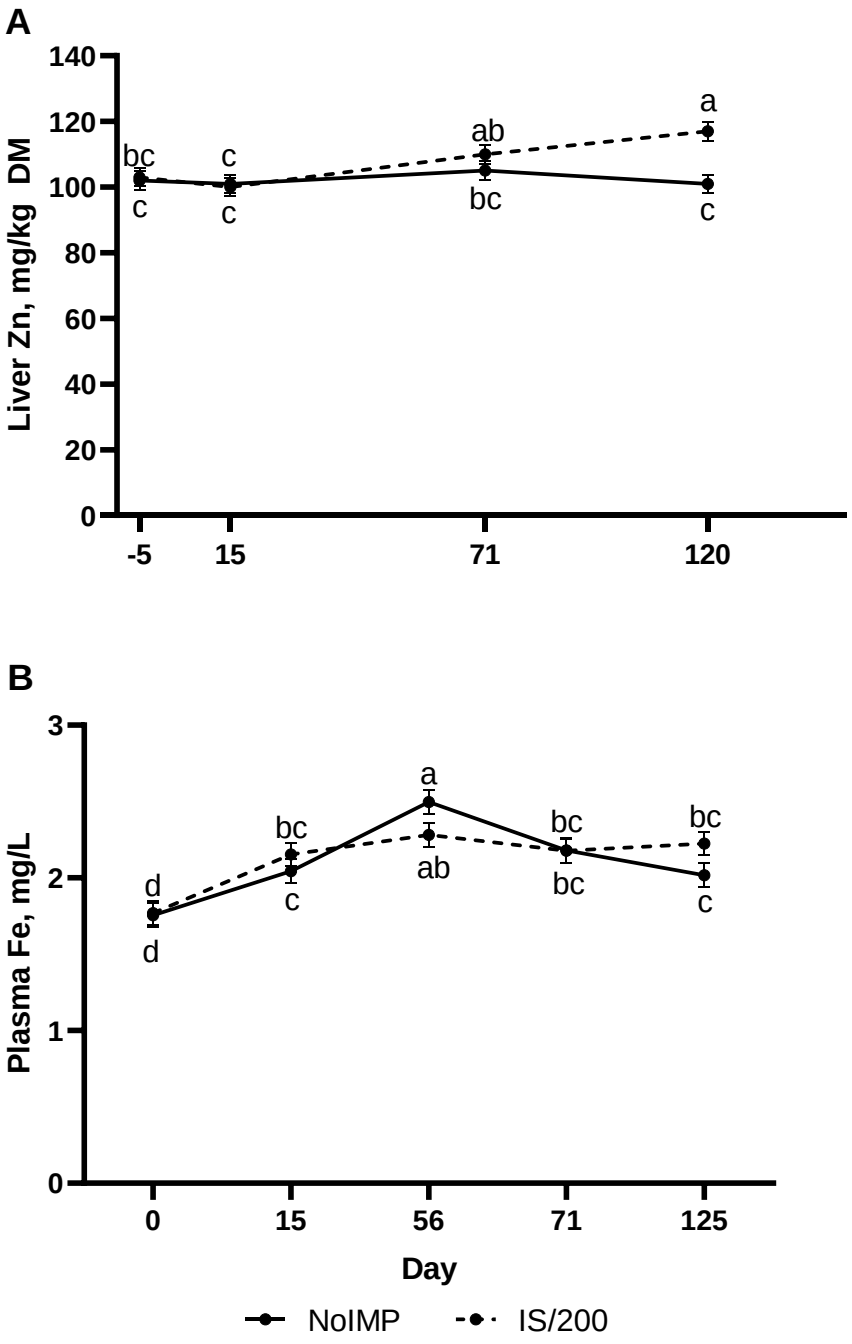


**Figure 2.** Steers were implanted with either no hormonal implant (**NoIMP**) or a Component TE-IS on d 0 followed by a Component TE-200 on d 57 (**IS/200**; Elanco). Liver biopsies were collected on d -5/-4, 14/15, 71/72, and 120/121. Blood was collected on d 0, 15, 56, 71/72, and 125 for plasma Fe analysis. Data were analyzed using repeated measures of the mixed procedure of SAS. Unlike superscripts indicate differences across all time points ($P \leq 0.05$). **A)** Liver Zn concentrations influenced by implant throughout the study (IMP × Day $P = 0.02$). **B)** Plasma Fe concentrations were influenced by implant treatment throughout trial (IMP × Day $P = 0.01$).

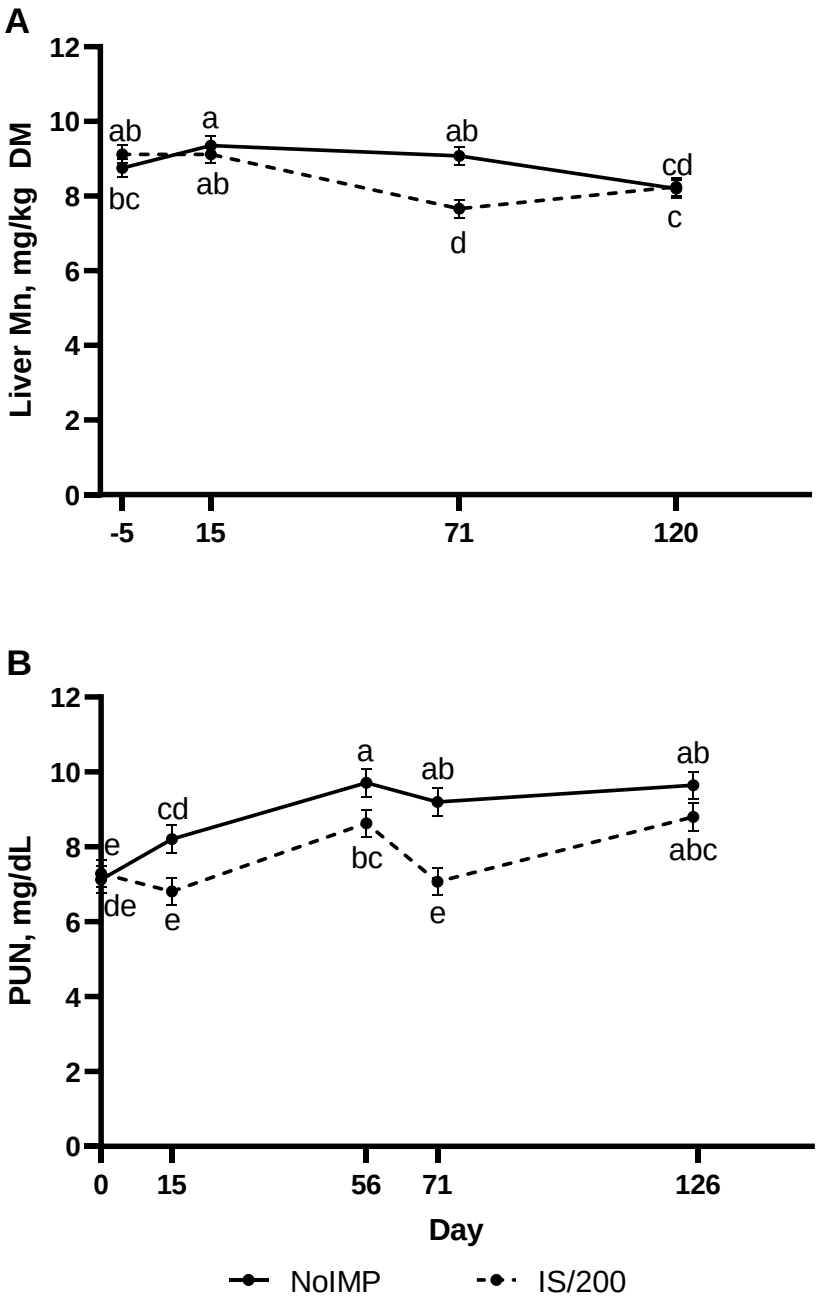


**Figure 3.** Steers were implanted with either no hormonal implant (**NoIMP**) or a Component TE-IS on d 0 followed by a Component TE-200 on d 57 (**IS/200**; Elanco). Liver biopsies were conducted on d -5/-4, 14/15, 71/72, and 120/121 for analysis of liver Mn concentrations. Blood was collected on d 0, 15, 56, 71/72, and 125 for plasma urea nitrogen (PUN) analysis. Data were analyzed using repeated measures of the mixed procedure of SAS. Unlike superscripts indicate differences across time points ($P \leq 0.05$). **A)** Liver Mn concentrations are influenced by implant treatment throughout study (IMP × Day $P = 0.001$). **B)** An IMP × Day effect ($P = 0.001$) was observed for PUN.

# CHAPTER 7. GENERAL CONCLUSIONS

Although trace mineral requirements of beef cattle have remained mostly unchanged for nearly 40 years (NRC, 1984), they are often supplemented at 2 to 3 times NASEM (2016) recommendations in feedlot cattle (Samuelson et al., 2016a). Niedermayer et al. (2018) discovered supplementation of these increased concentrations of trace minerals (Co, Cu, I, Mn, Se, and Zn) to steers improved hot carcass weight compared to steers supplemented NASEM (2016) recommendations. These data beg the question, are NASEM (2016) trace mineral recommendations in line with the nutrient requirements of modern beef cattle commonly administered technologies such as steroidal implants?

Steroidal implants increase the mature body weight (BW) of cattle by shifting the growth curve, resulting in greater protein accretion (Fredric N Owens et al., 1995). Steroidal implants improve cattle average daily gains by 16 to 20% (Duckett and Pratt, 2014). This substantial increase in gain may suggest requirements of cattle for nutrients such as Zn are increased with steroidal implant use. Indeed, steroidal implants increase the absorption and retention of Zn in lambs implanted with the estrogenic implant, zeranol (Hufstedler and Greene, 1995). Furthermore, plasma Zn concentrations decrease with steroidal implant use in steers (Messersmith, 2018). These data indicate implants influence Zn metabolism, and implanted cattle likely have a greater demand for Zn due to Zn's many roles in growth processes (Oberleas and Prasad, 1969; Duncan and Dreosti, 1976; Cousins et al., 2006a). Therefore, the studies presented throughout this dissertation examined the effects of Zn supplementation strategies on implant-induced growth.

Our research agrees supplemental Zn is beneficial to the growth of implanted cattle. A linear response to increasing Zn supplementation up to 150 mg Zn/kg dry matter (DM) from

$ZnSO_4$ on performance and muscle gene expression of implant signaling machinery was observed in implanted steers during peak hormonal payout of the steroidal implant (first 40 d after implant administration; Johnson et al., 1996). These Zn effects were not observed in non-implanted steers. These data are consistent with the linear increase in final BW due to increasing Zn supplementation up to 150 mg Zn/kg DM from a blend of $ZnSO_4$ and Zn amino-acid complex in steers fed ractopamine hydrochloride, but not steers receiving no beta agonist (Genther-Schroeder et al., 2016a). Therefore, cattle experiencing accelerated growth rates from growth-promoting technologies appear to have greater Zn demands than cattle not utilizing these technologies. Interestingly, examination of supplemental Zn concentration up to 180 mg Zn/kg DM from $ZnSO_4$ to implanted steers also yielded a linear response to Zn supplementation on d 10 BW, again, consistent with the peak hormonal payout of the implant. However, by d 98, the numerical difference between implanted steers supplemented 100 vs. 180 mg Zn/kg DM was minimal, suggesting supplementation of 100 mg Zn/kg DM may be optimal for improving implant-induced growth.

This beneficial growth response to supplementation of 100 mg Zn/kg DM from $ZnSO_4$ was also observed in heifers administered an extended-release implant or a two-implant strategy. During peak hormonal payout of these implant strategies, heifers supplemented 100 vs. 30 mg Zn/kg DM had greater performance. Furthermore, supplementation of blended Zn source treatments at 100 mg Zn/kg DM (100% $ZnSO_4$ or 70% $ZnSO_4$ + 30% basic ZnCl or Zn glycinate) to non-implanted and implanted steers resulted in an implant × Zn effect on final BW. Implanted steers supplemented 100% $ZnSO_4$ had greater final BW than the basic ZnCl blend treatment, with Zn glycinate blend intermediate. However, no differences in final BW due to Zn source were observed for non-implanted steers, similar to the performance of steers not receiving

a growth-promoting technology detailed earlier. These data align with our hypothesis that increasing supplemental Zn positively influences the performance of implanted cattle. However, Zn source may be more influential on performance outcomes when fed at concentrations closer to NASEM (2016; 30 mg Zn/kg DM) recommendations, as steers in the study described above were supplemented well above their Zn requirement.

Concurrent with the improved implant-induced performance of cattle due to Zn, plasma Zn concentrations were decreased in implanted cattle in the studies that included measurements of plasma Zn. However, though a sharp decline in plasma Zn concentrations was apparent for implanted steers 15 d post-implant administration, the plasma Zn response reported in Chapter 6 was convoluted by an implant strategy × zinc source × time effect. These data agree with the drop in plasma Zn concentrations within 2 to 13 d of implant administration observed by Messersmith (2018) and Reichhardt et al. (2021) and indicate steroidal implants transiently influence circulating Zn. Collectively, it appears steroidal implants increase cattle Zn demand, likely to accommodate Zn-dependent growth processes, particularly in the early days post administration of implants when peak hormone release is expected.

Additionally, steroidal implants decreased liver Mn concentrations during peak hormonal payout of the implant in all studies presented in this dissertation, consistent with the results of Messersmith (2018) and Niedermayer et al. (2018). These decreases correspond to lesser blood urea nitrogen concentrations due to implant administration and represent the decreased protein degradation associated with steroidal implants. Furthermore, liver Mn concentrations during peak hormonal payout of the implant were correlated with the activity of the terminal enzyme of the urea cycle, arginase, in the liver. These data provide an additional link between steroidal implants and protein degradation. Interestingly, steroidal implant potency also influences this

liver Mn response. Heifers administered the more potent two-implant strategy had lesser liver Mn concentrations than heifers administered the less potent extended-release implant during peak hormonal payout of each implant. Further work is needed to clarify the relationship between animal nitrogen metabolism and liver Mn concentrations, which may provide insight on the usefulness of liver Mn as a Mn status index for cattle.

Together these data indicate steroidal implants increase the Zn demand of cattle to support growth processes, especially during peak hormonal payout of the implant. However, more work is needed to determine Zn's mechanism of action on implant signaling machinery. Considering Zn is highly involved in post-translational modification of proteins, such as phosphorylation (Jung et al., 2009; Gao et al., 2014), implant signaling machinery should be evaluated on a protein basis next, as gene expression may not have fully encapsulated the effects of Zn in implant-induced growth. These data provide clear evidence that increased concentrations of supplemental Zn are most beneficial during peak hormonal payout of the steroidal implant. Future work should focus on the strategic supplementation of increased concentrations of Zn during periods of high growth, such as peak hormonal payout of implants. Although more work is warranted to determine the optimal concentration of supplemental Zn for implanted cattle, these data point towards 100 mg Zn/kg DM as an acceptable target due to minimal benefits noted above this rate of Zn supplementation. There remains room for refinement of the Zn requirement for beef feedlot cattle under different production scenarios, including varying genetic potential of the animal for growth and utilization of growth promoting technologies.

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