THESIS

EFFECT OF ZINC CONCENTRATION AND SOURCE ON PERFORMANCE AND CARCASS CHARACTERISTICS OF FEEDLOT STEERS.

Submitted by

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ABSTRACT

EFFECT OF ZINC CONCENTRATION AND SOURCE ON PERFORMANCE AND CARCASS CHARACTERISTICS OF FEEDLOT STEERS.

Three-hundred and sixty cross-bred steers (348.1 kg ± 28.9) were utilized to investigate the effects of zinc (Zn) concentration on performance and carcass characteristics of feedlot steers. Steers were blocked by weight and randomly assigned to one of the 5 supplemental Zn treatments (8 pens per treatment; 9 hd per pen). Treatments consisted of: 1) **Control-50 (CON-50**; 50 mg of supplemental Zn/kg DM from $ZnSO_4$); 2) **Methionine control** [**MetCON-50**; 50 mg of supplemental Zn/kg DM from $ZnSO_4$ plus MHA to equalize HMTBa concentrations across treatments; 3**) Organic-50 (ORG-50;** Control diet supplemented with 50 mg of Zn/kg DM from Mintrex Zn plus MHA to equalize HMTBa intake across treatments); 4) **Organic-100** (**ORG-100**; Control diet supplemented with 100 mg of Zn/kg DM from MINTREX Zn plus MHA to equalize HMTBa intake across treatments 2-5); and 5) **Organic-150** (**ORG-150**; Control diet supplemented with 150 mg of Zn/kg DM from Mintrex Zn). All steers were fed a typical high concentrate steam-flaked corn based finishing diet twice daily. Steers were individually weighed on d −1, 0, 144, and 145 and pen weighed on d 28, 56, 84, and 111. Ractopamine HCl was fed for the final 29 d of the finishing period to all treatments. On d 145, steers were transported to a commercial abattoir for slaughter. Initial and final body weight, ADG, DMI, and feed efficiency (g/f), were similar across treatments. However, there was a difference ($P < 0.04$) for ADG to be increased when MetCON-50 was compared to Con-50 on d-56 pen weigh and d-84 pen weigh (3.91, 4.42 ± 0.17 and 4.96, 4.29 ± 0.21 respectively). Average

daily gain was also increased (P <0.03) when the CON-50 treatment was compared to the ORG-50 treatment for the d-84 pen weigh period (4.96, 4.29 ± 0.21). There was a trend (P < 0.07) for gain:feed to be increased for the CON-50 when compared to MetCON-50 and ORG-50 for the d57-84 period (0.228, 0.198, 0.199 ± 0.01). Fat thickness, internal fat, hot carcass weight, KPH, marbling score, and dressing percentage were similar across treatments. Steers receiving ORG-150 had a greater (P < 0.03) yield grade compared to steers receiving ORG-50 (2.99 vs. 2.76 ± 0.08; respectively). Steers receiving 1080 Zn also had a greater (P < 0.01) yield grade compared to steers receiving ORG-100 (2.99 vs. 2.68 ± 0.08; respectively). There was a trend (P < 0.07) for steers receiving MetCON to have a greater yield grade compared to CON-50 (2.82 vs. 2.67 ± 0.08; respectively). In addition, liver biopsies and blood samples were analyzed for Zn, Cu, and Fe concentrations as well as alkaline phosphatase in the blood. Initial liver biopsies were similar in Cu and Fe among treatments. Whereas, the Zn concentrations were greater (P < 0.01) in the CON-50 group compared to the Methionine treatment group (218.7 vs. 170.2 ± 12.9). Day 111 liver Zn concentrations tended (P < 0.05) to be lower for CON-50 and MetCON-50 treatments compared to ORG-50 treatment, while no other differences were noted among treatments (117.8, 123.7 vs. 158.0 ± 16.1). Day-0 plasma results indicate levels of Zn, Cu, and Fe to be similar among treatments with a trend (P < 0.07) in Fe concentrations to be less in the MetCON-50 when compared to the CON-50 treatment (4.71 vs. 1.70 ± 0.85). As for the d-111 plasma analysis indicated no differences among treatments in regards to Zn, Cu, and Fe concentrations. The d-144 plasma analysis indicated no differences (P > .05) in Zn, Cu, and Fe concentrations among treatments. The alkaline phosphatase values for d-0, d-111, and d-144 were similar across treatments, yet the alkaline phosphatase means for each treatment increased over time. These data indicate that under conditions of this trial, increasing Zn concentration in the diet above

NRC recommendations has little impact on performance, however, may impact lipid partitioning in steers.

Key Words: carcass characteristics, feedlot, performance, ractopamine, Zn

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Properties of Zinc

Zinc, (Zn) a divalent cation, has an atomic number of 30 and is found in group IIB on the periodic table (McDowell, 1992). Zinc is a bluish white lustrous metal with the following classification properties; Atomic weight: 65.38, Electron Configuration: [Ar] $3d^{10}$ $4s^2$, Group: 12, Period in Periodic Table: 4, Density: 7.14gcm^{-3}, Melting point: 692.68 K, 419.53°C, 787.15 °F, Block in Periodic Table: d-block, Classification: Metallic (WebElements, 2012, McDowell, 1992). Zinc is the 24th most abundant element, and the largest mineable amounts are found in Australia, Asia, and the United States (Wikipedia, 2012). Typically Zn is stable in dry air, but the corrosion rate of Zn is increased as temperature and/or moisture increase along with the addition of carbon dioxide (Assembly of Life Sciences, 1979, Dorton, 2005). Once the corrosion process of Zn is initiated, it produces a light gray film that adheres tightly to the surface preventing further corrosion (Assembly of Life Sciences, Dorton, 2005). Due to this process Zn has been used to protect other metals from oxidation. Metals that are subject to corrosion such as iron, are given a protective coating of Zn. This common use of Zn is involved in the utilization of Zinc Oxide (ZnO) formed when Zn is exposed to air and forms a coating that protects the rest of the metal. This form of Zn is used in paints, some rubber products, cosmetics, pharmaceuticals, plastics, printing inks, soap and batteries to name a few.

Zinc is involved in diverse cellular processes, including catalysis and gene expression, and has been implicated as an inhibitor of apoptosis and of oxidative stress (Hambridge et al. 2000, Liuzzi et al. 2001). At least 90 enzymes and the hormone insulin have Zn present (Whitten, 1988). Zinc functions as the central metal ion for several enzymes such as DNA and RNA polymerase, alkaline phosphatase (AP), peptidases, carbonic anhydrases (CA), and alcohol dehydrogenases (ADH) (Kimura, 1993). The adrenal glands and pituitary glands

depend on Zn for proper function, as well as the pancreas and gonads (Whitten, 1988). Within the pancreas far more Zn is present than necessary for insulin activation. The remainder of Zn is the pancreas is utilized by carboxypeptidase (Vallee et. al. 1946).

Zinc functions within enzyme systems involved in nucleic acid metabolism, protein synthesis, and carbohydrate metabolism. (McDowell, 1992). An apparent role for Zn is as a component of Zn-fingers on binding proteins required to enhance transcription for specific genes. The concentrations of Zn in most mammalian tissues are in the order of 10 to 100mg/g wet weight, with little variation among species (McDowell, 1992). The amount of Zn concentration in a particular tissue varies depending on the specialization of that tissue (Mcdowell,1992).

Metabolism

Absorption: Zinc absorption in rats and ruminants is typically a reflection of their needs (Suttle et. al. 1982). Zinc-deficient animals absorb a higher percentage of administered Zn, when fed the same diet before and after testing, (Miller, 1970). Yet, the influence of Zn status on Zn homeostasis is differentially regulated depending on each tissue. Furthermore, site and extent of absorption can differ. For example McDowell reported that in a study by Arora et. al (1969) the absorption of Zn in sheep was greater in the rumen than in the small intestine. Also, when considering age, the percentage of Zn absorption was higher in younger calves than in older cattle (Miller et. al. 1968). Yet, the age effect on Zn absorption could be an indirect effect rather than the inability of the small intestinal tissues to absorb the Zn (Miller et. al. 1968). Miller (1968) demonstrated that in a diet adequate in Zn (38mg Zn/kg DM), 2.5 month old calves absorbed a much higher percentage of Zn than 4.5 month old calves. Yet, in a Zn deficient diet (2mg Zn/kg DM), there were no differences in Zn absorption with age (Miller et.

al. 1968). Perhaps, the fact that no difference in Zn absorption by age of animals receiving a Zn deficient diet indicates more than one mechanism regulating zinc absorption.

Even though the duodenum has been reported to be the major site for Zn absorption in the rat (Davies, 1980); absorption in ruminants may not only take place in the duodenum but also the rumen as discussed earlier. Once Zn has been absorbed into the intestinal mucosal cells (and possible the rumen papillae), a complex process occurs. This process can be broken down into four phases according to Cousins, (1982). The first phase involves the absorption of Zn into the intestinal mucosal cells. Zinc is transferred across the intestinal wall by a carrier mediated process (McDowell, 1992).

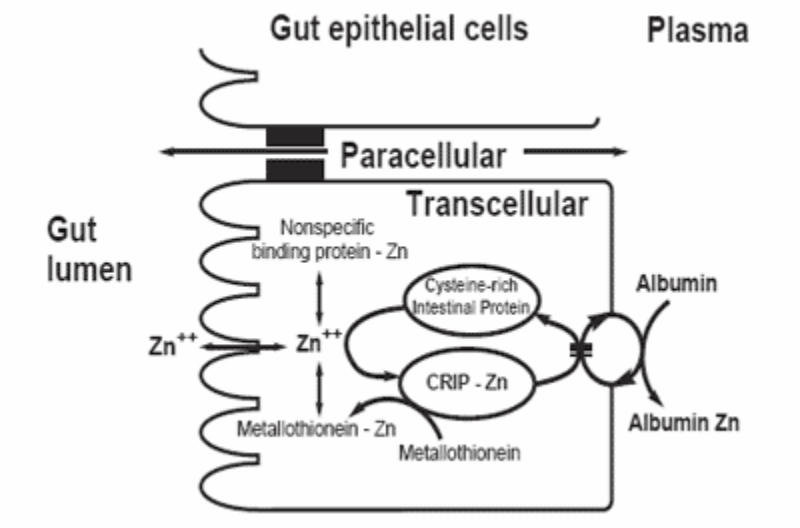

Figure 1. Zinc absoprttion through the gut lumen.J. NUTR. 122:89-95 (1992)

Absorption of Zn through the small intestine is regulated by a variety of low-molecular-weight binding ligands (McDowell, 1992). Metallothionein (a binding ligand) is a metal binding protein synthesized by hepatic and mucosal tissues, and can be influenced by dietary Zn and plasma Zn concentrations (Mcdowell,1992). The function of intestinal metallothionein is to limit the absorption of Zn within the intestinal mucosal cells when dietary Zn concentrations

are high (Cousins 1996, Underwood and Suttle, 1999). Elevated dietary Zn induces the production of metallothionein which then binds excess Zn and prevents further absorption (Davis and Cousins, 2000). When dietary Zn concentrations are low, metallothionein synthesis is decreased thus allowing for increased interactions of Zn and intracellular binding proteins such as Cysteine-rich intestinal binding protein (CRIP) that allow for the transport of Zn to the basolateral surface of the enterocyte.

Once in the enterocyte the absorption of Zn into the blood stream is dependent on Zn concentrations in the circulatory system along with the regulation of metallothionein in the enterocyte. When Zn concentrations in the blood are low, Zn transport into the blood will increase (Hambidge et. al. 1986). Yet, the reverse effect occurs when Zn concentrations are high in the blood; less Zn is absorbed from the intestinal cells into the blood stream. Transport of Zn from the intestinal lumen into the blood stream and from the blood stream into the lumen is a bidirectional process which the mechanism remain unknown (Hambidge et. al. 1986). Zinc that is absorbed through the rumen wall in ruminants can also be reabsorbed into the lumen of the small intestine. Within the lumen, Zn binds to the metallothionein (McDowell, 1999) unless the concentrations of Zn are lower in the blood. If Zn concentrations are lower in the blood, Zn will bind to cysteine rich binding protein (CRIP). Hempe and Cousins (1991) explain how CRIP moves Zn across the enterocyte to the basolateral side of the cell, then Zn is attached to a carrier molecule such as albumin. After Zn attaches to albumin it is transported throughout the body (Hempe and Cousins, 1991).

Zn absorption can also be regulated by other dietary factors. Chelated Zn, such as Zn methionine and zinc oxide, was metabolized differently after absorption in sheep (Spears and Samsell 1986). Therefore, suggesting that Zn is metabolized differently when absorbed as Zn

methionine rather than zinc oxide. Also, inositol hexaphosphates and pentaphosphates are the phytate forms that exert strong negative effects on Zn absorption (Lonnerdal, 2000). Yet, the amount of phytate found in the diet cannot interfere or limit the amount of Zn absorbed by acting as an inhibitor of Zn absorption in ruminants with a functional rumen (NRC, 2000). Phytate which can be found in cereal grains in the form of inositol hexophosphates and pentaphosphates, exert the negative effects on zinc absorption in non- ruminants (Lonnerdal, 2000). Also, McDowell explains that in another study (Cousin, 1978), there is evidence of dietary Zn and plasma Zn regulating the quantity of Zn absorbed by the body therefore playing a significant role in Zn homeostasis. Lonnerdal (2000), reports that with increasing amounts of Zn in meal, fractional Zn absorption (%) will decrease. Similarly, studies have shown that high levels of Zn had a negative effect on absorption when compared to a standard dose. Additionally the amount of protein in a meal has demonstrated to be positively correlated to zinc absorption (Sandstrom et al. 1980, Lonnerdal, 2000). However, fiber has been considered to have a negative effect on Zn absorption, yet, this is usually due to the fact that most fiber-containing foods also contain phytate (Lonnerdal, 2000). Lonnerdal (2000) states that it is unlikely that calcium per se has a negative effect on zinc absorption which agrees with previous work by Spencer et al. (1984) and Dawson-Hughes et al. (1986). Spencer and Dawson-Hughes added large amounts of calcium to a meal and found no effect on Zn absorption in human adults. Researchers have suggested that Zn homeostasis in the rat is a result of Zn secretion into the intestine (Evans et al. 1979), while others reported that the regulation of Zn metabolism is due to dietary factors involved in control over the absorption of Zn. The endogenous losses involved in metabolism could also alter absorption measurements of Zn. Influences on Zn homeostasis have been noticed when endogenous losses decrease as absorption increases

(Suttle et al. 1981). Suttle (1981) also demonstrated that regulation of Zn absorption changed rather than endogenous loss as Zn intakes were increased. Whether the absorption of Zn is up or down regulated, plasma Zn will dictate only part of Zn status within the animal.

Transport and Distribution: Once Zn enters the blood circulatory system a variety of factors will dictate its metabolism. Zinc is transported through the blood stream bound to either albumin, α_2-macroglobulin, and also as traces of metallothionein (Underwood and Suttle, 1999) and flows to the liver. Once in the liver Zn is primarily bound to metallothionein. Metallothionein in the liver is the major storage form of Zn, and can be mobilized during metabolic need (McDowell, 1992). There are four isoforms of metallothionein present in mammals: metallothionein 1 and 2 which have ubiquitous tissue distribution with particular abundance in liver, pancreas, intestine, and kidney, whereas metallothionein 3 and 4 are found principally in brain and skin (Davis and Cousins, 2000). The binding of Zn to liver metallothionein is relatively weak, thus giving liver metallothionein the ability to acquire and release Zn. Whereas the bond between enterocyte derived metallothionein and Zn is extremely tight.

Since Zn can be transported across the small intestine as a metalloprotein and transported in the body by albumin, the attachment of Zn to methionine may alter its mode of absorption and transport in the animal's body compared to Zn from Zn oxide (Greene et. al. 1988). The metabolism of Zn in the blood after it is absorbed is affected by the ligands involved which can vary depending on Zn status (McDowell 1992, Underwood and Suttle 1999). Only about two thirds of plasma Zn is bound to albumin in the portal blood stream (Underwood and Suttle, 1999). The other portion of plasma Zn can be bound to α_2-macroglobulin or to metallothionein for example. Even though the mechanisms of tissue uptake

of Zn have not yet been characterized, Zn complexed with albumin is readily available for uptake by tissues (McDowell, 1992). Yet, not all tissues that uptake Zn, make the Zn available to other tissues. For example, the uptake of Zn in bone and the central nervous system is relatively slow and firmly bound once acquired by these tissues, making the Zn unavailable to other tissues (McDowell, 1992).

The distribution of Zn throughout the body is well understood, but the mechanisms involved in Zn uptake by other tissues beyond the liver are not well known (Cousins, 1996, Underwood and Suttle, 1999). Subsequently Zn is released back into the blood stream after about 30-40% of the Zn entering through the hepatic venous supply is extracted from the liver (McDowell, 1992). The circulating Zn enters various extrahepatic tissues at differing rates, which consist of different rates of Zn turnover (Underwood and Suttle, 1999). Body tissues will exhibit different accumulation and turnover rates following oral administration and subsequent absorption (Miller et. al. 1970). After an oral dosing, plasma Zn concentrations reach their peak within 1 to 3 days followed by a rapid decline for 3 to 4 weeks and a subsequent very slow decrease (Miller et. al. 1970). Even though Zn tends to accumulate very slowly in some tissues, the amount in red blood cells, muscle, and bone continues to increase for several weeks after a single oral dose (Miller et. al. 1970). In the blood stream 80% is present in the erythrocytes, which contain about 1mg Zn per 10^6 cells (Underwood and Suttle, 1999). The pancreas, liver, kidney, and spleen have the most rapid accumulation and turnover of retained Zn (McKinney et al., 1962).

Most of the intracellular Zn is found in the cytosol (60-80%), with some Zn found in the crude nuclear fraction (10-20%) and small amounts in the microsomal and mitochondrial fractions (Saylor and Leach, 1980, McDowell, 1992). Zinc found in the cytosol is primarily

bound to proteins whereas other fractions of Zn may be found on the cell membrane (McDowell, 1992). Yet, Hempe et al., (1991) identified a low molecular mass, intracellular constituent from rat intestinal mucosa that binds Zn during transmucosal Zn transport. The low molecular mass was not metallothionein, based on the Cd-hemoglobin affinity assay (Hempe et al. 1991).

Storage and Excretion: Zinc storage within an animal is minimal, leading to complications during a dietary Zn deficiency. Although Zn is widely distributed throughout the body, animals have limited capacity for storing Zn in a form where it can be mobilized rapidly in order to prevent a deficiency (McDowell, 1992). Along with its importance in Zn absorption, metallothionein is also involved with being the major storage form of Zn within the liver (Richards and Cousins, 1976). Spears and Samsell (1986) reported that Zn retention was greater for lambs fed Zn methionine compared with those fed a control or Zn oxide-supplemented diet. Even though the absence of recognized stores exist, Zn may be redistributed from large pools found in bone and muscle during a deficiency (Underwood and Suttle, 1999). Also, when Zn is fed in large amounts the Zn content greatly increases in some tissues including blood, pancreas, kidney, bone, hair, and liver, but may have little effect on other tissues such as muscle (Miller et al., 1970).

***Zinc Deficiency and Toxicity*:**

Deficiency: A role for Zn in alterations of gene expression could also underlie a characteristic sign of Zn deficiency, namely, the incidence of parakaeratosis in the epidermis, esophagus, and buccal epithelium. According to Covey et al., (2005) if a calf becomes deficient in any nutrient then the recovery from these deficiencies would likely be delayed, impaired performance, and changes in carcass quality are likely**.** In cattle signs for a severe Zn deficiency are reduced growth, feed intake, and feed efficiency; listlessness; excessive salivation; reduced testicular growth; swollen feet with open, scaly lesions and some others as reported by the NRC (2000) from studies conducted by (Miller and Miller,1962, and Ott et al., 1965). Typically plasma or liver concentrations may be used to diagnose severe Zn deficiencies, but plasma Zn determination is of little value in detecting marginal deficiencies. A redistribution of Zn in the body temporarily results in low plasma concentrations due to stress or a disease, and noted as a characteristic of a severe deficiency (Hambridge et al., 1986). In a study by Engle et al., (1997) Holstein steers were allocated by BW to two groups, and fed a marginally Zn deficient diet or a Zn adequate diet. Results indicated that a marginal Zn deficiency decreased fractional protein accretion rate, increased ($P < .05$) urine excretion, and tended to increase ($P < 0.19$) Na and decrease ($P < 0.12$) K concentrations in the urine (Engle et al., 1997).

Toxicity: Zn toxicity when feeding beef cattle is not of great concern because the amount of Zn necessary to cause toxicity is much greater than the requirements. The NRC (1980) reports that the maximum tolerable concentration of zinc is 500 mg of Zn/kg DM. Whereas the requirement of Zn in beef cattle diets is 30 mg Zn/kg diet. In a survey conducted by Vasconcelos and Galyean (2007) some nutritionists recommended relatively high concentrations of certain minerals (e.g. Cu and Zn), which increased average values relative to

NRC (1996) recommendations. The (2007) Texas Tech University survey of nutritional recommendations of feedlot consulting nutritionists reported the mean for Zn to be 93 mg/kg DM (Vasconcelos and Galyean, 2007).

Ingestion of toxic concentrations of Zn resulted in high concentration of Zn accumulation in the blood and tissues of non-ruminants and lambs (Ott et al., 1996). These elevated concentrations of Zn in the blood and tissues generally result in reduced gain, feed consumption, and feed efficiency. The same latter effects were observed in feeder cattle consuming relatively high levels of zinc (Ott et al., 1966b), when a study of the effect of high levels of dietary zinc on blood components and tissue minerals was carried out (Ott et al., 1966).

Also the impact of over feeding any element can result in up regulation of the excretion of a metal such as Zn into the environment (Engle et al., 1997). An element that is poorly available to an animal and/or over fed is excreted into the environment. In areas of intense livestock concentration, heavy metal contamination of soil and ground water is being regulated. The allowable amounts of these elements may not necessarily be determined by animal need, but by waste content. Therefore, to minimize excretory losses of metals such as Zn, sources providing better utilization to the animal must be found.

Bioavailability of Mineral Sources: Currently, trace minerals are available in both organic and inorganic forms. Trace minerals defined as inorganic are those that are typically bound to sulfates, carbonates, chlorides, or oxides, while those defined as organic are bound to amino acids or protein complexes. The general premise behind increased bioavailability of organic trace minerals is that organic trace minerals are protected from many of the interactions (as previously mentioned) that can potentially make them unavailable for absorption (Hemken et

al., 1996). It has been theorized by some researchers that organic trace minerals remain intact in the gastrointestinal tract, through the sight of absorption, and perhaps beyond absorption.

A number of studies have been conducted using in vitro and in vivo techniques to determine the relative bioavailability of trace mineral sources. These experiments typically use an inorganic mineral as a benchmark (100%) and compare other mineral sources to it. Results have been variable, however under certain circumstances (as summarized below), organic mineral sources have been shown to be more bioavailable than inorganic sources. Furthermore, it is challenging to interpret data from different experiments because different researchers may have used various methods of supplementation, different sources of trace minerals, a variety of different cattle types, and a variety of reproductive variables. Moreover, breed of cattle, antagonists present in the diet, as well as physiological status of the animal must be taken into consideration when comparing the results from different experiments.

Beta agonists: When maximizing production efficiency within a specific feedlot management system, it is crucial to understand the growth mechanisms of finishing cattle (Winterholler et al., 2008). Ractopamine Hydrochloride is a β-adrenergic agonist approved for commercial use in beef cattle in the United States in 2003 by the USDA. Since the initiation of Ractompamine use in commercial beef cattle diets, research has indicated this compound generally increases protein accumulation, enhanced growth performance, and may affect adipose tissue deposition, depending on the dose and diet by ractopamine interactions (Dib et al., 2010; Abney et al., 2007).

Yet, even though the biological efficacy of ractopamine and a justification for its use has been well documented, thoughts on how to use this technology the best in commercial cattle feeding should be provoked (Pritchard, 2005). Currently, ractopamine is provided during

the last 28d to 42d prior to slaughter at 280mg * d^{-1} for feedlot steers. Feeding ractopamine at this rate can result in a 15 to 18 pound increase in hot carcass weight (HCW) over controls (Pritchard, 2005). Furthermore, ractopamine is biologically active when administered orally to cattle, and its response not only causes an economically but also biologically significant improvement in cumulative ADG and F/G (Pritchard, 2005). Therefore, potential concerns regarding the best use of ractopamine fed to commercial beef cattle is of much interest. Among these concerns is the recommended dosage accuracy for the full response of ractopmine. There are several factors involved when formulating for the adequate dosage of ractopamine such as the variation of individual DMI from steers in a single pen. Pritchard 2005, reports an example of a feedlot pen that has 498.95 kg steers consuming 8.16 kg DM and 635.03 kg steers consuming 12.7 kg DM that are to be sold in 30 d. These steers received diets formulated at 19g RAC/T assuming an average 9.53 kg DMI of all pens on that batch of feed. Consequently, the light steers then received 171mg*d^{-1}, and the big steers received 266 mg*d^{-1}. Pritchard (2005) also explains that the swine industry has had to increase CP and lysine to get the full effect of ractopamine. However, with increased muscle accretion perhaps dietary increasing dietary Zn during beta agonist feeding should be considered due to the role that Zn plays in protein metabolism.

Chapter II: EFFECT OF ZINC CONCENTRATION AND SOURCE ON PERFORMANCE AND CARCASS CHARACTERISTICS OF FEEDLOT STEERS.

Summary

Three-hundred and sixty cross-bred steers (348.1 kg ± 28.9) were utilized to investigate the effects of zinc (Zn) concentration on performance and carcass characteristics of feedlot steers. Steers were blocked by weight and randomly assigned to one of the 5 supplemental Zn treatments (8 pens per treatment; 9 hd per pen). Treatments consisted of: 1) **Control-50** (CON-50; 50 mg of supplemental Zn/kg DM from $ZnSO_4$); 2) **Methionine control** [**MetCON-50**; 50 mg of supplemental Zn/kg DM from $ZnSO_4$ plus MHA to equalize HMTBa concentrations across treatments; 3**) Organic-50 (ORG-50;** Control diet supplemented with 50 mg of Zn/kg DM from Mintrex Zn plus MHA to equalize HMTBa intake across treatments); 4) **Organic-100** (**ORG-100**; Control diet supplemented with 100 mg of Zn/kg DM from MINTREX Zn plus MHA to equalize HMTBa intake across treatments 2-5); and 5) **Organic-150** (**ORG-150**; Control diet supplemented with 150 mg of Zn/kg DM from Mintrex Zn). All steers were fed a typical high concentrate steam-flaked corn based finishing diet twice daily. Steers were individually weighed on d −1, 0, 144, and 145 and pen weighed on d 28, 56, 84, and 111. Ractopamine HCl was fed for the final 29 d of the finishing period to all treatments. On d 145, steers were transported to a commercial abattoir for slaughter. Initial and final body weight, ADG, DMI, and feed efficiency (g/f), were similar across treatments. However, there was a difference ($P < 0.04$) for ADG to be increased when MetCON-50 was compared to CON-50 on d-56 pen weigh and d-84 pen weigh (3.91, 4.42 ± 0.17 and 4.96, 4.29 ± 0.21 respectively). ADG was also increased ($P < 0.03$) when the CON-50 treatment was compared to the ORG-50

treatment for the d-84 pen weigh period (4.96, 4.29 ± 0.21). There was a trend (P< 0.07) for gain:feed to be increased for the CON-50 when compared to MetCON-50 and ORG-50 for the d57-84 period (0.228, 0.198, 0.199 ± 0.01). Fat thickness, internal fat, hot carcass weight, KPH, marbling score, and dressing percentage were similar across treatments. Steers receiving ORG-150 had a greater (P < 0.03) yield grade compared to steers receiving ORG-50 (2.99 vs. 2.76 ± 0.08; respectively). Steers receiving ORG-150 also had a greater (P < 0.01) yield grade compared to steers receiving ORG-100 (2.99 vs. 2.68 ± 0.08; respectively). There was a trend (P < 0.07) for steers receiving MetCON-50 to have a greater yield grade compared to CON-50 (2.82 vs. 2.67 ± 0.08; respectively). In addition, liver biopsies and blood samples were analyzed for Zn, Cu, and Fe concentrations as well as alkaline phosphatase in the blood. Initial liver biopsies were similar in Cu and Fe among treatments. Whereas, the Zn concentrations were greater (P < 0.01) in the CON-50 group compared to the MetCON-50 treatment group (218.7 vs. 170.2 ± 12.9). Day 111 liver Zn concentrations tended (P < 0.05) to be lower for CON-50 and MetCON-50 treatments compared to ORG-50 treatment, while no other differences were noted among treatments (117.8, 123.7 vs. 158.0 ± 16.1). Day-0 plasma results indicate levels of Zn, Cu, and Fe to be similar among treatments with a trend (P < 0.07) in Fe concentrations to be less in the MetCON-50 when compared to the CON-50 treatment (4.71 vs. 1.70 ± 0.85). As for the d-111 plasma analysis indicated no differences among treatments in regards to Zn, Cu, and Fe concentrations. The d-144 plasma analysis indicated no differences (P > .05) in Zn, Cu, and Fe concentrations among treatments. The alkaline phosphatase values for d-0, d-111, and d-144 were similar across treatments, yet the alkaline phosphatase means for each treatment increased over time. These data indicate that under conditions of this trial, increasing Zn concentration in

the diet above NRC recommendations has little impact on performance, however, may impact lipid partitioning in steers.

Key Words: carcass characteristics, feedlot, performance, ractopamine, Zn

Introduction

Ensuring the adequacy of trace mineral nutrition for feedlot cattle is an important consideration when formulating feedlot diets. The Beef Cattle National Research Council (NRC, 2000) recommends that beef cattle feedlot diets contain 30 mg Zn/kg DM; data reviewed for the publication of the 2000 NRC did not warrant a change in dietary Zn concentration in the feedlot from other phases, such as the cow calf and stocker sector, due to inconsistent growth responses with higher concentrations of Zn supplementation. Despite the NRC (2000) recommended level of 30 mg Zn/kg DM, a survey of consulting feedlot nutritionist indicated that feedlot diets are formulated to contain, on average, 93 ppm Zn with a range of 40 – 213 ppm (Vasconcelos and Galyean, 2007). Zinc was not the only nutrient supplemented above the NRC (2000) requirements that were reported by Vasconcelos and Galyean (2007), but also major nutrients and trace minerals typically fell within a range of 1 to 2 times the NRC (2000) recommendations for beef cattle.

Source (organic or inorganic) of Zn has been reported to influence production parameters in feedlot cattle. According to Spears et al., (1989) organic and inorganic forms of Zn are metabolized differently following absorption and under certain conditions, organic forms of Zn have been reported to enhance performance and improve health and reproduction, but the specific mechanisms underlying observed responses remain unclear (Spears, 1996; Galyean (1996) and results have been inconsistent (Greene et al., 1988; Malcolm-Callis et al.,

2000; Covey et al., 2005). Furthermore, Zn dose has also been of interest when considering best Zn supplementation practices. Even though, on average, consulting nutritionist formulate feedlot diets to contain Zn concentrations well in excess of the NRC (2000) requirements (Vasconcelos and Galyean, 2007), dose experiments in feedlot cattle are highly variable (Rhoads et al. 2003, Malcolm-Callis et al. 2000). Therefore, objectives of the present experiment were to investigate the effects of Zn concentration and source on performance, carcass characteristics, and mineral status of feedlot steers fed ractopamine hydrochloride the last 29 days on feed.

Materials and Methods

Prior to the initiation of this experiment, care, handling, and sampling of the animals defined in this research project was approved by the Colorado State University Animal Care and Use Committee.

Upon arrival to Colorado Beef in Lamar, CO, steers (n = 400; approximate pay-weight 340.2 kg) had an overnight access to long-stemmed grass hay and water. The next morning, steers were trailed (approximately 1.0 km) to the Colorado State University research feedlot - Southeast Colorado Research Center (SECRC) for processing. The processing procedures included: 1) application of ear tags (lot tags and electronic identification tags), 2) vaccinations with Presponse-SQ® (Fort Dodge Animal Health, Fort Dodge, IA), Pyramid II plus Type 2 BVD (Fort Dodge Animal Health, Fort Dodge, IA), and Promectin (Vedco, Inc, St. Joseph, MO), 3) administration of Safe-Guard (Intervet Inc. MN) to control internal parasites, and 4) growth implant administration (Revalor-XS; Merck Animal Health, NJ 20 mg of trenbolone acetate and 4 mg estradiol). All compounds were administered per the manufactures recommendation.

Three hundred sixty cross-bred steers (BW = 348.1 kg ± 28.9), selected from an initial group of approximately 400 head, were utilized in this experiment. The selection process was initiated by weighing all steers an assigning breed type scores on d -1. Breed scores specified to all steers were determined by using visual observations of hide color and assigning breed names. Steers exhibiting Brahman or dairy influence were excluded from further consideration for enrollment into this experiment. Steers were then ranked by body weight, and individuals that were beyond ± 2 SD from the mean were removed from the study. In addition, any individuals showing health problems or excessive Brahman, Longhorn, or Dairy breeding were excluded from the study. Upon initial processing, jugular blood samples were obtained from all steers for analysis of alkaline phosphatase activity as an indicator of Zn status. Alkaline phosphatase activity was determined within 24h of sample collection to serve as an additional blocking factor, if needed, to assist with similar alkaline phosphatase activity distributions across all pens of cattle. Remaining steers were assigned a random number from 1 to 1000 using Microsoft® Excel 2002. A sufficient number of steers with the lowest random numbers were removed from further consideration for the study leaving 360 eligible steers. Steers were ranked by body weight within breed type and divided into 8 weight block replicates. Within each breed type by weight block, each set group of 5 ranked steers were assigned to treatments 1 – 5 using the lowest to highest random number assigned to the steers, respectively. This was repeated for each group of 5 ranked steers within each breed type by weight block. By following these procedures, 8 weight block pen replicates, each one composed of nine steers and exhibiting a similar breed type distribution were used for each treatment of the experiment. Alkaline phosphatase activity was similar across treatments using the above randomization procedure and therefore was not used as a blocking factor.

The dietary Zn treatments consisted of: 1) **Control-50** (CON-50; 50 mg of supplemental Zn/kg DM from $ZnSO_4$); 2) **Methionine control** [**MetCON-50**; 50 mg of supplemental Zn/kg DM from $ZnSO_4$ plus MHA to equalize HMTBa concentrations across treatments; 3**) Organic-50 (ORG-50;** Control diet supplemented with 50 mg of Zn/kg DM from Mintrex Zn plus MHA to equalize HMTBa intake across treatments); 4) **Organic-100** (**ORG-100**; Control diet supplemented with 100 mg of Zn/kg DM from MINTREX Zn plus MHA to equalize HMTBa intake across treatments 2-5); and 5) **Organic-150** (**ORG-150**; Control diet supplemented with 150 mg of Zn/kg DM from Mintrex Zn). To maintain iso-concentrations of HMTBa across treatments 2-5, the following concentrations were utilized: Mintrex Zn contains 16.0% Zn and 80.0% HMTBa; MHA contains 84.0% HMTBa). Dietary Zn supplements were mixed as a component of the finished feed for the entire 145 d experiment.

The following day, steers were returned through the processing chute on d 0, individually weighed and tagged with visual tags identifying study number, and replicate, and animal number within each pen. As each individual steer left the chute they were sorted into treatment groups and the experiment was initiated.

Diets for all treatments were manufactured and fed two times per day starting at 0730 h and ending with the second feeding at approximately 1500 h. All steers were fed a typical high concentrate steam-flaked corn based finishing diet twice daily (Tables 1 and 2). Ractopamine HCl was fed for the final 29 d of the finishing period to all treatments. Feedings were consistent with the standard operating procedures at SECRC from d 1 through 145. On d 145, steers were transported to a commercial abattoir for slaughter.

Feedbunks for all treatments were evaluated each morning at 0630 h and each afternoon at 1600 h. Therefore, whenever feedbunks were observed empty for 2 consecutive mornings, the amount of feed delivered to each bunk was increased approximately 0.227 kg DM per head. Conversely, if excess feed was observed in the bunk for 2 consecutive mornings, the amount of feed delivered to the bunk was decreased an appropriate amount to entice the steers to clean the bunk. Furthermore, a starter and a series of step-up diets were used to acclimate steers to a steam-flaked corn based diet. The diets were formulated to meet or exceed the requirements for all nutrients listed by the NRC (2000). The starter and all step-up diets were each fed for 7 d. Whereas, the finishing diet was fed from d 21 through the end of the experiment and was formulated to contain 2% crude protein equivalent from non-protein nitrogen, 4% neutral detergent fiber solely from corn silage as the roughage source in the diet, 22046 IU per kg DM vitamin A, and 33.1IU per kg DM vitamin E. Thus, Optaflexx (Elanco; 200 mg/hd/d) was fed for the final 30 days of the finishing period to all treatment groups.

Representative samples of feed ingredients and rations were obtained weekly. Dry matter of feed ingredients and rations were determined weekly at SECRC by drying a portion of each sample in a forced-air drying oven (60°C) for 48h. Feed ingredients and ration samples were composited by month and sent to a commercial laboratory (SDK Labs, Hutchinson, KS) for routine nutrient analysis. Orts were obtained for DM analysis whenever feed became spoiled due to adverse weather as well as on weigh days.

The dry matter delivery for each treatment was calculated by multiplying the as-fed feed delivery recorded for each day by the average weekly dry matter concentration determined by the drying oven. Therefore, the DMI for each pen was calculated by subtracting the amount of DM refused from total DM delivered and dividing the result by head-days for the pen. Initial

body weights used for the experiment were the average of individual weights obtained during processing on d-1 and d-0. Whereas, the final individual body weights were obtained on 2 consecutive mornings immediately prior to the day of slaughter. Intermediate weights were obtained as pen weights for each treatment on d 28, 56, 84, and 111. Pen weights collected on d-111 were one week prior to the initiation of ractopamine supplementation. Each pen weigh period and total average daily gain were calculated by taking the total live weight gain and dividing by the number of days on feed for that given period. A four percent pencil shrink was applied to all weights prior to analysis. The net energy requirement for maintenance (NEm) and gain (NEg) for each pen of steers, from d 0 through slaughter, was calculated using equations for medium-framed steer calves published by NRC (2000). The net energy for maintenance and NEg derived from the diet for each pen was calculated from pen performance and pen requirements for NEm and NEg using the quadratic equation derivation of energy equations (further described by Zinn, 1992).

The individual cattle health observations were conducted on a daily basis. Cattle exhibiting signs of respiratory disease were assigned a score of 0 or 1 for each of the following symptoms: depressed appearance, nasal discharge, ocular discharge, rapid breathing, or coughing. Therefore steers scoring 2 or more respiratory points were removed the pen and rectal temperature was recorded. A steer that exhibited a rectal $\geq 39.72^{o}$ C was assigned an additional 2 respiratory score points. Steers that exhibited a combined score of 4 or more points were treated with the appropriate medications. Steers that received treatment were returned to their home pen and allowed to recover. Steers that were pulled a second time for the same disease were re-treated and then returned to their pen and allowed to recover. Subsequently a steer pulled a third time for the same disease was removed from the experiment. Also, steers

that were observed as injured or lame were removed from the pen, examined, treated, and an assessment was made to determine if the steers should be returned to their home pen. Steers that were deemed likely not able to recover were removed from the experiment. Any steer that was removed from the experiment for any reason was weighed in the squeeze chute or on the pen scale. The animal removal data including, date, pen number, steer number, body weight, diagnosis or reasons for removals, and disposition of the steer was recorded. Steers that died or that were euthanized during the course of the experiment were necropsied to determine the cause of death.

Steers were fed for 145 d then transported (266.2 km) to a commercial abattoir. On the day of slaughter steers were fed 30% of their daily feed allowance at 0700 h and trailed to Colorado Beef Feedyard for shipment at approximately 1130 h. The slaughter order, carcass tags, hot carcass weight (HCW), and liver scores were recorded on the day of slaughter. Carcass data were then collected following a 36 h chill (hot carcass weight, 12^{th} rib fat depth, longissimus muscle area, kidney-pelvic-and heart fat, calculated USDA yield grade, marbling score, and USDA quality grade).

Blood samples were obtained via jugular venipuncture (approximately 10 ml) from 3 steers/pen at the initiation, d-111, and termination of the experiment for serum alkaline phosphatase activity and plasma mineral concentrations. Jugular blood samples were obtained using three different vacutainer Serum tubes (Vacutainer® Serum; Plus; and Trace Element tubes; BD, Franklin Lakes, NJ) appropriate for each analysis.

Liver biopsies were obtained from the same 3 animals per pen at the beginning and d111 (on week prior to ractopamine phase) of the experiment. Briefly, liver biopsy sites were clipped of hair, scrubbed three times with Betadine then scrubbed with 70% ethyl alcohol

(altering Betadine and 70% ethyl alcohol), and the area was anesthetized with 5ml of lidocaine. The liver biopsy was obtained through a 1 cm incision made with a scalpel blade between the 11^{th} and 12^{th} ribs on a line from the tubercoxae to the point of the shoulder. A core sample of liver weighing approximately 50mg was taken by using the true-cut technique as described by Pearson and Craig (1980) using a modified JamShedi bone marrow biopsy punch (0.7 cm in diameter x 14 cm in length). Briefly, the biopsy probe was inserted into the liver and a negative pressure was applied with a 20cc syringe to aspirate the biopsy into the probe. All of the biopsy instruments were cold sterilized in 50% Nolvasan 50% deionized water in an enclosed stainless steel instrument container prior to use on each animal. Also, a new pair of sterile gloves was used for each biopsy. Once post biopsy, the instruments were placed back into the 50% Nolvasan and 50% deionized water solution and allowed to soak for approximately 10 min after every use. The biopsy probes were alternated between animals to allow for an increased retention time in the Nolvasan deionized water solution. Immediately after the liver biopsies were collected the steers received Banamine (Flunixin Megulamine; 1.1 mg/kg i.m.) and Oxytetracycline Hydrochloride (6.6-11.0 mg/lb body weight). Animals that were biopsied were then monitored twice daily (a.m./p.m.) for one week. If complications such as bleeding, decreased feed intake, and increased rectal temperature occur, the attending veterinarian was contacted immediately. For, this experiment no steers demonstrated signs of complications post liver biopsy.

Analytical Procedures: Alkaline phosphatase activity was analyzed for all three blood collections time points (d-0, 111, and 144). After each collection period, all tubes were centrifuged at 600 x *g* (4 ºC) using the Allegra® X-15R Centrifuge (Beckman Coulter Inc., North American Commercial Operation, Fullerton, CA). Alkaline phosphatase activity was

determined using a Phosphatase Assay Kit (G-Biosciences, St. Louis, MO. USA) designed to measure the activity of phosphatases in biological samples and to screen for agonists and inhibitors. This phosphatase assay uses *para*-nitrophenyl phosphate (*p*NPP), a chromogenic substrate for most phosphatases including alkaline phosphatase. The phosphatases in the blood sample remove the phosphate group to generate p-nitrophenol, which is deprotonated under alkaline conditions to produce p-nitrophenolate that can be read at 405nm using the Synergy HT instrument (manufactured by BioTek Instruments Inc. Highland Park, P.O Box 998, Winooski, Vermont 05404-0998, USA).

Liver biopsy samples were analyzed for mineral content by weighing the liver sample into a pre-weighed (pre-weighed crucibles were acid washed and dried in a forced-air drying oven prior to use) ashing crucible. Crucibles were placed into a forced-air drying oven at 60ºC for 24h. After removing crucibles from the drying oven they were placed in a desiccator (with 98% calcium sulfate and 2% calcium carbonate which served as the desiccant) for 25min. Samples were then reweighed and the DM of each sample was determined. Next samples were ashed in a muffle furnace (Thermolyne,) at 600˚C for approximately 12h. Crucibles were then placed in the oven by using tongs and leaving approximately 2cm of space from the walls. Once samples were ashed, the furnace was turned off for a cool down period of 45min. After the first 45min the furnace door was opened for a subsequent cool down period of 45min. Next, samples were removed from the oven by using tongs and placed in a desiccator for approximately 25min. Following the ashing procedure, 5ml of HCl (1 N HCL) solution (10:1 distilled H_2O to HCl) was added to each crucible and transferred to a test tube. Liver mineral concentrations were determined via inductively coupled plasma-atomic emission spectroscopy (ICP-AES) methods (Braselton et al., 1997) as described by Ahola et al. (2004) for Zn, Cu, Mo,

Mn, S, and Fe concentrations. Samples were diluted in distilled H2O to fit within a linear range of a standard curve generated by linear regression of known TM concentrations. Multielemental analysis was then carried out by the simultaneous/sequential ICP-AES, with cross flow nebulization, procedure. For plasma mineral analysis, 1.0 ml of plasma was added to pre-weighed crucibles and analyzed as described above.

Statistical Analysis: The experimental design was a randomized, complete block design with 5 treatments. Live animal performance, non-categorical carcass data and blood and tissue response variables were analyzed as a randomized complete block design using the MIXED procedure of SAS® and repeated measures where appropriate using pen as the experimental unit. Treatment was included in the models as a fixed classification variable. Weight block replicates were included in the model as a random class variable and pen was considered the experimental unit. The contrasts of interest for the main effects of source included; CON-50 (50mg Zn/kg DM) vs. MetCON-50(50mg Zn/kg DM); CON-50(50mg Zn/kg DM) vs. ORG-50(50mg Zn/kg DM); MetCON-50(50mg Zn/kg DM) vs. ORG-50(50mg Zn/kg DM); CON-50 (50mg Zn/kg DM) and MetCON-50(50mg Zn/kg DM) vs. ORG-50(50mg Zn/kg DM). The contrasts of interest for the main effects of dose included ORG-50(50mg Zn/kg DM) vs. ORG-100(100mg Zn/kg DM), ORG-50(50mg Zn/kg DM) vs. ORG-150(150mg Zn/kg DM); ORG-100(100mg Zn/kg DM) vs. ORG-150(150mg Zn/kg DM); and a linear effect using linear the SAS® IML procedure. Copper, Fe and Zn concentrations for the d 111 liver biopsy samples were analyzed model using d 0 liver biopsy respective mineral values as a covariate.

Categorical carcass, data, including USDA quality grade, USDA yield grade, and liver abscess data, were analyzed using PROC GLIMMIX of SAS using the same model as listed above. A binomial was assumed for the categorical data. Treatment means for categorical data

were reported as least square means ± SEM for the likelihood that an individual carcass within each pen was classified into each quality and yield grade category, hot carcass weight category, and the likelihood that an individual liver within each pen showed signs of abscesses. As for live performance data was evaluated for the feedlot phase prior to initiation of Optaflexx and for the Optaflexx phase separately.

Results and Discussion

Initial and final body weight and overall ADG, DMI, feed efficiency, and calculated energy recoveries were similar across treatments (Table 3). Brown et. al. (2004) reported steer DMI, ADG, and ADG:DMI before re-implanting or over the entire feeding period were not influenced ($P > 0.10$) by additional Zn from $ZnSO_4$, Zn from Zinpro 100®, and Zn from Availa Zn®. Similar results were also reported by Malcolm-Callis et el. (2000) were reported where ADG and gain:feed were similar in beef steers supplemented with Zn (as $ZnSO_4$) at 20, 100, or 200 mg/kg of dietary DM. In the present experiment ADG was greater ($P < 0.04$) for steers receiving the MetCON-50 when compared to the CON-50 during d 29-56 and during d 57-84 (Table 3). Average daily gain was also greater ($P <0.03$) in CON-50 steers when compared to ORG-50 supplemented steers during d 57-84. There was a trend ($P < 0.07$) for gain:feed to be increased for the ORG-50 supplemented steers compared to MetCON-50 supplemented steers from d 57-84 (Table 3). Dry matter intake was greater ($P < 0.03$) in the MetCON-50 treatment compared to the CON-50 supplemented steers from d 0-28 (Table 3). Furthermore DMI was higher ($P < 0.01$) for ORG-50 supplemented steers compare to CON-50 and MetCON-50 steers. Malcom-Callis et al. (2000) reported DMI to be higher ($P < 0.10$) in steers fed Zn in the form of a Zn amino acid complex compared to iso-amounts of $ZnSO_4$ in a

28 d receiving experiment, but observed no differences in DMI throughout the remainder of the finishing period.

Fat thickness, hot carcass weight, KPH, marbling score, and dressing percentage were similar across treatments (Table 5). Steers receiving ORG-150 had a greater ($P < 0.03$) yield grade compared to steers receiving ORG-50 (2.99 vs. 2.76 ± 0.08; respectively). Steers receiving ORG-150 also had a greater ($P < 0.01$) yield grade compared to steers receiving ORG-100 (2.99 vs. 2.68 ± 0.08; respectively). There was a trend ($P < 0.06$) for steers receiving MetCON-50 to have a greater yield grade compared to the CON-50 (2.82 vs. 2.67 ± 0.08; respectively). Longissiumus dorsi area was greater (P < 0.03) for steers receiving ORG-100 compared to ORG-150 (88.5 vs. 87.4 ± 0.21).

The proportion of cattle grading select was lower ($P < 0.02$) for CON-50 and tended ($P < 0.06$) to be lower for ORG-50 supplemented steers compared to MetCON-50 supplemented steers (Table 6). Furthermore, a trend ($P < 0.10$) for a linear dose effect in the proportion of cattle qualifying for YG 3 was observed. As does of organic Zn increased the proportion of YG 3 cattle tended to increase (Table 6). Overall, the proportion of cattle: grading low choice or higher, grading standard, receiving an YG of 1 or 2 or 4 or 5 and the proportion of cattle with normal or abscessed livers were similar across treatments.

Initial liver biopsies were similar in Cu and Fe concentrations across treatments (Table 7). Whereas, the Zn concentrations were greater ($P < 0.01$) in the CON-50 compared to the MetCON-50 supplemented cattle (Table 7). Thus, initial liver mineral concentrations were used as a covariate when analyzing d 111 liver mineral concentrations. Day 111 liver Zn concentrations tended (P < 0.05) to be lower for CON-50 and Met-50 treatments compared to the ORG-50 treatment, while no other differences were noted among treatments (Table 7).

Plasma concentrations of Zn, Cu, and Fe were similar across treatments on d 0 and 144 of the experiment (Table 7). Furthermore plasma alkaline phosphatase activity was similar across treatments on d 0, 111, and 144. Zinc absorption has been shown to be similar between Zn methionine and inorganic sources. However, evidence exists that Zn provided by Zn methionine is retained in the body more effectively than inorganic Zn (Brown et al. 2004, Spears, 1989).

In conclusion, these data indicate that under conditions of this experiment, source of Zn as well as increasing Zn concentration in the diet above NRC recommendations has little impact on performance, however, may impact lipid partitioning in steers.

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Appendix A

Table 1. Dry matter composition of basal diets.

	Diet, DM %				
Ingredients[a]	Starter	Step Up 1	Step Up 2	Finisher No/Opt.	Finisher w/Opt
Steam Flaked Corn	40.45	45.60	62.71	75.42	75.39
Corn Silage	22.07	25.08	15.73	9.78	9.78
Alfalfa hay	28.37	14.67	6.75	0.00	0.00
Corn Steep	3.00	3.00	3.00	3.00	3.00
DDG's	4.91	7.99	6.46	4.58	4.59
Tallow	0.00	1.70	2.72	3.79	3.79
Supplement	1.21	1.96	2.63	3.42	3.45
Theoretical Nutrients					
Dry matter, %	61.08	59.05	64.14	67.76	67.76
Crude Protein	13.50	13.50	13.50	13.50	13.50
Non-protein nitrogen[b]	1.000	1.500	2.500	3.500	3.500
Acid detergent fiber	19.63	15.16	9.654	5.294	5.295
Neutral detergent fiber	29.74	25.17	18.51	13.30	13.30
Eff-NDF[c]	22.99	17.59	12.18	8.016	8.014
ME, Mcal/kg[d]	1.425	1.411	0.981	0.661	0.662
NEg, Mcal/kg[e]	1.093	1.235	1.378	1.494	1.494
NEm, Mcal/kg[f]	1.781	1.922	2.067	2.186	2.186
Calcium	0.700	0.700	0.700	0.700	0.700
Phosphorus	0.375	0.379	0.372	0.359	0.359
Magnesium	0.230	0.229	0.211	0.194	0.195
Zinc, ppm	150.0	50.00	50.00	50.00	50.00
Copper, ppm	25.04	15.00	15.00	15.00	15.00
Cobalt, ppm	0.500	0.200	0.200	0.200	0.200

[a]DM percentage unless stated otherwise
[b]Crude protein equivalent
[c]Effective neutral detergent fiber
[d]Metabolizable energy
[e]Net energy for gain
[f]Net energy for maintenance

Table 2. Dry matter composition of the basal ration supplements.

	% of Supplement for Diets				
Ingredients[a]	Starter	Step Up 1	Step Up 2	Finisher No/Opt.	Finisher w/Opt
Limestone	41.2	49.46	49.24	45.38	45.12
Urea	27.0	25.71	32.65	35.56	35.33
Salt	20.7	12.79	9.50	7.31	7.26
Potassium Chloride	6.52	N/A	N/A	5.41	5.37
Ground Corn	N/A	4.04	2.76	1.99	1.98
Mineral Oil	1.98	4.01	2.74	1.99	1.98
Optaflexx 45®	N/A	N/A	N/A	N/A	0.61
Vitamin E	1.32	0.83	0.61	0.47	0.46
Rumensin	0.66	0.41	0.46	0.47	0.46
Manganese Sulfate	N/A	0.76	0.56	0.41	0.41
Zinc Sulfate	N/A	0.69	0.51	0.38	0.38
Sodium Selenite	N/A	0.63	0.47	0.35	0.35
Copper Sulfate	N/A	0.30	0.22	0.15	0.15
Tylan 100	N/A	0.25	0.18	0.12	0.12
EDDI	N/A	0.07	0.05	0.03	0.03
Vitamin A	0.17	0.05	0.04	0.00	0.00
Cobalt Carbonate	N/A	0.02	0.02	0.00	0.01

[a]Percentage of dry matter

Table 3. Effect of zinc concentration and source on performance and calculated energy recoveries of feedlot cattle.

Item	Treatment					SEM	P <			
	1[a]	2[a]	3[a]	4[a]	5[a]		1 vs 2	1 vs 3	3 vs 4	3 vs 5
Body Wt,kg										
Initial	336.6	337.0	337.5	337.4	337.9	8.75	0.75	0.48	0.89	0.77
D 28	383.7	383.3	381.1	380.3	381.3	7.98	0.91	0.43	0.58	0.87
D 56	433.3	439.4	435.0	434.8	439.3	8.12	0.26	0.74	0.93	0.18
D 84	493.7	487.7	489.4	488.9	494.7	8.98	0.56	0.70	0.85	0.09
D 111	536.5	542.2	534.7	534.9	541.7	8.21	0.37	0.78	0.96	0.22
D 145	594.8	600.8	588.0	586.3	597.5	10.93	0.42	0.44	0.80	0.18
Adg,kg/d										
D 0-28	1.68	1.65	1.56	1.53	1.68	0.07	0.78	0.22	0.66	0.90
D 29-56	1.77	2.00	1.93	1.95	1.77	0.08	0.04	0.16	0.86	0.17
D 57-84	2.25	1.95	1.94	1.93	1.98	0.10	0.03	0.03	0.92	0.72
D 85-111	1.61	1.72	1.61	1.64	1.68	0.12	0.38	0.99	0.87	0.72
D 112-145	1.71	1.72	1.57	1.65	1.64	0.13	0.94	0.31	0.62	0.64
D 0-145	1.78	1.82	1.73	1.72	1.79	0.04	0.47	0.33	0.81	0.18
DMI kg/d										
D 0-28	6.38	6.71	6.83	6.70	6.69	0.10	0.03	0.01	0.19	0.14
D 29-56	8.30	8.51	8.39	8.33	8.30	0.24	0.48	0.76	0.79	0.88
D 57-84	10.16	9.90	9.82	9.42	10.16	0.29	0.49	0.38	0.22	0.99
D 85-111	10.47	10.23	9.97	9.43	10.47	0.32	0.56	0.23	0.20	0.98
D 112-145	10.24	10.17	9.73	9.10	9.73	0.41	0.87	0.33	0.21	0.99
Gain:Feed										
D 0-28	0.263	0.247	0.228	0.229	0.234	0.011	0.26	0.03	0.90	0.50
D 29-56	0.215	0.230	0.217	0.234	0.250	0.012	0.34	0.89	0.28	0.05
D 57-84	0.228	0.198	0.199	0.205	0.202	0.010	0.07	0.09	0.52	0.77
D 85-111	0.162	0.180	0.168	0.175	0.155	0.010	0.05	0.32	0.43	0.70
D 112-145	0.168	0.170	0.161	0.179	0.168	0.011	0.84	0.54	0.29	0.64
D 0-145	0.196	0.200	0.193	0.199	0.201	0.005	0.49	0.68	0.29	0.19
Feed:Gain										
D 0-28	3.83	4.11	4.48	4.52	3.83	0.25	0.29	0.03	0.85	0.61
D 29-56	4.88	4.43	4.81	4.39	4.88	0.28	0.20	0.85	0.23	0.54
D 57-84	4.48	5.12	5.26	4.89	4.60	0.29	0.18	0.11	0.36	0.50
D 85-111	6.50	6.00	6.28	5.97	6.16	0.33	0.38	0.32	0.59	0.84
D 112-145	6.01	6.03	6.38	5.90	6.12	0.44	0.97	0.48	0.42	0.66
D 0-145	5.16	5.04	5.21	5.03	5.00	0.14	0.44	0.77	0.24	0.18
NEm, Mcal/kg DM	2.19	2.21	2.22	2.29	2.25	0.05	0.77	0.72	0.22	0.59
NEg, Mcal/kg DM	1.53	1.54	1.54	1.61	1.57	0.05	0.79	0.73	0.22	0.62

[a] Treatments: 1) CON-50 (50mg Zn/kg DM ZnSO4); 2) MetCON-50 (50mg Zn/kg DM ZnSO4 + MHA); 3) ORG-50 (50mg Zn/kg DM Mintrex Zn); 4) ORG-100 (100mg Zn/kg DM Mintrex Zn); 5) ORG-150 (150mg Zn/kg DM Mintrex Zn)

Table 4. Effect of zinc concentration and source on carcass characteristics of feedlot cattle.

Item	Treatment					SEM	P <			
	1[a]	2[a]	3[a]	4[a]	5[a]		1 vs 2	1 vs 3	3 vs 4	3 vs 5
Fat thickness, cm	1.09	1.17	1.12	1.12	1.22	0.02	0.06	0.38	0.93	0.21
Rib eye area, cm^2	88.5	87.8	87.3	88.5	87.4	0.21	0.57	0.26	0.41	0.13
KPH	1.86	1.84	1.87	1.85	1.89	0.03	0.56	0.91	0.73	0.71
Yield Grade	2.67	2.82	2.76	2.68	2.99	0.08	0.06	0.24	0.43	0.03
HCW	372.5	377.6	370.5	369.7	375.6	15.3	0.28	0.67	0.86	0.30
Marbling score	398.8	385.4	392.5	386.9	391.1	5.21	0.09	0.41	0.44	0.85
Dressing percentage	62.61	62.88	63.01	63.05	62.87	0.31	0.61	0.45	0.94	0.76

[a] Treatments: 1) CON-50 (50mg Zn/kg DM ZnSO4); 2) MetCON-50 (50mg Zn/kg DM ZnSO4 + MHA); 3) ORG-50 (50mg Zn/kg DM Mintrex Zn); 4) ORG-100 (100mg Zn/kg DM Mintrex Zn); 5) ORG-150 (150mg Zn/kg DM Mintrex Zn)

Table 5. Effect of zinc source and concentration on categorical carcass characteristics

	Treatment					P <				
Item	1[a]	2[a]	3[a]	4[a]	5[a]	1 vs 2	1 vs 3	1, 2 vs 3	3 vs 4	3 vs 5
n=	69	71	71	72	72					
Quality Grade										
≥ Low Choice	52.2 ± 6.01	31.9 ± 5.61	45.7 ± 5.95	37.5 ± 5.70	39.4 ± 5.80	0.25	0.59	0.68	0.51	0.59
Select	46.4 ± 6.11	68.1 ± 5.70	51.4 ± 6.08	61.1 ± 5.8	57.7 ± 5.97	0.02	0.56	0.42	0.46	0.59
≤ Standard[b]	1.4	0.00	2.8	1.4	2.8	n/a[b]	n/a[b]	n/a[b]	0.56	0.98
Liver Scores										
Normal[b]	87.1	76.9	74.29	80.62	77.6	n/a[b]	n/a[b]	n/a[b]	n/a[b]	n/a[b]
Abscesses[c]	8.6 ± 3.44	14.4 ± 4.38	20.0 ± 4.79	16.6 ± 4.56	16.8 ± 4.62	0.49	0.32	0.35	0.61	0.63
Other[d]	4.35 ± 2.46	8.69 ± 3.39	5.71 ± 2.77	2.78 ± 1.94	5.63 ± 2.74	0.49	0.78	0.92	0.55	0.99
Yield Grade										
1 or 2	65.6 ± 6.36	60.6 ± 6.51	66.4 ± 6.65	69.7 ± 6.01	54.3 ± 7.10	0.68	0.93	0.73	0.68	0.16
3	34.3 ± 6.63	30.7 ± 6.33	26.3 ± 6.27	28.6 ± 6.46	40.1 ± 7.20	0.66	0.33	0.39	0.76	0.11
4 or 5[e]	0.01	8.69	7.30	1.69	5.56	n/a[b]	n/a[b]	n/a[b]	0.51	0.78

[a] Treatments: 1) CON-50 (50mg Zn/kg DM ZnSO4); 2) MetCON-50 (50mg Zn/kg DM ZnSO4 + MHA); 3) ORG-50 (50mg Zn/kg DM Mintrex Zn); 4) ORG-100 (100mg Zn/kg DM Mintrex Zn); 5) ORG-150 (150mg Zn/kg DM Mintrex Zn)

[b] Did not converge during PROC GLIMMIX analysis. Results reported as the percentage of individual carcasses for each treatment.

[c] The percentage likelihood that an individual liver showed symptoms of liver abscesses.

[d] Other condemnations - telang (abnormal dialations of red, blue, or purple superficial capillaries, arterioles, or venules typically localized just below the outer surface) and contaminated.

[e] Did not converge during PROC GLIMMIX analysis. Results reported as the percentage of individual carcasses for each treatment.

Table 6. Effects of zinc concentration and source on liver and plasma mineral concentrations and plasma

	Treatment						P <			
Liver	1[a]	2[a]	3[a]	4[a]	5[a]	SEM	1 vs 2	1 vs 3	3 vs 4	3 vs 5
Day 0										
Zn	218.7	170.2	208.1	206.6	201.3	12.9	0.01	0.50	0.93	0.69
Cu	106.2	89.5	94.3	111.1	123.2	28.7	0.61	0.72	0.73	0.56
Fe	426.7	465.6	522.4	489.8	471.6	43.6	0.51	0.12	0.56	0.37
Day 111										
Zn	117.8	123.7	158.0	128.4	143.0	16.1	0.81	0.08	0.23	0.53
Cu	95.3	82.6	77.5	99.1	104.8	24.5	0.64	0.50	0.49	0.37
Fe	486.3	411.4	412.1	420.2	430.0	42.7	0.22	0.24	0.84	0.70
Plasma Day 0[b]										
Zn	1.46	1.03	0.87	0.72	0.91	0.42	0.20	0.08	0.59	0.88
Cu	0.11	0.05	0.11	0.09	0.21	0.07	0.38	0.99	0.83	0.36
Fe	4.71	1.70	2.43	1.93	2.38	0.85	0.03	0.09	0.64	0.96
Day 111										
Zn	1.36	1.14	0.78	1.06	1.13	0.25	0.55	0.14	0.50	0.36
Cu	0.08	0.14	0.26	0.36	0.27	0.12	0.63	0.18	0.56	0.95
Fe	4.83	5.87	3.55	4.07	4.54	1.07	0..61	0.51	0.75	0.48
Day 144										
Zn	1.82	2.71	2.57	2.16	2.24	0.47	0.22	0.31	0.55	0.65
Cu	0.30	0.27	0.13	0.15	0.30	0.10	0.83	0.27	0.89	0.23
Fe	11.4	11.2	15.2	11.4	11.0	2.03	0.92	0.19	0.21	0.17
Alkaline Phosphatase										
Day 0	50.43	50.08	50.13	50.66	50.79	2.10	.9074	.9206	.8580	.8255
Day 111	67.81	60.16	59.09	68.51	64.64	6.25	.4018	.3402	.2930	.5322
Day 144	60.23	64.35	70.54	74.20	82.84	6.72	.6104	.2100	.7434	.2779

[a] Treatments: 1) 50mg Zn/kg DM ZnSO4; 2) 50mg Zn/kg DM ZnSO4 + MHA; 3) 50mg Zn/kg DM Mintrex Zn; 4) 100mg Zn/kg DM Mintrex Zn; 5) 150mg Zn/kg DM Mintrex Zn.

[b] Copper, Fe and Zn concentrations for the d 111 liver biopsy samples were analyzed model using d 0 liver biopsy respective mineral values as a covariate

Appendix B

SAS code used to analyze Performance data.

```
options ls=100 ps=150;
data adg;
input pen trt rep vt time adg;
cards;
;
proc sort;
by pen time;
run;
proc mixed scoring=2;
class pen rep trt time;
model adg= trt time trt*time/ddfm=kenwardroger;
repeated /subject=pen(trt) type=ar(1) r rcorr;
random rep;
lsmeans  trt time trt*time/pdiff;
contrast 'trt 1 vs trt 2' trt 1 -1 0;
contrast 'trt 1 vs trt 3' trt 1 0 -1;
contrast 'trt 2 vs trt 3' trt 0 1 -1;
contrast 'trt 1 2 vs trt 3' trt -1 -1 2;
run;


options ls=100 ps=150;
data adg;
input pen trt rep vt time adg;
cards;
;
proc sort;
by pen time;
run;
proc mixed scoring=2;
class pen rep trt time;
model adg= trt time trt*time/ddfm=kenwardroger;
repeated /subject=pen(trt) type=ar(1) r rcorr;
random rep;
lsmeans  trt time trt*time/pdiff;
contrast 'trt 3 vs trt 4' trt 1 -1 0;
contrast 'trt 3 vs trt 5' trt 1 0 -1;
contrast 'trt 4 vs trt 5' trt 0 1 -1;
contrast 'linear' trt -0.663392 -0.080533 0.7439255;
run;


options ls=100 ps=150;
data bw;
input pen trt rep vt time bw;
cards;
;
proc sort;
by pen time;
run;
proc mixed scoring=2;
class pen rep trt time;
```

```
model bw= trt time trt*time/ddfm=kenwardroger;
random rep;
repeated /subject=pen(trt) type=ar(1) r rcorr;
lsmeans  trt time trt*time/pdiff;
contrast 'trt 1 vs trt 2' trt 1 -1 0;
contrast 'trt 1 vs trt 3' trt 1 0 -1;
contrast 'trt 2 vs trt 3' trt 0 1 -1;
contrast 'trt 1 2 vs trt 3' trt -1 -1 2;
run;


options ls=100 ps=150;
data bw;
input pen trt rep vt time bw;
cards;
;
proc sort;
by pen time;
run;
proc mixed scoring=2;
class pen rep trt time;
model bw= trt time trt*time/ddfm=kenwardroger;
repeated /subject=pen(trt) type=ar(1) r rcorr;
random rep;
lsmeans  trt time trt*time/pdiff;
contrast 'trt 3 vs trt 4' trt 1 -1 0;
contrast 'trt 3 vs trt 5' trt 1 0 -1;
contrast 'trt 4 vs trt 5' trt 0 1 -1;
contrast 'linear' trt -0.663392 -0.080533 0.7439255;
run;


options ls=100 ps=150;
data dmi;
input pen trt rep vt time dmi;
if time=0 then delete;
cards;
;
proc sort;
by pen time;
proc mixed scoring=2;
class pen rep trt time;
model dmi= trt time trt*time/ddfm=kenwardroger;
random rep;
repeated /subject=pen(trt) type=ar(1) r rcorr;
lsmeans  trt time trt*time/pdiff;
contrast 'trt 1 vs trt 2' trt 1 -1 0;
contrast 'trt 1 vs trt 3' trt 1 0 -1;
contrast 'trt 2 vs trt 3' trt 0 1 -1;
contrast 'trt 1 2 vs trt 3' trt -1 -1 2;
run;



options ls=100 ps=150;
data dmi;
input pen trt rep vt time dmi;
```

```
if time=0 then delete;
cards;
;
proc sort;
by pen time;
proc mixed scoring=2;
class pen rep trt time;
model dmi= trt time trt*time/ddfm=kenwardroger;
random rep;
repeated /subject=pen(trt) type=ar(1) r rcorr;
lsmeans  trt time trt*time/pdiff;
contrast 'trt 3 vs trt 4' trt 1 -1 0;
contrast 'trt 3 vs trt 5' trt 1 0 -1;
contrast 'trt 4 vs trt 5' trt 0 1 -1;
contrast 'linear' trt -0.663392 -0.080533 0.7439255;
run;


options ls=100 ps=150;
data feed to gain;
input pen trt rep vt time ftg;
cards;
;
proc sort;
by pen time;
proc mixed scoring=2;
class pen rep trt time;
model ftg= trt time trt*time/ddfm=kenwardroger;
random rep;
repeated /subject=pen(trt) type=ar(1) r rcorr;
lsmeans  trt time trt*time/pdiff;
contrast 'trt 1 vs trt 2' trt 1 -1 0;
contrast 'trt 1 vs trt 3' trt 1 0 -1;
contrast 'trt 2 vs trt 3' trt 0 1 -1;
contrast 'trt 1 2 vs trt 3' trt -1 -1 2;
run;


options ls=100 ps=150;
data feed to gain;
input pen trt rep vt time ftg;
cards;
;
proc sort;
by pen time;
proc mixed scoring=2;
class pen rep trt time;
model ftg= trt time trt*time/ddfm=kenwardroger;
random rep;
repeated /subject=pen(trt) type=ar(1) r rcorr;
lsmeans  trt time trt*time/pdiff;
contrast 'trt 3 vs trt 4' trt 1 -1 0;
contrast 'trt 3 vs trt 5' trt 1 0 -1;
contrast 'trt 4 vs trt 5' trt 0 1 -1;
contrast 'linear' trt -0.663392 -0.080533 0.7439255;
run;
```

```
options ls=100 ps=150;
data gain to feed;
input pen trt rep vt time gtf;
cards;
;
proc sort;
by pen time;
proc mixed scoring=2;
class pen rep trt time;
model gtf= trt time trt*time/ddfm=kenwardroger;
random rep;
repeated /subject=pen(trt) type=ar(1) r rcorr;
lsmeans  trt time trt*time/pdiff;
contrast 'trt 1 vs trt 2' trt 1 -1 0;
contrast 'trt 1 vs trt 3' trt 1 0 -1;
contrast 'trt 2 vs trt 3' trt 0 1 -1;
contrast 'trt 1 2 vs trt 3' trt -1 -1 2;
run;

options ls=100 ps=150;
data gain to feed;
input pen trt rep vt time gtf;
cards;
;
proc sort;
by pen time;
proc mixed scoring=2;
class pen rep trt time;
model gtf= trt time trt*time/ddfm=kenwardroger;
random rep;
repeated /subject=pen(trt) type=ar(1) r rcorr;
lsmeans  trt time trt*time/pdiff;
contrast 'trt 3 vs trt 4' trt 1 -1 0;
contrast 'trt 3 vs trt 5' trt 1 0 -1;
contrast 'trt 4 vs trt 5' trt 0 1 -1;
contrast 'linear' trt -0.663392 -0.080533 0.7439255;
run;
```

SAS code used to analyze NEm and NEg

```
data NEm;
input pen trt rep vt time NEm NEg;
cards;
;
proc sort;
by pen time;
run;
proc mixed scoring=2;
class pen rep trt time;
model NEm= trt time trt*time/ddfm=kenwardroger;
repeated /subject=pen(trt) type=ar(1) r rcorr;
random rep;
lsmeans  trt time trt*time/pdiff;
contrast 'trt 1 vs trt 2' trt 1 -1 0;
contrast 'trt 1 vs trt 3' trt 1 0 -1;
contrast 'trt 2 vs trt 3' trt 0 1 -1;
contrast 'trt 1 2 vs trt 3' trt -1 -1 2;
run;


data NEm;
input pen trt rep vt time NEm NEg;
cards;
;
proc sort;
by pen time;
run;
proc mixed scoring=2;
class pen rep trt time;
model NEm= trt time trt*time/ddfm=kenwardroger;
repeated /subject=pen(trt) type=ar(1) r rcorr;
random rep;
lsmeans  trt time trt*time/pdiff;
contrast 'trt 3 vs trt 4' trt 1 -1 0;
contrast 'trt 3 vs trt 5' trt 1 0 -1;
contrast 'trt 4 vs trt 5' trt 0 1 -1;
contrast 'linear' trt -0.663392 -0.080533 0.7439255;
run;
```

```
data NEg;
input pen trt rep vt time NEm NEg;
cards;
;
proc sort;
by pen time;
run;
proc mixed scoring=2;
class pen rep trt time;
model NEg= trt time trt*time/ddfm=kenwardroger;
repeated /subject=pen(trt) type=ar(1) r rcorr;
random rep;
lsmeans  trt time trt*time/pdiff;
contrast 'trt 1 vs trt 2' trt 1 -1 0;
contrast 'trt 1 vs trt 3' trt 1 0 -1;
contrast 'trt 2 vs trt 3' trt 0 1 -1;
contrast 'trt 1 2 vs trt 3' trt -1 -1 2;
run;


data NEg;
input pen trt rep vt time NEm NEg;
cards;
;
proc sort;
by pen time;
run;
proc mixed scoring=2;
class pen rep trt time;
model NEg= trt time trt*time/ddfm=kenwardroger;
repeated /subject=pen(trt) type=ar(1) r rcorr;
random rep;
lsmeans  trt time trt*time/pdiff;
contrast 'trt 3 vs trt 4' trt 1 -1 0;
contrast 'trt 3 vs trt 5' trt 1 0 -1;
contrast 'trt 4 vs trt 5' trt 0 1 -1;
contrast 'linear' trt -0.663392 -0.080533 0.7439255;
run;
```

SAS code used to analyze Carcass data.

```
options ls=100 ps=150;
data carcass;
input pen trt rep et ft rea if yg hcw kph ms dp qg;
cards;
;
proc sort;
by pen;
proc mixed scoring=2;
class pen rep trt;
model ft=trt/ddfm=kenwardroger;
random rep;
repeated /subject=pen(trt) type=ar(1) r rcorr;
lsmeans  trt/pdiff;
contrast 'trt 1 vs trt 2' trt 1 -1 0;
contrast 'trt 1 vs trt 3' trt 1 0 -1;
contrast 'trt 2 vs trt 3' trt 0 1 -1;
contrast 'trt 1 2 vs trt 3' trt -1 -1 2;
run;
proc mixed scoring=2;
class pen rep trt;
model rea=trt/ddfm=kenwardroger;
random rep;
repeated /subject=pen(trt) type=ar(1) r rcorr;
lsmeans  trt/pdiff;
contrast 'trt 1 vs trt 2' trt 1 -1 0;
contrast 'trt 1 vs trt 3' trt 1 0 -1;
contrast 'trt 2 vs trt 3' trt 0 1 -1;
contrast 'trt 1 2 vs trt 3' trt -1 -1 2;
run;
 proc mixed scoring=2;
class pen rep trt;
model if= trt/ddfm=kenwardroger;
random rep;
repeated /subject=pen(trt) type=ar(1) r rcorr;
lsmeans  trt/pdiff;
contrast 'trt 1 vs trt 2' trt 1 -1 0;
contrast 'trt 1 vs trt 3' trt 1 0 -1;
contrast 'trt 2 vs trt 3' trt 0 1 -1;
contrast 'trt 1 2 vs trt 3' trt -1 -1 2;
run;
proc mixed scoring=2;
class pen rep trt;
model yg= trt/ddfm=kenwardroger;
random rep;
repeated /subject=pen(trt) type=ar(1) r rcorr;
lsmeans  trt/pdiff;
contrast 'trt 1 vs trt 2' trt 1 -1 0;
contrast 'trt 1 vs trt 3' trt 1 0 -1;
contrast 'trt 2 vs trt 3' trt 0 1 -1;
contrast 'trt 1 2 vs trt 3' trt -1 -1 2;
run;
proc mixed scoring=2;
```

```
class pen rep trt;
model hcw= trt/ddfm=kenwardroger;
random rep;
repeated /subject=pen(trt) type=ar(1) r rcorr;
lsmeans  trt/pdiff;
contrast 'trt 1 vs trt 2' trt 1 -1 0;
contrast 'trt 1 vs trt 3' trt 1 0 -1;
contrast 'trt 2 vs trt 3' trt 0 1 -1;
contrast 'trt 1 2 vs trt 3' trt -1 -1 2;
run;
proc mixed scoring=2;
class pen rep trt;
model kph= trt/ddfm=kenwardroger;
random rep;
repeated /subject=pen(trt) type=ar(1) r rcorr;
lsmeans  trt/pdiff;
contrast 'trt 1 vs trt 2' trt 1 -1 0;
contrast 'trt 1 vs trt 3' trt 1 0 -1;
contrast 'trt 2 vs trt 3' trt 0 1 -1;
contrast 'trt 1 2 vs trt 3' trt -1 -1 2;
run;
proc mixed scoring=2;
class pen rep trt;
model ms= trt/ddfm=kenwardroger;
random rep;
repeated /subject=pen(trt) type=ar(1) r rcorr;
lsmeans  trt/pdiff;
contrast 'trt 1 vs trt 2' trt 1 -1 0;
contrast 'trt 1 vs trt 3' trt 1 0 -1;
contrast 'trt 2 vs trt 3' trt 0 1 -1;
contrast 'trt 1 2 vs trt 3' trt -1 -1 2;
run;
proc mixed scoring=2;
class pen rep trt ;
model dp= trt/ddfm=kenwardroger;
random rep;
repeated /subject=pen(trt) type=ar(1) r rcorr;
lsmeans  trt/pdiff;
contrast 'trt 1 vs trt 2' trt 1 -1 0;
contrast 'trt 1 vs trt 3' trt 1 0 -1;
contrast 'trt 2 vs trt 3' trt 0 1 -1;
contrast 'trt 1 2 vs trt 3' trt -1 -1 2;
run;
proc mixed scoring=2;
class pen rep trt;
model qg= trt/ddfm=kenwardroger;
random rep;
repeated /subject=pen(trt) type=ar(1) r rcorr;
lsmeans  trt/pdiff;
contrast 'trt 1 vs trt 2' trt 1 -1 0;
contrast 'trt 1 vs trt 3' trt 1 0 -1;
contrast 'trt 2 vs trt 3' trt 0 1 -1;
contrast 'trt 1 2 vs trt 3' trt -1 -1 2;
run;
```

```
options ls=100 ps=150;
data carcass;
input pen trt rep et ft rea if yg hcw kph ms dp qg;
cards;
;
proc sort;
by pen;
proc mixed scoring=2;
class pen rep trt;
model ft=trt/ddfm=kenwardroger;
random rep;
repeated /subject=pen(trt) type=ar(1) r rcorr;
lsmeans  trt/pdiff;
contrast 'trt 3 vs trt 4' trt 1 -1 0;
contrast 'trt 3 vs trt 5' trt 1 0 -1;
contrast 'trt 4 vs trt 5' trt 0 1 -1;
contrast 'linear' trt -0.663392 -0.080533 0.7439255;
run;
proc mixed scoring=2;
class pen rep trt;
model rea=trt/ddfm=kenwardroger;
random rep;
repeated /subject=pen(trt) type=ar(1) r rcorr;
lsmeans  trt/pdiff;
contrast 'trt 3 vs trt 4' trt 1 -1 0;
contrast 'trt 3 vs trt 5' trt 1 0 -1;
contrast 'trt 4 vs trt 5' trt 0 1 -1;
contrast 'linear' trt -0.663392 -0.080533 0.7439255;
run;
 proc mixed scoring=2;
class pen rep trt;
model if= trt/ddfm=kenwardroger;
random rep;
repeated /subject=pen(trt) type=ar(1) r rcorr;
lsmeans  trt/pdiff;
contrast 'trt 3 vs trt 4' trt 1 -1 0;
contrast 'trt 3 vs trt 5' trt 1 0 -1;
contrast 'trt 4 vs trt 5' trt 0 1 -1;
contrast 'linear' trt -0.663392 -0.080533 0.7439255;
run;
proc mixed scoring=2;
class pen rep trt;
model yg= trt/ddfm=kenwardroger;
random rep;
repeated /subject=pen(trt) type=ar(1) r rcorr;
lsmeans  trt/pdiff;
contrast 'trt 3 vs trt 4' trt 1 -1 0;
contrast 'trt 3 vs trt 5' trt 1 0 -1;
contrast 'trt 4 vs trt 5' trt 0 1 -1;
contrast 'linear' trt -0.663392 -0.080533 0.7439255;
run;
proc mixed scoring=2;
class pen rep trt;
model hcw= trt/ddfm=kenwardroger;
random rep;
```

```
repeated /subject=pen(trt) type=ar(1) r rcorr;
lsmeans  trt/pdiff;
contrast 'trt 3 vs trt 4' trt 1 -1 0;
contrast 'trt 3 vs trt 5' trt 1 0 -1;
contrast 'trt 4 vs trt 5' trt 0 1 -1;
contrast 'linear' trt -0.663392 -0.080533 0.7439255;
run;
proc mixed scoring=2;
class pen rep trt;
model kph= trt/ddfm=kenwardroger;
random rep;
repeated /subject=pen(trt) type=ar(1) r rcorr;
lsmeans  trt/pdiff;
contrast 'trt 3 vs trt 4' trt 1 -1 0;
contrast 'trt 3 vs trt 5' trt 1 0 -1;
contrast 'trt 4 vs trt 5' trt 0 1 -1;
contrast 'linear' trt -0.663392 -0.080533 0.7439255;
run;
proc mixed scoring=2;
class pen rep trt;
model ms= trt/ddfm=kenwardroger;
random rep;
repeated /subject=pen(trt) type=ar(1) r rcorr;
lsmeans  trt/pdiff;
contrast 'trt 3 vs trt 4' trt 1 -1 0;
contrast 'trt 3 vs trt 5' trt 1 0 -1;
contrast 'trt 4 vs trt 5' trt 0 1 -1;
contrast 'linear' trt -0.663392 -0.080533 0.7439255;
run;
proc mixed scoring=2;
class pen rep trt ;
model dp= trt/ddfm=kenwardroger;
random rep;
repeated /subject=pen(trt) type=ar(1) r rcorr;
lsmeans  trt/pdiff;
contrast 'trt 3 vs trt 4' trt 1 -1 0;
contrast 'trt 3 vs trt 5' trt 1 0 -1;
contrast 'trt 4 vs trt 5' trt 0 1 -1;
contrast 'linear' trt -0.663392 -0.080533 0.7439255;
run;
proc mixed scoring=2;
class pen rep trt;
model qg= trt/ddfm=kenwardroger;
random rep;
repeated /subject=pen(trt) type=ar(1) r rcorr;
lsmeans  trt/pdiff;
contrast 'trt 3 vs trt 4' trt 1 -1 0;
contrast 'trt 3 vs trt 5' trt 1 0 -1;
contrast 'trt 4 vs trt 5' trt 0 1 -1;
contrast 'linear' trt -0.663392 -0.080533 0.7439255;
run;
```

```
options ls=100 ps=150;
data carcass categ;
other=telang+contaminated;
input pen str trt rep  premchoice choice pc select standard liver telang
contaminated denom;
other=telang+contaminated;
cards;
;
proc print;
run;
proc sort; by trt rep pen;
proc means noprint sum;
by trt rep pen;
var pc select standard liver other denom;
output out=carcass sum=pc select standard liver other denom;
proc print;
proc glimmix data=carcass;
class rep trt;
model pc/denom=trt/s ddfm=kr error=binomial link=logit;
random rep;
lsmeans trt/cl pdiff ilink;
contrast 'trt 1 vs trt 2' trt 1 -1 0;
contrast 'trt 1 vs trt 3' trt 1 0 -1;
contrast 'trt 2 vs trt 3' trt 0 1 -1;
contrast 'trt 1 2 vs trt 3' trt -1 -1 2;
proc glimmix data=carcass;
class rep trt;
model select/denom=trt/s ddfm=kr error=binomial link=logit;
random rep;
lsmeans trt/cl pdiff ilink;
contrast 'trt 1 vs trt 2' trt 1 -1 0;
contrast 'trt 1 vs trt 3' trt 1 0 -1;
contrast 'trt 2 vs trt 3' trt 0 1 -1;
contrast 'trt 1 2 vs trt 3' trt -1 -1 2;
proc glimmix data=carcass;
class rep trt;
model standard/denom=trt/s ddfm=kr error=binomial link=logit;
random rep;
lsmeans trt/cl pdiff ilink;
contrast 'trt 1 vs trt 2' trt 1 -1 0;
contrast 'trt 1 vs trt 3' trt 1 0 -1;
contrast 'trt 2 vs trt 3' trt 0 1 -1;
contrast 'trt 1 2 vs trt 3' trt -1 -1 2;
proc glimmix data=carcass;
class rep trt;
model liver/denom=trt/s ddfm=kr error=binomial link=logit;
random rep;
lsmeans trt/cl pdiff ilink;
contrast 'trt 1 vs trt 2' trt 1 -1 0;
contrast 'trt 1 vs trt 3' trt 1 0 -1;
contrast 'trt 2 vs trt 3' trt 0 1 -1;
contrast 'trt 1 2 vs trt 3' trt -1 -1 2;
proc glimmix data=carcass;
class rep trt;
model other/denom=trt/s ddfm=kr error=binomial link=logit;
```

```
random rep;
lsmeans trt/cl pdiff ilink;
contrast 'trt 1 vs trt 2' trt 1 -1 0;
contrast 'trt 1 vs trt 3' trt 1 0 -1;
contrast 'trt 2 vs trt 3' trt 0 1 -1;
contrast 'trt 1 2 vs trt 3' trt -1 -1 2;
run;


options ls=100 ps=150;
data yg categ;
input pen eartag trt rep ygoneortwo ygthree ygfourorfive denom;
cards;
;
proc sort; by trt rep pen;
proc means noprint sum;
by trt rep pen;
var ygoneortwo ygthree ygfourorfive denom;
output out=carcassind sum=ygoneortwo ygthree ygfourorfive denom;
proc print;
proc glimmix data=carcassind;
class rep trt;
model ygoneortwo/denom=trt/s ddfm=kr error=binomial link=logit;
random rep;
lsmeans trt/cl pdiff ilink;
contrast 'trt 1 vs trt 2' trt 1 -1 0;
contrast 'trt 1 vs trt 3' trt 1 0 -1;
contrast 'trt 2 vs trt 3' trt 0 1 -1;
contrast 'trt 1 2 vs trt 3' trt -1 -1 2;
proc glimmix data=carcassind;
class rep trt;
model ygthree/denom=trt/s ddfm=kr error=binomial link=logit;
random rep;
lsmeans trt/cl pdiff ilink;
contrast 'trt 1 vs trt 2' trt 1 -1 0;
contrast 'trt 1 vs trt 3' trt 1 0 -1;
contrast 'trt 2 vs trt 3' trt 0 1 -1;
contrast 'trt 1 2 vs trt 3' trt -1 -1 2;
proc glimmix data=carcassind;
class rep trt;
model ygfourorfive/denom=trt/s ddfm=kr error=binomial link=logit;
random rep;
lsmeans trt/cl pdiff ilink;
contrast 'trt 1 vs trt 2' trt 1 -1 0;
contrast 'trt 1 vs trt 3' trt 1 0 -1;
contrast 'trt 2 vs trt 3' trt 0 1 -1;
contrast 'trt 1 2 vs trt 3' trt -1 -1 2;
run;


options ls=100 ps=150;
data yg categ;
input pen eartag trt rep ygoneortwo ygthree ygfourorfive denom;
cards;
;
proc sort; by trt rep pen;
proc means noprint sum;
```

```
by trt rep pen;
var ygoneortwo ygthree ygfourorfive denom;
output out=carcassind sum=ygoneortwo ygthree ygfourorfive denom;
proc print;
proc glimmix data=carcassind;
class rep trt;
model ygoneortwo/denom=trt/s ddfm=kr error=binomial link=logit;
random rep;
lsmeans trt/cl pdiff ilink;
contrast 'trt 3 vs trt 4' trt 1 -1 0;
contrast 'trt 3 vs trt 5' trt 1 0 -1;
contrast 'trt 4 vs trt 5' trt 0 1 -1;
contrast 'linear' trt -0.663392 -0.080533 0.7439255;
proc glimmix data=carcassind;
class rep trt;
model ygthree/denom=trt/s ddfm=kr error=binomial link=logit;
random rep;
lsmeans trt/cl pdiff ilink;
contrast 'trt 3 vs trt 4' trt 1 -1 0;
contrast 'trt 3 vs trt 5' trt 1 0 -1;
contrast 'trt 4 vs trt 5' trt 0 1 -1;
contrast 'linear' trt -0.663392 -0.080533 0.7439255;
proc glimmix data=carcassind;
class rep trt;
model ygfourorfive/denom=trt/s ddfm=kr error=binomial link=logit;
random rep;
lsmeans trt/cl pdiff ilink;
contrast 'trt 3 vs trt 4' trt 1 -1 0;
contrast 'trt 3 vs trt 5' trt 1 0 -1;
contrast 'trt 4 vs trt 5' trt 0 1 -1;
contrast 'linear' trt -0.663392 -0.080533 0.7439255;
run;
```

SAS code used to analyze Liver Biopsy data.

```
options ls=100 ps=150;
data liver;
input Pen str TRT REP AvgZn1 AvgZn2 RationZn DMI;
Znintake= RationZn*DMI;
cards;
;
proc print;
proc sort;
by pen;
proc mixed scoring=2;
class pen rep trt;
model AvgZn1=trt/ddfm=kenwardroger;
random rep;
repeated /subject=pen(trt) type=ar(1) r rcorr;
lsmeans  trt/pdiff;
contrast 'trt 1 vs trt 2' trt 1 -1 0;
contrast 'trt 1 vs trt 3' trt 1 0 -1;
contrast 'trt 2 vs trt 3' trt 0 1 -1;
contrast 'trt 1 2 vs trt 3' trt -1 -1 2;
run;
```

```
options ls=100 ps=150;
data liver;
input Pen str TRT REP AvgZn1 AvgZn2 RationZn DMI;
Znintake= RationZn*DMI;
cards;
;
proc print;
proc sort;
by pen;
proc mixed scoring=2;
class pen rep trt;
model AvgZn1=trt/ddfm=kenwardroger;
random rep;
repeated /subject=pen(trt) type=ar(1) r rcorr;
lsmeans  trt/pdiff;
contrast 'trt 3 vs trt 4' trt 1 -1 0;
contrast 'trt 3 vs trt 5' trt 1 0 -1;
contrast 'trt 4 vs trt 5' trt 0 1 -1;
contrast 'linear' trt -0.663392 -0.080533 0.7439255;
run;
```

SAS code used to analyze Alkaline Phosphatase data.

```
options ls=100 ps=150;
data AlkalineP;
input pen str trt rep AlkP1 Unitsml;
cards;
;
proc print;
proc sort;
by pen;
proc mixed scoring=2;
class pen rep trt;
model AlkP1=trt/ddfm=kenwardroger;
random rep;
repeated /subject=pen(trt) type=ar(1) r rcorr;
lsmeans  trt/pdiff;
contrast 'trt 1 vs trt 2' trt 1 -1 0;
contrast 'trt 1 vs trt 3' trt 1 0 -1;
contrast 'trt 2 vs trt 3' trt 0 1 -1;
contrast 'trt 1 2 vs trt 3' trt -1 -1 2;
run;
```

```
options ls=100 ps=150;
data AlkalineP;
input pen str trt rep AlkP1 Unitsml;
cards;
;
proc print;
proc sort;
by pen;
proc mixed scoring=2;
class pen rep trt;
model AlkP1=trt/ddfm=kenwardroger;
random rep;
repeated /subject=pen(trt) type=ar(1) r rcorr;
lsmeans  trt/pdiff;
contrast 'trt 3 vs trt 4' trt 1 -1 0;
contrast 'trt 3 vs trt 5' trt 1 0 -1;
contrast 'trt 4 vs trt 5' trt 0 1 -1;
contrast 'linear' trt -0.753846 0.1052858 0.6485605;
run;
```

SAS code used to analyze Plasma Zn data.

```
options ls=100 ps=150;
data plasma;
input Pen str TRT REP AvgCu AvgFe AvgZn1 RationZn DMI;
Znintake= RationZn*DMI;
cards;
;
proc print;
proc sort;
by pen;
proc mixed scoring=2;
class pen rep trt;
model AvgZn1=trt/ddfm=kenwardroger;
random rep;
repeated /subject=pen(trt) type=ar(1) r rcorr;
lsmeans  trt/pdiff;
contrast 'trt 1 vs trt 2' trt 1 -1 0;
contrast 'trt 1 vs trt 3' trt 1 0 -1;
contrast 'trt 2 vs trt 3' trt 0 1 -1;
contrast 'trt 1 2 vs trt 3' trt -1 -1 2;
run;


options ls=100 ps=150;
data plasma;
input Pen str TRT REP AvgCu AvgFe AvgZn1 RationZn DMI;
Znintake= RationZn*DMI;
cards;
;
proc print;
proc sort;
by pen;
proc mixed scoring=2;
class pen rep trt;
model AvgZn1=trt/ddfm=kenwardroger;
random rep;
repeated /subject=pen(trt) type=ar(1) r rcorr;
lsmeans  trt/pdiff;
contrast 'trt 3 vs trt 4' trt 1 -1 0;
contrast 'trt 3 vs trt 5' trt 1 0 -1;
contrast 'trt 4 vs trt 5' trt 0 1 -1;
contrast 'linear' trt -0.663392 -0.080533 0.7439255;
run;
```