THESIS

INFLUENCE OF TRACE MINERAL CONCENTRATION AND SOURCE ON YEARLING FEEDLOT STEER PERFORMANCE, CARCASS CHARACTERISTICS, AND TRACE MINERAL STATUS

Submitted by

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ABSTRACT

INFLUENCE OF TRACE MINERAL CONCENTRATION AND SOURCE ON YEARLING FEEDLOT STEER PERFORMANCE, CARCASS CHARACTERISTICS, AND TRACE MINERAL STATUS

Effects of trace mineral (**TM**) concentration and source on yearling feedlot steer performance, carcass characteristics, and liver TM status, were determined utilizing 360 crossbred steers (initial BW=350 ± 4.0 kg). Steers were blocked by initial BW and randomly assigned to one of 4 treatments (10pens/treatment; 9 hd/pen). Treatments consisted of: 1) negative control (**NC**), no supplemental TM (basal diet contained 7.65 mg Cu/kg DM, 50.5 mg Zn/kg DM, 27.7 mg Mn/kg DM, and 0.12 mg Co/kg DM); 2) basal diet supplemented with 10 mg Cu/kg DM from $CuSO_4$, 30 mg Zn/kg DM from $ZnSO_4$, 20 mg Mn/kg DM from $MnSO_4$, 0.50 mg I/kg DM from EDDI, 0.10 mg Se/kg DM from Na_2O_3Se, and 0.10 mg Co/kg DM from $CoCO_3$ (**NRC**); 3) basal diet supplemented with inorganic forms of Cu, Zn, Mn, EDDI, Se and Co at consulting nutritionist recommendations (**CNI**, 20, 100, 50, 0.50, 0.20, and 0.20 mg of mineral/kg DM, respectively); and 4) basal diet supplemented with 66.6% inorganic and 33.4% organic Cu, Zn, Mn and Co, and inorganic forms of I and Se at iso-concentration to consulting nutritionist recommendations of treatment 3 (**CNO**). All steers were fed a high concentrate, steam-flaked, corn-based diet for 154 d. Steers were individually weighed on d -1, 0, 35, 121, 153, and 154. Continuous data were analyzed on a pen mean basis using a mixed model appropriate for a randomized block design (fixed effects = treatment and time; random effect = replicate). Categorical data were analyzed utilizing GLIMMIX (fixed effect = treatment; random effect = replicate). Initial and final BW, ADG, DMI, F:G and G:F ratios and calculated net

energy recoveries were similar ($P > 0.23$) across treatments. Subcutaneous adipose tissue depth, HCW, KPH, yield grade, marbling score, and quality grade were similar across treatments ($P > 0.17$). Final liver Zn, Mn, Se, and Co concentrations were similar across treatments ($P > 0.37$). Under the conditions of this experiment, it appears that basal dietary concentrations of Cu, Zn, Mn, and Co were adequate for growth and performance of finishing yearling feedlot steers. For experiment 2, an *in vitro* analysis was utilized to determine "releasability" of trace minerals from the basal diets fed in experiment 1. Three tubes for each treatment ration as listed for experiment 1 were incubated for 0, 6, 12, and 24 h in a 3:1 ratio of modified McDougall (1984) buffer and rumen fluid taken from steers fed high a concentrate finishing diet. Dry matter disappearance percentage was similar across treatments ($P > 0.49$). Percent Cu released was significantly different ($P > 0.02$) for treatment where NC was less than Suppl, and percent Zn released was also significant ($P > 0.0004$) for treatment NC was also lower than Suppl. From the simulated conditions of the second experiment, it appears there are differences in the ration "releaseabilities" of Cu and Zn from the simulated abomasal and ruminal conditions from treatment diets.

Key words: Beef cattle, feedlot, *in vitro*, trace mineral

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DEDICATION

This thesis is dedicated to Wildman, you have always been there, whenever and wherever I have gone for various jobs and schools. You always seemed to understand throughout the whole journey and never failed when I needed your help. Thanks for the ride, Wildman.

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CHAPTER I

REVIEW OF LITERATURE

Minerals can be divided into two main categories: 1) macro-minerals and 2) micro or trace minerals. In general, macro minerals are required at concentrations greater than 100 mg of mineral/kg of the diet and are often expressed as a percentage, while trace minerals are required at concentrations much lower than 100 mg/kg (McDowell, 1992, NRC 2000). Macro minerals such as phosphorus, potassium, sulfur, magnesium, sodium, and chloride and trace minerals such as copper, zinc, iodine, manganese, selenium, cobalt, and iron are considered essential for beef cattle (NRC, 2000). If not supplied in correct amounts or ratios in the diet, specific metabolic diseases and/or toxicities can be produced. Figure 1 below (Spears, 2008) illustrates the importance of proper trace mineral intake as it relates to animal performance.

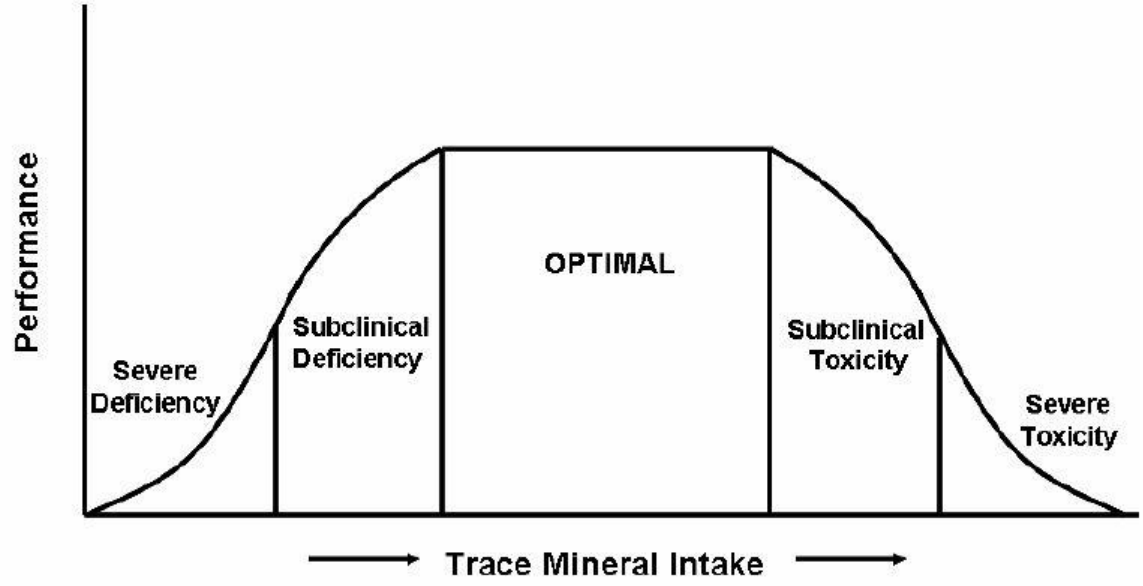

Figure 1. Animal performance as affected by trace mineral intake (Spears, 2008).

Improper management of trace minerals can lead to imbalances, deficiencies, toxicities and overall decreased production efficiency. Ultimately, this literature review will explore the general description and characteristics, metabolism, physiological function, deficiency and toxicity of copper, cobalt, zinc, manganese, selenium, and iodine, along with discussion of inorganic and organic forms of trace minerals.

COPPER:

Copper (Cu) has an atomic number of 29, an atomic weight of 63.55, a melting point of 1,083.4^{0}C, a boiling point of 2567^{0}C, and is insoluble in water (Lide, 1993; McDowell, 1992). Copper was first reported to be biologically relevant when Hart et al. (1928) discovered that Cu played a role in the creation of hemoglobin in mammals. Prior to this finding, there was awareness that Cu existed in plant and animal material but the primary function or functions of Cu had yet to be determined. It is now known that Cu is crucial for the proper function of multiple metalloenzymes involved in numerous metabolic processes, vital for proper growth and the prevention of a variety of clinical and pathological disorders in animals (Underwood and Suttle, 1999). For ruminants, Cu was discovered to be essential in 1931 when a Cu deficiency in grazing cattle was reported in Florida (Becker et al., 1965).

Metabolism:

The absorption and retention of Cu is greatly influenced by the different chemical forms in which the mineral is consumed, by dietary concentration of known Cu antagonists (i.e. sulfur, Mo, Fe, and Zn), and by the pH of the gastrointestinal tract (Underwood, 1971). The absorption of Cu can take place in virtually all sections of the gastrointestinal tract (McDowell, 1992). However, the majority of Cu absorption takes place in the first portion of the small intestine. This process of Cu absorption and movement into the blood is illustrated in Figure 2 by

Kozlowski et al. (2009) shown below.

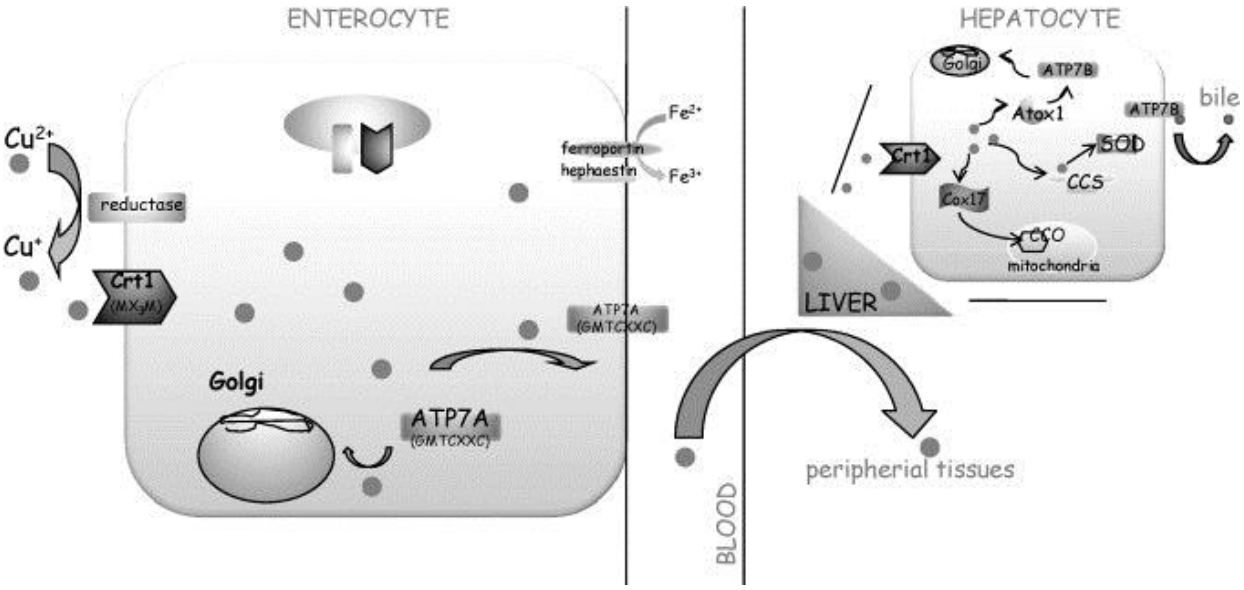

Figure 2. Copper absorption and movement into the blood (Kozlowski et al. 2009).

The process of Cu absorption can take place via active (saturable) or passive transport (unsaturable), involving two steps, 1) uptake by the mucosal cells and 2) transcellular transport as noted by Bronner and Yost (1985) in rats. Transport of absorbed Cu in the circulatory system is facilitated by Cu binding to serum albumin and free amino acids where it is transported to the liver. By way of a two-step process, Cu is taken up by the liver; first associating to glutathione then being transferred to metallothionein prior to being separated for biliary secretion, synthesis of ceruloplasmin and/or storage (Bremner, 1993). Ceruloplasmin is the main transporter for tissue-specific dispersion of Cu from the liver to preferred organs. The liver is essential for Cu metabolism, and is a good indicator of intake and an animal's Cu status (McDowell, 1992). When Cu is ingested in excessive concentrations the unabsorbed portions are excreted via the bile and through the feces. Alternatively, small amounts of Cu can be excreted via urine in variable amounts (Underwood, 1971). Endogenous losses of Cu include milk secretion, sloughing of enterocytes, and perspiration (McDowell, 1992).

Copper deficiency in mammals can influence several metabolic processes including lipid metabolism, hemoglobin synthesis, reproduction, growth, cardiovascular function, hair pigmentation and bone metabolism (McDowell, 1992; Underwood, 1971). The first sign of Cu deficiency is a slow deprivation of Cu stores in the body and involves a decrease in Cu concentration in the blood plasma, eventually falling below the concentration required to sustain a normal rate of hematopoiesis (Underwood, 1971). On the other hand, an animal suffering from Cu toxicity can experience various symptoms such as increased salivation, abdominal pain, nausea, convulsions, paralysis, collapse, and in some circumstances, death (McDowell, 1992). The recommended concentration for Cu in beef cattle diets has been established at 10 mg Cu/kg DM. This is considered sufficient if concentrations of Mo and S are not excessive. The maximum concentration of Cu in diets for beef cattle has been set at 40 mg Cu/kg DM. Cattle can tolerate higher Cu concentrations for a couple weeks or even months (NRC, 2005). However, feeding Cu in concentrations slightly less than 40 mg Cu/kg over extended periods could result in Cu toxicosis (Bradley, 1993). High concentrations of dietary Zn can protect against Cu toxicity. High concentrations of Zn will promote high concentrations of metallothionein found in the intestinal mucosa which is then bound to Cu more intensely than it is to Zn. Beyond absorption, it is unclear if high concentrations of dietary Zn interfere with Cu metabolism; however, it is known that Cu metalloenzymes are inhibited by excessive Zn (Ammerman et al., 1995).

Physiological functions of copper:

In addition to other minerals, Cu is essential for the creation of hemoglobin. Hemoglobin does not specifically contain Cu; however, ceruloplasmin is a Cu dependent enzyme responsible for the incorporation of Fe into hemoglobin. Copper is also a necessary component for the cross-

linking of connective tissue in collagen and elastin via the Cu dependent enzyme lysyl oxidase. This enzyme assists with the addition of a hydroxyl group to lysine therefore permitting cross-links between collagen fibers (McDowell, 1992). Copper also plays a role in pigmentation. A lack of pigmentation in animals is usually credited with an absence of tyrosinase. A possible explanation is the change from tyrosine to melanin; this reaction is catalyzed by polyphenyl oxidases that contain Cu (Underwood, 1971).

In areas such as Western Australia, Allcroft and Parker (1949) reported decreased fertility in cows as a result of Cu deficiency, and it is common to observe cows with depressed or deferred estrus in those areas (Underwood, 1971). Copper also functions as an integral part of copper-zinc superoxide dismutase which acts as an antioxidant to protect cells from oxidative stress. Furthermore, the same enzyme has been reported to assist with immunity (O'Dell et al., 1979). A more recent function of Cu was described by Lei (1991) where Cu deficient rats experienced alterations in lipid and lipoprotein metabolism.

COBALT:

Cobalt (Co) has the atomic number of 27 and its estimated atomic weight is 58.93. Cobalt has a melting point of 1495^0C and a boiling point of 2879^0C (Lide, 1993). Cobalt was first found in plant tissues in the 1800s but was later confirmed in animal tissues in the early 1900s. Underwood and Filmer (1935) in Australia were the first to report that Co was a necessary nutrient for ruminants. Furthermore, in 1937 in Florida, the possibility of Co deficiency was realized, when cattle were thought to be "salt sick" and Co deficiency was partially liable for the ailment. Researchers soon learned this could be avoided if Co, in addition to Fe and Zn, were sufficiently provided to the animals (Becker et al., 1965).

Metabolism:

In general, the ruminant animal makes poor utilization of dietary Co. However, the microorganisms in the rumen are well adapted to utilizing Co. In the rumen, microorganisms metabolize Co into two forms: an active form (cobalamins) and an additional biologically inactive compound that is very similar to vitamin B_{12} (corrinoids) that the ruminant is unable to utilize or absorb (Gawthorne, 1970). Synthesis of vitamin B_{12} in the rumen responds rapidly to dietary changes in Co; however, the effectiveness of the sequestration of Co into B_{12} decreases as the intake of Co increases (Underwood and Suttle, 1999). As noted earlier, ruminants are unsuccessful at absorbing large quantities of soluble Co from the rumen when compared to the absorption of Co from a non-ruminant digestive tract (Smith and Marston, 1970; McDowell, 1992).

For the ruminant animal, most of the absorption of vitamin B_{12} takes place in the lower section of the small intestine. Significant quantities of vitamin B_{12} in addition to Co are released into the duodenum and reabsorbed again in the ileum. The lack of absorption could be contributed to the quick binding of Co in the rumen by microbes, while still accounting for a small portion of total Co available. The movement of vitamin B_{12} across the intestinal wall involves specific transporter compounds (a combination of intrinsic and nonintrinsic factors), which are capable of binding the vitamin (McDowell, 1992). The primary route for excretion of Co and vitamin B_{12} is in fecal matter, although some amounts are also expelled in the urine (Smith and Marston, 1970). Small amounts of Co can be lost through sweat and hair (McDowell, 1992).

The first signs of a Co deficiency are usually characterized by biochemical changes of Co and vitamin B_{12} in tissues and fluids (Somers and Gawthorne, 1969). At the onset of Co

deprivation in ruminants, the quantity of Co and vitamin B_{12} begin to decrease in ruminal fluid (Underwood and Suttle, 1999). An animal suffering from severe Co deficiency often shows signs of extensive emaciation and intense lethargy, taking the appearance of a starved animal additionally, the mucous membranes are faded and the skin is pale and delicate (Underwood, 1971). According to Filmer (1933), a Co deficient animal experiences a complete lack of body fat, while a fatty liver still exists, the spleen becomes hemosidereized and there is underdeveloped erythrogenic tissue of the bone marrow. Cobalt deficiency has also been reported to decrease the conversion of propionate carbons to glucose (Marston et al., 1961). In general, propionic acid is produced from fermentation of carbohydrates in the rumen. Once absorbed, propionate is converted to methylmalonyl-CoA and then can be converted to succinyl-CoA with the assistance of B_{12}. This allows the carbons from propionate to enter the gluconeogenic pathway in the liver. With Co deficiency, B_{12} concentrations decrease leading to a buildup of methylmalonyl-CoA and decreases in succinyl-CoA, ultimately impairing glucose synthesis. To compensate for the decrease in glucose synthesis, lipolysis of subcutaneous adipose tissue is initiated to supply the liver with carbons to generate reducing equivalents to maintain hepatic ATP concentration (Marston et al., 1972; Gawthorne, 1968). However, since lipids are not gluconeogenic, the liver eventually becomes inundated with lipids and the animal develops fatty liver disease. Figure 3 below illustrates the biochemical changes prior to clinical symptoms for a Co and vitamin B_{12} deficient ruminant leading to MMA buildup, and white liver disease (Underwood and Suttle, 1999).

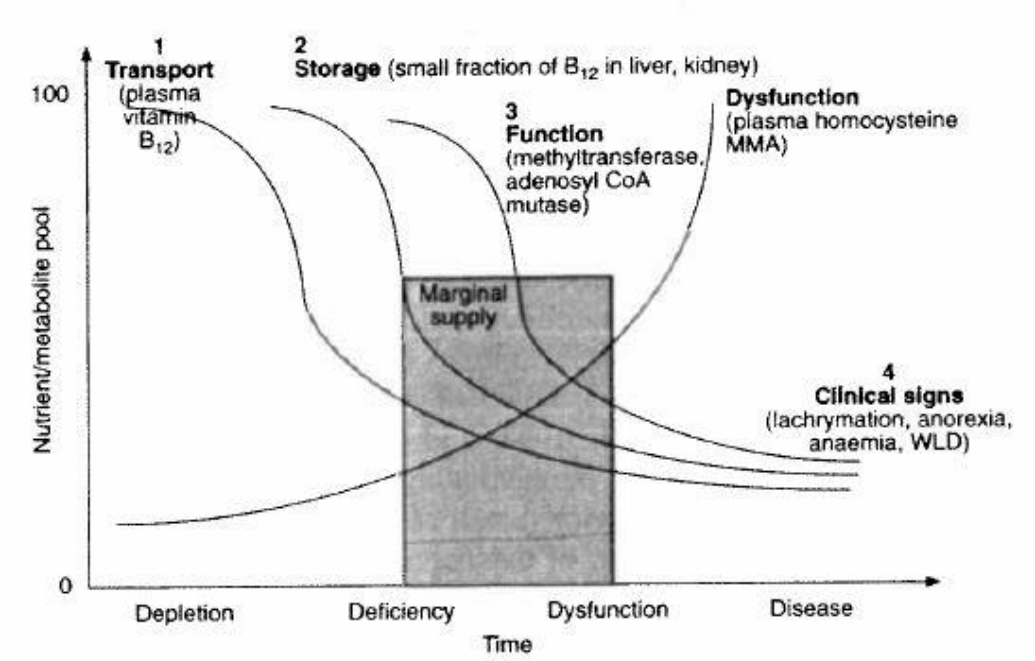

Figure 3. Biochemical changes prior to clinical symptoms for a Co and vitamin B12 deficient ruminant.

Ultimately, the liver disease continues due to mild fatty infiltration of hepatocytes to an extent where bile-ducts greatly multiply, enzymes escape from damaged liver cells and bilirubin concentrations increase (Kennedy et al., 1997). Furthermore, microbial fermentation can be altered leading to reduced propionate production when cattle are fed high concentrate finishing diets (Tiffany and Spears, 2005).

Cobalt toxicity is rare as cattle can handle up to 100 times the dietary recommendation. It would take extreme concentrations of Co to impair liver function; absorption of Co is reduced by accelerated requisition of Co by rumen microorganisms (McDowell, 1992). The efficiency of conversion from Co to vitamin B_{12} is directly correlated with Co intake. Furthermore, when adequate amounts of Co were fed to sheep, about 3% was sufficiently converted to B_{12} in comparison to 13±5% when diets were deficient in Co (Smith and Marston, 1970). Signs of toxicity are similar to a deficient animal such as suppressed appetite, a reduction in body weight, anemia, and an increase in hemoglobin content in erythrocytes (NRC, 1980). The toxicity

caused by excessive ingestion of Co seems to result from a mineral antagonism, where the reduction in red blood cell numbers is caused by a depression in Fe absorption due to excess dietary Co (McDowell, 1992). The interaction between Co and Fe is mutually antagonistic, suggesting that Co and Fe share, at least a portion of, the same absorption pathway (Thomson et al., 1971) More times than not, Co toxicity is the consequence of an incorrectly formulated trace mineral mix (McDowell, 1992).

Physiological functions of cobalt:

The primary function of Co is its contribution to vitamin B_{12} structure. This component is also known as a cobalamin and is usually found in two active forms: adenosylcobalamin (coenzyme for methlytransferase) and methylcobalamin (coenzyme for mutase) as reviewed by McDowell, 1992. Methylcobalamin is central in transporting methyl groups from one molecule to another. In addition, methyltransferase enzymes also donate methyl groups (Underwood and Suttle, 1999). For ruminants, methylcobalamin is essential for microorganisms and is required for methane, acetate, and methionine production by the rumen bacteria (Poston and Stadman, 1975). Adenosylcobalamin is crucial, as it assists in energy metabolism by enabling the development of glucose as it helps methylmalonyl-coenzyme A (CoA) mutase to yield succinate from propionate, mainly in the liver (Underwood and Suttle, 1999). In the rumen, the microorganisms also depend on this same reaction in reverse, where propionate production from succinate proceeds by the same mutase (Babior, 1975).

McDowell (1989) indicates there are four primary functions of cobalamin, one is discussed above. The other three include: cobalamin enzymes that participate in purine pyrimidine synthesis, the synthesis of proteins from amino acids, and carbohydrate and fat

metabolism. Cobalamin has also been reported to be involved in the maintenance of the nervous system and red blood cell synthesis (McDowell, 1992).

ZINC:

Zinc (Zn) has an atomic number 30, an atomic weight of 65.38, a melting point of 419.58^0C and a boiling point of 907^0C (Lide, 1993). Zinc has been known to be required by mammals for a little over a century in addition to requirements by higher plants (Underwood and Suttle, 1999). Some of the first evidence that Zn was needed for growth and fitness of rats and mice was completed by Todd et al. (1934), leading to additional experiments on pigs, calves, lambs, and poultry. In 1955, the dietary interest in Zn increased when the element was found to be deficient in swine diets. The timetable continued when it was determined there were nutritional inadequacies in poultry in 1958, followed by cattle in 1960, and finally humans in 1961 which led to increased research into this essential element (McDowell, 1992).

Metabolism:

Absorption of elemental Zn primarily takes place in the small intestine. The mechanisms by which absorption take place are not fully understood. The quantity and chemical form of Zn can affect absorption along with the concentration of other elements and dietary constituents (Underwood, 1971). It has also been reported that more Zn absorption takes place in the rumen rather than the small intestine in sheep (Arora et al., 1969). On the other hand, Cousins (1985) reported that absorption of Zn is restricted to the small intestine with most absorbed in the duodenum in non-ruminant species (Naveh et al., 1988).

Initially, Zn absorption requires Zn transport from the lumen of the intestine into a mucosal cell. A carrier-mediated transport mechanism is responsible for the transfer of Zn through the brush border, that likely includes an interaction with Zn in a chelated composition

(Solomons and Cousins, 1984). The transport of Zn inside the intestinal mucosal cell is controlled by a metal-binding protein known as metallothionein, which is generated in the liver. The synthesis of metallothionein is regulated by dietary Zn and plasma Zn concentrations, thus influencing the amount of Zn that enters the body, as result, it greatly contributes to Zn homeostasis (Cousins, 1978). Many dietary constituents can alter or reduce the absorption of Zn, including phytate, Ca, P, Cu, Cd, and Cr (Miller and Cuhna, 1979). One of the most crucial determinants of Zn absorption is the Zn concentration of the diet (McDowell, 1992). When Zn is consumed or injected the primary route for excretion is through the feces, whereas a smaller amount expelled through the urine (Miller, 1969). Endogenous fecal Zn is consequential of gastrointestinal, pancreatic and biliary discharge (McDowell, 1992).

With Zn deficiency, it is common to see a reduction in feed consumption, growth and feed efficiency in a developing animal, and eventually parakeratosis and loss of hair occur (McDowell, 1992). A Zn deficiency negatively impacts the primary and secondary sex organs in males, in addition, to every segment of the reproductive cycle in the female (Underwood, 1971). Along with deficient animals growing at a reduced rate, feed passes through the digestive tract more slowly. Other deficiency symptoms include decreased ability to fight infection and stress, reduced milk production, and diminished reproductive efficiency (Miller, 1970).

In general, livestock can tolerate high concentrations of dietary Zn. Tolerance level depends on the species, with additional influential factors, such as composition of the diet including the concentrations of Ca, Cu, Fe, and Cd (Underwood and Suttle, 1999). High concentrations of dietary Zn can contribute to a deficiency of other minerals such as Fe and Cu. No detrimental physiological alterations were observed in most studies with supplemental Zn below 600 mg Zn/kg DM. Ultimately, toxicosis is usually observed when diet concentrations

reach 1,000 mg Zn/kg DM greatly exceeding the recommended amount of 30 mg Zn/kg DM (NRC, 1980).

Physiological functions of zinc:

Zinc is involved in enzyme function in several ways: 1) as a part of the structural integrity of the enzyme (metalloenzymes) and 2) as an activator of certain enzymes. Structurally, Zn helps to stabilize the quaternary arrangement of certain enzymes (McDowell, 1992). Also, Zn will bind to certain cysteine and histidine residues of certain proteins ultimately assisting with structural integrity of the enzyme. The Zn metalloenzymes that have been extensively researched are carbonic anhydrase, carboxypeptidase A and similar peptidases, alkaline phosphatase, alcohol dehydrogenase, and cytosolic superoxide dismutase in mammals (McDowell, 2003).

There is also a fair amount of Zn found in the blood and it is also present in plasma in addition to erythrocytes, leukocytes, and platelets. Modified concentrations of Zn in the blood are often a result of dietary intake variance (Underwood, 1971). Zinc is involved in hormone metabolism as well, ranging from formation, repository, and secretion of certain hormones. Furthermore, Zn assists with the effectiveness of receptor sites and end-organ sensitivity. Some noteworthy effects of Zn deficiency on the creation and secretion of hormones involve testosterone, insulin and adrenal corticosteroids (McDowell, 2003).

Zinc is essential for the maintenance of growth rate. As a result, growth retardation is common in Zn deficient animals along with diminishing amino acid utilization or protein creation. One of the first signs of a Zn deficiency is a reduction in appetite (McDowell, 2003). Zinc is critical in maintaining the immune system. In monogastrics, lack of Zn led to diminished thymus weight and circulating lymphocyte concentrations. Disturbed electrolyte equilibrium has

also been established in animals suffering from Zn deficiency. Similarly, Zn aids in maintaining normal concentrations of vitamin A. It has been determined that a Zn metalloenzyme "alcohol dehydrogenase" is responsible for the transformation of the vitamin A alcohol (retinol) to vitamin A aldehyde (retinal), a practice crucial for normal vision (McDowell, 1992). Motor development, neuropsychological behavior, and attention activity can be different in the presence of a Zn inadequacy therefore affecting cognitive maturation. Zinc plays a role in skin maintenance as evidenced by parakeratotic lesions observed with Zn deficiency (McDowell, 2003). Finally, Zn plays an indispensable role in digestion, glycolysis, DNA production, nucleic acid synthesis, and protein uptake (Underwood and Suttle, 1999).

MANGANESE:

Manganese has an atomic number of 25, an estimated atomic weight of 54.94, a melting point of 1244 ± 3^0C, and a boiling point of 1962^0C (Lide, 1993). Manganese is necessary for animals. A deficiency is unlikely to occur naturally in diets formulated to contain typical feed ingredients for ruminants, monogastrics, and poultry. In 1931, Mn was determined to be an essential element for growth and reproduction in rats and mice (McDowell, 1992). Interest in researching Mn nutrition was motivated by the findings of Wilgus et al., (1936) indicating that an insufficiency of Mn contributed to a disease that plagued chickens called perosis or slipped tendon. This study helped to create a sufficient base for the fundamental functions of Mn, but it wasn't until 1951 that a deficiency in ruminants was documented (Bentley and Phillips, 1951).

Metabolism:

Manganese absorption, across all animals, is generally low (McDowell, 1992). Of the total concentration of Mn in the diet, cattle absorb 1-4% (Abrams et al., 1977), less than 1% is absorbed by birds (Turk et al., 1982), and rats and humans absorb approximately 3-4% of dietary

Mn (Greenberg and Campbell, 1940; Hurley and Keen, 1987). According to Howes et al., (1973), young calves absorb Mn in greater amounts than older cattle. Manganese seems to be universally absorbed across all sections of the small intestine from a combination of two processes, uptake from the lumen of the gut and transport across the mucosal cells (Keen and Zidenberg-Cherr, 1990). Other elements such as Ca, P, and Fe can influence that rate of absorption (McDowell, 1992). Relative to birds, the composition of Ca can induce a Mn deficiency by decreasing the amount that is soluble in the gut (Davies and Nightingale, 1975). Likewise, Mn competes with Co and Fe for binding sites, as a result, a surplus of dietary Fe or Co could produce a Mn deficiency, and excess Mn or Co could lead to Fe deprivation (McDowell, 1992). While the mechanism is not completely understood, an avian coccidiosis infection in chicks has been reported to enhance Mn utilization. Absorption of Cu, Fe, and Co were additionally increased, where the coccidial infection exasperated toxicities; therefore, dramatically increasing tissue mineral concentrations of the trace minerals when compared to the healthy and uninfected chicks (Southern and Baker, 1983). Once absorbed, Mn is rapidly attached to α_2-macro-globulin before crossing the liver where it is detached (Hurley and Keen, 1987). Homeostasis is originally accomplished by regulating absorption of Mn (Underwood and Suttle, 1999). Most orally consumed Mn is excreted through the feces at a rate of about 95-98%, whereas only 0.1-3% is removed through the urine (Thomas, 1970).

Indicators of Mn deficiency include a wide range of symptoms regarding reduced growth, skeletal irregularities, altered reproduction performance, loss of body control in newborns, and imperfections in fat and carbohydrate metabolism; however, the degree and severity of the symptoms depend on the extent of the deficiency along with the growth period of the animal when the deficiency takes place (McDowell, 1992). It is unlikely that under natural

circumstances a ruminant will experience a Mn deficiency; it is much more common for a ruminant to experience deficiencies of other trace minerals such as Co, Cu, I, Se, or Zn. Typically, forages have more than enough Mn (McDowell, 2003). It is true that Mn deprivation is more common in avian species (Underwood and Suttle, 1999); however, under experimentally induced deficiency in ruminants, reduced skeletal growth and reproductive functions were observed (McDowell, 1992).

Maximum tolerable concentration of Mn for sheep and cattle is set at 1,000 mg Mn/kg DM (NRC, 1980). Manganese was fed to calves at 1,000 mg Mn/kg DM for a period of 100 d with no detrimental effects; concentrations greater than 2,000 mg Mn/kg were needed to negatively impact growth and feed intake (Cunningham et al., 1966). Toxicity from excessive Mn ingestion most likely results from an antagonism with other minerals, most notably, Fe. In addition, hemoglobin concentrations are reduced in animals experiencing Mn toxicity (McDowell, 1992).

Physiological functions of manganese:

A crucial function of Mn is to maintain reproductive performance. One study reported different phases of Mn deficiency in rodent females; first, young are born with ataxia, second, young die quickly after being born, and third, there is a disruption in estrus and limited reproduction all together. Similarly, male rodents deficient in Mn were also sterile (Shils and McCollum, 1943). Ruminant reproduction is also affected by Mn deficiency. In a study by Bentley and Philips (1951), they observed sterility in roughly 10% of cattle herds fed Mn at concentrations of 20 mg/kg or less. Overall, the Mn tissue dispersal studies in ruminants observed that Mn aids in corpus luteum function (Hidiroglou, 1975).

Manganese is required for the generation of the organic matrix in the bone, which is primarily constructed from mucopolysaccharide. Altered mucopolysaccharide creation linked with a Mn deficiency has been associated with the stimulation of glucosyltransferases (Leach, 1971). These enzymes are necessary for the generation of polysaccharide and glycoprotein where Mn is often the most crucial metal ion required for the process (McDowell, 1992).

For years, there has been a known correlation between Mn and choline. Manganese and choline can be utilized to lessen the symptoms of fatty liver in rats, when the condition is caused by a Mn deficiency (McDowell, 1992). Irregular fat deposition and accumulation can be prevented as a result of the lipotropic actions of choline, and easily adaptable methyl groups are supplied by choline for the creation of methionine. The majority of lipids contain choline; however, little information is available on the bioavailability of choline in feed stuffs (NRC, 2000). Conversely, in pigs, Mn deprivation can cause greater fat storage and backfat thickness (McDowell, 1992). For poultry, perosis can be avoided, although both Mn and choline are required. The biosynthesis of choline also includes Mn. Still, the deviations in liver ultrastructure noted by a choline deficiency are comparable to that of a Mn deficiency (Bruni and Hegsted, 1970). Combined deficiencies in Mn and choline can have a negative influence on membrane structural integrity. Additionally, Mn assists with cholesterol biosynthesis (Davis et al., 1990).

The consumption of glucose is negatively modified during a Mn shortage. This has been illustrated in defects of the pancreas where the organs fail to develop properly, indicating that Mn is associated with insulin synthesis or transport (McDowell, 1992). Immunological capacity is influenced by Mn. Irregularities in cell operation and structure are present during a Mn deficiency, specifically concerning the mitochondria (Hurley and Keen, 1987). Manganese plays

a dual role as enzyme initiator and a component of metalloenzymes. Enzymes encompassing Mn involve arginase, pyruvate carboxylase, and Mn-superoxide dismutase (McDowell, 1992).

SELENIUM:

Selenium (Se) has the atomic number of 34, an atomic weight of 78.96, melting point of 217^0C and a boiling point of 684.9 ± 1^0C (Lide, 1993). In the mid-1930s, Se was classified as a lethal mineral in certain feeds due to the observations of animals losing hair, nails and hooves. Presently, Se is known to be essential for laboratory and food animals, in addition to humans. Selenium was originally reported to be cancer causing in laboratory animals; however, it is now thought to mitigate certain cancer threats in humans (McDowell, 1992). The finding that Se has a necessary physiological function in higher animals, regardless of the fact it exists at lower concentrations in plant tissue when compared to other elements, was not made until 1957 (Underwood and Suttle, 1999). Intake of feedstuffs with varying concentrations of Se from toxic (> 5 mg/kg) and to deficient (<0.1 mg/kg) illustrated a problem for animals, specifically grazing animals (McDowell, 1992). Shortly thereafter, it became clear that areas in the world affected by Se deficiency exceeds the number of areas that are affected by Se excess (Underwood, 1971).

Metabolism:

The majority of Se is absorbed in the small intestine, more specifically in the duodenum. Ruminal absorption of Se is poor (Wright and Bell, 1966). Overall, ruminants absorb less Se than monogastrics, where 77% orally dispensed selenite was retained in non-ruminants compared to 29% for ruminants (Wright and Bell, 1966). It has been proposed that Se may be absorbed in lower quantities in ruminants due to selenite being transformed to insoluble compounds in the rumen (McDowell, 1992). Similarly, the degree of Se absorption in the gastrointestinal tract and the magnitude and circulation throughout the body depends on animal type, the chemical

arrangement, and the quantity of Se consumed (Underwood, 1971). Rats absorbed 92, 91, and 81% of selenite, selenomethionine, and selenocystine, respectively, making some Se components highly available from the gastrointestinal tract (Thomson et al., 1975). Until the absorbed Se penetrates tissues, it is transported in the plasma (aided by protein). It is speculated that transport in rats is by way of a selenocysteine-containing plasma protein otherwise known as selenoprotein P (Motsenbocker and Tappel, 1982). When dietary concentrations for Se are sufficient, the kidney contains the highest concentrations, followed by the liver, spleen, and finally the pancreas. The nervous system tissues contain low amounts; conversely, wool and hair will have much higher concentrations. When the dietary intake becomes suppressed the kidney still contains higher Se concentrations than liver; however, once adequate Se intake is restored the liver typically contains greater concentrations than the kidney (Oh et al., 1976).

Selenium is typically stored at higher concentrations in animal tissues when Se is provided in an organic form rather than inorganic Se in the diet (McDowell, 1992). The quantity of Se secured depends on tissue demands, where Se uptake is noticed more in deficient animals rather than animals with excess Se. Additionally, no differences in absorption were distinguished when Se was fed from varying sources at concentrations less than 0.10 mg Se/kg DM (Gary et al., 1973).

A primary sign of Se deficiency, found in recently born ruminants, is white muscle disease (WMD) which includes deterioration of striated muscle. This is usually present in two different patterns; the first present at birth is muscular dystrophy where the young die shortly after birth after a period of abrupt physical overexertion. Second, the young experience delayed white muscle disease which advances after birth. This is usually observed most commonly at 3 to 6 wk of age but may transpire up to 4 mo after parturition for lambs. Conversely, it may

exhibit itself in calves anywhere from 1 to 4 mo of age. The characteristics of WMD commonly include loss of strength, stiffness, and muscle degeneration along with trouble standing (McDowell, 1992).

Calves deficient in Se can also experience higher mortality rates and decreased weaning weights (Spears et al., 1986), a reduction in hemoglobin and the presence of heinz bodies (Morris et al., 1984). Furthermore, they may experience weight loss and diarrhea, otherwise known as unthriftiness (Underwood, 1981). Impaired reproductive performance involving retained placenta was illustrated as a result of Se deficiency in areas of the United States, Brazil, and Scotland (McDowell, 1992).

Toxicity of Se can result from consumption of plants that are naturally abundant in Se (NRC, 2000). Very few elements can be considered toxic when solely absorbed from feedstuffs, other than Se. Two conditions caused by Se toxicity are known as alkali disease and blind staggers, both contributing to large numbers of livestock fatalities. The first, alkali disease, has been associated with grazed forages with concentrations of Se ranging from 5 to 40 mg/kg. The second form, blind staggers, requires significant quantities of Se to be ingested. Severe Se poisoning is usually the result of consumption of Se-accumulator type plants that vary in Se content ranging from 100 to 9,000 mg Se/kg DM (McDowell, 1992). Due to the lack of experimental evidence in ruminants, a maximum tolerable concentration for ruminants seems realistic at values of 4 to 5 mg Se/kg DM (McDowell, 1992). However, the current maximum tolerable concentration for Se has been established at 2 mg/kg for beef cattle (NRC, 1980).

Physiological functions of selenium:

There is a close relationship between Se and vitamin E, aiding in the defense of biological membranes from oxidation. A deficiency in both of these nutrients leads to tissue

failure (McDowell, 1992). Selenium is an important element in the makeup of glutathione peroxidase (GSH-Px) that contains 4 g-atoms Se for every mole of enzyme (Rotruck et al., 1973). The membrane contains a fat-soluble antioxidant, like vitamin E, where Se is a constituent of GSH-Px that eliminates peroxides before cellular membranes are damaged (McDowell, 1992). Selenium has known effects on vitamin E. First, Se helps protect the pancreas facilitating standard lipid digestion. Second, through GSH-Px, Se decreases the proportion of vitamin E necessary to support fat membrane quality. Third, it contributes to the preservation of vitamin E in the plasma, although it is unknown how this occurs. However, vitamin E decreases the metabolic need for Se by an animal by conserving Se in the active form, inhibiting Se losses (Scott et al., 1976). Other functions of Se have been proposed where a selenoprotein of spermatozoa assists with energy production; as an enzyme, it contributes to RNA as Se can be combined into purine or pyrimidine bases; it may ensure a precise function in production of prostaglandin and vital fatty acid digestion; and satisfactory immune acknowledgements in livestock require Se and vitamin E (NRC, 1983). In the chick, mitochondria and microsomes continue to yield antibodies along with additional defense mechanisms and sufficient Se and vitamin E ensure prevention of deficiency along with protection of organelles accountable for averting disease, radiation and added stresses (Scott et al., 1976).

IODINE:

Iodine (I) has the atomic number of 50, and its estimated atomic weight is 126.90. Iodine has a melting point of 113.5^0C and a boiling point of 184.35^0C (Lide, 1993). Iodine is distinctive because it is a component of the hormones thyroxine and triiodothyronine in the thyroid (McDowell, 1992). A specific characteristic of I deficiency is known as an amplified

thyroid gland and is also recognized as 'goiter'. Efforts to solve and manage goiter due to I deficiency or toxicity have dated back hundreds of years; however, it was not until the 19th century where it was revealed that I was sufficient in treating goiter in Europe. Later on, it became apparent that in I deficient animals, thyroid dysfunction was similar to humans and could be treated with thyroid gland extracts. Ultimately, I was revealed to be a regular component in an animal's body with especially high concentrations in the thyroid gland (Harington, 1934).

Metabolism:

Iodine exists mostly as inorganic iodide in common feeds and water, and it typically is absorbed through the gastrointestinal tract and is carried in the circulatory system by an insecure attachment to plasma proteins (McDowell, 1992). Assessment of rate constants indicate I flow from the gastrointestinal tract to central pool (or plasma), was less than half the speed of movement from central pool to the gastrointestinal tract. Because of the larger quantity of I in the gastrointestinal tract rather than the central pool, net absorption of I is possible (Miller et al., 1975). Average I absorbed from the rumen accounts for 70 to 80%, along with 10% absorbed in the omasum (Barua et al., 1964). The absorption that occurs in the rumen may be altered by the chemical form of I. When supplemented with Ca iodate, a more rapid reduction to I resulted in the abomasum when compared to the rumen (Moss and Miller, 1970). Cows tend to secrete less I in milk compared to other animals, in addition, they possess a proficient I recycling system through the gastrointestinal tract allowing for I preservation (Miller et al., 1975). Figure 4 below illustrates the mechanism by which I recycling occurs in ruminants as demonstrated by Miller et al. (1975).

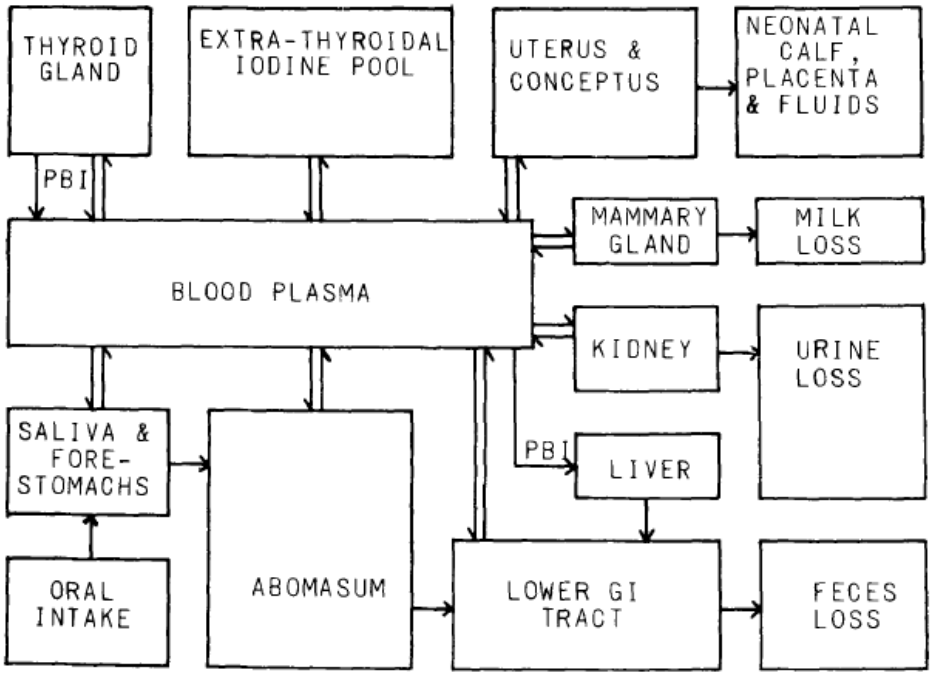

Figure 4. The mechanism by which Iodine recycling occurs in ruminants addapted from J. Dairy Sci. 58:1578–1593.

With specific forms of I, like diiodosalicylic acid (Aschbacher et al., 1966), and milk protein-bound iodine (PBI; Swanson et al., 1965), are likely absorbed in the rumen in arrangements that are different from how I is metabolized. This was illustrated when thyroid uptake was decreased and urinary excretion amplified as a result of the organic forms administered. Metabolism differed for I and milk PBI when only these two forms were positioned in the rumen (Swanson et al., 1965).

A clinical I deficiency is noticeable when goiter is present in young ruminants. The deficiency has also been characterized by lack of strength, being born blind, without hair, or even dead (McDowell, 1992). Goiter is a less extreme expression of I deficiency when compared to nonexistent hair or wool (Underwood, 1981). It is also possible that I deficiency can result in compromised brain evolution in young lambs (Potter et al., 1982). Reduced conception rates along with infertility or sterility, a consequence of variable or inhibited estrus, are results of

thyroid malfunction and are recognized as increased losses of I during peak lactation when breeding typically happens (Hemken, 1970). It could take up to a year for adult cattle to exhibit signs of deficiency on a low I diet (Swanson, 1972). On the other hand, a more extended deficiency could illustrate signs of diminished feed intake, milk fat, mild production, and possibly signs of hypothyroidism (Hemken et al., 1971). Additionally, cattle that are deficient in I may be more sensitive to stress and have a higher occurrence of ketosis (Hemken, 1970).

Indications of toxicosis in beef cattle were expressed when the diet had concentrations of I in the range of 50 to 100 mg I/kg DM. Lactating cows are less sensitive to excessive concentrations of I than younger animals. In milk replacer, the pre-ruminant calf was able to withstand up to 50 mg I/kg DM for 5 weeks after birth (Jenkins and Hidiroglou, 1990). Decreased appetite, dismal and lethargic presence, immoderate tears, scaliness, and sloughing of skin, trouble swallowing, and a hacking cough are all signs of I toxicity. Consumption of copious amounts of I for prolonged time periods led to compromised operation of humoral and cell-mediated immune systems; therefore, reducing the body's ability to create antibodies in response to a disease challenge (Haggard et al., 1980). Currently, the maximum tolerable concentration for beef cattle has been established at 50 mg I/kg DM. In one study, I in the form of ethylenediamine dihydroiodide (**EDDI**) was fed at concentrations above 50 mg/kg without detrimental effects to either the calves or lactating cows (NRC, 1980).

Physiological functions of iodine:

The primary known function of I is to generate thyroid hormones, thyroxine and triiodothyronine. Iodine makes up about 65% of thyroxine. These thyroid hormones play many crucial functions like thermoregulation, transitional metabolism, reproduction, growth and development circulation, muscle function, along with regulating oxidation rate of cells

(McDowell, 1992). They also have additional functions, first, they effect physical and mental growth and variation or development of tissues; second, they influence some endocrine glands, specifically the hypophysis and gonads; third, they affect neuromuscular functioning; fourth, they influence integument and its outgrowths, hair, fur and feathers; and finally, they affect metabolism of nutrients involving numerous minerals and water (Scott et al., 1976). Among different species, the biological influence of thyroid hormones differs, especially with different phases of development on variable tissues. Thyroid hormones are crucial to the fetus, neonate and young animals for cellular distinction, growth, and maturation (Shambaugh, 1978), likely facilitated through gene expression and demonstrated by enlarged creation of new proteins and enzymes or initiation of remaining enzymes (Oppenheimer et al., 1976). Specific proteins are proposed to be more closely monitored by thyroid hormones, especially those related to the epidermis and hair production, and cartilage metabolism. At the molecular level, thyroid hormones are considered to be either, the hormones that commence their operation a at nucleus site, or those that act as the plasma membrane with extranuclear organelles (McDowell, 1992).

ORGANIC VERSUS INORGANIC TRACE MINERALS:

It is common to hear discussion on the utilization of inorganic and organic trace minerals (**TM**s), and the benefits and disadvantages associated with their use in terms of cost and bioavailability to the animal. There is insufficient information that organic TMs have improved absorption over there inorganic counterparts. Positive responses to specific organic TMs have been measured where the total quantity absorbed may not be as essential as the form in which the mineral was absorbed (Spears, 1996). Effectiveness of absorption for TMs varies between ruminants and nonruminants in regards to dietary factors that influence bioavailability. This is mostly due to the fact that ruminants have microbial fermentation taking place in the rumen and

reticulum prior to feeds being passed to abomasum and small intestine. Since the ruminant diet is typically high in fibrous components, significant microbial action is required in the rumen (Spears, 2003). Originally, TM supplements to animals were in the form of inorganic salts. Lately, the current trend has been to utilize organic or chelated minerals in the ruminant diet.

Organic TMs can be categorized as complexes, chelates or proteinates. The summary of these are described by Spears (1996). Chelation involves a unique complex that is formed amongst a ligand and a metal ion. If a chelating agent or ligand is comprised of 2 functional groups where both are able to donate a pair of electrons to couple with a metal and can form a heterocyclic ring arrangement, then it is considered a chelate (Kratzer and Vohra, 1986). To lead to increased absorption, the metal chelate or complex needs to be structurally sound in the digestive tract where the metal would be shielded from the creation of complexes with various dietary constituents that restrict absorption. This presumes that the metal chelate or compound be absorbed in its current form or altered to a chemical form that could also be absorbed. This would be effective as TMs are typically found in the body as organic compounds or chelates and are not usually found in the form of free inorganic ions. Therefore, inorganic minerals rely on the animal and its ability to transform them to an active organic form. In reality, TMs in most feedstuffs are found as organic chelates or compounds. Currently, under certain conditions, ruminants will respond (improved growth, milk production, immune response, and reproduction) to TM chelates or complexes. Presently, it is not possible to conclude whether responses observed in ruminants are a result of organic mineral supplementation or rather an increased dietary mineral intake for that animal (Spears, 1996). The same author suggests that further research is required to determine under what conditions responses for performance or health might be expected, resolve optimal supplementation levels for trace minerals of the organic

classification, justify if responses are sufficient to cover mineral costs, and define the mechanism at which trace mineral supplements increase ruminant performance.

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CHAPTER II

COMPARISON OF NRC AND INDUSTRY DIETARY TRACE MINERAL STANDARDS FOR YEARLING FEEDLOT STEERS

SUMMARY

Two experiments were conducted to determine the effects of trace mineral (**TM**) concentration and source on: 1) yearling feedlot steer performance, carcass characteristics, and liver TM status and 2) *in vitro* "releasibility" of Cu and Zn. For experiment 1, 360 crossbred steers (initial BW=350 ± 4.0 kg) were utilized. Steers were blocked by initial BW and randomly assigned to one of 4 treatments (10pens/treatment; 9 hd/pen). Treatments consisted of: 1) negative control (**NC**), no supplemental TM (basal diet contained 7.65 mg Cu/kg DM, 50.5 mg Zn/kg DM, 27.7 mg Mn/kg DM, and 0.12 mg Co/kg DM); 2) basal diet supplemented with 10 mg Cu/kg DM from $CuSO_4$, 30 mg Zn/kg DM from $ZnSO_4$, 20 mg Mn/kg DM from $MnSO_4$, 0.50 mg I/kg DM from EDDI, 0.10 mg Se/kg DM from Na_2O_3Se, and 0.10 mg Co/kg DM from $CoCO_3$ (**NRC**); 3) basal diet supplemented with inorganic forms of Cu, Zn, Mn, EDDI, Se and Co at consulting nutritionist recommendations (**CNI**, 20, 100, 50, 0.50, 0.20, and 0.20 mg of mineral/kg DM, respectively); and 4) basal diet supplemented with 66.6% inorganic and 33.4% organic Cu, Zn, Mn and Co, and inorganic forms of I and Se at iso-concentration to consulting nutritionist recommendations of treatment 3 (**CNO**). All steers were fed a high concentrate, steam-flaked, corn-based diet for 154 d. Steers were individually weighed on d -1, 0, 35, 121, 153, and 154. Continuous data were analyzed on a pen mean basis using a mixed model appropriate for a randomized block design (fixed effects = treatment and time; random effect = replicate). Categorical data were analyzed utilizing GLIMMIX (fixed effect = treatment; random effect = replicate). For experiment 2, an *in vitro* analysis was utilized to determine "releasability" of Cu and Zn from diets fed in experiment 1. Total mixed ration samples collected throughout

Experiment 1 were composited, dried at 60°C for 24 h, ground through a 2mm screen, and utilized as substrate. Samples were incubated for 0, 6, 12, and 24 h (in triplicate) in a 3:1 ratio of modified McDougall (1984) buffer and rumen fluid obtained from rumen fistulated steers fed high a concentrate finishing diet. After *in vitro* fermentation, tubes were centrifuged, and undigested residue was quantified to determine DMD. The residue was them subjected to a pepsin HCl solution for 1 h to simulate abomasal conditions. Were again centrifuged and undigested residue was quantified. Final abomasal undigested residues were analyzed for Cu and Zn, In experiment 1, initial and final BW, ADG, DMI, F:G and G:F ratios and calculated net energy recoveries were similar ($P > 0.23$) across treatments. Subcutaneous adipose tissue depth, HCW, KPH, yield grade, marbling score, and quality grade were similar across treatments ($P > 0.17$). Final liver Zn, Mn, Se, and Co concentrations were similar across treatments ($P > 0.37$). For experiment 2, DMD was similar across treatments ($P > 0.49$). Percent Cu ($P > 0.02$) and Zn ($P > 0.0004$) released after simulated rumen and abomasal digestion was less for NC compare to NRC, CNI, and CNO diets. Under the conditions of this experiment, it appears that basal dietary concentrations of Cu, Zn, Mn, and Co were adequate for growth and performance of finishing yearling feedlot steers and that Cu and Zn released from supplemented diets was greater that NC.

Key words: Beef cattle, feedlot, *in vitro*, trace mineral

INTRODUCTION

Trace minerals, such as copper (**Cu**), zinc (**Zn**), and manganese (**Mn**), have long been known as essential elements for cattle. Typical feedlot diets are normally fortified with trace minerals because feedstuffs utilized in feedlot rations normally contain low concentrations of

essential trace minerals and/or may contain high concentrations of known trace mineral antagonists. Vasconcelos and Gaylean (2007) conducted a survey of 28 consulting feedlot nutritionists, representing approximately 70% of the feedlot cattle on feed. Averages for trace mineral concentrations supplemented to feedlot cattle diets were: 17.61 mg Cu/kg DM, 92.95 mg Zn/kg DM, 47.86 mg Mn/kg DM, 51.73 mg Fe/kg DM, 0.24 mg Se/kg DM, 0.75 mg I/kg DM, and 0.38 mg Co/kg DM. Most of these average values are well above the outlined recommendations for beef cattle by the NRC (2000). The NRC (2000) recommends 10 mg Cu/kg DM, 30 mg Zn/kg DM, 20 mg Mn/kg DM, 50 mg Fe/kg DM, 0.10 mg Se/kg DM, 0.50 mg I/kg DM, and 0.10 mg Co/kg DM to meet the dietary requirements for beef cattle. Some of the concentrations recommended for supplementation by consulting nutritionists are as high as 3 times the NRC (2000) recommendation. Although TMs are a small component of total diet cost of a typical feedlot ration, a significant decrease in net income can take place when animal performance is altered by the presence of deficiencies, excesses, or imbalances of trace minerals (Miller, 1975). Furthermore, due to the lack of literature containing trace mineral feedlot experiments with a negative control, the objectives of these experiments were to: 1) determine the effects of trace mineral source and concentration on yearling feedlot steer performance, carcass characteristics, and trace mineral status, and 2) investigate *in vitro* "releasability" of Cu and Zn from typical feedlot diets supplemented with different forms and concentrations of Cu and Zn.

MATERIALS AND METHODS

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

Experiment 1: Upon arrival all steers (total: 429, 364.9 ± 36.7 kg) were housed at Colorado Beef Feedlot (Lamar, CO) and allowed free-choice grass hay and water access overnight. For processing the next morning, cattle were trailed approximately 1.0 km to Colorado State University's Southeast Colorado Research Center (**SECRC**). At processing, all cattle were weighed, assigned a breed type score, and received an individual unique electronic identification tag, 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), for parasite control, Safe-Guard (Intervet Inc., MN), and growth implanted with Revalor-XS (Merck Animal Health, NJ 20 mg of trenbolone acetate and 4 mg estradiol). Applications of all products were conducted in accordance with labeled instructions. Of the original 429 crossbred steers, 360 (initial BW=350 ± 4.0 kg) were utilized for the following experiment. Selection criteria were as follows: based on the assigned breed score, and those that appeared to contain excessive Brahman, dairy, or Longhorn genetics were eliminated from additional consideration for enrollment in the experiment. Furthermore, steers that were ± 2 SD from the mean BW and steers illustrating health problems were excluded from the study. All remaining steers were randomized using Microsoft® Excel 2007 and allocated a number from 1 to 1,000. To arrive at 360 qualified steers for this experiment, forty additional steers were pulled from the study via lowest random numbers.

After categorizing by body weight within assigned breed type, steers were divided into 10, weight block replicates. Additionally, in each weight block by breed type, every group set of 4 steers was ranked and assigned to treatment 1 – 4, once again through lowest to highest random number in Excel, respectively. This process was applied to each group of 4 ranked

steers for each breed by weight block. Ultimately for each treatment, 10 weight block replicates containing 9 steers with similar dispersion for breed type were formed.

The experiment was initiated on d 0 when steers were individually weighed and tagged with visual numbers corresponding to trial number, replicate, and animal number in each pen. Upon exiting the chute steers were sorted into respective treatment groups and relocated to their respective pen.

Diets were manufactured and delivered twice a d starting at 0730 h and finishing with the second round at approximately 1100 h. Steers were fed standard starter, step-up 1, step-up 2, and finish diets (Tables 1, 2, and 3) for the duration of the experiment. For the final 29 d of the experiment all steers were fed Ractopamine HCl (Optaflexx, Elanco Animal Health). Throughout the experiment from d 0 to d 154, feedings were consistent with SECRC standard operating procedures.

Dietary treatments consisted of: 1) negative control (**NC**), no supplemental TM (basal finishing diet contained 7.65 mg Cu/kg DM, 50.5 mg Zn/kg DM, 27.7 mg Mn/kg DM, and 0.12 mg Co/kg DM); 2) basal diet supplemented with 10 mg Cu/kg DM from $CuSO_4$, 30 mg Zn/kg DM from $ZnSO_4$, 20 mg Mn/kg DM from $MnSO_4$, 0.50 mg I/kg DM from EDDI, 0.10 mg Se/kg DM from Na_2O_3Se, and 0.10 mg Co/kg DM from $CoCO_3$ (**NRC**); 3) basal diet supplemented with inorganic forms of Cu, Zn, Mn, EDDI, Se and Co at consulting nutritionist recommendations (**CNI**, 20, 100, 50, 0.50, 0.20, and 0.20 mg of mineral/kg DM, respectively); and 4) basal finishing diet supplemented with 66.6% inorganic and 33.4% organic Cu, Zn, Mn and Co, and inorganic forms of I and Se at iso-concentration to consulting nutritionist recommendations of treatment 3 (**CNO**). Supplements were mixed with corresponding treatment trace mineral premix. For the NC diet, ground corn was used in place of the trace

mineral premix (Table 3). Predicted supplemental concentrations of TMs for all treatments are contained in Table 4.

At 0630 h and again at 1± h feed bunks were evaluated for all treatments. After 2 sequential d of observing empty bunks at 0630 h, feed delivery was increased by 0.227 kg DM per head. Equally, if 2 sequential observations were made for surplus feed, a reduction of a suitable amount was made to encourage steers to clean the bunk. Traditional starter and step-up diets were used to transition steers to a high concentrate steam-flaked corn diet. All diets were designed to meet or exceed basic requirements as noted by NRC (2000). On average, starter and step-up diets were fed for 7 d. At d 21 through the end of trial steers were fed finishing diet, with the addition of Optaflexx (Elanco; 200 mg/hd/d) for the last 29 d to all treatments. The finish diet was formulated to utilize non-protein nitrogen to account for 3.5% of the crude protein; corn silage was utilized to provide 4% neutral detergent fiber as the roughage source in the diet. Weekly dry matter was determined by SECRC personnel from a portion of representative samples from rations and feed ingredients in a forced-air drying oven for 48 h at 60°C. Monthly composited feed ingredient, ration, supplement, and trace mineral premix samples were sent to an established laboratory (SDK Labs, Hutchinson, KS) for trace mineral analysis. On weigh d, and in the event of spoiled feed due to weather, orts were collected for DM analysis. For each treatment, as fed delivery was recorded for each day. It was multiplied by the average dry matter concentration value calculated from the results of the samples in the drying oven. To determine the dry matter intake (**DMI**) for each pen, the DM refused was subtracted from the total DM delivered and divided by the head-days for that pen.

Steer weights recorded on d -1 and 0 were used to calculate initial weight. Final weight was determined from the 2 weights recorded prior to shipping. Individual weights during the

trial were recorded on d 35 and 120, while pen weights were recorded on d 28. Average daily gain for each weigh period was calculated by taking live weight gain and dividing by the number of d on feed. Roughly 1 wk prior to the addition of Ractopamine HCl, individual weights were collected. Preceding analysis, a pencil shrink of 4% was applied to all weights. The net energy maintenance (NEm) and net energy gain (NEg) from the diet was calculated from pen performance and requirements and the description of the quadratic equation is outlined by Zinn (1992). The series of equations utilized for NEm and NEg for each of the 40 pens are for medium framed steer calves available from the NRC (2000).

On a daily basis, individual cattle were health checked and observations recorded. For respiratory disease, cattle that showed signs of depressed appearance, nasal discharge, ocular discharge, rapid breathing or coughing were given a score of 0 or 1 for each symptom. Once steers had a score of 2 or higher, they were pulled and a rectal temperature was taken. If the steer's temperature exceeded 39.72°C, it received 2 more points. Steers that received 4 points or more were treated and returned to their original pen to recover. If a steer was re-pulled for the same respiratory disease, he was treated and again returned to his home pen for recovery. If a third pull occurred for a steer with the same respiratory disease, he was eliminated from the study. Overall, health was assessed and if any steer was unsound or injured, he was inspected and treated if necessary, and the decision made on whether the steer should return to its original pen. In the event that a steer was considered unrecoverable, he was eliminated from the experiment. Once elimination was deemed necessary, the steer was weighed via individual or pen scale, followed by data recording of removal date, pen number, steer number, body weight, disposition, and diagnosis and explanation for removal. Necropsy was performed on steers that

either died or were euthanized to conclude cause of death. A total of 3 steers died during the experiment.

On day 155 steers were fed 30% of their allotted daily feed at 0700 h, and at 1100 h, were moved to the Colorado Beef Feedyard for shipping. Slaughter order, carcass tag, hot carcass weight, liver score and liver samples were obtained for each animal post-harvest. Liver samples were placed in pre-labeled whirlpak bags and immediately frozen for later analysis. Additional carcass data was collected after a 36 h chill, like hot carcass weight, 12^{th} rib fat depth, kidney-pelvic and heart fat, marbling score, longissimus muscle area, and USDA quality and yield grade.

Experiment 2: Three crossbred ruminally and duodenally fistulated feedlot steers (approximately 580 kg and 1.5 yr. of age) were utilized to examine *in vitro* "releaseability" of Cu and Zn from each diet fed in experiment 1. Rumen fluid was collected from each steer approximately 2 h post feeding of a high concentrate, steam-flaked, corn-based finishing diet (basal diet contained: 15.71 mg Cu/kg DM, 32.38 mg Zn/kg DM, 16.05 mg Mn/kg DM, and 0.34 mg Co/kg DM). Rumen fluid was filtered through 4 layers of cheesecloth twice before being added to a pre-warmed (39°C) thermos. Equal amounts of rumen fluid were collected from each steer and combined (approximately 1.0 L per steer). A modified McDougall's (McDougall, 1948) buffer solution (19.60 g $NaHCO_3$, 7.40 g Na_2HPO_4, 1.14 g KCl, 0.94 g NaCl, 0.24 $MgS0_4*7H_20$ per 2 L H_20) was mixed at a ratio of 3 to 1 buffer to rumen fluid. Rumen fluid pH was measured before and after addition of McDougall buffer. Preceding rumen fluid collection, treatment diet samples (total mixed rations) that were composited monthly throughout experiment 1 were dried in a forced air oven at 60°C for 24 h. Samples were then ground through a 2 mm Wiley mill, and 50 g from each monthly sample were

composited within treatment. Pre-labeled 50 ml conical tubes were weighed and 0.53 ± 0.02 g of ration (in triplicate for each diet within each time period; 0, 6, 12, and 24 h) per dietary treatment was added to each digestion tube and weight was recorded. A water bath large enough to hold all tubes for the duration of the experiment was maintained at 39°C. All glass ware, tubes with substrate, and other needed items were placed in a dry oven to maintain temperature at 39°C 12 h prior to use. The combined mixture of buffer and rumen fluid was flushed with CO_2, and 30 ml of buffer rumen fluid mixture was added to each conical tube containing dietary substrate and no substrate (blank). Trace mineral concentrations (Cu, Mn, Co, and Zn) of blanks (rumen fluid-McDougall's solution mixture) were subtracted from mineral concentrations of tubes containing dietary substrate at each time point prior to statistical analysis. Conical tubes were capped with a rubber stopper fitted with a rubber one way valve to maintain anaerobic conditions. After all samples were placed in the water bath, the incubation time started (0700 h). During the *in vitro* simulated rumen incubations, samples were gently swirled approximately every 4 h. At the appropriate time points, tubes were removed from the water bath and placed in a refrigerator to stop microbial fermentation and quickly moved from the Ag Research Development and Education Center to campus prior to centrifugation.

Analytical Procedures:

Experiment 1: Of the 355 liver samples collected at harvest, 3 liver samples from each pen were selected for trace mineral analysis. Prior to analysis, all liver samples were snap-frozen in liquid nitrogen and homogenized in a coffee grinder. Individual liver samples were weighed into pre-weighed acid washed crucibles and were dried in a forced air oven at 60° C for 24 h. After the drying period, samples were re-weighed. Samples were then placed in a

muffle furnace and ashed at 600°C for 12 h. Samples were then removed and placed in a desiccator for an additional 30 min to cool. Finally, samples were weighed and re-suspended with 5 ml of HCl (12 N HCl). Each crucible was warmed on a heating plate under a hood and rinsed with 2 ml HCl, another 2 ml was added and removed before a final 1 ml of HCl was used to rinse each crucible into a pre-labeled test tube for analysis. Trace mineral concentrations of the liver samples were determined through inductively coupled plasma-atomic emission spectroscopy (ICP-AES) methods (Braselton et al., 1997) for Cu, Co, Mn, Se, and Zn as further described by Ahola et al. (2004).

Experiment 2: After incubation, samples were centrifuged at 1,000 x *g* for 20 min at room temperature. Supernatant was decanted into a new pre-weighed conical tube and placed in the refrigerator at 5°C. The remaining pellet was placed in a -80°C freezer. All liquid supernatant samples were measured out into 1.0 ml amounts into pre-weighed ash crucibles and placed in a drying oven at 60°C overnight. The remaining pellet after *in vitro* fermentation was also placed in a forced air drying oven at 60°C for 48 h to determine *in vitro* dry matter disappearance (**DMD**). Following removal from the drying oven, a 3 ml mixture of 5% pepsin (1:10,000) and 1 N HCl was added to each tube. Tubes were then allowed to incubate in a forced air drying oven at 39°C for 1 h with gentle swirling every 15 min. After incubation, samples were centrifuged at 1300 x *g* for 20 min, and the supernatant decanted and placed into a pre-labeled 15 ml conical tube and re-centrifuged at 1750 x *g* for an additional 20 min. The two liquid portions from each sample were combined into one tube and then 1.0 ml portions of the pepsin supernatant were weighed out into pre-weighed ash crucibles. The remaining pellet after pepsin-HCl digestion and centrifugation was weighed into an ashing crucible and placed into a forced air drying oven for 24 h at 60°C and reweighed upon removal. All ash crucibles

were removed from the drying oven and placed into an ash oven for 24 h at 600°C. Samples were removed from the ashing oven and placed in a desiccator and allowed to cool for approximately 1 h. Samples were then re-suspended with 5 ml of 12 N HCl. Trace mineral concentrations of the rumen fluid, pepsin supernatant, and remaining undigested pellet samples after pepsin-HCl digestion were determined through inductively coupled plasma-atomic emission spectroscopy (ICP-AES) methods (Braselton et al., 1997) for Cu, Co, Mn, Se, and Zn as further described by Ahola et al. (2004).

Statistical Procedures:

Experiment 1: Data were analyzed as a randomized complete block design with 4 treatments. Liver TM concentrations, non-categorical carcass data, and live animal performance response variables were analyzed utilizing the PROC MIXED procedure in SAS (Statistical Analysis System, 9.2, Cary, NC). Pen was used as the experimental unit. Treatment was considered a fixed class variable, where weight block replicates were included as a random class variable. Initial weight was included in the model as a covariant when $P < 0.10$. Contrasts for main effects were: 1) no supplemental TM vs. Suppl (NRC, CNI, and CNO); 2) NRC (supplement as NRC recommended values) vs. consulting nutritionist (both CNI and CNO); and 3) CNI (consulting nutritionist values inorganic) vs. CNO (consulting nutritionist values organic).

Categorical carcass traits involving liver abscess data and USDA quality and yield grade were analyzed in the same model as listed above using binomial distribution PROC GLIMMIX procedure in SAS (Statistical Analysis System, 9.2, Cary, NC). Treatment means are reported both as raw pen means ± SEM, and as least square means ± SEM for categorical data, indicating the likelihood that an individual within pen would classify into each category

like quality and yield grade, hot carcass weight size category and likelihood of an individual liver showing signs of abscesses.

Experiment 2: Data were analyzed as a completely randomized design with 4 treatments. Trace mineral concentrations were analyzed using the PROC MIXED procedure in SAS (Statistical Analysis System, 9.3, Cary, NC). Classification variables included treatment and time. Differences between treatment means were determined by utilizing LSMEANS/PDIFF statements along with a SLICE statement. Contrasts for main effects across treatments were illustrated by NC (no supplemental TM) vs. Suppl (NRC, CNI, and CNO); NRC (supplement as NRC recommended values) vs. consulting nutritionist (**CN**) both CNI and CNO; and CNI (consulting nutritionist values inorganic) vs. CNO (consulting nutritionist values organic).

RESULTS AND DISCUSSION

EXPERIMENT 1

Performance: While initial BW across treatments were similar ($P < 0.49$), a treatment effect ($P < 0.005$) was observed on d 35 for BW (Table 5). Steers receiving NC had greater ($P < 0.02$) BW compared to steers receiving supplemental TMs, and steers receiving NRC concentrations of trace minerals had greater 35 d BW than steers receiving CN concentrations of supplemental trace minerals. However, final BW (d 154) were similar across treatments. Average daily gain for d 0-35 was affected ($P < 0.005$) by treatment. Steers receiving NC had greater ($P < 0.02$) ADG compared to steers receiving supplemental TMs, and steers receiving NRC concentrations of trace minerals had greater ADG than steers receiving CN concentrations of supplemental trace minerals. However, overall ADG (d 0 - 154) were similar across treatments. Dry matter intakes were similar across all treatments for all time periods.

Table 6 contains the least squares means for the effects of trace mineral concentrations and source on feed to gain (**F:G**) and gain to feed (**G:F**) ratios. Overall (d 0-154), F:G and G:F ratios were similar across treatments (Table 6). However, a treatment effect ($P < 0.05$) was observed for F:G and G:F for d 0-35. Steers receiving NC had a lower F:G ($P < 0.03$) and a greater G:F ($P < 0.04$) than steers supplemented with trace minerals. Trace mineral analysis of the total mixed ration indicate that treatment structure (supplemented mineral intake) was maintained throughout the entire experiment.

Net energy recoveries for NEm and NEg are shown in Table 7. Overall, net energy recoveries were similar across treatments. However, treatment tended ($P < 0.07$) to influence period 2 NEm and NEg recoveries. In period 1, steers receiving NC tended ($P < 0.08$) to have greater NEm and NEg recoveries than steers receiving supplemental trace minerals.

In relation to Cu supplementation of finishing cattle, highly variable results have been reported. Ward and Spears (1997) observed a positive effect, Engle and Spears (2000) observed a negative effect, and Engle and Spears (2001) observed no effect on performance of cattle compared to non-supplemented controls. Greater final BW and a tendency for greater ADG in organic vs. inorganic supplemented steers has been reported (Lee et al., 2002), while it has also been reported that Cu source had no effect on steer finishing phase performance (Engle and Spears, 2000). Zinc supplementation of finishing cattle had no effect on ADG, DMI, or G:F (Spears and Kegley, 2002) when compared to non-supplemented controls; but one study reported DMI decreased with increasing Zn concentration (Malcolm-Callis et al., 2000). Greene et al. (1988) determined there was no difference in ADG for the entire 112-d trial, along with no difference in DMI for control vs. Zn supplemented steers. In one study, a tendency was reported for ADG ($P = 0.10$) and G:F ($P < 0.07$) to be greater in organic supplemented cattle compared to

those receiving inorganic Zn (Spears and Kegley, 2002). Multiple researchers have concluded that Zn source had no impact on performance (Malcolm-Callis et al., 2000; Greene et al., 1988).

Carcass Characteristics:

Table 8 displays the results for effect of trace mineral concentration and source on carcass merit. Hot carcass weight, dressing percentage (DP), subcutaneous adipose tissue depth, percent kidney, pelvic, heart (KPH) fat ($P < 0.79$), longissimus muscle area, marbling, muscling, liver abscesses, calculated yield grade, actual yield grade, and the distributions of yield grades were similar across treatments. There was a tendency for CNI supplemented steers to have greater ($P < 0.07$) quality grade distribution in ≥ low choice (CH) compared to CNO supplemented steers. Among treatments, there was a treatment effect ($P < 0.02$) for quality grade distribution. Steers receiving CNO had a greater ($P < 0.005$) likelihood for carcasses falling into the select category when compared to steers receiving CNI (Table 8).

These data are supported by the work of Rhoads et al. (2003) who compared inorganic and organic supplementation of finishing cattle. They reported no difference between carcass traits (HCW, DP, REA, YG, backfat, KPH, or marbling) across treatments. Engle et al. (2000) found that backfat was lower ($P < 0.05$) in Cu supplemented steers over control steers. Additionally, the authors also found that HCW was decreased ($P < 0.05$) for Cu supplemented steers compared to control steers. They also reported finding no difference between dressing percentage, KPH, quality grade and yield grade. Engle and Spears (2001) reported finishing steers that received 40 mg Cu/kg tended ($P < 0.10$) to have less backfat than steers that received 10 mg Cu/kg DM; however, controls did not differ from Cu supplemented steers. Again, supporting earlier results, they reported no difference between dressing percentage, HCW, KPH, yield and quality grade. Furthermore, Spears and Kegley (2002) found that backfat was lower in

control steers compared to steers supplemented with inorganic and organic sources of Zn. Malcolm-Callis et al. (2000) determined that increasing the Zn concentration to 200 mg/kg did not affect carcass traits such as DP, HCW, KPH, and marbling. Similarly, Zn source did not affect the same traits in addition to yield grade.

Trace Mineral Status:

Trace mineral concentrations in liver samples obtained at the end of experiment 1 are reported in Table 9. Cobalt, Mn, and Zn concentrations were similar across treatments. There was a tendency for a treatment effect ($P < 0.08$) for liver Cu concentrations. Steers receiving NRC supplemented diets tended ($P < 0.09$) to have lower concentrations of Cu in the liver compared to CNI and CNO. Finally, there was a tendency ($P < 0.07$) for CNI to have greater ($P < 0.07$) liver Cu concentrations compared to CNO supplemented steers.

Similar data presented by Engle and Spears (2000) indicated that finishing steers on the control diet had numerically lower final liver Zn concentrations compared to Cu supplemented steers. In another instance, Engle and Spears (2001) found that liver Cu concentrations were lower ($P < 0.001$) for control steers vs. supplemented steers and also significantly lower ($P < 0.001$) for steers receiving 10 mg Cu/kg DM compared to steers supplemented with 40 mg Cu/kg DM. Other work by Rhoads et al. (2003) indicated that liver mineral concentrations of steers supplemented with Zn, Cu, Mn, and Cu were not impacted across dose treatment. They also observed that steers receiving the organic form of Zn at 1.5 times the NRC (1996) recommended concentration had greater liver Zn concentration than those that were supplemented with the inorganic form of Zn.

Because it has become an accepted practice to supplement TMs at 2 or 3 times the NRC (2000) recommended concentrations, a common question is “what are the cost differences

associated with supplementing at different levels and different forms?" The data presented in Table 10 illustrates the cost difference between treatments, calculated on a per head basis over the 154 d feedlot experiment. By design, the NC treatment has no added cost, when in comparison to the cost of the CNO treatment for Zn at $3.04 per head. This could have significant cost impact on a large feedyard, especially when considering all TMs.

EXPERIMENT 2:

Least square means for trace mineral concentrations of supernatant from simulated rumen and abomasal digestion were analyzed (data not shown). There were no treatment by time interactions for any response variables measured. Overall, the µg of Co, Cu, Mn, and Zn released · g DMD^{-1} were similar across all treatments. In general, µg of Co and Cu released · g DMD^{-1} were greater under simulated rumen conditions whereas µg Mn and Zn released · g DMD^{-1} appeared to be greater under simulated abomasal conditions and this data is summarized in Table 11. It appears that when Co and Cu are exposed to ruminial conditions a greater proportion of these minerals are rereleased whereas Mn and Zn are released when conditions are acidic.

Table 12 shows the least square mean estimates for overall DMD and percentage of Cu and Zn released under simulated ruminal and abomasal conditions. Dry matter disappearance was similar across treatments. However, there was an overall treatment effect for percent released of Cu ($P < 0.02$) and Zn ($P < 0.0004$). Percent of Cu and Zn released under ruminal and abomasal simulated conditions were higher ($P < 0.05$) for supplemented diets when compared to NC as well as NRC vs. CNI and CNO supplemented diets. No difference ($P < 0.42$) in release of Cu and Zn was detected for CNI vs. CNO supplemented diets. Based on the mineral analysis of the 4 treatment rations, the basal concentrations for Cu were NC, 5.74;

NRC, 8.48; CNI, 12.56; and CNO, 12.73; and Zn concentrations were NC, 38.11; NRC, 27.66; CNI, 76.86; and CNO, 84.68 mg/kg. From these concentrations, we can estimate that 52.20% of the 5.74 total mg Cu/kg or (0.5220 x 5.74) 3 mg/kg was soluble or available to the steers receiving the NC diet. Similarly, it can be estimated that 4.88, 8.0, and 8.63 mg/kg were released under simulated ruminal and abomasal digestion from NRC, CNI, and CNO diets, respectively. Following the same procedure for Zn, it can be estimated that 28.5, 37.9, 66.2, and 73.2 mg/kg were released under simulated ruminal and abomasal digestion from NC, NRC, CNI, and CNO diets, respectively. Ammerman and Miller (1972) summarized an experiment where Cu was supplemented to rats as Cu oxide, Cu pyrophosphate, Cu concentrate (74.1% Cu), and Cu sulfate at 250 and 300 mg/kg. It was estimated that Cu oxide, Cu concentrate, and Cu pyrophosphate are approximately 25, 33 and 50% available, respectively utilizing liver Cu storage as a criterion. Additional work reported by these authors indicates that Zn from Zn carbonate, Zn oxide, Zn chloride, or Zn proteinates were similarly available when growth in chicks was recorded.

It should also be noted that the values for trace mineral concentrations utilized for experiment 1 and 2 were completed at 2 different locations 1) a commercial laboratory where analysis and results obtained were utilized for experiment 1; and 2) in house analysis prior to the initiation of the *in vitro* analysis for trace mineral concentrations of the total mixed ration for each treatment and were utilized for the data analysis for experiment 2.

IMPLICATIONS

Overall, there was little difference among treatments for the carcass characteristics, performance, and trace mineral status of yearling feedlot steers. The yearling feedlot steers utilized in this experiment were considered “low-risk” cattle and may have contributed to the

lack of performance differences across treatments. As mentioned earlier, the cost per head of supplementing no additional trace minerals is much less than the cost associated with the inorganic and organic treatments. While the second experiment would indicate differences in TM "releasability" for Cu and Zn from the basal treatment diets for inorganic and organic, the benefit of these differences may be in question when compared to the feedlot performance experiment (Exp. 1). If the dietary requirements of the individual animal are being met, there may be little to benefit from increased "realeasability" from ruminal and abomasal digestion from the organic TM treatment found in the second experiment.

Table 1. Dry matter nutrient composition of basal diets (Experiments 1 and 2).

Item[1]	Starter	Step – 1	Step – 2	Finish w/o Opt	Finish w/Opt
Ingredient					
Corn Silage	22.07	25.07	20.23	14.67	14.67
Steam Flaked Corn	40.45	45.59	58.05	70.67	70.62
Alfalfa	28.37	14.67	7.06	–	–
Corn Steep	3.00	3.00	3.00	3.00	3.00
Dried Distiller's Grains	4.91	8.00	6.32	4.50	4.52
Tallow	–	1.74	2.76	3.80	3.80
Supplement[2]	1.20	1.91	2.58	3.36	3.38
Theoretical Nutrients					
Dry Matter, % as-fed[3]	61.09	59.05	61.38	64.41	64.41
Crude Protein	13.50	13.50	13.50	13.50	13.50
Non-protein nitrogen[4]	1.00	1.50	2.50	3.50	3.50
Acid detergent fiber	19.63	15.16	10.73	6.35	6.35
Neutral detergent fiber	29.74	25.17	19.98	14.78	14.78
Crude fiber	14.77	11.75	8.04	4.30	4.30
eNDF[5]	22.99	17.60	13.32	9.11	9.11
fNDF[6]	24.00	18.00	12.00	6.00	6.00
NEm, Mcal/kg[7]	1.78	1.92	2.05	2.17	2.17
NEg, Mcal/kg[8]	1.09	1.24	1.38	1.47	1.47
Calcium	0.70	0.70	0.70	0.70	0.70
Phosphorus	0.37	0.38	0.37	0.35	0.35
Potassium	1.31	1.06	0.85	0.70	0.70
Magnesium	0.23	0.23	0.21	0.20	0.20

[1]Percentage of dry matter unless stated otherwise.
[2]See table 2 for supplement composition.
[3]Dry matter content of initial formulation, percentage as-fed.
[4]Crude protein equivalent.
[5]Effective neutral detergent fiber. Calculated from NRC (2000).
[6]Neutral detergent fiber from only the forage component of the diet.
[7]Net energy for maintenance.
[8]Net energy for gain.

Table 2. As-fed ingredient composition of supplements used for Experiments 1 and 2.

Item[1]	Starter	Step – 1	Step – 2	Finish w/o Opt	Finish w/ Opt
Calcium Carbonate	41.2553	40.5609	49.5186	46.2892	45.9595
Urea	27.2657	36.5692	33.4121	36.2797	36.0190
Salt	20.6364	13.2361	9.6184	7.3785	7.3254
Trace mineral pre-mix[2]	6.6036	5.9540	4.0592	3.1135	3.0909
Mineral oil	2.0107	1.9857	2.0002	2.0000	2.0001
Vitamin E premix[3]	1.3756	0.8822	0.6412	0.4921	0.4885
Rumensin 90[4]	0.6878	0.4415	0.4808	0.4921	0.4885
Vitamin A premix[5]	0.1649	0.1059	0.0771	0.0591	0.0587
Tylan 100[6]	–	0.2644	0.1924	0.1475	0.1465
Potassium Chloride	–	–	–	3.7481	3.7223
Optaflexx 45[7]	–	–	–	–	0.7005

[1] Percentage of as-fed.
[2] See table 3 for trace mineral composition for different treatments.
[3] 198,414 IU/kg vitamin E activity.
[4] Monensin, 198.4 g/kg.
[5] 110,230,000 IU/kg vitamin A activity.
[6] Tylosin, 220.4 g/kg.
[7] Ractopamine hydrochloride, 100 g/kg.

Table 3. As-fed ingredient composition of the different trace mineral premix treatments used in Experiments 1 and 2.

	Treatments[2]			
Item[1]	NC	NRC	CNI	CNO
Zinc sulfate	–	8.1448	27.1491	18.1899
Availa zinc[3]	–	–	–	31.8053
Manganese sulfate	–	6.0237	15.0593	10.0897
Alltech manganese[4]	–	–	–	15.9026
Sodium selenite	–	6.0237	12.0474	12.0474
Copper sulfate	–	3.8246	7.6492	5.1249
Availa Copper[5]	–	–	–	6.3611
Cobalt carbonate	–	0.2095	0.4190	0.41904
Ground Corn	100	75.7137	37.6159	–
Iodine (EDDI premix)	–	0.0600	0.0600	0.0600

[1] Percentage of as-fed.
[2] NC = Negative Control, no supplemented trace minerals; NRC = National Research Council, 2000 recommended supplemented concentrations of copper, zinc, manganese, iodine, selenium, and cobalt trace minerals; CNI = Consulting nutritionist survey (Vasconcelos and Galyean, 2007) mode concentrations of copper, zinc, manganese, iodine, selenium, and cobalt supplemented to the basal diet using inorganic sources; CNO = Consulting nutritionist survey (Vasconcelos and Galyean, 2007) mode concentrations of copper, zinc, manganese, iodine, selenium, and cobalt supplemented to the basal diet using were added to the basal diet with 66.6% from inorganic sources and 33.4% from organic sources.
[3] Organic zinc.
[4] Organic manganese.
[5] Organic copper.

Table 4. Predicted supplemental trace mineral concentrations for each treatment in addition to the basal diet.

	Treatments[2]			
Trace Mineral[1]	NC[3]	NRC	CNI	CNO
Zinc (I)	–	30	100	66.6
Zinc (O)	–	–	–	33.4
Copper (I)	–	10	20	13.4
Copper (O)	–	–	–	6.6
Manganese (I)	–	20	50	33.5
Manganese (O)	–	–	–	16.5
Cobalt (I)	–	0.10	0.20	0.20
Iodine (I)	–	0.50	0.50	0.50
Selenium (I)	–	0.10	0.20	0.20

[1] Percentage of as-fed. I=inorganic and O=organic.
[2] NC = Negative Control, no supplemented trace minerals; NRC = National Research Council, 2000 recommended supplemented concentrations of copper, zinc, manganese, iodine, selenium, and cobalt trace minerals; CNI = Consulting nutritionist survey (Vasconcelos and Galyean, 2007) mode concentrations of copper, zinc, manganese, iodine, selenium, and cobalt supplemented to the basal diet using inorganic sources; CNO = Consulting nutritionist survey (Vasconcelos and Galyean, 2007) mode concentrations of copper, zinc, manganese, iodine, selenium, and cobalt supplemented to the basal diet using were added to the basal diet with 66.6% from inorganic sources and 33.4% from organic sources.
[3] Ground corn was used instead of trace mineral premix.

Table 5. Least squares means of the effects of trace mineral concentration and source on body weight, average daily gain and dry matter intake (Experiment 1).

Item[1]	Treatment[2]					*P* <			
	NC	NRC	CNI	CNO	SEM[3]	TRT[4]	NC vs. Suppl	NRC vs. CN	CNI vs. CNO
Initial weight, kg	350	349	351	351	8.3	0.49	0.79	0.13	0.97
D35 weight[5], kg	423	421	420	416	1.9	0.005	0.02	0.02	0.15
D121 weight[5], kg	559	553	554	558	4.2	0.69	0.34	0.84	0.48
Final weight[5], kg	601	597	597	596	4.4	0.79	0.34	0.95	0.76
ADG Period 1[5], kg	2.10	2.05	1.96	1.86	0.054	0.005	0.02	0.02	0.15
ADG Period 2, kg	1.57	1.53	1.56	1.65	0.036	0.19	0.85	0.13	0.13
ADG Period 3[5], kg	1.29	1.29	1.34	1.17	0.078	0.44	0.83	0.68	0.12
ADG, d 0 – 154, kg	1.63	1.60	1.61	1.59	0.028	0.79	0.34	0.96	0.76
DMI Period 1, kg	7.66	7.69	7.62	7.48	0.18	0.82	0.74	0.51	0.56
DMI Period 2[5], kg	9.63	9.83	9.82	9.84	0.17	0.61	0.19	1.00	0.96
DMI Period 3, kg	9.37	9.37	9.62	9.53	0.25	0.87	0.63	0.51	0.81
DMI, d 0 – 154, kg	9.12	9.25	9.27	9.24	0.142	0.78	0.32	0.97	0.83

[1] ADG = Average daily gain. DMI = Dry matter intake. Period 1 = d 0 – 35, Period 2 = d 36 – 120, Period 3 = d 121 – 154.
[2] NC = Negative Control, no supplemented trace minerals; NRC = National Research Council, 2000 recommended supplemented concentrations of copper, zinc, manganese, iodine, selenium, and cobalt trace minerals; CNI = Consulting nutritionist survey (Vasconcelos and Galyean, 2007) mode concentrations of copper, zinc, manganese, iodine, selenium, and cobalt supplemented to the basal diet using inorganic sources; CNO = Consulting nutritionist survey (Vasconcelos and Galyean, 2007) mode concentrations of copper, zinc, manganese, iodine, selenium, and cobalt supplemented to the basal diet using were added to the basal diet with 66.6% from inorganic sources and 33.4% from organic sources.
[3] Standard error of the mean.
[4] TRT = probability for treatment. NC vs. Suppl = Negative control vs. supplemented (NRC, CNI, and CNO). NRC vs. CN = NRC vs. consulting nutritionist (both CNI and CNO). CNI vs. CNO = Consulting nutritionist inorganic vs. consulting nutritionist organic.
[5] Initial weight used as a covariant, $P < 0.10$.

Table 6. Least squares means of the effects of trace mineral concentration and source on feed efficiency and gain efficiency (Experiment 1).

	Treatment[2]					*P* <			
Item[1]	NC	NRC	CNI	CNO	SEM[3]	TRT[4]	NC vs. Suppl.	NRC vs. CN	CNI vs. CNO
FG Period 1[5]	3.67	3.78	3.93	4.06	0.098	0.04	0.03	0.08	0.36
FG Period 2[5]	6.12	6.40	6.29	6.01	0.106	0.06	0.36	0.06	0.07
FG Period 3	7.54	7.55	7.34	8.33	0.433	0.31	0.66	0.55	0.09
FG, d 0 – 154[5]	5.59	5.79	5.78	5.82	0.084	0.24	0.05	0.87	0.75
GF Period 1[5]	0.275	0.268	0.258	0.250	0.0063	0.04	0.04	0.07	0.37
GF Period 2[5]	0.164	0.156	0.160	0.167	0.0029	0.05	0.45	0.05	0.06
GF Period 3	0.137	0.137	0.139	0.123	0.0070	0.22	0.57	0.37	0.07
GF, d 0 – 154[5]	0.179	0.174	0.174	0.173	0.0025	0.26	0.05	0.91	0.84

[1] FG = Feed to gain ratio; GF = Gain to feed ratio. Period 1 = d 0 – 35, Period 2 = d 36 – 120, Period 3 = d 121 – 154.
[2] NC = Negative Control, no supplemented trace minerals; NRC = National Research Council, 2000 recommended supplemented concentrations of copper, zinc, manganese, iodine, selenium, and cobalt trace minerals; CNI = Consulting nutritionist survey (Vasconcelos and Galyean, 2007) mode concentrations of copper, zinc, manganese, iodine, selenium, and cobalt supplemented to the basal diet using inorganic sources; CNO = Consulting nutritionist survey (Vasconcelos and Galyean, 2007) mode concentrations of copper, zinc, manganese, iodine, selenium, and cobalt supplemented to the basal diet using were added to the basal diet with 66.6% from inorganic sources and 33.4% from organic sources.
[3] Standard error of the mean.
[4] TRT = probability for treatment. NC vs. Suppl = Negative control vs. supplemented (NRC, CNI, and CNO). NRC vs. CN = NRC vs. consulting nutritionist (both CNI and CNO). CNI vs. CNO = Consulting nutritionist inorganic vs. consulting nutritionist organic.
[5] Initial weight used as a covariant, $P < 0.10$.

Table 7. Least squares means of the effects of trace mineral concentration and source on calculated net energy recovery for feedlot steers (Experiment 1).

	Treatment[2]					*P* <			
Item[1]	NC	NRC	CNI	CNO	SEM[3]	TRT[4]	NC vs. Suppl.	NRC vs. CN	CNI vs. CNO
NEm Period 1[5]	2.55	2.52	2.45	2.41	0.04	0.12	0.08	0.12	0.51
NEm Period 2[5]	2.06	1.99	2.01	2.07	0.02	0.07	0.16	0.12	0.08
NEm Period 3	2.11	2.11	2.09	1.98	0.05	0.16	0.40	0.20	0.09
NEm, d 0 – 154	2.16	2.11	2.11	2.10	0.02	0.23	0.05	0.83	0.91
NEg Period 1[5]	1.83	1.80	1.74	1.71	0.04	0.12	0.08	0.12	0.51
NEg Period 2	1.40	1.34	1.35	1.40	0.02	0.07	0.16	0.12	0.08
NEg Period 3	1.44	1.44	1.43	1.32	0.05	0.16	0.40	0.20	0.09
NEg, d 0 – 154	1.48	1.44	1.44	1.43	0.02	0.23	0.05	0.83	0.91

[1] NEm = Net energy for maintenance recovery, Mcal/kg DM and NEg = Net energy for gain recovery, Mcal/kg DM. Period 1 = d 0 – 35, Period 2 = d 36 – 120, Period 3 = d 121 – 154.

[2] NC = Negative Control, no supplemented trace minerals; NRC = National Research Council, 2000 recommended supplemented concentrations of copper, zinc, manganese, iodine, selenium, and cobalt trace minerals; CNI = Consulting nutritionist survey (Vasconcelos and Galyean, 2007) mode concentrations of copper, zinc, manganese, iodine, selenium, and cobalt supplemented to the basal diet using inorganic sources; CNO = Consulting nutritionist survey (Vasconcelos and Galyean, 2007) mode concentrations of copper, zinc, manganese, iodine, selenium, and cobalt supplemented to the basal diet using were added to the basal diet with 66.6% from inorganic sources and 33.4% from organic sources.

[3] Standard error of the mean.

[4] TRT = probability for treatment. NC vs. Suppl = Negative control vs. supplemented (NRC, CNI, and CNO). NRC vs. CN = NRC vs. consulting nutritionist (both CNI and CNO). CNI vs. CNO = Consulting nutritionist inorganic vs. consulting nutritionist organic.

[5] Initial weight used as a covariant, $P < 0.10$.

Table 8. Least squares means of the effects of trace mineral concentration and source on carcass merit (Experiment 1).

	Treatment[2]					*P* <			
Item[1]	NC	NRC	CNI	CNO	SEM[3]	TRT[4]	NC vs. Suppl.	NRC vs. CN	CNI vs. CNO
HCW[5], kg	383.6	377.5	381.5	378.9	2.89	0.46	0.21	0.45	0.53
Hot carcass weight distribution									
272 – 430 kg	88.56	93.85	91.75	96.96	2.962	0.18	0.07	0.71	0.15
431 – 453 kg	6.29	3.50	3.50	1.71	2.192	0.38	0.12	0.60	0.42
≥ 454 kg[6]	1.1	1.1	0.0	0.0					
DP	63.74	63.26	63.78	63.63	0.224	0.35	0.48	0.12	0.63
Fat depth, cm	1.30	1.32	1.27	1.27	0.043	0.70	0.58	0.30	0.94
KPH	2.49	2.50	2.45	2.48	0.041	0.79	0.79	0.40	0.61
REA, sq. cm	91.48	88.90	91.35	90.71	0.475	0.41	0.41	0.16	0.70
Muscling	0.238	0.236	0.240	0.240	0.0031	0.78	0.84	0.31	0.99
Calc. YG[7]	3.00	3.07	2.92	2.93	0.082	0.50	0.80	0.14	0.91
USDA Yield Grade distribution[8]									
YG 1 & 2	43.32	42.21	52.23	47.77	5.439	0.51	0.51	0.24	0.56
YG 3	41.11	43.33	37.78	40.00	5.171	0.89	0.92	0.62	0.82
YG 4 & 5	10.00	11.11	4.44	6.67	2.819	0.60	0.53	0.35	0.64
Marbling	445.8	431.1	437.1	412.3	12.52	0.26	0.18	0.67	0.16
Rel. Marb.	0.138	-0.129	0.135	-0.148	0.1190	0.17	0.19	0.41	0.11
Lean Mat.	A61	A66	A62	A60	2.02	0.30	0.58	0.10	0.50
Skel. Mat.	A60	A61	A62	A60	1.92	0.94	0.81	0.93	0.65
Overall Mat.	A61	A64	A63	A60	2.12	0.53	0.51	0.27	0.47
Quality grade	9.9	9.8	9.8	9.6	0.14	0.50	0.27	0.88	0.30
USDA Quality Grade distribution[8]									
≥ Avg. CH	27.71	18.82	22.15	15.49	4.454	0.23	0.08	0.97	0.27
≥ Low CH	57.79	51.11	60.02	45.54	5.459	0.20	0.38	0.80	0.07
Select	34.40	44.43	27.72	50.00	5.312	0.02	0.33	0.34	0.005
Standard	4.24	3.17	7.46	3.17	2.269	0.46	1.00	0.52	0.22
Liver absc.[9]	16.66	9.99	9.99	15.55	3.561	0.39	0.23	0.55	0.28

[1] HCW = Hot carcass weight; DP = Dressing percentage; KPH = Kidney, pelvic, and heart fat, %; REA = Ribeye area; Muscling = Ribeye area per kg carcass weight; Calc. YG = Yield grade calculated from carcass measurements; Marbling score units, 400 = Small[00], 500 = modest[00]; Relative marbling = [(Individual marbling – average marbling)/(standard deviation for marbling)] – [(Individual YG – average YG)/(standard deviation for YG)]. Values > 0 indicate that marbling score is relatively high compared with yield grade. Values < 0 indicate that marbling score is relatively low compared with yield grade; Quality grade: 10 = Select, 11 = low Choice, 12 = average Choice, 13 = Prime.

[2] NC = Negative Control, no supplemented trace minerals; NRC = National Research Council, 2000 recommended supplemented concentrations of copper, zinc, manganese, iodine, selenium, and cobalt trace minerals; CNI = Consulting nutritionist survey (Vasconcelos and Galyean, 2007) mode concentrations of copper, zinc, manganese, iodine, selenium, and cobalt supplemented to the basal diet using inorganic sources; CNO = Consulting nutritionist survey (Vasconcelos and Galyean, 2007) mode concentrations of copper, zinc, manganese, iodine, selenium, and cobalt supplemented to the basal diet using were added to the basal diet with 66.6% from inorganic sources and 33.4% from organic sources.

[3] Standard error of the mean.

[4] TRT = probability for treatment. NC vs. Suppl = Negative control vs. supplemented (NRC, CNI, and CNO). NRC vs. CN = NRC vs. consulting nutritionist (both CNI and CNO). CNI vs. CNO = Consulting nutritionist inorganic vs. consulting nutritionist organic.

[5] Initial weight used as a covariant, P < 0.10.

[6] Convergence criteria not met using PROC GLIMMIX. Results shown as the percentage of individual carcasses for each treatment in a specific category.

[7] USDA Yield Grade calculated from carcass data

[8] Likelihood that an individual carcass within a pen qualifies for a specific category.

[9] Likelihood of an individual liver within a pen of showing signs of liver abscesses.

Table 9. Least squares means of the effects of trace mineral concentration and source on liver mineral concentrations (Experiment 1).

	Treatment[2]					*P* <			
Item[1]	NC	NRC	CNI	CNO	SEM[3]	TRT[4]	NC vs. Suppl.	NRC vs. CN	CNI vs. CNO
Cobalt	0.40	0.40	0.39	0.38	0.015	0.60	0.32	0.43	0.61
Copper	172.78	166.80	214.70	178.06	13.815	0.08	0.39	0.09	0.07
Manganese	13.47	13.74	13.29	12.95	0.454	0.64	0.78	0.26	0.59
Zinc	127.68	129.70	134.70	128.57	3.016	0.37	0.35	0.60	0.16

[1] Trace mineral concentration in mg/kg on a dry matter basis.
[2] NC = Negative Control, no supplemented trace minerals; NRC = National Research Council, 2000 recommended supplemented concentrations of copper, zinc, manganese, iodine, selenium, and cobalt trace minerals; CNI = Consulting nutritionist survey (Vasconcelos and Galyean, 2007) mode concentrations of copper, zinc, manganese, iodine, selenium, and cobalt supplemented to the basal diet using inorganic sources; CNO = Consulting nutritionist survey (Vasconcelos and Galyean, 2007) mode concentrations of copper, zinc, manganese, iodine, selenium, and cobalt supplemented to the basal diet using were added to the basal diet with 66.6% from inorganic sources and 33.4% from organic sources.
[3] Standard error of the mean.
[4] TRT = probability for treatment. NC vs. Suppl = Negative control vs. supplemented (NRC, CNI, and CNO). NRC vs. CN = NRC vs. consulting nutritionist (both CNI and CNO). CNI vs. CNO = Consulting nutritionist inorganic vs. consulting nutritionist organic.

Table 10. Calculated costs of trace minerals for all treatments including inorganic and organic forms.

	Treatment[2]			
Item[1]	NC	NRC	CNI	CNO
Zinc	$0	$0.25	$0.84	$3.04
Copper	$0	$0.19	$0.38	$0.90
Manganese	$0	$0.13	$0.33	$1.02
Cobalt	$0	$0.02	$0.03	$0.03
Iodine	$0	$0.06	$0.06	$0.06
Selenium	$0	$0.07	$0.14	$0.14
Total $/hd/154d	$0	$0.72	$1.79	$5.20

[1] Price calculated per head for entire experiment (154 days on feed).
[2] NC = Negative Control, no supplemented trace minerals; NRC = National Research Council, 2000 recommended supplemented concentrations of copper, zinc, manganese, iodine, selenium, and cobalt trace minerals; CNI = Consulting nutritionist survey (Vasconcelos and Galyean, 2007) mode concentrations of copper, zinc, manganese, iodine, selenium, and cobalt supplemented to the basal diet using inorganic sources; CNO = Consulting nutritionist survey (Vasconcelos and Galyean, 2007) mode concentrations of copper, zinc, manganese, iodine, selenium, and cobalt supplemented to the basal diet using were added to the basal diet with 66.6% from inorganic sources and 33.4% from organic sources.

Table 11. Least square means for trace mineral concentrations of supernatant from simulated rumen and abomasal digestion conditions (Experiment 2) [1].

	Treatment[2]					*P* <			
Item	NC	NRC	CNI	CNO	SEM[3]	TRT[4]	NC vs. Suppl	NRC vs. CN	CNI vs. CNO
Cobalt, rumen	2.91	2.16	2.56	2.30	0.278	0.27	0.09	0.45	0.53
Cobalt, abomasum	1.36	1.23	0.75	1.12	0.542	0.44	0.31	0.39	0.35
Copper, rumen	6.37	4.91	6.60	5.80	0.767	0.42	0.52	0.18	0.46
Copper, abomasum	4.97	5.20	3.69	5.52	1.207	0.74	0.91	0.70	0.30
Manganese, rumen	31.72	24.41	30.55	27.82	5.329	0.78	0.52	0.47	0.72
Manganese, abomasum	44.17	47.15	41.40	43.60	7.997	0.97	0.99	0.64	0.85
Zinc, rumen	6.48	6.57	6.76	6.51	1.141	1.00	0.93	0.97	0.89
Zinc, abomasum	29.06	27.91	20.10	32.33	7.123	0.68	0.78	0.85	0.25

[1] Trace mineral expressed as µg of trace mineral released · g DMD^{-1}.

[2] NC = Negative Control, no supplemented trace minerals; NRC = National Research Council, 2000 recommended supplemented concentrations of copper, zinc, manganese, iodine, selenium, and cobalt trace minerals; CNI = Consulting nutritionist survey (Vasconcelos and Galyean, 2007) mode concentrations of copper, zinc, manganese, iodine, selenium, and cobalt supplemented to the basal diet using inorganic sources; CNO = Consulting nutritionist survey (Vasconcelos and Galyean, 2007) mode concentrations of copper, zinc, manganese, iodine, selenium, and cobalt supplemented to the basal diet using were added to the basal diet with 66.6% from inorganic sources and 33.4% from organic sources.

[3] Standard error of the mean.

[4] TRT = probability for treatment. NC vs. Suppl = Negative control vs. supplemented (NRC, CNI, and CNO). NRC vs. CN = NRC vs. consulting nutritionist (both CNI and CNO). CNI vs. CNO = Consulting nutritionist inorganic vs. consulting nutritionist organic.

Table 12. Least squares means of the effects of trace mineral concentration and source dry matter disappearance and percent of copper and zinc released under simulated ruminal and abomasal conditions (Experiment 2).

	Treatment[2]					*P* <			
Item[1]	NC	NRC	CNI	CNO	SEM[3]	TRT[4]	NC vs. Suppl	NRC vs. CN	CNI vs. CNO
DMD[5]	71.41	72.72	77.03	71.29	2.920	0.49	0.59	0.67	0.19
Copper	52.20	57.51	63.72	67.81	3.377	0.02	0.008	0.05	0.42
Zinc	74.73	79.57	86.10	86.45	1.976	0.0004	0.0003	0.008	0.91

[1] Trace mineral expressed as a % released, when DMD was used as a covariate.
[2] NC = Negative Control, no supplemented trace minerals; NRC = National Research Council, 2000 recommended supplemented concentrations of copper, zinc, manganese, iodine, selenium, and cobalt trace minerals; CNI = Consulting nutritionist survey (Vasconcelos and Galyean, 2007) mode concentrations of copper, zinc, manganese, iodine, selenium, and cobalt supplemented to the basal diet using inorganic sources; CNO = Consulting nutritionist survey (Vasconcelos and Galyean, 2007) mode concentrations of copper, zinc, manganese, iodine, selenium, and cobalt supplemented to the basal diet using were added to the basal diet with 66.6% from inorganic sources and 33.4% from organic sources.
[3] Standard error of the mean.
[4] TRT = probability for treatment. NC vs. Suppl = Negative control vs. supplemented (NRC, CNI, and CNO). NRC vs. CN = NRC vs. consulting nutritionist (both CNI and CNO). CNI vs. CNO = Consulting nutritionist inorganic vs. consulting nutritionist organic.
[5] Dry matter disappearance.

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Appendix A

Table 13. Unadjusted raw pen means showing the effect of trace mineral concentration and source on body weight, average daily gain and dry matter intake.

	Treatment[2]			
Item[1]	NC	NRC	CNI	CNO
Initial weight, kg	349.9 ± 8.12	348.8 ± 8.05	350.9 ± 8.22	351.0 ± 8.81
D35 weight[5], kg	423.4 ± 6.98	421.0 ± 7.41	419.4 ± 6.77	416.2 ± 7.25
D121 weight[5], kg	558.7 ± 8.82	553.0 ± 9.00	553.9 ± 7.14	557.7 ± 8.07
Final weight[5], kg	601.2 ± 9.81	595.5 ± 10.14	598.3 ± 9.43	596.5 ± 8.57
ADG Period 1[5], kg	2.10 ± 0.005	2.06 ± 0.073	1.96 ± 0.067	1.86 ± 0.069
ADG Period 2, kg	1.57 ± 0.035	1.54 ± 0.033	1.56 ± 0.025	1.65 ± 0.048
ADG Period 3[5], kg	1.29 ± 0.090	1.29 ± 0.093	1.34 ± 0.084	1.17 ± 0.073
ADG, d 0 – 154, kg	1.63 ± 0.015	1.60 ± 0.033	1.61 ± 0.022	1.59 ± 0.037
DMI Period 1, kg	7.66 ± 0.169	7.69 ± 0.220	7.62 ± 0.156	7.49 ± 0.163
DMI Period 2[5], kg	9.62 ± 0.218	9.81 ± 0.212	9.84 ± 0.168	9.85 ± 0.186
DMI Period 3, kg	9.36 ± 0.251	9.35 ± 0.372	9.63 ± 0.227	9.54 ± 0.206
DMI, d 0 – 154, kg	9.12 ± 0.167	9.23 ± 0.192	9.28 ± 0.157	9.25 ± 0.140

[1] Pen means ± standard error of the mean. FG = Feed to gain ratio; GF = Gain to feed ratio. Period 1 = d 0 – 35, Period 2 = d 36 – 120, Period 3 = d 121 – 154.

[2] NC = Negative Control, no supplemented trace minerals; NRC = National Research Council, 2000 recommended supplemented concentrations of copper, zinc, manganese, iodine, selenium, and cobalt trace minerals; CNI = Consulting nutritionist survey (Vasconcelos and Galyean, 2007) mode concentrations of copper, zinc, manganese, iodine, selenium, and cobalt supplemented to the basal diet using inorganic sources; CNO = Consulting nutritionist survey (Vasconcelos and Galyean, 2007) mode concentrations of copper, zinc, manganese, iodine, selenium, and cobalt supplemented to the basal diet using were added to the basal diet with 66.6% from inorganic sources and 33.4% from organic sources.

Table 14. Unadjusted pen means and standard errors showing the effect of trace mineral concentration and source on feed:gain and gain:feed ratio.

	Treatment[2]			
Item[1]	NC	NRC	CNI	CNO
FG Period 1	3.66 ± 0.10	3.78 ± 0.14	3.94 ± 0.17	4.07 ± 0.16
FG Period 2	6.12 ± 0.09	6.40 ± 0.07	6.30 ± 0.13	6.01 ± 0.16
FG Period 3	7.53 ± 0.45	7.55 ± 0.55	7.34 ± 0.33	8.33 ± 0.38
FG, d 0 – 154	5.59 ± 0.07	5.77 ± 0.10	5.78 ± 0.09	5.82 ± 0.13
GF Period 1	0.27 ± 0.007	0.27 ± 0.010	0.26 ± 0.010	0.25 ± 0.010
GF Period 2	0.16 ± 0.002	0.16 ± 0.001	0.16 ± 0.003	0.17 ± 0.004
GF Period 3	0.14 ± 0.007	0.14 ± 0.008	0.14 ± 0.006	0.12 ± 0.006
GF, d 0 – 154	0.18 ± 0.002	0.17 ± 0.002	0.17 ± 0.003	0.17 ± 0.004

[1] Pen means ± standard error of the mean. FG = Feed to gain ratio; GF = Gain to feed ratio. Period 1 = d 0 – 35, Period 2 = d 36 – 120, Period 3 = d 121 – 154.

[2] NC = Negative Control, no supplemented trace minerals; NRC = National Research Council, 2000 recommended supplemented concentrations of copper, zinc, manganese, iodine, selenium, and cobalt trace minerals; CNI = Consulting nutritionist survey (Vasconcelos and Galyean, 2007) mode concentrations of copper, zinc, manganese, iodine, selenium, and cobalt supplemented to the basal diet using inorganic sources; CNO = Consulting nutritionist survey (Vasconcelos and Galyean, 2007) mode concentrations of copper, zinc, manganese, iodine, selenium, and cobalt supplemented to the basal diet using were added to the basal diet with 66.6% from inorganic sources and 33.4% from organic sources.

Table 15. Unadjusted pen means and standard errors showing the effects of trace mineral concentration and source on carcass merit.

	Treatments[2]			
Item[1]	NC	NRC	CNI	CNO
HCW[3], kg	383.4 ± 6.25	376.7 ± 6.35	381.9 ± 6.44	379.5 ± 5.38
Hot carcass weight distribution[4]				
272 – 430 kg	90.8	94.4	95.3	97.8
431 – 453 kg	8.0	4.5	4.7	2.2
≥ 454 kg	1.1	1.1	0.0	0.0
Dressing percent	63.74± 0.146	63.26 ± 0.308	63.78 ± 0.214	63.63 ± 0.197
Fat depth, cm	1.30 ± 0.048	1.32 ± 0.051	1.27 ± 0.046	1.27 ± 0.025
Ribeye area, sq. cm	91.48 ± 1.271	88.77 ± 1.168	91.42 ± 1.619	90.77 ± 1.394
Muscling[5]	0.238 ± 0.0057	0.236 ± 0.0028	0.240 ± 0.0023	0.240 ± 0.0017
KPH[6]	2.49 ± 0.039	2.50 ± 0.043	2.45 ± 0.042	2.47 ± 0.041
Calc. YG[7], units	3.00 ± 0.117	3.07 ± 0.090	2.92 ± 0.056	2.93 ± 0.044
USDA Yield Grade distribution[4]				
YG 1 and 2	45.9	43.7	55.3	50.6
YG 3	43.5	44.8	40.0	32.4
YG 4 and 5	10.6	11.5	4.7	7.1
Marbling[8], units	445.8 ± 17.23	431.1 ± 8.62	437.1 ± 10.67	412.3 ± 11.29
Rel. marbling[9]	0.140 ± 0.1221	-0.129 ± 0.1147	0.135 ± 0.1350	-0.148 ± 0.1018
Lean maturity	A61 ± 0.8	A66 ± 3.2	A62 ± 1.8	A60 ± 1.47
Skel. Maturity	A60 ± 1.1	A61 ± 2.3	A62 ± 2.6	A60 ± 1.2
Maturity	A60 ± 1.0	A64 ± 3.2	A63 ± 2.3	A60 ± 1.1
Quality grade[10]	9.9 ± 0.19	9.8 ± 0.092	9.8 ± 0.12	9.6 ± 0.14
USDA Quality grade distribution[4]				
≥ Low Choice	59.8	51.7	62.8	46.1
≥ Avg. Choice	28.7	19.1	23.2	15.7
Select	35.6	44.9	29.1	50.6
Standard	4.6	3.4	8.1	3.4
Liver abscess rate[11]	17.2	10.1	10.5	15.9

[1] Pen means ± standard error of the mean.
[2] NC = Negative Control, no supplemented trace minerals; NRC = National Research Council, 2000 recommended supplemented concentrations of copper, zinc, manganese, iodine, selenium, and cobalt trace minerals; CNI = Consulting nutritionist survey (Vasconcelos and Galyean, 2007) mode concentrations of copper, zinc, manganese, iodine, selenium, and cobalt supplemented to the basal diet using inorganic sources; CNO = Consulting nutritionist survey (Vasconcelos and Galyean, 2007) mode concentrations of copper, zinc, manganese, iodine, selenium, and cobalt supplemented to the basal diet using were added to the basal diet with 66.6% from inorganic sources and 33.4% from organic sources.
[3] Hot carcass weight.
[4] Percentage of individual carcasses qualifying for each category.
[5] Ribeye area per kg HCW.
[6] Kidney, pelvic, and heart fat, %.
[7] USDA Yield Grade calculated from carcass data.
[8] Marbling score units: 400 = Small[00]; 500 = Modest[00].
[9] Relative marbling = [(Ind. marb. – Avg. marb.)/(Std. dev for marb.)] – [(Ind. YG – Avg. YG)/(Std. dev. for YG)]. Values > 0 indicate high marbling score relative to YG. Values < 0 indicate low marbling score relative to YG.
[10] Quality grade: 10 = Select, 11 = low Choice, 12 = average Choice, 13 = Prime.
[11] Percentage of individual livers showing signs of liver abscesses.

Table 16. Unadjusted pen means and standard error of the means for effect of trace mineral concentration and source for liver concentration of trace minerals.

	Treatment[2]			
Item[1]	NC	NRC	CNI	CNO
Cobalt	0.40 ± 0.014	0.37 ± 0.014	0.39 ± 0.014	0.38 ± 0.017
Copper	172.78 ± 10.371	166.80 ± 11.377	214.70 ± 19.551	178.06 ± 12.011
Manganese	13.47 ± 0.338	13.74 ± 0.371	13.29 ± 0.633	12.95 ± 0.416
Zinc	127.68 ± 1.928	129.70 ± 2.793	134.70 ± 2.585	128.58 ± 4.264

[1] Trace mineral concentration in mg/kg on a dry matter basis.
[2] NC = Negative Control, no supplemented trace minerals; NRC = National Research Council, 2000 recommended supplemented concentrations of copper, zinc, manganese, iodine, selenium, and cobalt trace minerals; CNI = Consulting nutritionist survey (Vasconcelos and Galyean, 2007) mode concentrations of copper, zinc, manganese, iodine, selenium, and cobalt supplemented to the basal diet using inorganic sources; CNO = Consulting nutritionist survey (Vasconcelos and Galyean, 2007) mode concentrations of copper, zinc, manganese, iodine, selenium, and cobalt supplemented to the basal diet using were added to the basal diet with 66.6% from inorganic sources and 33.4% from organic sources.

Table 17. Unadjusted means and standard error of the means for actual mineral concentrations for the total mixed ration.

	Treatment[2]			
Item[1]	NC	NRC	CNI	CNO
Cobalt	0.12 ± 0.06	0.10 ± 0.049	0.26 ± 0.076	0.18 ± 0.080
Copper	7.65 ± 0.98	14.92 ± 1.208	25.14 ± 2.272	23.49 ± 2.541
Manganese	27.7 ± 1.86	40.66 ± 2.750	69.93 ± 5.421	66.88 ± 4.681
Zinc	50.5 ± 5.00	65.21 ± 9.206	151.01 ± 16.581	149.11 ± 10.260

[1] Trace mineral concentration in mg/kg on a dry matter basis.
[2] NC = Negative Control, no supplemented trace minerals; NRC = National Research Council, 2000 recommended supplemented concentrations of copper, zinc, manganese, iodine, selenium, and cobalt trace minerals; CNI = Consulting nutritionist survey (Vasconcelos and Galyean, 2007) mode concentrations of copper, zinc, manganese, iodine, selenium, and cobalt supplemented to the basal diet using inorganic sources; CNO = Consulting nutritionist survey (Vasconcelos and Galyean, 2007) mode concentrations of copper, zinc, manganese, iodine, selenium, and cobalt supplemented to the basal diet using were added to the basal diet with 66.6% from inorganic sources and 33.4% from organic sources.

Table 18. Least square means of trace mineral concentrations for the total mixed ration.

	Treatment[2]					*P* <			
Item[1]	NC	NRC	CNI	CNO	SEM[3]	TRT[4]	NC vs. Suppl.	NRC vs. CN	CNI vs. CNO
Cobalt	0.14	0.10	0.29	0.20	0.074	0.28	0.52	0.11	0.37
Copper	7.16	14.14	24.82	22.92	2.27	0.01	0.01	0.01	0.40
Manganese	26.39	40.14	70.17	67.37	4.61	0.01	0.01	0.01	0.60
Zinc	60.96	62.53	152.31	145.93	13.61	0.01	0.01	0.01	0.68

[1] Trace mineral concentration in mg/kg on a dry matter basis.
[2] NC = Negative Control, no supplemented trace minerals; NRC = National Research Council, 2000 recommended supplemented concentrations of copper, zinc, manganese, iodine, selenium, and cobalt trace minerals; CNI = Consulting nutritionist survey (Vasconcelos and Galyean, 2007) mode concentrations of copper, zinc, manganese, iodine, selenium, and cobalt supplemented to the basal diet using inorganic sources; CNO = Consulting nutritionist survey (Vasconcelos and Galyean, 2007) mode concentrations of copper, zinc, manganese, iodine, selenium, and cobalt supplemented to the basal diet using were added to the basal diet with 66.6% from inorganic sources and 33.4% from organic sources.
[3] Standard error of the mean.
[4] TRT = probability for treatment. NC vs. Suppl = Negative control vs. supplemented (NRC, CNI, and CNO). NRC vs. CN = NRC vs. consulting nutritionist (both CNI and CNO). CNI vs. CNO = Consulting nutritionist inorganic vs. consulting nutritionist organic.

Table 19. Unadjusted means and standard error of the means for actual mineral concentrations for the supplement.

	Treatment[2]			
Item[1]	NC	NRC	CNI	CNO
Cobalt	1.5 ± 0.43	3.1 ± 0.37	6.3 ± 0.61	6.8 ± 0.75
Copper	99.8 ± 28.08	332.9 ± 36.48	685.8 ± 59.63	660.7 ± 68.39
Manganese	392.0 ± 149.59	763.9 ± 64.28	1680.0 ± 136.77	1657.8 ± 136.56
Zinc	743.6 ± 351.23	1045.8 ± 116.20	3584.4 ± 429.32	3977.8 ± 521.58

[1] Trace mineral concentration in mg/kg on a dry matter basis.
[2] NC = Negative Control, no supplemented trace minerals; NRC = National Research Council, 2000 recommended supplemented concentrations of copper, zinc, manganese, iodine, selenium, and cobalt trace minerals; CNI = Consulting nutritionist survey (Vasconcelos and Galyean, 2007) mode concentrations of copper, zinc, manganese, iodine, selenium, and cobalt supplemented to the basal diet using inorganic sources; CNO = Consulting nutritionist survey (Vasconcelos and Galyean, 2007) mode concentrations of copper, zinc, manganese, iodine, selenium, and cobalt supplemented to the basal diet using were added to the basal diet with 66.6% from inorganic sources and 33.4% from organic sources.

Table 20. Least square means of trace mineral concentrations for the supplement.

	Treatment[2]					*P* <			
Item[1]	NC	NRC	CNI	CNO	SEM[3]	TRT[4]	NC vs. Suppl.	NRC vs. CN	CNI vs. CNO
Cobalt	1.51	3.06	6.32	6.82	0.586	0.01	0.01	0.01	0.54
Copper	94.71	327.76	680.64	655.53	55.352	0.01	0.01	0.01	0.73
Manganese	392.00	763.89	1680.00	1657.78	126.360	0.01	0.01	0.01	0.91
Zinc	743.56	1045.78	3584.44	3977.78	385.110	0.01	0.01	0.01	0.48

[1] Trace mineral concentration in mg/kg on a dry matter basis.
[2] NC = Negative Control, no supplemented trace minerals; NRC = National Research Council, 2000 recommended supplemented concentrations of copper, zinc, manganese, iodine, selenium, and cobalt trace minerals; CNI = Consulting nutritionist survey (Vasconcelos and Galyean, 2007) mode concentrations of copper, zinc, manganese, iodine, selenium, and cobalt supplemented to the basal diet using inorganic sources; CNO = Consulting nutritionist survey (Vasconcelos and Galyean, 2007) mode concentrations of copper, zinc, manganese, iodine, selenium, and cobalt supplemented to the basal diet using were added to the basal diet with 66.6% from inorganic sources and 33.4% from organic sources.
[3] Standard error of the mean.
[4] TRT = probability for treatment. NC vs. Suppl = Negative control vs. supplemented (NRC, CNI, and CNO). NRC vs. CN = NRC vs. consulting nutritionist (both CNI and CNO). CNI vs. CNO = Consulting nutritionist inorganic vs. consulting nutritionist organic.

Table 21. Unadjusted means and standard error of the means for actual trace mineral concentrations for the premix.

	Treatment[2]			
Item[1]	NC[3]	NRC	CNI	CNO
Cobalt	0	112 ± 2	186 ± 3	194 ± 18
Copper	2.51	12,180 ± 764	24,060 ± 902	19,840 ± 1117
Manganese	7.89	22,240 ± 1686	54,200 ± 4352	43,540 ± 4099
Zinc	24.2	39,780 ± 2266	111,740 ± 10945	103,540 ± 10581

[1] Trace mineral concentration in mg/kg on a dry matter basis.
[2] NC = Negative Control, no supplemented trace minerals; NRC = National Research Council, 2000 recommended supplemented concentrations of copper, zinc, manganese, iodine, selenium, and cobalt trace minerals; CNI = Consulting nutritionist survey (Vasconcelos and Galyean, 2007) mode concentrations of copper, zinc, manganese, iodine, selenium, and cobalt supplemented to the basal diet using inorganic sources; CNO = Consulting nutritionist survey (Vasconcelos and Galyean, 2007) mode concentrations of copper, zinc, manganese, iodine, selenium, and cobalt supplemented to the basal diet using were added to the basal diet with 66.6% from inorganic sources and 33.4% from organic sources.
[3] Ground corn used in place of trace mineral, reference value in mg/kg from Table 11-1 entry 38, NRC (2000).

Table 22. Least square means of trace mineral concentrations for the premix.

	Treatment[2]					*P* <		
Item[1]	NC	NRC	CNI	CNO	SEM[3]	TRT[4]	NRC vs. CN	CNI vs. CNO
Cobalt	–	112.0	186.4	194.0	10.92	0.01	0.01	0.63
Copper	–	12180.0	24060.0	19840	939.08	0.01	0.01	0.01
Manganese	–	22240.0	54200.0	43540.0	3586.88	0.01	0.01	0.07
Zinc	–	3978.00	111740.0	103540.0	8886.46	0.01	0.01	0.50

[1] Trace mineral concentration in mg/kg on a dry matter basis.
[2] Negative Control (NC), no supplemented trace minerals. NRC 2000 recommended (NRC), recommended supplemented concentrations of copper, zinc, manganese, iodine, selenium, and cobalt trace minerals. Consulting nutritionist survey mode inorganic (CNI), supplemented concentrations of copper, zinc, manganese, iodine, selenium, and cobalt trace minerals to basal diet using inorganic sources. Consulting nutritionist survey mode organic (CNO), supplemented copper, zinc, manganese, iodine, selenium, and cobalt where concentrations of copper, zinc, and manganese were added to the basal diet with 66.6% from inorganic sources and 33.4% from organic sources.
[3] Standard error of the mean.
[4] TRT = probability for treatment. NC vs. Suppl = Negative control vs. supplemented (NRC, CNI, and CNO). NRC vs. CN = NRC vs. consulting nutritionist (both CNI and CNO). CNI vs. CNO = Consulting nutritionist inorganic vs. consulting nutritionist organic.

Table 23. Least square means for trace mineral concentrations of supernatant from simulated rumen and abomasal digestion and final pellet[1].

	Treatment[2]					*P* <			
Item	NC	NRC	CNI	CNO	SEM[3]	TRT[4]	NC vs. Suppl	NRC vs. CN	CNI vs. CNO
Cobalt, rumen[5]	0.29	0.30	0.33	0.33	0.330	0.85	0.42	0.41	0.95
Cobalt, abomasum	0.33	0.28	0.23	0.31	0.044	0.46	0.35	0.80	0.10
Cobalt, pellet	0.60	0.62	0.61	0.60	0.069	0.03	0.89	0.83	0.96
Copper, rumen[5]	1.18	1.07	1.16	1.24	0.092	0.80	0.88	0.28	0.55
Copper, abomasum	1.35	1.29	1.19	1.68	0.156	0.28	0.82	0.52	0.03
Copper, pellet[6]	7.92	10.06	13.08	12.06	0.982	0.04	0.03	0.02	0.38
0, h	4.40	7.80	9.03	8.10	1.685	0.27	0.002	0.42	0.40
6, h	6.45	9.26	8.85	12.86	1.685	0.06	0.12	0.50	0.14
12, h	9.97	11.57	15.81	15.42	1.685	0.12	0.02	0.03	0.86
24, h	10.87	11.62	18.65	11.86	1.685	0.007	0.26	0.20	0.05
Manganese, rumen[5]	6.31	6.48	7.05	7.06	0.625	0.74	0.49	0.45	1.00
Manganese, abomasum	10.80	10.69	11.62	12.46	1.049	0.20	0.52	0.38	0.51
Manganese, pellet	21.02	26.90	31.44	28.48	2.023	0.001	0.02	0.17	0.28
Zinc, rumen	1.43	1.40	1.99	1.38	0.929	0.17	0.53	0.31	0.10
Zinc, abomasum[5]	7.59	6.29	6.57	7.36	0.890	0.69	0.39	0.50	0.63
Zinc, pellet	27.76	25.53	28.40	30.86	2.191	0.18	0.87	0.12	0.40

[1] Trace mineral expressed as mg of trace mineral·kg^{-1} DM.

[2] NC = Negative Control, no supplemented trace minerals; NRC = National Research Council, 2000 recommended supplemented concentrations of copper, zinc, manganese, iodine, selenium, and cobalt trace minerals; CNI = Consulting nutritionist survey (Vasconcelos and Galyean, 2007) mode concentrations of copper, zinc, manganese, iodine, selenium, and cobalt supplemented to the basal diet using inorganic sources; CNO = Consulting nutritionist survey (Vasconcelos and Galyean, 2007) mode concentrations of copper, zinc, manganese, iodine, selenium, and cobalt supplemented to the basal diet using were added to the basal diet with 66.6% from inorganic sources and 33.4% from organic sources.

[3] Standard error of the mean.

[4] TRT = probability for treatment. NC vs. Suppl = Negative control vs. supplemented (NRC, CNI, and CNO). NRC vs. CN = NRC vs. consulting nutritionist (both CNI and CNO). CNI vs. CNO = Consulting nutritionist inorganic vs. consulting nutritionist organic.

[5] Initial trace mineral concentration for time period zero used as a covariant, $P < 0.05$.

[6] The *p*-value for a significant ($P < 0.05$) treatment by time interaction is reported.

Table 24. Least squares means, % released over 24 h period from simulated ruminal fluid.

Item[1]	Treatment[2]					*P* <			
	NC	NRC	CNI	CNO	SEM[3]	TRT[4]	NC vs. Suppl	NRC vs. CN	CNI vs. CNO
Cobalt	104.87	80.61	74.99	71.05	9.379	0.13	0.03	0.53	0.78
Copper	24.53	13.60	7.68	7.72	1.668	0.0004	0.0001	0.03	0.98
Manganese	25.39	17.95	16.68	10.51	1.962	0.005	0.002	0.11	0.06
Zinc	4.25	2.74	3.10	1.69	1.000	0.40	0.17	0.79	0.35

[1] Percentage of trace mineral released from the ruminal fluid.
[2] NC = Negative Control, no supplemented trace minerals; NRC = National Research Council, 2000 recommended supplemented concentrations of copper, zinc, manganese, iodine, selenium, and cobalt trace minerals; CNI = Consulting nutritionist survey (Vasconcelos and Galyean, 2007) mode concentrations of copper, zinc, manganese, iodine, selenium, and cobalt supplemented to the basal diet using inorganic sources; CNO = Consulting nutritionist survey (Vasconcelos and Galyean, 2007) mode concentrations of copper, zinc, manganese, iodine, selenium, and cobalt supplemented to the basal diet using were added to the basal diet with 66.6% from inorganic sources and 33.4% from organic sources.
[3] Standard error of the mean.
[4] TRT = probability for treatment. NC vs. Suppl = Negative control vs. supplemented (NRC, CNI, and CNO). NRC vs. CN = NRC vs. consulting nutritionist (both CNI and CNO). CNI vs. CNO = Consulting nutritionist inorganic vs. consulting nutritionist organic.

Table 25. Least squares means, % released over 24 h time period from simulated abomasal digestion.

Item[1]	Treatment[2]					*P* <			
	NC	NRC	CNI	CNO	SEM[3]	TRT[4]	NC vs. Suppl	NRC vs. CN	CNI vs. CNO
Cobalt	69.58	81.60	45.70	122.27	11.648	0.02	0.35	0.88	0.002
Copper	13.30	12.91	5.92	15.70	2.369	0.09	0.54	0.49	0.02
Manganese	24.90	25.59	21.44	23.38	1.860	0.45	0.53	0.20	0.49
Zinc	11.64	10.46	5.57	8.45	2.692	0.46	0.30	0.33	0.48

[1] Percentage of trace mineral released from simulated abomoasal digestion.
[2] NC = Negative Control, no supplemented trace minerals; NRC = National Research Council, 2000 recommended supplemented concentrations of copper, zinc, manganese, iodine, selenium, and cobalt trace minerals; CNI = Consulting nutritionist survey (Vasconcelos and Galyean, 2007) mode concentrations of copper, zinc, manganese, iodine, selenium, and cobalt supplemented to the basal diet using inorganic sources; CNO = Consulting nutritionist survey (Vasconcelos and Galyean, 2007) mode concentrations of copper, zinc, manganese, iodine, selenium, and cobalt supplemented to the basal diet using were added to the basal diet with 66.6% from inorganic sources and 33.4% from organic sources.
[3] Standard error of the mean.
[4] TRT = probability for treatment. NC vs. Suppl = Negative control vs. supplemented (NRC, CNI, and CNO). NRC vs. CN = NRC vs. consulting nutritionist (both CNI and CNO). CNI vs. CNO = Consulting nutritionist inorganic vs. consulting nutritionist organic.

Table 26. Least squares means, % released over 24 h time period, undigested residue (pellet).

Item[1]	Treatment[2]					*P* <			
	NC	NRC	CNI	CNO	SEM[3]	TRT[4]	NC vs. Suppl	NRC vs. CN	CNI vs. CNO
Cobalt	239.95	165.89	190.54	215.06	42.63	0.66	0.35	0.50	0.70
Copper	193.68	137.37	148.19	93.28	17.90	0.03	0.02	0.47	0.07
Manganese	69.49	50.10	66.10	39.44	6.837	0.05	0.06	0.76	0.03
Zinc	83.96	57.00	43.55	30.19	9.888	0.03	0.008	0.14	0.37

[1] Percentage of trace mineral released from pellet.
[2] NC = Negative Control, no supplemented trace minerals; NRC = National Research Council, 2000 recommended supplemented concentrations of copper, zinc, manganese, iodine, selenium, and cobalt trace minerals; CNI = Consulting nutritionist survey (Vasconcelos and Galyean, 2007) mode concentrations of copper, zinc, manganese, iodine, selenium, and cobalt supplemented to the basal diet using inorganic sources; CNO = Consulting nutritionist survey (Vasconcelos and Galyean, 2007) mode concentrations of copper, zinc, manganese, iodine, selenium, and cobalt supplemented to the basal diet using were added to the basal diet with 66.6% from inorganic sources and 33.4% from organic sources.
[3] Standard error of the mean.
[4] TRT = probability for treatment. NC vs. Suppl = Negative control vs. supplemented (NRC, CNI, and CNO). NRC vs. CN = NRC vs. consulting nutritionist (both CNI and CNO). CNI vs. CNO = Consulting nutritionist inorganic vs. consulting nutritionist organic.

Table 27. Least squares means, total % released over entire 24 h time period.

	Treatment[2]					*P* <			
Item[1]	NC	NRC	CNI	CNO	SEM[3]	TRT[4]	NC vs. Suppl	NRC vs. CN	CNI vs. CNO
Cobalt	-74.45	-62.20	-20.69	-93.32	18.216	0.11	0.48	0.82	0.03
Copper	62.17	73.50	86.40	76.58	3.880	0.02	0.006	0.14	0.12
Manganese	49.70	56.46	72.51	66.12	2.901	0.003	0.002	0.007	0.16
Zinc	84.10	86.79	91.33	89.86	3.513	0.51	0.24	0.41	0.78
DMD rumen fluid,%	40.85	42.46	40.24	40.52	0.410	0.003	0.64	0.0003	0.64
DMD pepsin,%	69.95	71.00	75.98	59.45	6.181	0.33	0.88	0.67	0.08

[1] Total trace mineral released expressed as (basal ration – (RF+PEP)/basal ration)*100.

[2] NC = Negative Control, no supplemented trace minerals; NRC = National Research Council, 2000 recommended supplemented concentrations of copper, zinc, manganese, iodine, selenium, and cobalt trace minerals; CNI = Consulting nutritionist survey (Vasconcelos and Galyean, 2007) mode concentrations of copper, zinc, manganese, iodine, selenium, and cobalt supplemented to the basal diet using inorganic sources; CNO = Consulting nutritionist survey (Vasconcelos and Galyean, 2007) mode concentrations of copper, zinc, manganese, iodine, selenium, and cobalt supplemented to the basal diet using were added to the basal diet with 66.6% from inorganic sources and 33.4% from organic sources.

[3] Standard error of the mean.

[4] TRT = probability for treatment. NC vs. Suppl = Negative control vs. supplemented (NRC, CNI, and CNO). NRC vs. CN = NRC vs. consulting nutritionist (both CNI and CNO). CNI vs. CNO = Consulting nutritionist inorganic vs. consulting nutritionist organic.

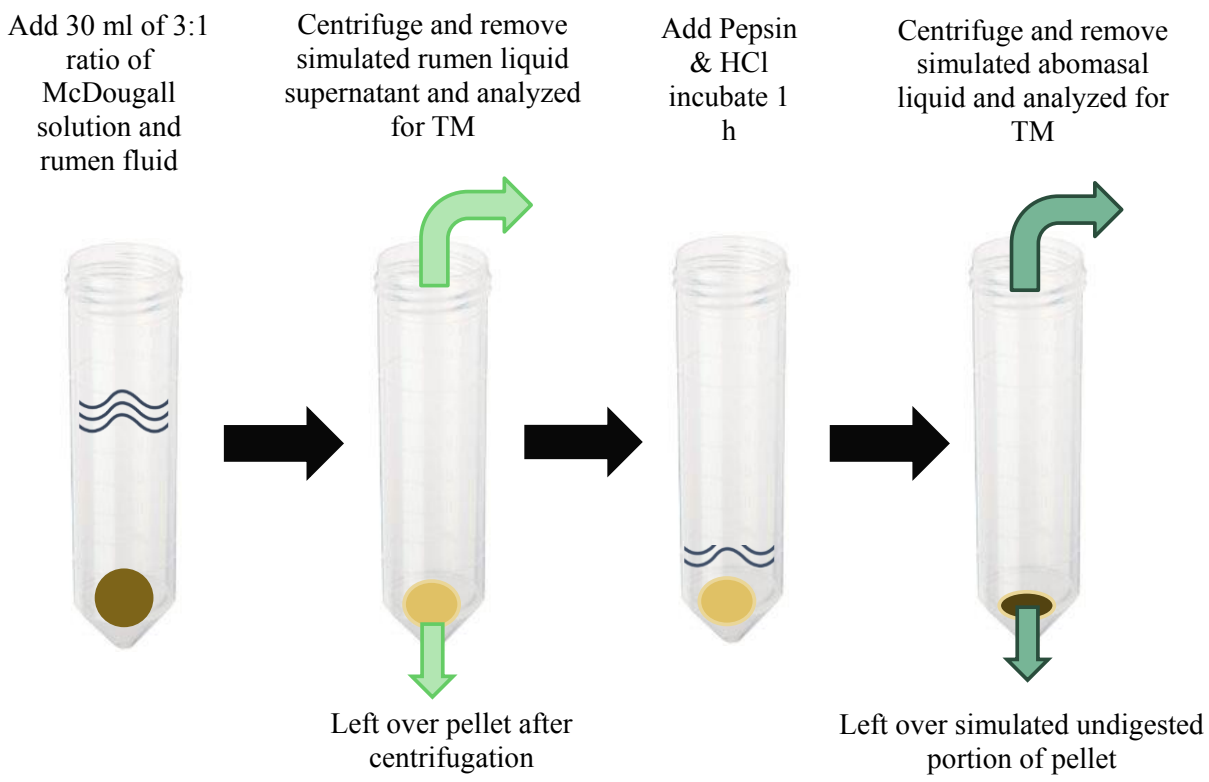

Figure 5. General design of *in vitro* Experiment (2).

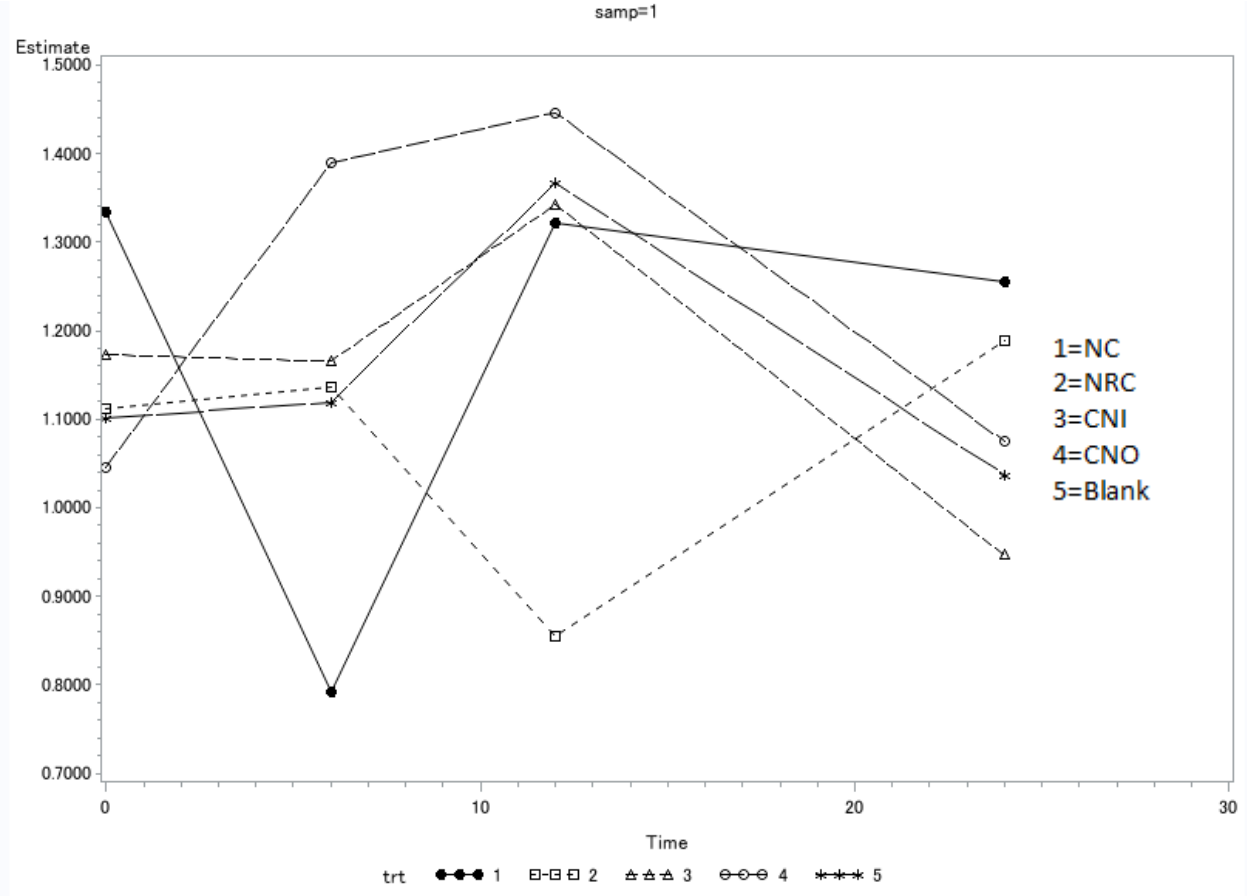

Figure 6. Least squares means estimates utilizing an initial concentration covariate for Cu mg/kg for ruminal fluid over time.

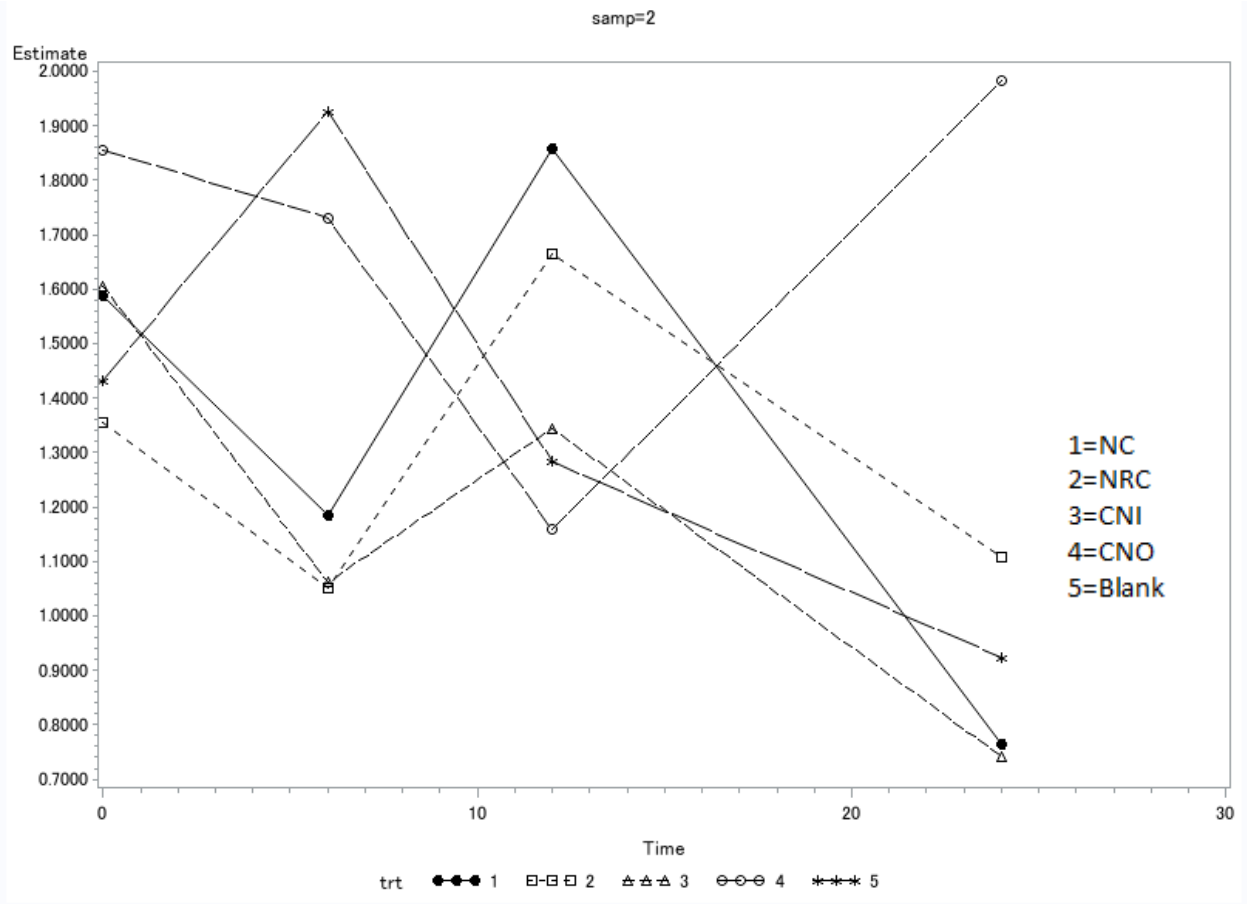

Figure 7. Least squares means estimates utilizing an initial concentration covariate for Cu mg/kg for abomasal over time.

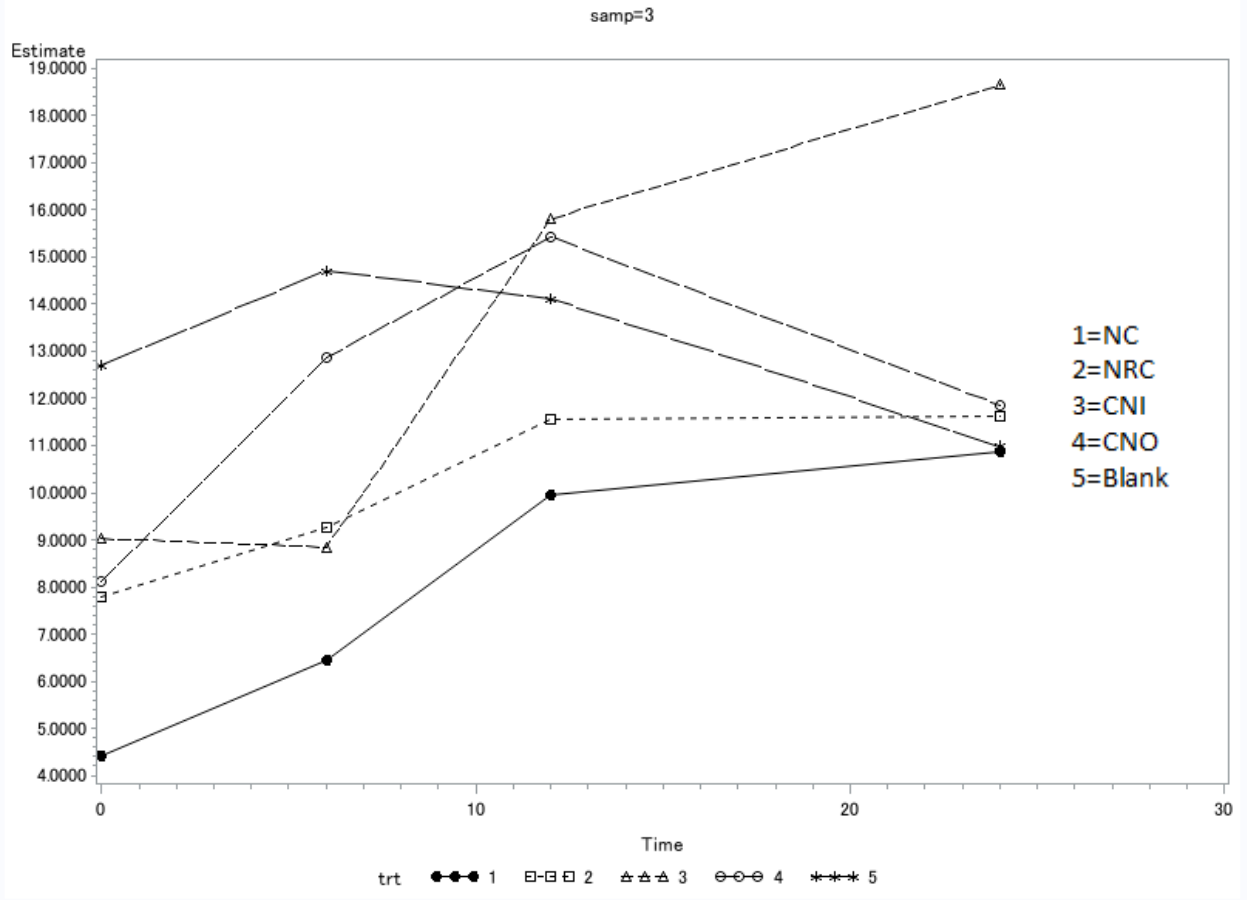

Figure 8. Least squares means estimates utilizing an initial concentration covariate for Cu mg/kg for remaining pellet over time.

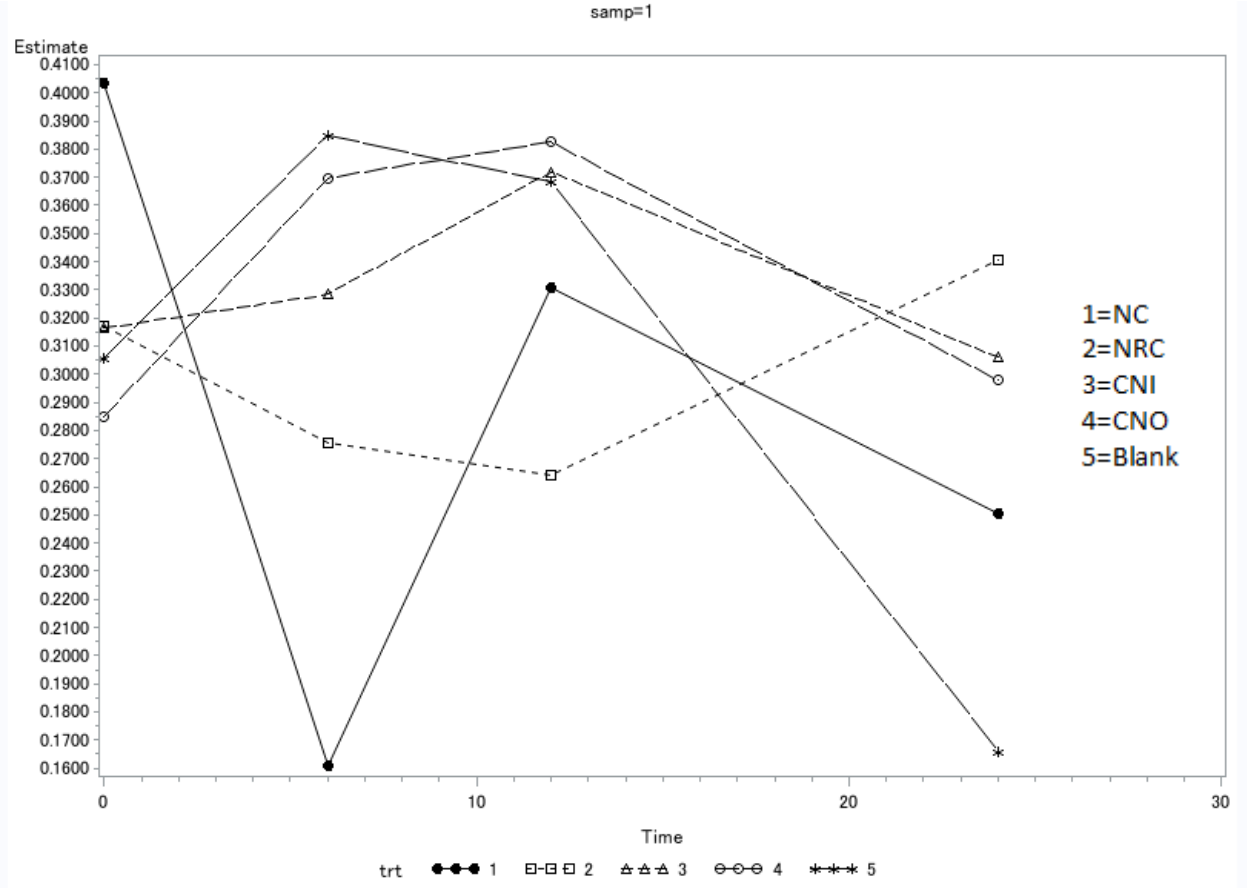

Figure 9. Least squares means estimates utilizing an initial concentration covariate for Co mg/kg for ruminal fluid over time.

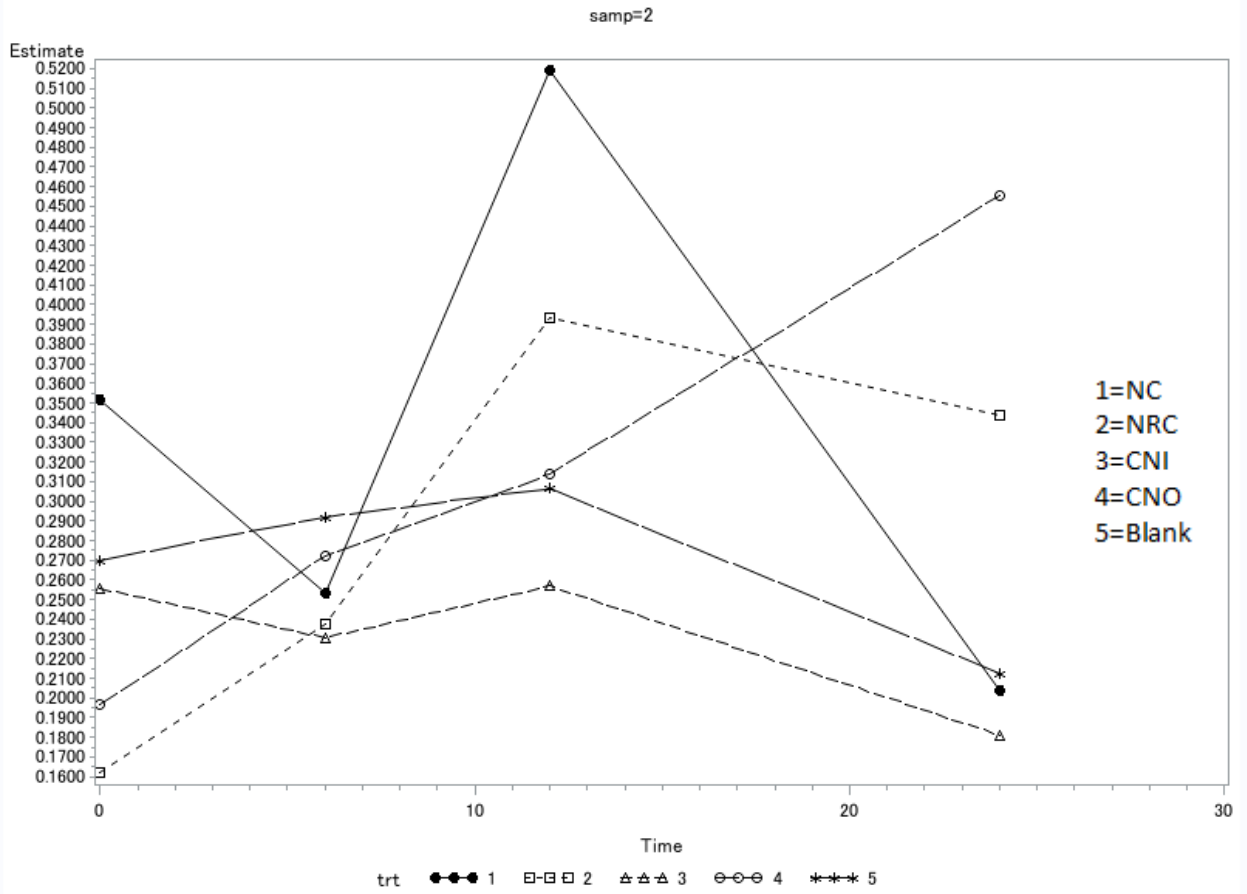

Figure 10. Least squares means estimates utilizing an initial concentration covariate for Co mg/kg for abomasal over time.

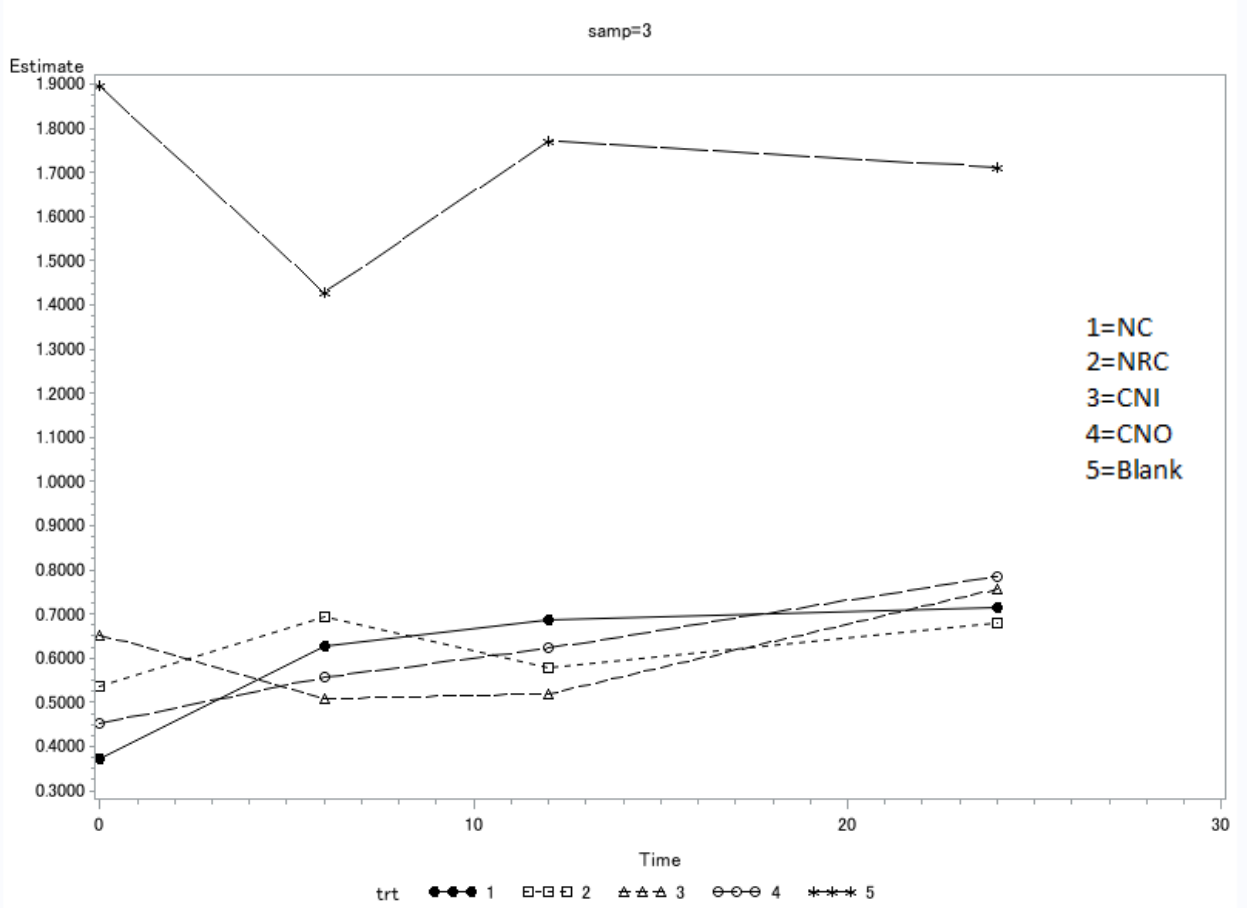

Figure 11. Least squares means estimates utilizing an initial concentration covariate for Co mg/kg for remaining pellet over time.

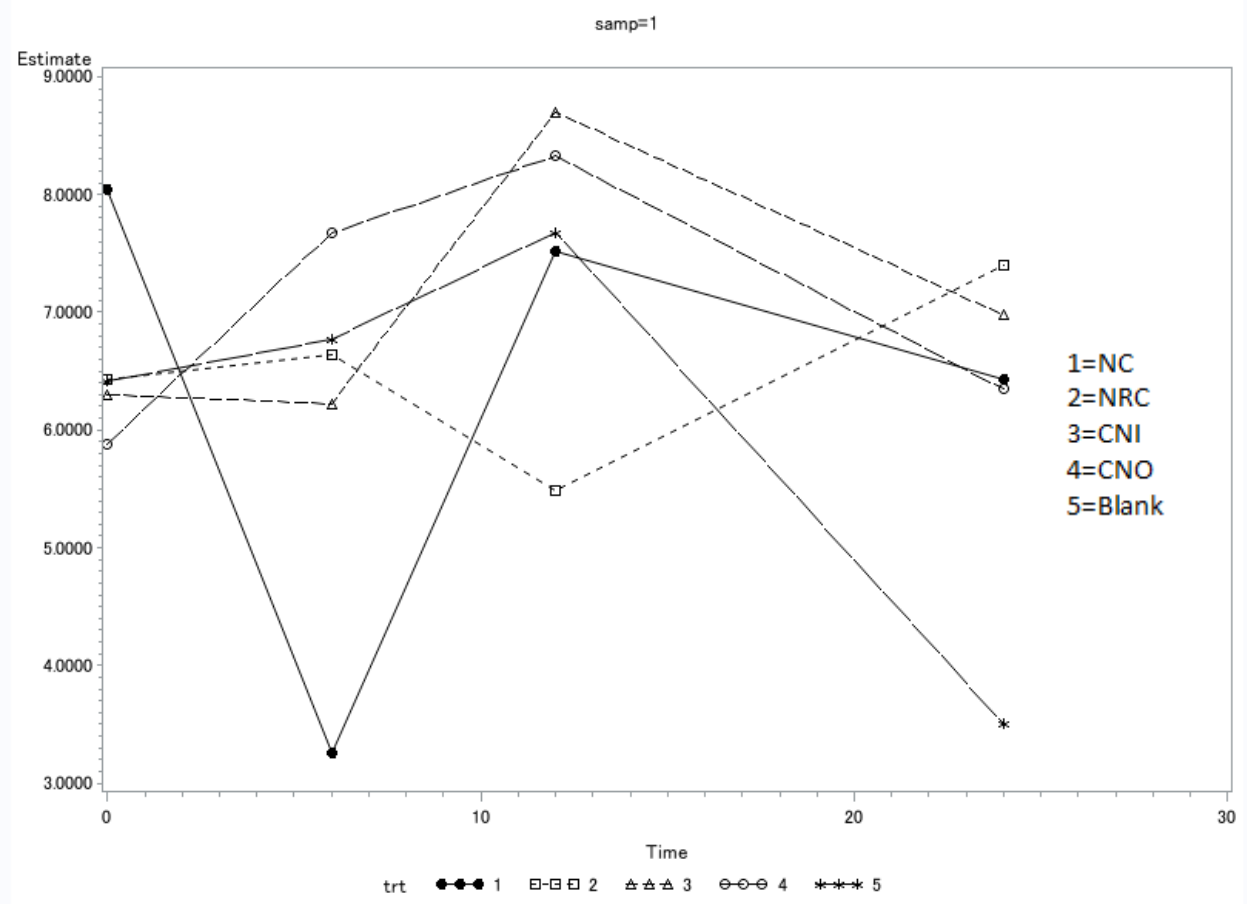

Figure 12. Least squares means estimates utilizing an initial concentration covariate for Mn mg/kg for ruminal fluid over time.

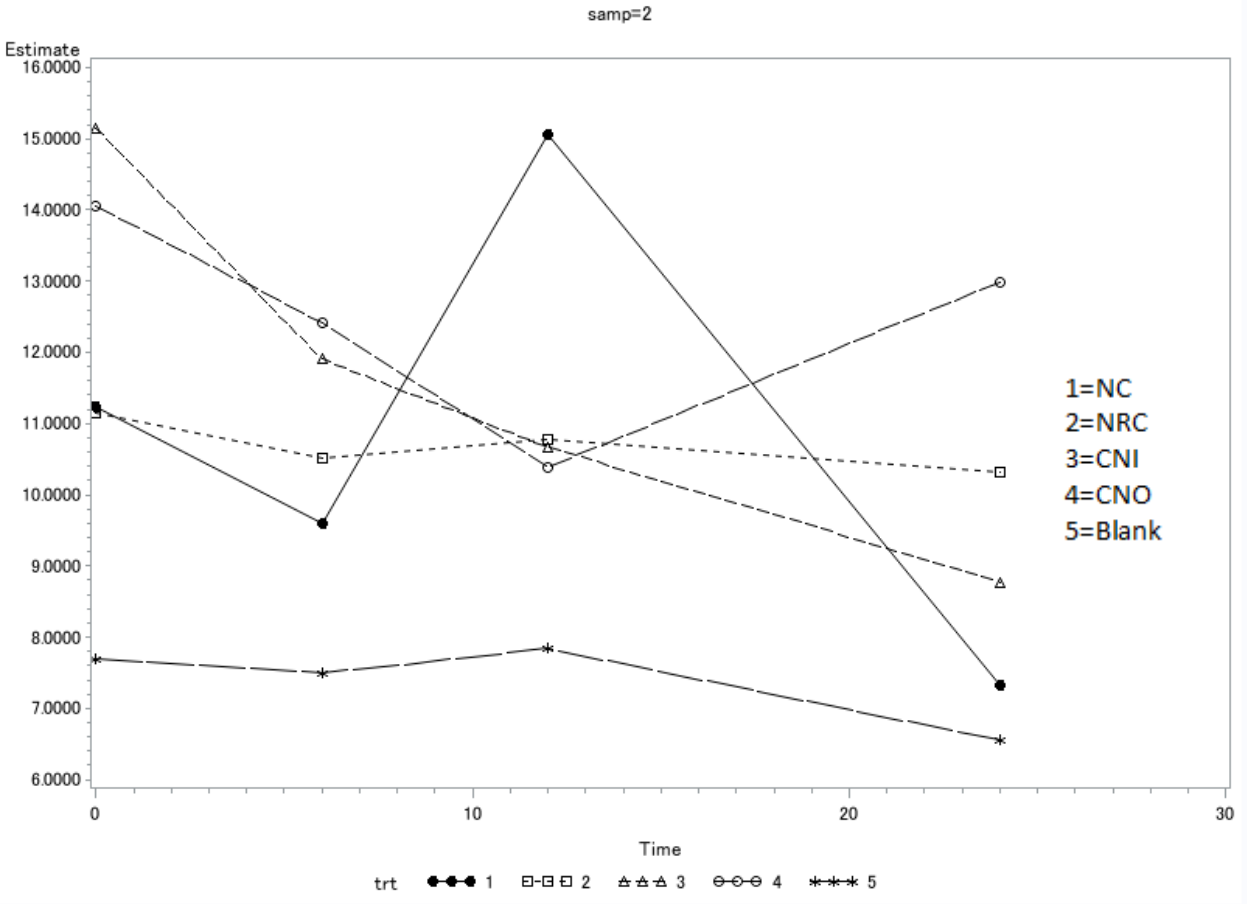

Figure 13. Least squares means estimates utilizing an initial concentration covariate for Mn mg/kg for abomasal over time.

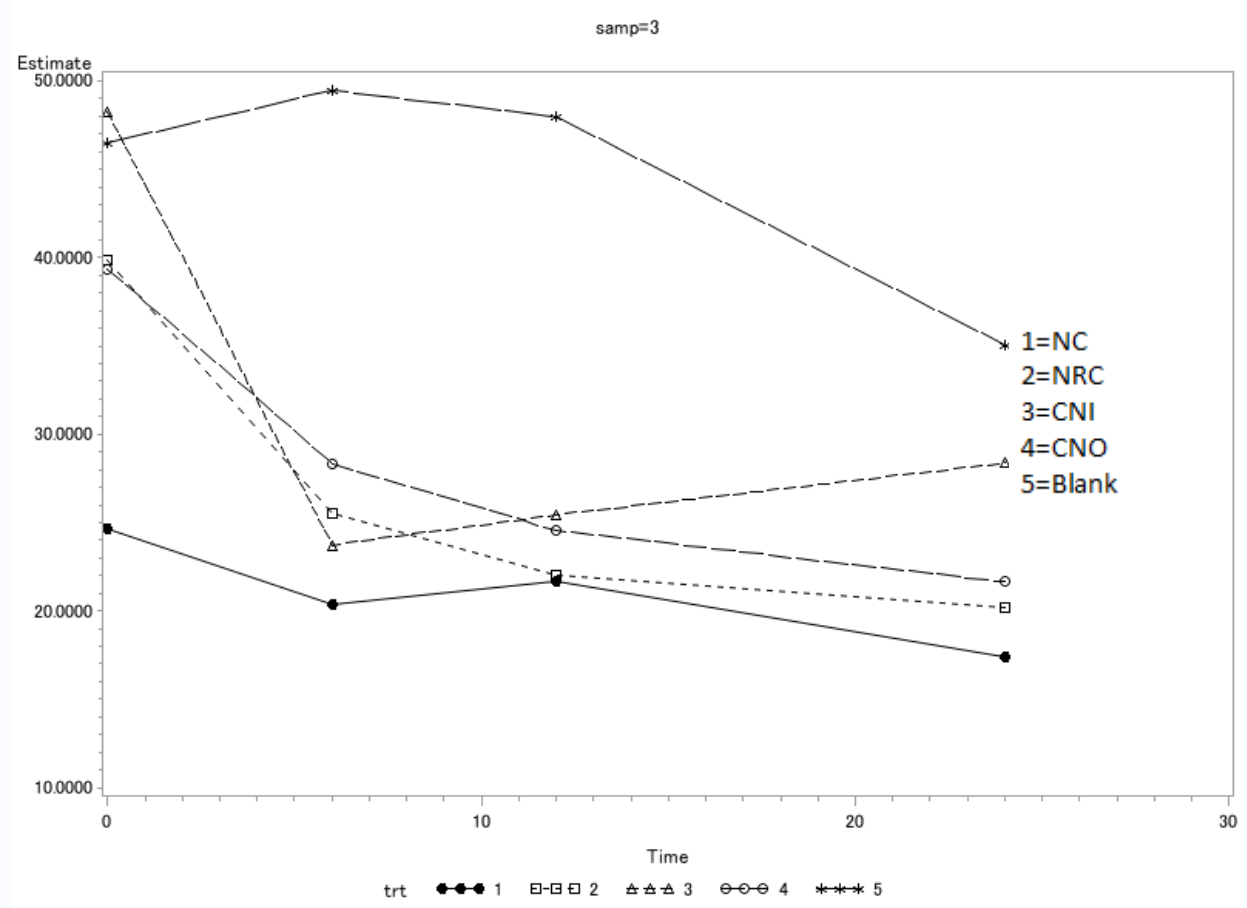

Figure 14. Least squares means estimates utilizing an initial concentration covariate for Mn mg/kg for remaining pellet over time.

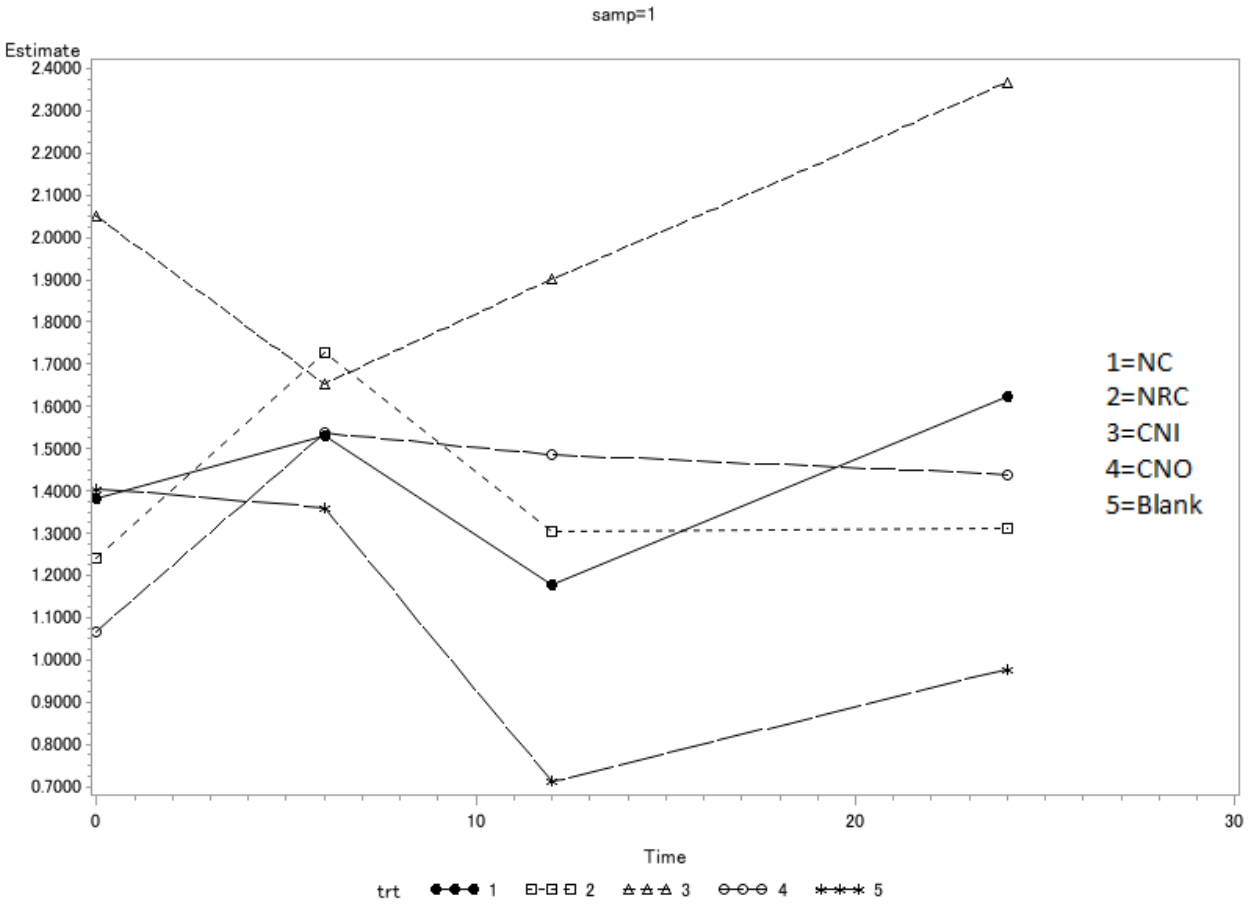

Figure 15. Least squares means estimates utilizing an initial concentration covariate for Zn mg/kg for ruminal fluid over time.

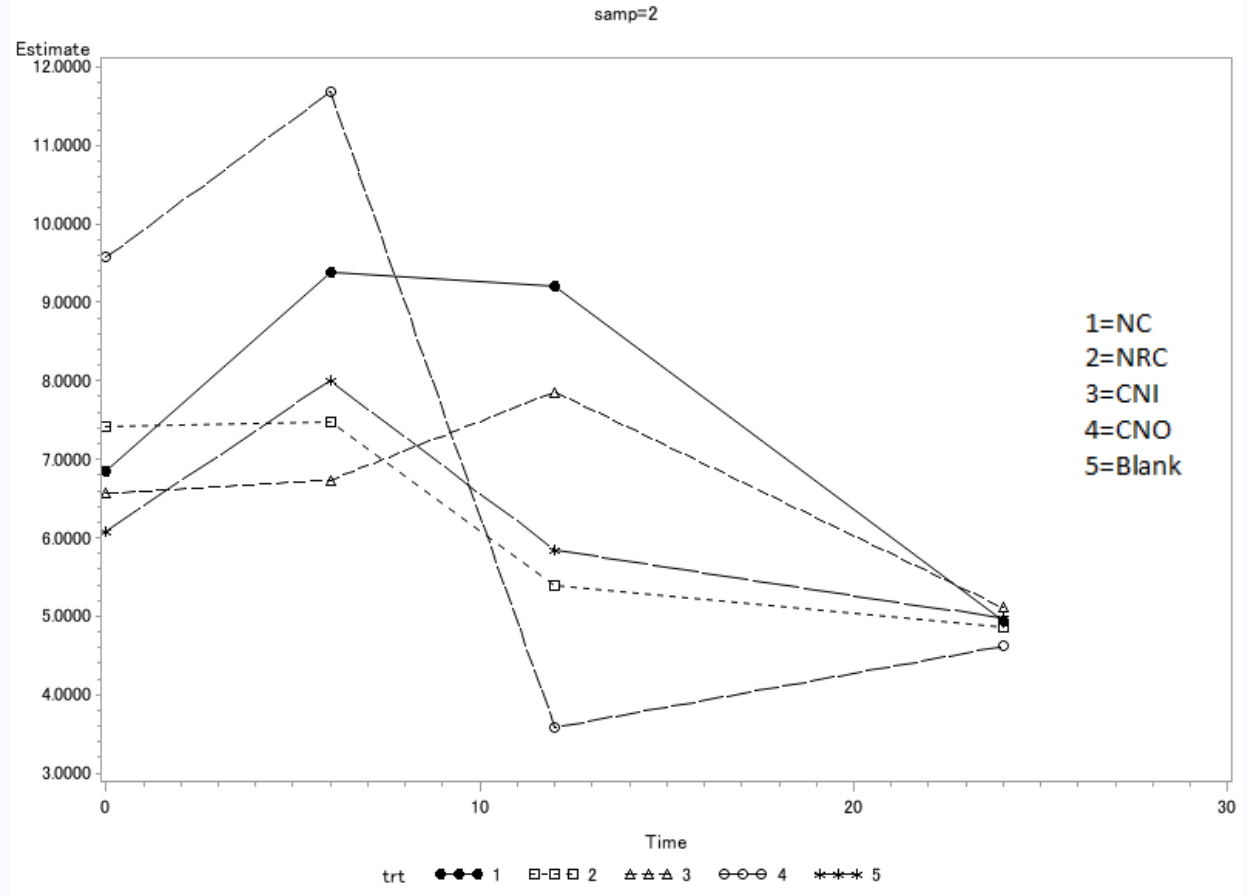

Figure 16. Least squares means estimates utilizing an initial concentration covariate for Zn mg/kg for abomasal over time.

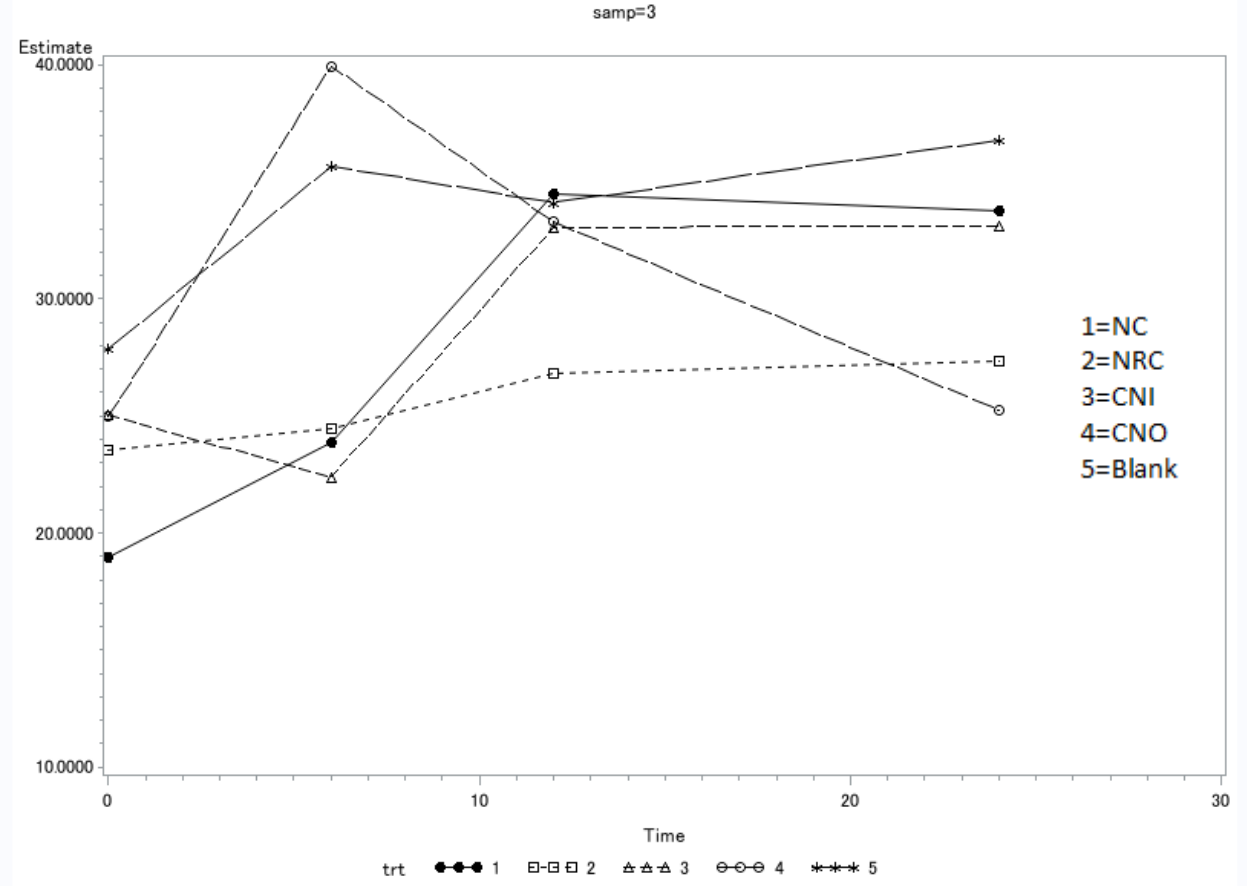

Figure 17. Least squares means estimates utilizing an initial concentration covariate for Zn mg/kg for remaining pellet over time.

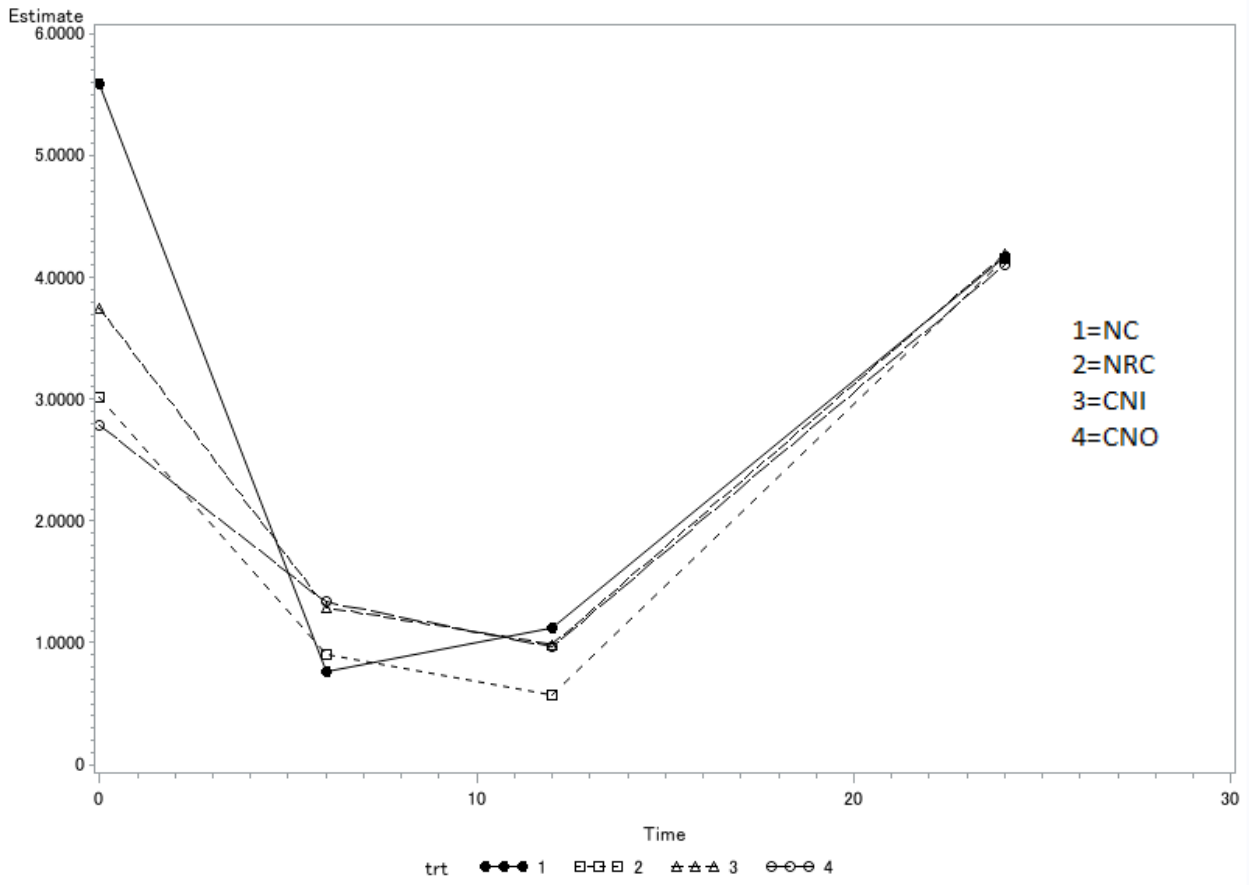

Figure 18. Least squares means estimates for µg of Co released · g DMD^{-1} for ruminal fluid over time.

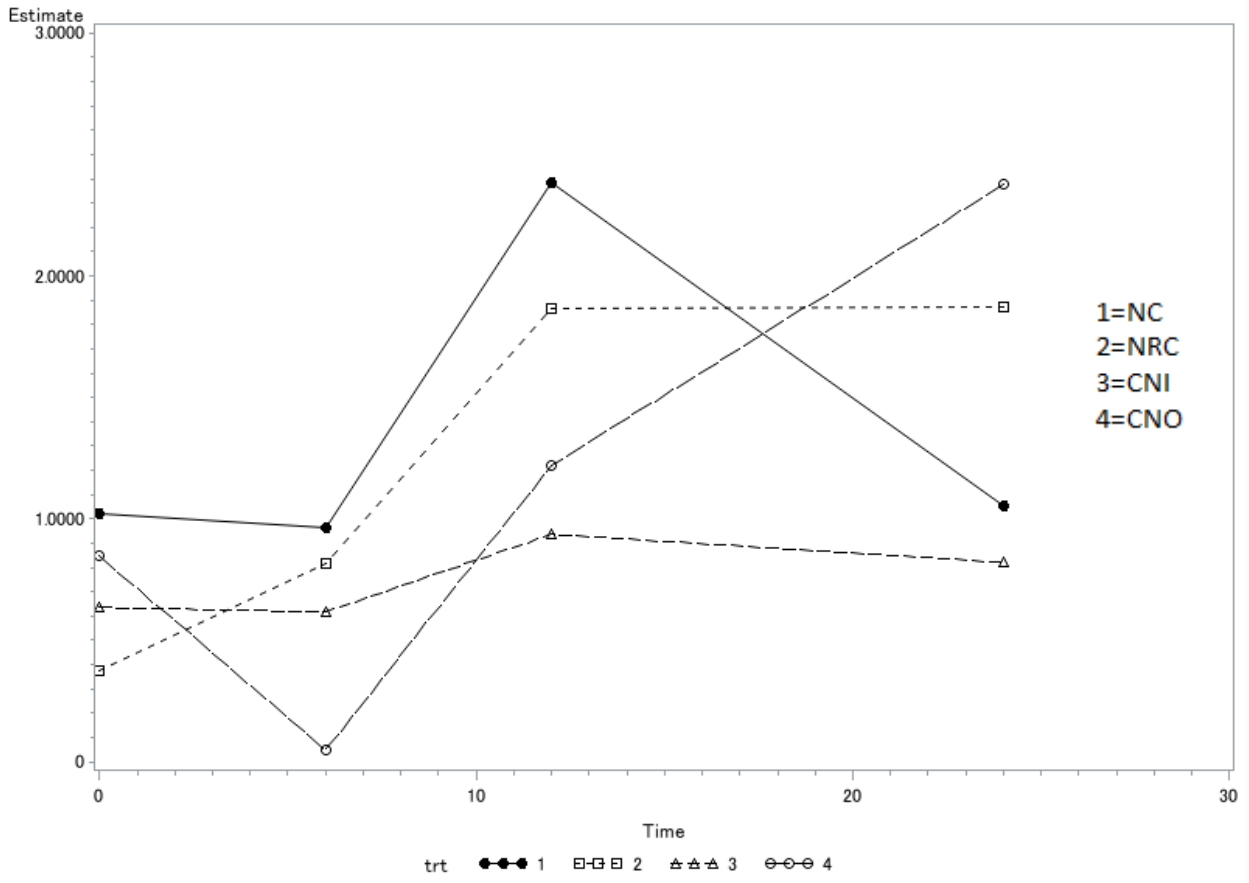

Figure 19. Least squares means estimates for µg of Co released · g DMD^{-1} for abomasal over time.

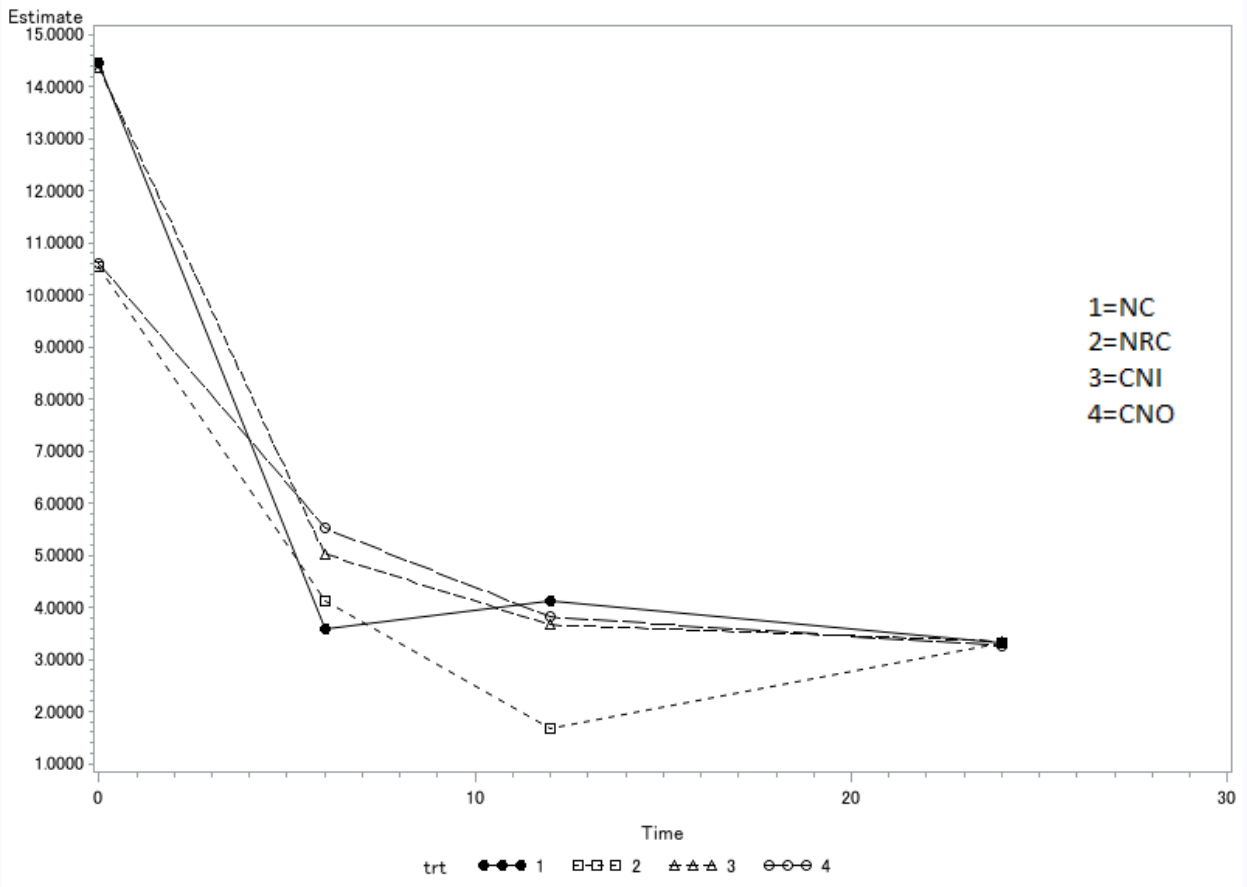

Figure 20. Least squares means estimates for µg of Cu released · g DMD^{-1} for ruminal fluid over time.

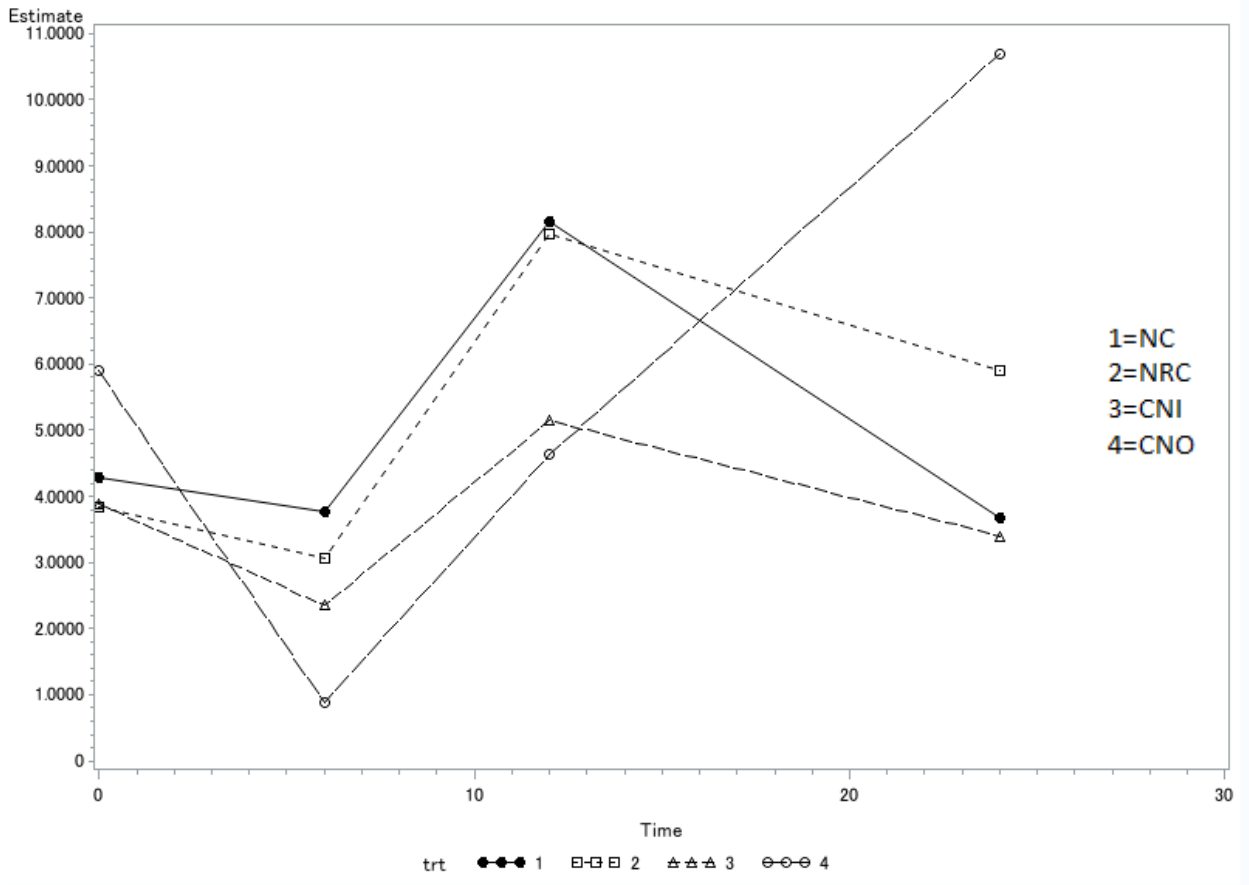

Figure 21. Least squares means estimates for µg of Cu released · g DMD^{-1} for abomasal over time.

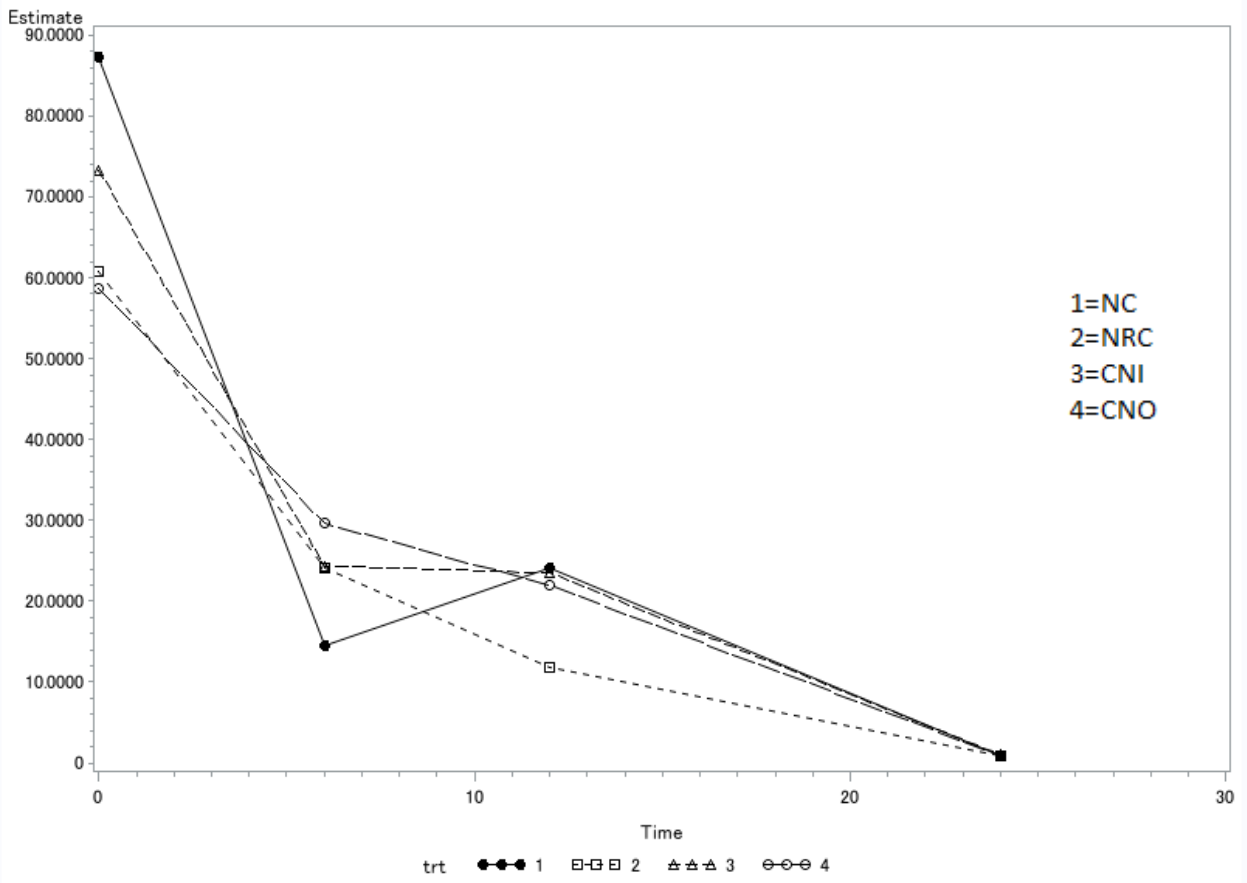

Figure 22. Least squares means estimates for µg of Mn released · g DMD^{-1} for ruminal fluid over time.

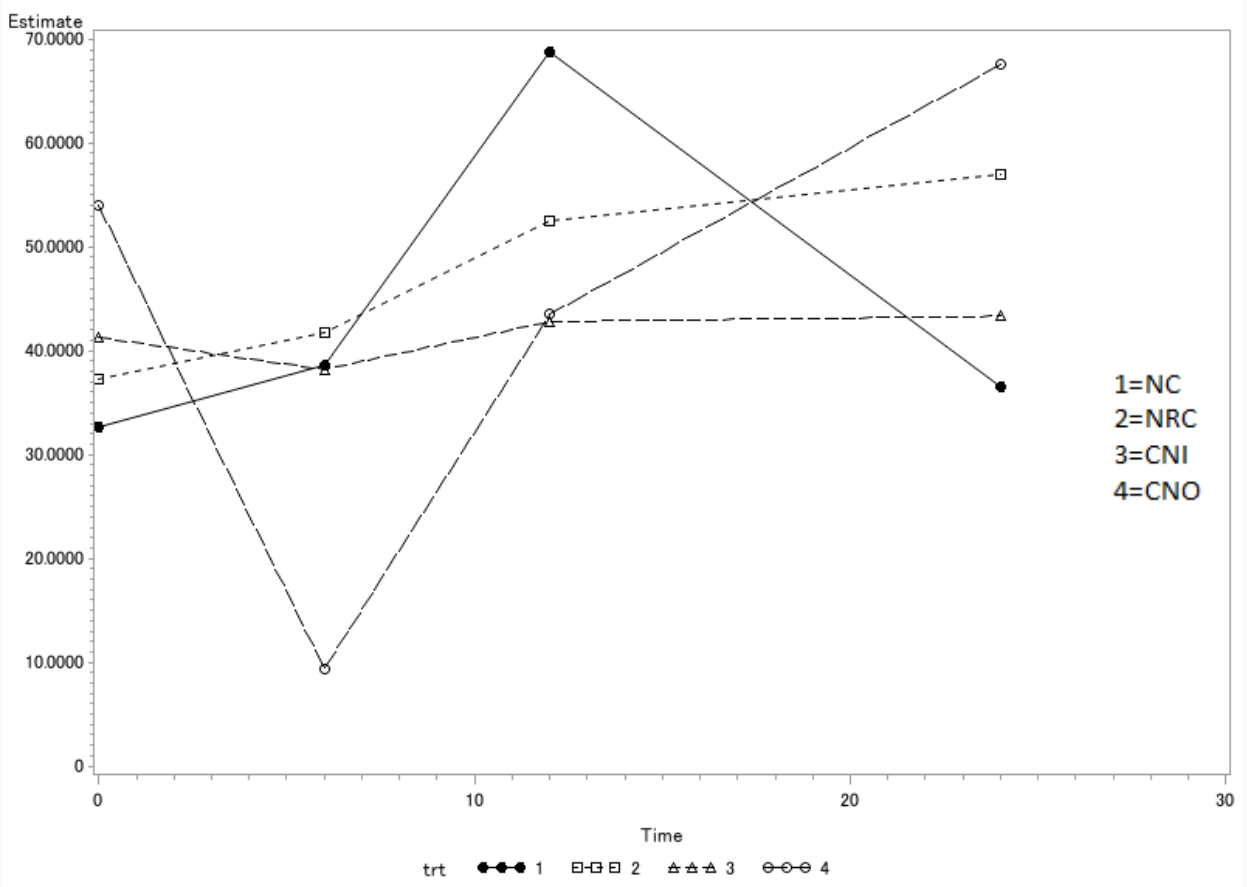

Figure 23. Least squares means estimates for µg of Mn released · g DMD^{-1} for abomasal over time.

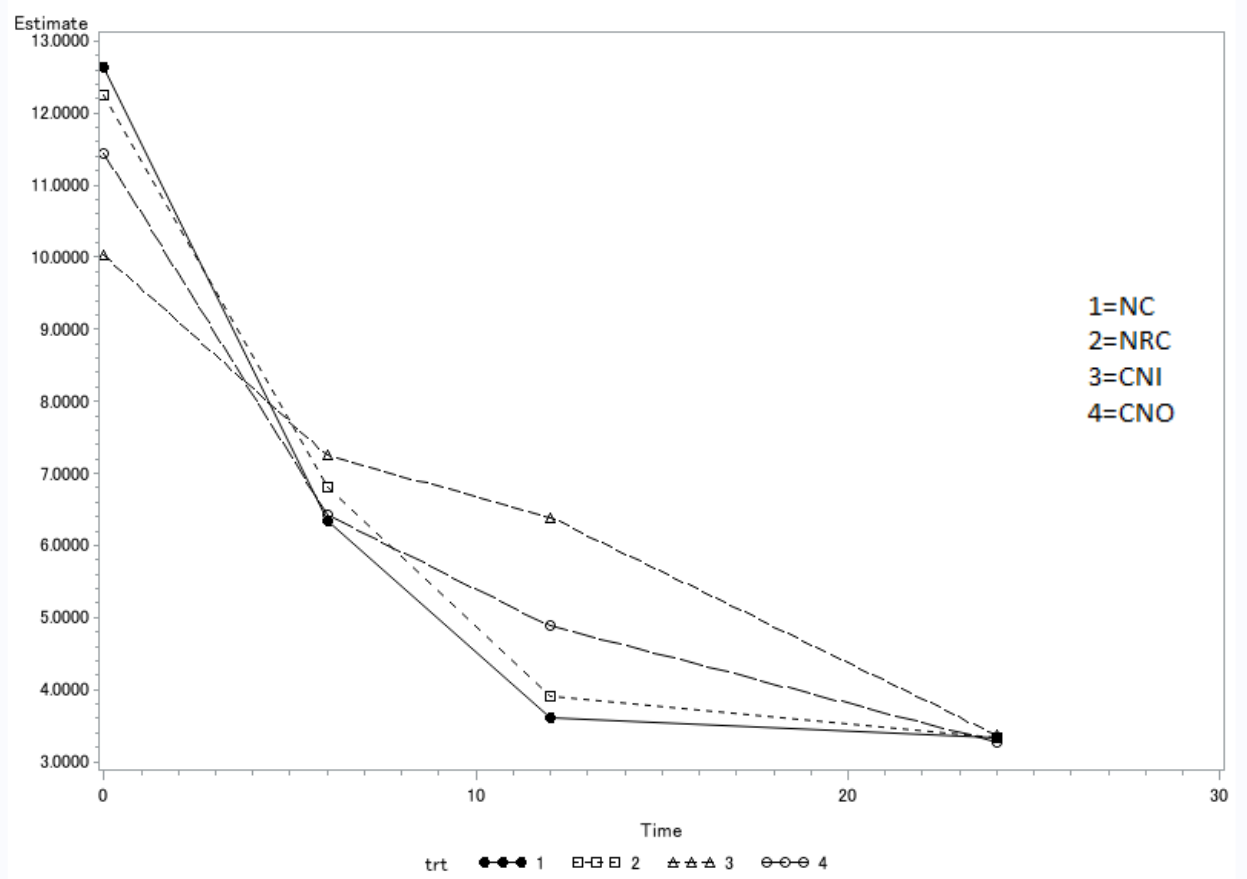

Figure 24. Least squares means estimates for µg of Zn released · g DMD^{-1} for ruminal fluid over time.

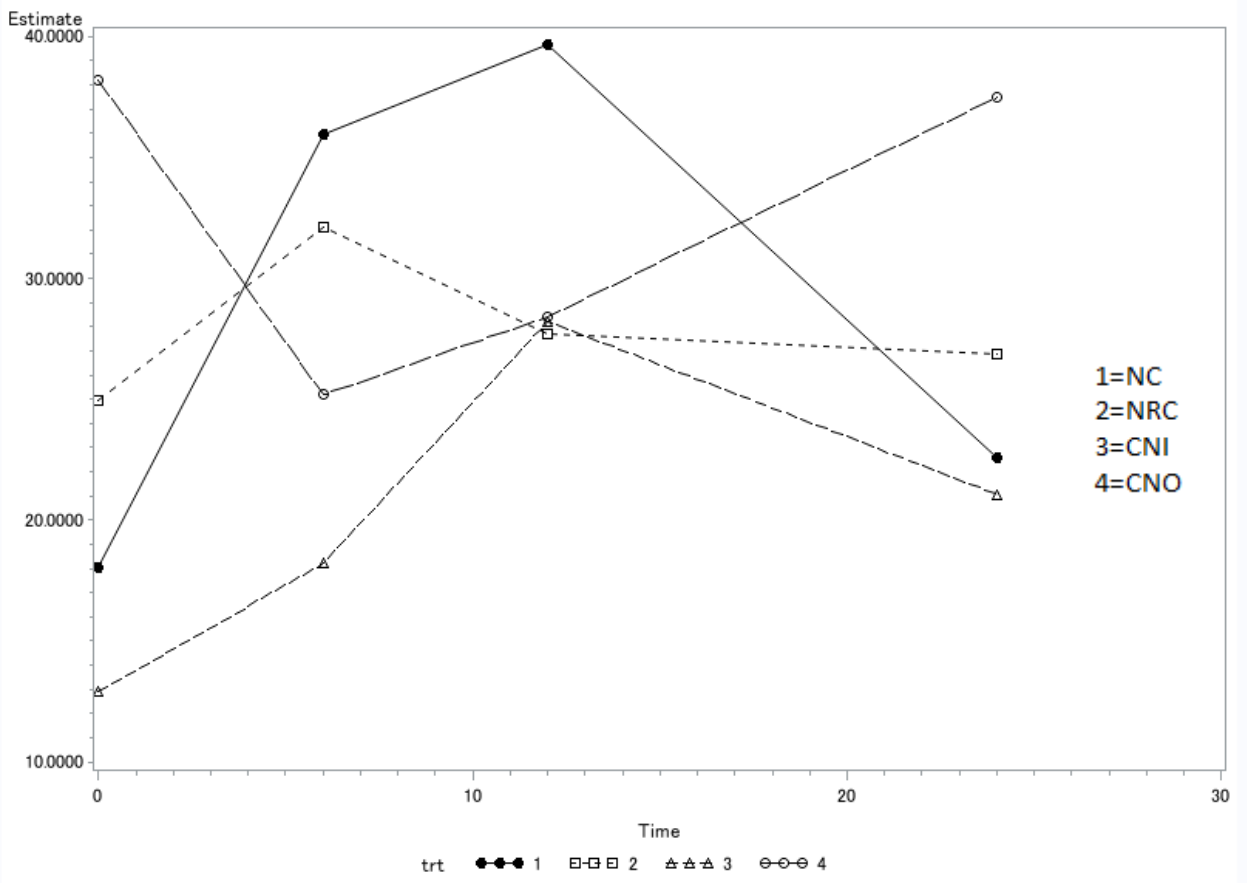

Figure 25. Least squares means estimates for µg of Zn released · g DMD^{-1} for abomasal over time.

Appendix B

EXPERIMENT 1:

RATION AND DRY MATTER INTAKE ANALYSIS SAS CODE.

```
data asfed; input pen asfed diet dm   dmdel per  head  dof   dmi;
cards;

Proc sort; by pen per;
proc means noprint sum; by pen per; var dmdel head;
output out=pendmcon sum=dmdel head;
data perdmi; set pendmcon;
dmi=dmdel/head;
data per1; set perdmi; if per=1;
rename dmi=dmi1;
rename dmdel=dmdel1;
rename head=head1;

proc sort; by pen;
data per2; set perdmi; if per=2;
rename dmi=dmi2;
rename dmdel=dmdel2;
rename head=head2;

proc sort; by pen;
data per3; set perdmi; if per=3;
rename dmi=dmi3;
rename dmdel=dmdel3;
rename head=head3;
proc sort; by pen;
proc sort data=asfed; by pen;
proc means noprint sum; by pen; var dmdel head;
output out=pendmtot sum=dmdel head;
data totdmi; set pendmtot;
dmi=dmdel/head;
proc sort; by pen;
data intake; merge per1 per2 per3 totdmi; by pen;
proc print;
run;

proc mixed data=fg;
class trt rep;
model inwt=trt/s ddfm=kr;
random rep;
lsmeans trt/pdiff;
contrast 'NC vs supplemented' trt 3 -1 -1 -1;
contrast 'NRC vs Con Nutri' trt 0 2 -1 -1;
contrast 'InOrg vs Org' trt 0 0 1 -1;

proc mixed data=fg;
class trt rep;
model finwt=trt/s ddfm=kr;
```

```
random rep;
lsmeans trt/pdiff;
contrast 'NC vs supplemented' trt 3 -1 -1 -1;
contrast 'NRC vs Con Nutri' trt 0 2 -1 -1;
contrast 'InOrg vs Org' trt 0 0 1 -1;

proc mixed data=fg;
class trt rep;
model finwt=inwt trt/s ddfm=kr;
random rep;
lsmeans trt/pdiff;
contrast 'NC vs supplemented' trt 3 -1 -1 -1;
contrast 'NRC vs Con Nutri' trt 0 2 -1 -1;
contrast 'InOrg vs Org' trt 0 0 1 -1;

proc mixed data=fg;
class trt rep;
model d35=trt/s ddfm=kr;
random rep;
lsmeans trt/pdiff;
contrast 'NC vs supplemented' trt 3 -1 -1 -1;
contrast 'NRC vs Con Nutri' trt 0 2 -1 -1;
contrast 'InOrg vs Org' trt 0 0 1 -1;

proc mixed data=fg;
class trt rep;
model d35=inwt trt/s ddfm=kr;
random rep;
lsmeans trt/pdiff;
contrast 'NC vs supplemented' trt 3 -1 -1 -1;
contrast 'NRC vs Con Nutri' trt 0 2 -1 -1;
contrast 'InOrg vs Org' trt 0 0 1 -1;

proc mixed data=fg;
class trt rep;
model d121=trt/s ddfm=kr;
random rep;
lsmeans trt/pdiff;
contrast 'NC vs supplemented' trt 3 -1 -1 -1;
contrast 'NRC vs Con Nutri' trt 0 2 -1 -1;
contrast 'InOrg vs Org' trt 0 0 1 -1;

proc mixed data=fg;
class trt rep;
model d121=inwt trt/s ddfm=kr;
random rep;
lsmeans trt/pdiff;
contrast 'NC vs supplemented' trt 3 -1 -1 -1;
contrast 'NRC vs Con Nutri' trt 0 2 -1 -1;
contrast 'InOrg vs Org' trt 0 0 1 -1;

proc mixed data=fg;
```

```
class trt rep;
model adg1=trt/s ddfm=kr;
random rep;
lsmeans trt/pdiff;
contrast 'NC vs supplemented' trt 3 -1 -1 -1;
contrast 'NRC vs Con Nutri' trt 0 2 -1 -1;
contrast 'InOrg vs Org' trt 0 0 1 -1;

proc mixed data=fg;
class trt rep;
model adg1=inwt trt/s ddfm=kr;
random rep;
lsmeans trt/pdiff;
contrast 'NC vs supplemented' trt 3 -1 -1 -1;
contrast 'NRC vs Con Nutri' trt 0 2 -1 -1;
contrast 'InOrg vs Org' trt 0 0 1 -1;

proc mixed data=fg;
class trt rep;
model dmi1=trt/s ddfm=kr;
random rep;
lsmeans trt/pdiff;
contrast 'NC vs supplemented' trt 3 -1 -1 -1;
contrast 'NRC vs Con Nutri' trt 0 2 -1 -1;
contrast 'InOrg vs Org' trt 0 0 1 -1;

proc mixed data=fg;
class trt rep;
model dmi1=inwt trt/s ddfm=kr;
random rep;
lsmeans trt/pdiff;
contrast 'NC vs supplemented' trt 3 -1 -1 -1;
contrast 'NRC vs Con Nutri' trt 0 2 -1 -1;
contrast 'InOrg vs Org' trt 0 0 1 -1;

proc mixed data=fg;
class trt rep;
model fg1=trt/s ddfm=kr;
random rep;
lsmeans trt/pdiff;
contrast 'NC vs supplemented' trt 3 -1 -1 -1;
contrast 'NRC vs Con Nutri' trt 0 2 -1 -1;
contrast 'InOrg vs Org' trt 0 0 1 -1;

proc mixed data=fg;
class trt rep;
model fg1=inwt trt/s ddfm=kr;
random rep;
lsmeans trt/pdiff;
contrast 'NC vs supplemented' trt 3 -1 -1 -1;
contrast 'NRC vs Con Nutri' trt 0 2 -1 -1;
contrast 'InOrg vs Org' trt 0 0 1 -1;
```

```
proc mixed data=fg;
class trt rep;
model gf1=trt/s ddfm=kr;
random rep;
lsmeans trt/pdiff;
contrast 'NC vs supplemented' trt 3 -1 -1 -1;
contrast 'NRC vs Con Nutri' trt 0 2 -1 -1;
contrast 'InOrg vs Org' trt 0 0 1 -1;

proc mixed data=fg;
class trt rep;
model gf1=inwt trt/s ddfm=kr;
random rep;
lsmeans trt/pdiff;
contrast 'NC vs supplemented' trt 3 -1 -1 -1;
contrast 'NRC vs Con Nutri' trt 0 2 -1 -1;
contrast 'InOrg vs Org' trt 0 0 1 -1;

proc mixed data=fg;
class trt rep;
model adg2=trt/s ddfm=kr;
random rep;
lsmeans trt/pdiff;
contrast 'NC vs supplemented' trt 3 -1 -1 -1;
contrast 'NRC vs Con Nutri' trt 0 2 -1 -1;
contrast 'InOrg vs Org' trt 0 0 1 -1;

proc mixed data=fg;
class trt rep;
model adg2=inwt trt/s ddfm=kr;
random rep;
lsmeans trt/pdiff;
contrast 'NC vs supplemented' trt 3 -1 -1 -1;
contrast 'NRC vs Con Nutri' trt 0 2 -1 -1;
contrast 'InOrg vs Org' trt 0 0 1 -1;

proc mixed data=fg;
class trt rep;
model dmi2=trt/s ddfm=kr;
random rep;
lsmeans trt/pdiff;
contrast 'NC vs supplemented' trt 3 -1 -1 -1;
contrast 'NRC vs Con Nutri' trt 0 2 -1 -1;
contrast 'InOrg vs Org' trt 0 0 1 -1;

proc mixed data=fg;
class trt rep;
model dmi2=inwt trt/s ddfm=kr;
random rep;
lsmeans trt/pdiff;
contrast 'NC vs supplemented' trt 3 -1 -1 -1;
```

```
contrast 'NRC vs Con Nutri' trt 0 2 -1 -1;
contrast 'InOrg vs Org' trt 0 0 1 -1;

proc mixed data=fg;
class trt rep;
model fg2=trt/s ddfm=kr;
random rep;
lsmeans trt/pdiff;
contrast 'NC vs supplemented' trt 3 -1 -1 -1;
contrast 'NRC vs Con Nutri' trt 0 2 -1 -1;
contrast 'InOrg vs Org' trt 0 0 1 -1;

proc mixed data=fg;
class trt rep;
model fg2=inwt trt/s ddfm=kr;
random rep;
lsmeans trt/pdiff;
contrast 'NC vs supplemented' trt 3 -1 -1 -1;
contrast 'NRC vs Con Nutri' trt 0 2 -1 -1;
contrast 'InOrg vs Org' trt 0 0 1 -1;

proc mixed data=fg;
class trt rep;
model gf2=trt/s ddfm=kr;
random rep;
lsmeans trt/pdiff;
contrast 'NC vs supplemented' trt 3 -1 -1 -1;
contrast 'NRC vs Con Nutri' trt 0 2 -1 -1;
contrast 'InOrg vs Org' trt 0 0 1 -1;

proc mixed data=fg;
class trt rep;
model gf2=inwt trt/s ddfm=kr;
random rep;
lsmeans trt/pdiff;
contrast 'NC vs supplemented' trt 3 -1 -1 -1;
contrast 'NRC vs Con Nutri' trt 0 2 -1 -1;
contrast 'InOrg vs Org' trt 0 0 1 -1;

proc mixed data=fg;
class trt rep;
model adg3=trt/s ddfm=kr;
random rep;
lsmeans trt/pdiff;
contrast 'NC vs supplemented' trt 3 -1 -1 -1;
contrast 'NRC vs Con Nutri' trt 0 2 -1 -1;
contrast 'InOrg vs Org' trt 0 0 1 -1;

proc mixed data=fg;
class trt rep;
model adg3=inwt trt/s ddfm=kr;
random rep;
```

```
lsmeans trt/pdiff;
contrast 'NC vs supplemented' trt 3 -1 -1 -1;
contrast 'NRC vs Con Nutri' trt 0 2 -1 -1;
contrast 'InOrg vs Org' trt 0 0 1 -1;

proc mixed data=fg;
class trt rep;
model dmi3=trt/s ddfm=kr;
random rep;
lsmeans trt/pdiff;
contrast 'NC vs supplemented' trt 3 -1 -1 -1;
contrast 'NRC vs Con Nutri' trt 0 2 -1 -1;
contrast 'InOrg vs Org' trt 0 0 1 -1;

proc mixed data=fg;
class trt rep;
model dmi3=inwt trt/s ddfm=kr;
random rep;
lsmeans trt/pdiff;
contrast 'NC vs supplemented' trt 3 -1 -1 -1;
contrast 'NRC vs Con Nutri' trt 0 2 -1 -1;
contrast 'InOrg vs Org' trt 0 0 1 -1;

proc mixed data=fg;
class trt rep;
model fg3=trt/s ddfm=kr;
random rep;
lsmeans trt/pdiff;
contrast 'NC vs supplemented' trt 3 -1 -1 -1;
contrast 'NRC vs Con Nutri' trt 0 2 -1 -1;
contrast 'InOrg vs Org' trt 0 0 1 -1;

proc mixed data=fg;
class trt rep;
model fg3=inwt trt/s ddfm=kr;
random rep;
lsmeans trt/pdiff;
contrast 'NC vs supplemented' trt 3 -1 -1 -1;
contrast 'NRC vs Con Nutri' trt 0 2 -1 -1;
contrast 'InOrg vs Org' trt 0 0 1 -1;

proc mixed data=fg;
class trt rep;
model gf3=trt/s ddfm=kr;
random rep;
lsmeans trt/pdiff;
contrast 'NC vs supplemented' trt 3 -1 -1 -1;
contrast 'NRC vs Con Nutri' trt 0 2 -1 -1;
contrast 'InOrg vs Org' trt 0 0 1 -1;

proc mixed data=fg;
class trt rep;
```

```
model gf3=inwt trt/s ddfm=kr;
random rep;
lsmeans trt/pdiff;
contrast 'NC vs supplemented' trt 3 -1 -1 -1;
contrast 'NRC vs Con Nutri' trt 0 2 -1 -1;
contrast 'InOrg vs Org' trt 0 0 1 -1;

proc mixed data=fg;
class trt rep;
model adg=trt/s ddfm=kr;
random rep;
lsmeans trt/pdiff;
contrast 'NC vs supplemented' trt 3 -1 -1 -1;
contrast 'NRC vs Con Nutri' trt 0 2 -1 -1;
contrast 'InOrg vs Org' trt 0 0 1 -1;

proc mixed data=fg;
class trt rep;
model adg=inwt trt/s ddfm=kr;
random rep;
lsmeans trt/pdiff;
contrast 'NC vs supplemented' trt 3 -1 -1 -1;
contrast 'NRC vs Con Nutri' trt 0 2 -1 -1;
contrast 'InOrg vs Org' trt 0 0 1 -1;

proc mixed data=fg;
class trt rep;
model dmi=trt/s ddfm=kr;
random rep;
lsmeans trt/pdiff;
contrast 'NC vs supplemented' trt 3 -1 -1 -1;
contrast 'NRC vs Con Nutri' trt 0 2 -1 -1;
contrast 'InOrg vs Org' trt 0 0 1 -1;

proc mixed data=fg;
class trt rep;
model dmi=inwt trt/s ddfm=kr;
random rep;
lsmeans trt/pdiff;
contrast 'NC vs supplemented' trt 3 -1 -1 -1;
contrast 'NRC vs Con Nutri' trt 0 2 -1 -1;
contrast 'InOrg vs Org' trt 0 0 1 -1;

proc mixed data=fg;
class trt rep;
model fg=trt/s ddfm=kr;
random rep;
lsmeans trt/pdiff;
contrast 'NC vs supplemented' trt 3 -1 -1 -1;
contrast 'NRC vs Con Nutri' trt 0 2 -1 -1;
contrast 'InOrg vs Org' trt 0 0 1 -1;
```

```
proc mixed data=fg;
class trt rep;
model fg=inwt trt/s ddfm=kr;
random rep;
lsmeans trt/pdiff;
contrast 'NC vs supplemented' trt 3 -1 -1 -1;
contrast 'NRC vs Con Nutri' trt 0 2 -1 -1;
contrast 'InOrg vs Org' trt 0 0 1 -1;

proc mixed data=fg;
class trt rep;
model gf=trt/s ddfm=kr;
random rep;
lsmeans trt/pdiff;
contrast 'NC vs supplemented' trt 3 -1 -1 -1;
contrast 'NRC vs Con Nutri' trt 0 2 -1 -1;
contrast 'InOrg vs Org' trt 0 0 1 -1;

proc mixed data=fg;
class trt rep;
model gf=inwt trt/s ddfm=kr;
random rep;
lsmeans trt/pdiff;
contrast 'NC vs supplemented' trt 3 -1 -1 -1;
contrast 'NRC vs Con Nutri' trt 0 2 -1 -1;
contrast 'InOrg vs Org' trt 0 0 1 -1;
run;
```

CARCASS ANALYSIS SAS CODE.

```
proc mixed covtest data=pencarc;
class trt rep;
model hcw=trt/s ddfm=kr;
random rep;
lsmeans trt/cl pdiff;
contrast 'neg control vs supple TM' trt 3 -1 -1 -1;
contrast 'nrc vs vasc' trt 0 2 -1 -1;
contrast 'inorg vs org' trt 0 0 1 -1;

proc mixed covtest data=pencarc;
class trt rep;
model hcw=inwt trt/s ddfm=kr;
random rep;
lsmeans trt/cl pdiff;
contrast 'neg control vs supple TM' trt 3 -1 -1 -1;
contrast 'nrc vs vasc' trt 0 2 -1 -1;
contrast 'inorg vs org' trt 0 0 1 -1;

proc mixed covtest data=pencarc;
class trt rep;
```

```
model dp=trt/s ddfm=kr;
random rep;
lsmeans trt/cl pdiff;
contrast 'neg control vs supple TM' trt 3 -1 -1 -1;
contrast 'nrc vs vasc' trt 0 2 -1 -1;
contrast 'inorg vs org' trt 0 0 1 -1;

proc mixed covtest data=pencarc;
class trt rep;
model dp=inwt trt/s ddfm=kr;
random rep;
lsmeans trt/cl pdiff;
contrast 'neg control vs supple TM' trt 3 -1 -1 -1;
contrast 'nrc vs vasc' trt 0 2 -1 -1;
contrast 'inorg vs org' trt 0 0 1 -1;

proc mixed covtest data=pencarc;
class trt rep;
model fat=trt/s ddfm=kr;
random rep;
lsmeans trt/cl pdiff;
contrast 'neg control vs supple TM' trt 3 -1 -1 -1;
contrast 'nrc vs vasc' trt 0 2 -1 -1;
contrast 'inorg vs org' trt 0 0 1 -1;

proc mixed covtest data=pencarc;
class trt rep;
model fat=inwt trt/s ddfm=kr;
random rep;
lsmeans trt/cl pdiff;
contrast 'neg control vs supple TM' trt 3 -1 -1 -1;
contrast 'nrc vs vasc' trt 0 2 -1 -1;
contrast 'inorg vs org' trt 0 0 1 -1;

proc mixed covtest data=pencarc;
class trt rep;
model rea=trt/s ddfm=kr;
random rep;
lsmeans trt/cl pdiff;
contrast 'neg control vs supple TM' trt 3 -1 -1 -1;
contrast 'nrc vs vasc' trt 0 2 -1 -1;
contrast 'inorg vs org' trt 0 0 1 -1;

proc mixed covtest data=pencarc;
class trt rep;
model rea=inwt trt/s ddfm=kr;
random rep;
lsmeans trt/cl pdiff;
contrast 'neg control vs supple TM' trt 3 -1 -1 -1;
contrast 'nrc vs vasc' trt 0 2 -1 -1;
contrast 'inorg vs org' trt 0 0 1 -1;
```

```
proc mixed covtest data=pencarc;
class trt rep;
model musc=trt/s ddfm=kr;
random rep;
lsmeans trt/cl pdiff;
contrast 'neg control vs supple TM' trt 3 -1 -1 -1;
contrast 'nrc vs vasc' trt 0 2 -1 -1;
contrast 'inorg vs org' trt 0 0 1 -1;

proc mixed covtest data=pencarc;
class trt rep;
model musc=inwt trt/s ddfm=kr;
random rep;
lsmeans trt/cl pdiff;
contrast 'neg control vs supple TM' trt 3 -1 -1 -1;
contrast 'nrc vs vasc' trt 0 2 -1 -1;
contrast 'inorg vs org' trt 0 0 1 -1;

proc mixed covtest data=pencarc;
class trt rep;
model kph=trt/s ddfm=kr;
random rep;
lsmeans trt/cl pdiff;
contrast 'neg control vs supple TM' trt 3 -1 -1 -1;
contrast 'nrc vs vasc' trt 0 2 -1 -1;
contrast 'inorg vs org' trt 0 0 1 -1;

proc mixed covtest data=pencarc;
class trt rep;
model kph=inwt trt/s ddfm=kr;
random rep;
lsmeans trt/cl pdiff;
contrast 'neg control vs supple TM' trt 3 -1 -1 -1;
contrast 'nrc vs vasc' trt 0 2 -1 -1;
contrast 'inorg vs org' trt 0 0 1 -1;

proc mixed covtest data=pencarc;
class trt rep;
model yg=trt/s ddfm=kr;
random rep;
lsmeans trt/cl pdiff;
contrast 'neg control vs supple TM' trt 3 -1 -1 -1;
contrast 'nrc vs vasc' trt 0 2 -1 -1;
contrast 'inorg vs org' trt 0 0 1 -1;

proc mixed covtest data=pencarc;
class trt rep;
model yg=inwt trt/s ddfm=kr;
random rep;
lsmeans trt/cl pdiff;
contrast 'neg control vs supple TM' trt 3 -1 -1 -1;
contrast 'nrc vs vasc' trt 0 2 -1 -1;
```

```
contrast 'inorg vs org' trt 0 0 1 -1;

proc mixed covtest data=pencarc;
class trt rep;
model marb=trt/s ddfm=kr;
random rep;
lsmeans trt/cl pdiff;
contrast 'neg control vs supple TM' trt 3 -1 -1 -1;
contrast 'nrc vs vasc' trt 0 2 -1 -1;
contrast 'inorg vs org' trt 0 0 1 -1;

proc mixed covtest data=pencarc;
class trt rep;
model marb=inwt trt/s ddfm=kr;
random rep;
lsmeans trt/cl pdiff;
contrast 'neg control vs supple TM' trt 3 -1 -1 -1;
contrast 'nrc vs vasc' trt 0 2 -1 -1;
contrast 'inorg vs org' trt 0 0 1 -1;

proc mixed covtest data=pencarc;
class trt rep;
model relmarb=trt/s ddfm=kr;
random rep;
lsmeans trt/cl pdiff;
contrast 'neg control vs supple TM' trt 3 -1 -1 -1;
contrast 'nrc vs vasc' trt 0 2 -1 -1;
contrast 'inorg vs org' trt 0 0 1 -1;

proc mixed covtest data=pencarc;
class trt rep;
model relmarb=inwt trt/s ddfm=kr;
random rep;
lsmeans trt/cl pdiff;
contrast 'neg control vs supple TM' trt 3 -1 -1 -1;
contrast 'nrc vs vasc' trt 0 2 -1 -1;
contrast 'inorg vs org' trt 0 0 1 -1;

proc mixed covtest data=pencarc;
class trt rep;
model lmat=trt/s ddfm=kr;
random rep;
lsmeans trt/cl pdiff;
contrast 'neg control vs supple TM' trt 3 -1 -1 -1;
contrast 'nrc vs vasc' trt 0 2 -1 -1;
contrast 'inorg vs org' trt 0 0 1 -1;

proc mixed covtest data=pencarc;
class trt rep;
model lmat=inwt trt/s ddfm=kr;
random rep;
lsmeans trt/cl pdiff;
```

```
contrast 'neg control vs supple TM' trt 3 -1 -1 -1;
contrast 'nrc vs vasc' trt 0 2 -1 -1;
contrast 'inorg vs org' trt 0 0 1 -1;

proc mixed covtest data=pencarc;
class trt rep;
model skmat=trt/s ddfm=kr;
random rep;
lsmeans trt/cl pdiff;
contrast 'neg control vs supple TM' trt 3 -1 -1 -1;
contrast 'nrc vs vasc' trt 0 2 -1 -1;
contrast 'inorg vs org' trt 0 0 1 -1;

proc mixed covtest data=pencarc;
class trt rep;
model skmat=inwt trt/s ddfm=kr;
random rep;
lsmeans trt/cl pdiff;
contrast 'neg control vs supple TM' trt 3 -1 -1 -1;
contrast 'nrc vs vasc' trt 0 2 -1 -1;
contrast 'inorg vs org' trt 0 0 1 -1;

proc mixed covtest data=pencarc;
class trt rep;
model ovmat=trt/s ddfm=kr;
random rep;
lsmeans trt/cl pdiff;
contrast 'neg control vs supple TM' trt 3 -1 -1 -1;
contrast 'nrc vs vasc' trt 0 2 -1 -1;
contrast 'inorg vs org' trt 0 0 1 -1;

proc mixed covtest data=pencarc;
class trt rep;
model ovmat=inwt trt/s ddfm=kr;
random rep;
lsmeans trt/cl pdiff;
contrast 'neg control vs supple TM' trt 3 -1 -1 -1;
contrast 'nrc vs vasc' trt 0 2 -1 -1;
contrast 'inorg vs org' trt 0 0 1 -1;

proc mixed covtest data=pencarc;
class trt rep;
model qg=trt/s ddfm=kr;
random rep;
lsmeans trt/cl pdiff;
contrast 'neg control vs supple TM' trt 3 -1 -1 -1;
contrast 'nrc vs vasc' trt 0 2 -1 -1;
contrast 'inorg vs org' trt 0 0 1 -1;

proc mixed covtest data=pencarc;
class trt rep;
model qg=inwt trt/s ddfm=kr;
```

```
random rep;
lsmeans trt/cl pdiff;
contrast 'neg control vs supple TM' trt 3 -1 -1 -1;
contrast 'nrc vs vasc' trt 0 2 -1 -1;
contrast 'inorg vs org' trt 0 0 1 -1;
run;
```

PERFORMANCE ANALYSIS SAS CODE.

```
data asfed; input pen asfed diet dm   dmdel per  head dof   dmi;
cards;
Proc sort; by pen per;
proc means noprint sum; by pen per; var dmdel head;
output out=pendmcon sum=dmdel head;
data perdmi; set pendmcon;
dmi=dmdel/head;
data per1; set perdmi; if per=1;
rename dmi=dmi1;
rename dmdel=dmdel1;
rename head=head1;

proc sort; by pen;
data per2; set perdmi; if per=2;
rename dmi=dmi2;
rename dmdel=dmdel2;
rename head=head2;

proc sort; by pen;
data per3; set perdmi; if per=3;
rename dmi=dmi3;
rename dmdel=dmdel3;
rename head=head3;
proc sort; by pen;
proc sort data=asfed; by pen;
proc means noprint sum; by pen; var dmdel head;
output out=pendmtot sum=dmdel head;
data totdmi; set pendmtot;
dmi=dmdel/head;
proc sort; by pen;
data intake; merge per1 per2 per3 totdmi; by pen;
proc print;
run;

data indwgt; input pen     str   trt   rep   inwt  d121  finwt;
cards;
proc sort; by pen;
proc means noprint; by pen; var trt rep inwt d121 finwt;
output out=penwt mean=trt rep inwt d121 finwt;
proc sort; by pen;
data step1; input pen d35;
```

```
cards;
proc sort; by pen;
data weight; merge penwt step1;
data adg; set weight;
adg1=(d35-inwt)/35;
adg2=(d121-d35)/86;
adg3=(finwt-d121)/33;
adg=(finwt-inwt)/154;
proc sort; by pen;
proc sort data=intake; by pen;
data step2; merge adg intake; by pen;
data fg; set step2;
fg1=dmi1/adg1;
gf1=adg1/dmi1;
fg2=dmi2/adg2;
gf2=adg2/dmi2;
fg3=dmi3/adg3;
gf3=adg3/dmi3;
fg=dmi/adg;
gf=adg/dmi;
proc print;
proc means n mean stderr min max;
proc sort; by trt;
proc means n mean stderr min max; by trt;
run;
```

NEM AND NEG CODE EQUATIONS.

```
data nem; set fg;
avgwt1=(inwt+d35)/2;
avgwt2=(d35+d121)/2;
avgwt3=(d121+finwt)/2;
avgwt=(inwt+finwt)/2;
avgwt1k=avgwt1/2.204;
eqsbw1=avgwt1k*(478/(finwt/2.204));
ebw1=0.891*eqsbw1;
ebg1=0.956*(adg1/2.204);
nemr1=.077*(avgwt1k**.75);
negr1=.0635*(ebw1**.75)*(ebg1**1.097);
qa1=0.877*(dmi1/2.204);
qb1=(0.877*-nemr1)+(-.41*(dmi1/2.204))-negr1;
qc1=(-.41*-nemr1);
qneg1=-qb1;
qbsq1=qb1**2;
q4ac1=4*qa1*qc1;
qsqrt1=(qbsq1-q4ac1)**0.50;
qnum1=qneg1+qsqrt1;
qden1=2*qa1;
cnemkg1=qnum1/qden1;
cnegkg1=(cnemkg1*0.877)-0.41;
```

```
cnem1=cnemkg1/2.204*100;
cneg1=cnegkg1/2.204*100;
avgwt2k=avgwt2/2.204;
eqsbw2=avgwt2k*(478/(finwt/2.204));
ebw2=0.891*eqsbw2;
ebg2=0.956*(adg2/2.204);
nemr2=.077*(avgwt2k**.75);
negr2=.0635*(ebw2**.75)*(ebg2**1.097);
qa2=0.877*(dmi2/2.204);
qb2=(0.877*-nemr2)+(-.41*(dmi2/2.204))-negr2;
qc2=(-.41*-nemr2);
qneg2=-qb2;
qbsq2=qb2**2;
q4ac2=4*qa2*qc2;
qsqrt2=(qbsq2-q4ac2)**0.50;
qnum2=qneg2+qsqrt2;
qden2=2*qa2;
cnemkg2=qnum2/qden2;
cnegkg2=(cnemkg2*0.877)-0.41;
cnem2=cnemkg2/2.204*100;
cneg2=cnegkg2/2.204*100;
avgwt3k=avgwt3/2.204;
eqsbw3=avgwt3k*(478/(finwt/2.204));
ebw3=0.891*eqsbw3;
ebg3=0.956*(adg3/2.204);
nemr3=.077*(avgwt3k**.75);
negr3=.0635*(ebw3**.75)*(ebg3**1.097);
qa3=0.877*(dmi3/2.204);
qb3=(0.877*-nemr3)+(-.41*(dmi3/2.204))-negr3;
qc3=(-.41*-nemr3);
qneg3=-qb3;
qbsq3=qb3**2;
q4ac3=4*qa3*qc3;
qsqrt3=(qbsq3-q4ac3)**0.50;
qnum3=qneg3+qsqrt3;
qden3=2*qa3;
cnemkg3=qnum3/qden3;
cnegkg3=(cnemkg3*0.877)-0.41;
cnem3=cnemkg3/2.204*100;
cneg3=cnegkg3/2.204*100;
avgwtk=avgwt/2.204;
eqsbw=avgwtk*(478/(finwt/2.204));
ebw=0.891*eqsbw;
ebg=0.956*(adg/2.204);
nemr=.077*(avgwtk**.75);
negr=.0635*(ebw**.75)*(ebg**1.097);
qa=0.877*(dmi/2.204);
qb=(0.877*-nemr)+(-.41*(dmi/2.204))-negr;
qc=(-.41*-nemr);
qneg=-qb;
qbsq=qb**2;
q4ac=4*qa*qc;
```

```
qsqrt=(qbsq-q4ac)**0.50;
qnum=qneg+qsqrt;
qden=2*qa;
cnemkg=qnum/qden;
cnegkg=(cnemkg*0.877)-0.41;
cnem=cnemkg/2.204*100;
cneg=cnegkg/2.204*100;
proc print;
proc means;
proc sort; by trt;
proc means n mean stderr min max; by trt;
run;
```

NEM AND NEG MIXED MODEL ANALYSIS SAS CODE.

```
proc mixed data=nem;
class trt rep;
model cnem1=trt/s ddfm=kr;
random rep;
lsmeans trt/pdiff;
contrast 'NC vs supplemented' trt 3 -1 -1 -1;
contrast 'NRC vs Con Nutri' trt 0 2 -1 -1;
contrast 'InOrg vs Org' trt 0 0 1 -1;

proc mixed data=nem;
class trt rep;
model cnem1=inwt trt/s ddfm=kr;
random rep;
lsmeans trt/pdiff;
contrast 'NC vs supplemented' trt 3 -1 -1 -1;
contrast 'NRC vs Con Nutri' trt 0 2 -1 -1;
contrast 'InOrg vs Org' trt 0 0 1 -1;

proc mixed data=nem;
class trt rep;
model cnem2=trt/s ddfm=kr;
random rep;
lsmeans trt/pdiff;
contrast 'NC vs supplemented' trt 3 -1 -1 -1;
contrast 'NRC vs Con Nutri' trt 0 2 -1 -1;
contrast 'InOrg vs Org' trt 0 0 1 -1;

proc mixed data=nem;
class trt rep;
model cnem2=inwt trt/s ddfm=kr;
random rep;
lsmeans trt/pdiff;
contrast 'NC vs supplemented' trt 3 -1 -1 -1;
contrast 'NRC vs Con Nutri' trt 0 2 -1 -1;
contrast 'InOrg vs Org' trt 0 0 1 -1;proc mixed data=nem;
```

```
class trt rep;
model cnem3=trt/s ddfm=kr;
random rep;
lsmeans trt/pdiff;
contrast 'NC vs supplemented' trt 3 -1 -1 -1;
contrast 'NRC vs Con Nutri' trt 0 2 -1 -1;
contrast 'InOrg vs Org' trt 0 0 1 -1;

proc mixed data=nem;
class trt rep;
model cnem3=inwt trt/s ddfm=kr;
random rep;
lsmeans trt/pdiff;
contrast 'NC vs supplemented' trt 3 -1 -1 -1;
contrast 'NRC vs Con Nutri' trt 0 2 -1 -1;
contrast 'InOrg vs Org' trt 0 0 1 -1;proc mixed data=nem;
class trt rep;
model cnem=trt/s ddfm=kr;
random rep;
lsmeans trt/pdiff;
contrast 'NC vs supplemented' trt 3 -1 -1 -1;
contrast 'NRC vs Con Nutri' trt 0 2 -1 -1;
contrast 'InOrg vs Org' trt 0 0 1 -1;

proc mixed data=nem;
class trt rep;
model cnem=inwt trt/s ddfm=kr;
random rep;
lsmeans trt/pdiff;
contrast 'NC vs supplemented' trt 3 -1 -1 -1;
contrast 'NRC vs Con Nutri' trt 0 2 -1 -1;
contrast 'InOrg vs Org' trt 0 0 1 -1;proc mixed data=nem;
class trt rep;
model cneg1=trt/s ddfm=kr;
random rep;
lsmeans trt/pdiff;
contrast 'NC vs supplemented' trt 3 -1 -1 -1;
contrast 'NRC vs Con Nutri' trt 0 2 -1 -1;
contrast 'InOrg vs Org' trt 0 0 1 -1;

proc mixed data=nem;
class trt rep;
model cneg1=inwt trt/s ddfm=kr;
random rep;
lsmeans trt/pdiff;
contrast 'NC vs supplemented' trt 3 -1 -1 -1;
contrast 'NRC vs Con Nutri' trt 0 2 -1 -1;
contrast 'InOrg vs Org' trt 0 0 1 -1;proc mixed data=nem;
class trt rep;
model cneg2=trt/s ddfm=kr;
random rep;
lsmeans trt/pdiff;
```

```
contrast 'NC vs supplemented' trt 3 -1 -1 -1;
contrast 'NRC vs Con Nutri' trt 0 2 -1 -1;
contrast 'InOrg vs Org' trt 0 0 1 -1;

proc mixed data=nem;
class trt rep;
model cneg2=inwt trt/s ddfm=kr;
random rep;
lsmeans trt/pdiff;
contrast 'NC vs supplemented' trt 3 -1 -1 -1;
contrast 'NRC vs Con Nutri' trt 0 2 -1 -1;
contrast 'InOrg vs Org' trt 0 0 1 -1;proc mixed data=nem;
class trt rep;
model cneg3=trt/s ddfm=kr;
random rep;
lsmeans trt/pdiff;
contrast 'NC vs supplemented' trt 3 -1 -1 -1;
contrast 'NRC vs Con Nutri' trt 0 2 -1 -1;
contrast 'InOrg vs Org' trt 0 0 1 -1;

proc mixed data=nem;
class trt rep;
model cneg3=inwt trt/s ddfm=kr;
random rep;
lsmeans trt/pdiff;
contrast 'NC vs supplemented' trt 3 -1 -1 -1;
contrast 'NRC vs Con Nutri' trt 0 2 -1 -1;
contrast 'InOrg vs Org' trt 0 0 1 -1;proc mixed data=nem;
class trt rep;
model cneg=trt/s ddfm=kr;
random rep;
lsmeans trt/pdiff;
contrast 'NC vs supplemented' trt 3 -1 -1 -1;
contrast 'NRC vs Con Nutri' trt 0 2 -1 -1;
contrast 'InOrg vs Org' trt 0 0 1 -1;

proc mixed data=nem;
class trt rep;
model cneg=inwt trt/s ddfm=kr;
random rep;
lsmeans trt/pdiff;
contrast 'NC vs supplemented' trt 3 -1 -1 -1;
contrast 'NRC vs Con Nutri' trt 0 2 -1 -1;
contrast 'InOrg vs Org' trt 0 0 1 -1;
run;
```

LIVER TM ANALYSIS SAS CODE.

```
data liver; input pen trt   rep   Co    Cu    Mn    Se    Zn;
cards;
```

```
;
proc sort; by trt;
proc means n mean stderr min max; by trt;
proc sort; by rep;
proc means n mean stderr min max; by rep;

proc mixed covtest data=liver;
class trt rep;
model co=trt/s ddfm=kr;
random rep;
lsmeans trt/cl pdiff;
contrast 'neg control vs supple TM' trt 3 -1 -1 -1;
contrast 'nrc vs vasc' trt 0 2 -1 -1;
contrast 'inorg vs org' trt 0 0 1 -1;

proc mixed covtest data=liver;
class trt rep;
model cu=trt/s ddfm=kr;
random rep;
lsmeans trt/cl pdiff;
contrast 'neg control vs supple TM' trt 3 -1 -1 -1;
contrast 'nrc vs vasc' trt 0 2 -1 -1;
contrast 'inorg vs org' trt 0 0 1 -1;proc mixed covtest data=liver;
class trt rep;
model zn=trt/s ddfm=kr;
random rep;
lsmeans trt/cl pdiff;
contrast 'neg control vs supple TM' trt 3 -1 -1 -1;
contrast 'nrc vs vasc' trt 0 2 -1 -1;
contrast 'inorg vs org' trt 0 0 1 -1;proc mixed covtest data=liver;
class trt rep;
model se=trt/s ddfm=kr;
random rep;
lsmeans trt/cl pdiff;
contrast 'neg control vs supple TM' trt 3 -1 -1 -1;
contrast 'nrc vs vasc' trt 0 2 -1 -1;
contrast 'inorg vs org' trt 0 0 1 -1;proc mixed covtest data=liver;
class trt rep;
model mn=trt/s ddfm=kr;
random rep;
lsmeans trt/cl pdiff;
contrast 'neg control vs supple TM' trt 3 -1 -1 -1;
contrast 'nrc vs vasc' trt 0 2 -1 -1;
contrast 'inorg vs org' trt 0 0 1 -1;
run;
```

FEED ANALYSIS SAS CODE.

RATION:

```
data ration; input Month ID CP   NPN   aNDF  Fat   Ca    P     K     Mg
     Su;
cards;
proc sort; by id;
proc means n mean stderr min max; by id;

proc mixed;
class id month;
model CP=id/ddfm=kr;
random month;
lsmeans id/cl pdiff;

proc mixed;
class id month;
model NPN=id/ddfm=kr;
random month;
lsmeans id/cl pdiff;

proc mixed;
class id month;
model aNDF=id/ddfm=kr;
random month;
lsmeans id/cl pdiff;

proc mixed;
class id month;
model Fat=id/ddfm=kr;
random month;
lsmeans id/cl pdiff;

proc mixed;
class id month;
model Ca=id/ddfm=kr;
random month;
lsmeans id/cl pdiff;

proc mixed;
class id month;
model P=id/ddfm=kr;
random month;
lsmeans id/cl pdiff;

proc mixed;
class id month;
model K=id/ddfm=kr;
random month;
lsmeans id/cl pdiff;

proc mixed;
class id month;
model Mg=id/ddfm=kr;
random month;
```

```
lsmeans id/cl pdiff;proc mixed;
class id month;
model Su=id/ddfm=kr;
random month;
lsmeans id/cl pdiff;

run;

data ration; input Month IDAl    Co    Cu    Fe    Mn    Mo    Zn;
cards;
proc sort; by id;
proc means n mean stderr min max; by id;

proc mixed;
class id month;
model al=id/ddfm=kr;
random month;
lsmeans id/cl pdiff;
contrast 'trt 1 vs 2-4' id 3 -1 -1 -1;
contrast 'trt 2 vs 3-4' id 0 2 -1 -1;
contrast 'trt 3 vs 4' id 0 0 1 -1;

proc mixed;
class id month;
model co=id/ddfm=kr;
random month;
lsmeans id/cl pdiff;
contrast 'trt 1 vs 2-4' id 3 -1 -1 -1;
contrast 'trt 2 vs 3-4' id 0 2 -1 -1;
contrast 'trt 3 vs 4' id 0 0 1 -1;

proc mixed;
class id month;
model cu=id/ddfm=kr;
random month;
lsmeans id/cl pdiff;
contrast 'trt 1 vs 2-4' id 3 -1 -1 -1;
contrast 'trt 2 vs 3-4' id 0 2 -1 -1;
contrast 'trt 3 vs 4' id 0 0 1 -1;

proc mixed;
class id month;
model fe=id/ddfm=kr;
random month;
lsmeans id/cl pdiff;
contrast 'trt 1 vs 2-4' id 3 -1 -1 -1;
contrast 'trt 2 vs 3-4' id 0 2 -1 -1;
contrast 'trt 3 vs 4' id 0 0 1 -1;

proc mixed;
class id month;
model mn=id/ddfm=kr;
```

```
random month;
lsmeans id/cl pdiff;
contrast 'trt 1 vs 2-4' id 3 -1 -1 -1;
contrast 'trt 2 vs 3-4' id 0 2 -1 -1;
contrast 'trt 3 vs 4' id 0 0 1 -1;

proc mixed;
class id month;
model mo=id/ddfm=kr;
random month;
lsmeans id/cl pdiff;
contrast 'trt 1 vs 2-4' id 3 -1 -1 -1;
contrast 'trt 2 vs 3-4' id 0 2 -1 -1;
contrast 'trt 3 vs 4' id 0 0 1 -1;

proc mixed;
class id month;
model zn=id/ddfm=kr;
random month;
lsmeans id/cl pdiff;
contrast 'trt 1 vs 2-4' id 3 -1 -1 -1;
contrast 'trt 2 vs 3-4' id 0 2 -1 -1;
contrast 'trt 3 vs 4' id 0 0 1 -1;

run;
```

SUPPLEMENT:

```
data supp; input Month ID   CP    NPN   Ca    K     Mg    P;
cards;
proc sort; by id;
proc means n mean stderr min max; by id;

proc mixed;
class id month;
model CP=id/ddfm=kr;
random month;
lsmeans id/cl pdiff;

proc mixed;
class id month;
model NPN=id/ddfm=kr;
random month;
lsmeans id/cl pdiff;

proc mixed;
class id month;
model Ca=id/ddfm=kr;
random month;
lsmeans id/cl pdiff;

proc mixed;
```

```
class id month;
model K=id/ddfm=kr;
random month;
lsmeans id/cl pdiff;

proc mixed;
class id month;
model Mg=id/ddfm=kr;
random month;

proc mixed;
class id month;
model P=id/ddfm=kr;
random month;
lsmeans id/cl pdiff;

run;

data supp; input Month ID  Al    Co    Cu    Fe    Mn    Mo    Zn;
cards;
proc sort; by id;
proc means n mean stderr min max; by id;

proc mixed;
class id month;
model al=id/ddfm=kr;
random month;
lsmeans id/cl pdiff;
contrast 'trt NC vs supp' id 3 -1 -1 -1;
contrast 'trt NRC vs CN' id 0 2 -1 -1;
contrast 'trt CNI vs CNO' id 0 0 1 -1;

proc mixed;
class id month;
model co=id/ddfm=kr;
random month;
lsmeans id/cl pdiff;
contrast 'trt NC vs supp' id 3 -1 -1 -1;
contrast 'trt NRC vs CN' id 0 2 -1 -1;
contrast 'trt CNI vs CNO' id 0 0 1 -1;

proc mixed;
class id month;
model cu=id/ddfm=kr;
random month;
lsmeans id/cl pdiff;
contrast 'trt NC vs supp' id 3 -1 -1 -1;
contrast 'trt NRC vs CN' id 0 2 -1 -1;
contrast 'trt CNI vs CNO' id 0 0 1 -1;

proc mixed;
class id month;
```

```
model fe=id/ddfm=kr;
random month;
lsmeans id/cl pdiff;
contrast 'trt NC vs supp' id 3 -1 -1 -1;
contrast 'trt NRC vs CN' id 0 2 -1 -1;
contrast 'trt CNI vs CNO' id 0 0 1 -1;

proc mixed;
class id month;
model mn=id/ddfm=kr;
random month;
lsmeans id/cl pdiff;
contrast 'trt NC vs supp' id 3 -1 -1 -1;
contrast 'trt NRC vs CN' id 0 2 -1 -1;
contrast 'trt CNI vs CNO' id 0 0 1 -1;

proc mixed;
class id month;
model mo=id/ddfm=kr;
random month;
lsmeans id/cl pdiff;
contrast 'trt NC vs supp' id 3 -1 -1 -1;
contrast 'trt NRC vs CN' id 0 2 -1 -1;
contrast 'trt CNI vs CNO' id 0 0 1 -1;

proc mixed;
class id month;
model zn=id/ddfm=kr;
random month;
lsmeans id/cl pdiff;
contrast 'trt NC vs supp' id 3 -1 -1 -1;
contrast 'trt NRC vs CN' id 0 2 -1 -1;
contrast 'trt CNI vs CNO' id 0 0 1 -1;

run;
```

TRACE MINERAL PREMIX:

```
data premix; input Month ID   Al    Co    Cu    Fe    Mn    Mo    Zn;
cards;
proc sort; by id;
proc means n mean stderr min max; by id;

proc mixed;
class id month;
model al=id/ddfm=kr;
random month;
lsmeans id/cl pdiff;
contrast 'trt NRC vs CN' id 2 -1 -1;
contrast 'trt CNI vs CNO' id 0 1 -1;

proc mixed;
```

```
class id month;
model co=id/ddfm=kr;
random month;
lsmeans id/cl pdiff;
contrast 'trt NRC vs CN' id 2 -1 -1;
contrast 'trt CNI vs CNO' id 0 1 -1;

proc mixed;
class id month;
model cu=id/ddfm=kr;
random month;
lsmeans id/cl pdiff;
contrast 'trt NRC vs CN' id 2 -1 -1;
contrast 'trt CNI vs CNO' id 0 1 -1;

proc mixed;
class id month;
model fe=id/ddfm=kr;
random month;
lsmeans id/cl pdiff;
contrast 'trt NRC vs CN' id 2 -1 -1;
contrast 'trt CNI vs CNO' id 0 1 -1;

proc mixed;
class id month;
model mn=id/ddfm=kr;
random month;
lsmeans id/cl pdiff;
contrast 'trt NRC vs CN' id 2 -1 -1;
contrast 'trt CNI vs CNO' id 0 1 -1;

proc mixed;
class id month;
model mo=id/ddfm=kr;
random month;
lsmeans id/cl pdiff;
contrast 'trt NRC vs CN' id 2 -1 -1;
contrast 'trt CNI vs CNO' id 0 1 -1;

proc mixed;
class id month;
model zn=id/ddfm=kr;
random month;
lsmeans id/cl pdiff;
contrast 'trt NRC vs CN' id 2 -1 -1;
contrast 'trt CNI vs CNO' id 0 1 -1;

run;
```

EXPERIMENT 2:

IN VITRO ANALYSIS SAS CODE

```
data invitro;
input trt  ID    Time  samp  sb    Co    CovCo0     Cu    CovCu0     Mn
      CovMn0     Zn    CovZn0;
cards;

proc print;
quit;

proc sort data=Invitro;
by samp;
run;

proc mixed method = reml data=invitro covtest cl;
by samp;
class trt time;
model cu = CovCu0 trt|time / residual outp=diagnostics;
lsmeans trt/ pdiff;
contrast 'nc vs supp' trt 3 -1 -1 -1;
contrast 'nrc vs cn' trt 0 2 -1 -1;
contrast 'cni vs cno' trt 0 0 1 -1;
lsmeans trt*time/pdiff;
slice time*trt/ sliceby=time pdiff;
ods output lsmeans=lsmeans;
run;

proc sort data=diagnostics;
by samp studentresid;
run;

proc gplot data=lsmeans;
by samp;
plot Estimate*Time=Trt;
symbol1 v=dot color=black l=1 i=join;
symbol2 v=square color=black l=2 i=join;
symbol3 v=triangle color=black l=3 i=join;
symbol4 v=circle color=black l=4 i=join;
symbol5 v=star color=black l=5 i=join;
run;

proc mixed method = reml data=invitro covtest cl;
by samp;
class trt time;
model co = CovCo0 trt|time / residual outp=diagnostics;
lsmeans trt/ pdiff;
contrast 'nc vs supp' trt 3 -1 -1 -1;
contrast 'nrc vs cn' trt 0 2 -1 -1;
contrast 'cni vs cno' trt 0 0 1 -1;
```

```
lsmeans trt*time/pdiff;
slice time*trt/ sliceby=time pdiff;
ods output lsmeans=lsmeans;
run;

proc sort data=diagnostics;
by samp studentresid;
run;

proc gplot data=lsmeans;
by samp;
plot Estimate*Time=Trt;
symbol1 v=dot color=black l=1 i=join;
symbol2 v=square color=black l=2 i=join;
symbol3 v=triangle color=black l=3 i=join;
symbol4 v=circle color=black l=4 i=join;
symbol5 v=star color=black l=5 i=join;
run;

proc mixed method = reml data=invitro covtest cl;
by samp;
class trt time;
model mn = CovMn0 trt|time / residual outp=diagnostics;
lsmeans trt/ pdiff;
contrast 'nc vs supp' trt 3 -1 -1 -1;
contrast 'nrc vs cn' trt 0 2 -1 -1;
contrast 'cni vs cno' trt 0 0 1 -1;
lsmeans trt*time/pdiff;
slice time*trt/ sliceby=time pdiff;
ods output lsmeans=lsmeans;
run;

proc sort data=diagnostics;
by samp studentresid;
run;

proc gplot data=lsmeans;
by samp;
plot Estimate*Time=Trt;
symbol1 v=dot color=black l=1 i=join;
symbol2 v=square color=black l=2 i=join;
symbol3 v=triangle color=black l=3 i=join;
symbol4 v=circle color=black l=4 i=join;
symbol5 v=star color=black l=5 i=join;
run;

proc mixed method = reml data=invitro covtest cl;
by samp;
class trt time;
model zn = CovZn0 trt|time / residual outp=diagnostics;
lsmeans trt/ pdiff;
contrast 'nc vs supp' trt 3 -1 -1 -1;
```

```
contrast 'nrc vs cn' trt 0 2 -1 -1;
contrast 'cni vs cno' trt 0 0 1 -1;
lsmeans trt*time/pdiff;
slice time*trt/ sliceby=time pdiff;
ods output lsmeans=lsmeans;
run;

proc sort data=diagnostics;
by samp studentresid;
run;

proc gplot data=lsmeans;
by samp;
plot Estimate*Time=Trt;
symbol1 v=dot color=black l=1 i=join;
symbol2 v=square color=black l=2 i=join;
symbol3 v=triangle color=black l=3 i=join;
symbol4 v=circle color=black l=4 i=join;
symbol5 v=star color=black l=5 i=join;
run;


data invitro;
input trt  ID    Time  TotalRelCo TotalRelCu TotalRelMn TotalRelZn;
cards;

proc print;
quit;
proc mixed method = reml data=invitro covtest cl;
class trt time;
model TotalRelCo = trt / residual outp=diagnostics;
lsmeans trt/pdiff;
contrast 'nc vs supp' trt 3 -1 -1 -1;
contrast 'nrc vs cn' trt 0 2 -1 -1;
contrast 'cni vs cno' trt 0 0 1 -1;
ods output lsmeans=lsmeans;
run;

proc mixed method = reml data=invitro covtest cl;
class trt time;
model TotalRelCu = trt / residual outp=diagnostics;
lsmeans trt/pdiff;
contrast 'nc vs supp' trt 3 -1 -1 -1;
contrast 'nrc vs cn' trt 0 2 -1 -1;
contrast 'cni vs cno' trt 0 0 1 -1;
ods output lsmeans=lsmeans;
run;

proc mixed method = reml data=invitro covtest cl;
class trt time;
model TotalRelMn = trt / residual outp=diagnostics;
lsmeans trt/pdiff;
```

```
contrast 'nc vs supp' trt 3 -1 -1 -1;
contrast 'nrc vs cn' trt 0 2 -1 -1;
contrast 'cni vs cno' trt 0 0 1 -1;
ods output lsmeans=lsmeans;
run;

proc mixed method = reml data=invitro covtest cl;
class trt time;
model TotalRelZn = trt / residual outp=diagnostics;
lsmeans trt/pdiff;
contrast 'nc vs supp' trt 3 -1 -1 -1;
contrast 'nrc vs cn' trt 0 2 -1 -1;
contrast 'cni vs cno' trt 0 0 1 -1;
ods output lsmeans=lsmeans;
run;


data invitro;
input trt  ID    Time  DMDig CoblankRF  CoblankPep CublankRF  CublankPep
     MnblankRF  MnblankPep ZnblankRF  ZnblankPep;
datalines;

proc mixed method = reml data=invitro covtest cl;
class trt time;
model DMDig = trt|time / residual outp=diagnostics;
lsmeans trt/ pdiff;
contrast 'nc vs supp' trt 3 -1 -1 -1;
contrast 'nrc vs cn' trt 0 2 -1 -1;
contrast 'cni vs cno' trt 0 0 1 -1;
lsmeans trt*time/pdiff;
run;

proc mixed method = reml data=invitro covtest cl;
class trt time;
model CoblankRF = trt|time / residual outp=diagnostics;
lsmeans trt/ pdiff;
contrast 'nc vs supp' trt 3 -1 -1 -1;
contrast 'nrc vs cn' trt 0 2 -1 -1;
contrast 'cni vs cno' trt 0 0 1 -1;
lsmeans trt*time/pdiff;
slice time*trt/ sliceby=time pdiff;
ods output lsmeans=lsmeans;
run;
proc sort data=diagnostics;
by studentresid;
run;

proc gplot data=lsmeans;
plot Estimate*Time=Trt;
symbol1 v=dot color=black l=1 i=join;
symbol2 v=square color=black l=2 i=join;
symbol3 v=triangle color=black l=3 i=join;
```

```
symbol4 v=circle color=black l=4 i=join;
run;

proc mixed method = reml data=invitro covtest cl;
class trt time;
model PepCoblnk = trt|time / residual outp=diagnostics;
lsmeans trt/ pdiff;
contrast 'nc vs supp' trt 3 -1 -1 -1;
contrast 'nrc vs cn' trt 0 2 -1 -1;
contrast 'cni vs cno' trt 0 0 1 -1;
lsmeans trt*time/pdiff;
slice time*trt/ sliceby=time pdiff;
ods output lsmeans=lsmeans;
run;
proc sort data=diagnostics;
by studentresid;
run;

proc gplot data=lsmeans;
plot Estimate*Time=Trt;
symbol1 v=dot color=black l=1 i=join;
symbol2 v=square color=black l=2 i=join;
symbol3 v=triangle color=black l=3 i=join;
symbol4 v=circle color=black l=4 i=join;
run;

proc mixed method = reml data=invitro covtest cl;
class trt time;
model CublankRF = trt|time / residual outp=diagnostics;
lsmeans trt/ pdiff;
contrast 'nc vs supp' trt 3 -1 -1 -1;
contrast 'nrc vs cn' trt 0 2 -1 -1;
contrast 'cni vs cno' trt 0 0 1 -1;
lsmeans trt*time/pdiff;
slice time*trt/ sliceby=time pdiff;
ods output lsmeans=lsmeans;
run;
proc sort data=diagnostics;
by studentresid;
run;

proc gplot data=lsmeans;
plot Estimate*Time=Trt;
symbol1 v=dot color=black l=1 i=join;
symbol2 v=square color=black l=2 i=join;
symbol3 v=triangle color=black l=3 i=join;
symbol4 v=circle color=black l=4 i=join;
run;

proc mixed method = reml data=invitro covtest cl;
class trt time;
model PepCublnk = trt|time / residual outp=diagnostics;
```

```
lsmeans trt/ pdiff;
contrast 'nc vs supp' trt 3 -1 -1 -1;
contrast 'nrc vs cn' trt 0 2 -1 -1;
contrast 'cni vs cno' trt 0 0 1 -1;
lsmeans trt*time/pdiff;
slice time*trt/ sliceby=time pdiff;
ods output lsmeans=lsmeans;
run;
proc sort data=diagnostics;
by studentresid;
run;

proc gplot data=lsmeans;
plot Estimate*Time=Trt;
symbol1 v=dot color=black l=1 i=join;
symbol2 v=square color=black l=2 i=join;
symbol3 v=triangle color=black l=3 i=join;
symbol4 v=circle color=black l=4 i=join;
run;

proc mixed method = reml data=invitro covtest cl;
class trt time;
model MnblankRF = trt|time / residual outp=diagnostics;
lsmeans trt/ pdiff;
contrast 'nc vs supp' trt 3 -1 -1 -1;
contrast 'nrc vs cn' trt 0 2 -1 -1;
contrast 'cni vs cno' trt 0 0 1 -1;
lsmeans trt*time/pdiff;
slice time*trt/ sliceby=time pdiff;
ods output lsmeans=lsmeans;
run;
proc sort data=diagnostics;
by studentresid;
run;

proc gplot data=lsmeans;
plot Estimate*Time=Trt;
symbol1 v=dot color=black l=1 i=join;
symbol2 v=square color=black l=2 i=join;
symbol3 v=triangle color=black l=3 i=join;
symbol4 v=circle color=black l=4 i=join;
run;

proc mixed method = reml data=invitro covtest cl;
class trt time;
model PepMnblnk = trt|time / residual outp=diagnostics;
lsmeans trt/ pdiff;
contrast 'nc vs supp' trt 3 -1 -1 -1;
contrast 'nrc vs cn' trt 0 2 -1 -1;
contrast 'cni vs cno' trt 0 0 1 -1;
lsmeans trt*time/pdiff;
slice time*trt/ sliceby=time pdiff;
```

```
ods output lsmeans=lsmeans;
run;
proc sort data=diagnostics;
by studentresid;
run;

proc gplot data=lsmeans;
plot Estimate*Time=Trt;
symbol1 v=dot color=black l=1 i=join;
symbol2 v=square color=black l=2 i=join;
symbol3 v=triangle color=black l=3 i=join;
symbol4 v=circle color=black l=4 i=join;
run;

proc mixed method = reml data=invitro covtest cl;
class trt time;
model ZnblankRF = trt|time / residual outp=diagnostics;
lsmeans trt/ pdiff;
contrast 'nc vs supp' trt 3 -1 -1 -1;
contrast 'nrc vs cn' trt 0 2 -1 -1;
contrast 'cni vs cno' trt 0 0 1 -1;
lsmeans trt*time/pdiff;
slice time*trt/ sliceby=time pdiff;
ods output lsmeans=lsmeans;
run;
proc sort data=diagnostics;
by studentresid;
run;

proc gplot data=lsmeans;
plot Estimate*Time=Trt;
symbol1 v=dot color=black l=1 i=join;
symbol2 v=square color=black l=2 i=join;
symbol3 v=triangle color=black l=3 i=join;
symbol4 v=circle color=black l=4 i=join;
run;

proc mixed method = reml data=invitro covtest cl;
class trt time;
model PepZnblnk = trt|time / residual outp=diagnostics;
lsmeans trt/ pdiff;
contrast 'nc vs supp' trt 3 -1 -1 -1;
contrast 'nrc vs cn' trt 0 2 -1 -1;
contrast 'cni vs cno' trt 0 0 1 -1;
lsmeans trt*time/pdiff;
slice time*trt/ sliceby=time pdiff;
ods output lsmeans=lsmeans;
run;
proc sort data=diagnostics;
by studentresid;
run;
```

```
proc gplot data=lsmeans;
plot Estimate*Time=Trt;
symbol1 v=dot color=black l=1 i=join;
symbol2 v=square color=black l=2 i=join;
symbol3 v=triangle color=black l=3 i=join;
symbol4 v=circle color=black l=4 i=join;
run;

proc mixed method = reml data=invitro covtest cl;
class trt time;
model DMD2 = trt|time / residual outp=diagnostics;
lsmeans trt/ pdiff;
contrast 'nc vs supp' trt 3 -1 -1 -1;
contrast 'nrc vs cn' trt 0 2 -1 -1;
contrast 'cni vs cno' trt 0 0 1 -1;
lsmeans trt*time/pdiff;
run;

data Invitro;
input trt  ID    Time  samp  Co    Cotrtavg   Corel Cu    Cutrtavg   Curel
      Mn    Mntrtavg   Mnrel Zn    Zntrtavg   Znrel;
cards;

proc print;
quit;
proc sort data=Invitro;
by samp;
run;

proc mixed method = reml data=invitro covtest cl;
by samp;
class trt time;
model Corel = trt / residual outp=diagnostics;
lsmeans trt/pdiff;
contrast 'nc vs supp' trt 3 -1 -1 -1;
contrast 'nrc vs cn' trt 0 2 -1 -1;
contrast 'cni vs cno' trt 0 0 1 -1;
ods output lsmeans=lsmeans;
run;

proc mixed method = reml data=invitro covtest cl;
by samp;
class trt time;
model Curel = trt / residual outp=diagnostics;
lsmeans trt/pdiff;
contrast 'nc vs supp' trt 3 -1 -1 -1;
contrast 'nrc vs cn' trt 0 2 -1 -1;
contrast 'cni vs cno' trt 0 0 1 -1;
ods output lsmeans=lsmeans;
run;

proc mixed method = reml data=invitro covtest cl;
```

```
by samp;
class trt time;
model Mnrel = trt / residual outp=diagnostics;
lsmeans trt/pdiff;
contrast 'nc vs supp' trt 3 -1 -1 -1;
contrast 'nrc vs cn' trt 0 2 -1 -1;
contrast 'cni vs cno' trt 0 0 1 -1;
ods output lsmeans=lsmeans;
run;

proc mixed method = reml data=invitro covtest cl;
by samp;
class trt time;
model Znrel = trt / residual outp=diagnostics;
lsmeans trt/pdiff;
contrast 'nc vs supp' trt 3 -1 -1 -1;
contrast 'nrc vs cn' trt 0 2 -1 -1;
contrast 'cni vs cno' trt 0 0 1 -1;
ods output lsmeans=lsmeans;
run;

data invitro;
input Trt  ID   samp  Time  Znperrel   Cuperrel;
cards;

;
run;
proc print;
quit;

proc mixed method = reml data=invitro covtest cl;
class trt time;
model Znperrel = trt time / residual outp=diagnostics;
lsmeans trt / pdiff;
lsmeans time;
contrast 'nc vs supp' trt 3 -1 -1 -1;
contrast 'nrc vs cn' trt 0 2 -1 -1;
contrast 'cni vs cno' trt 0 0 1 -1;
ods output lsmeans=lsmeans;
run;

proc gplot data=lsmeans;
plot Estimate*trt=time;
symbol1 v=dot color=black l=1 i=join;
symbol2 v=square color=black l=2 i=join;
symbol3 v=triangle color=black l=3 i=join;
symbol4 v=circle color=black l=4 i=join;
run;

proc mixed method = reml data=invitro covtest cl;
class trt time;
```

```
model Cuperrel = trt time / residual outp=diagnostics;
lsmeans trt / pdiff;
lsmeans time;
contrast 'nc vs supp' trt 3 -1 -1 -1;
contrast 'nrc vs cn' trt 0 2 -1 -1;
contrast 'cni vs cno' trt 0 0 1 -1;
ods output lsmeans=lsmeans;
run;

proc gplot data=lsmeans;
plot Estimate*trt=time;
symbol1 v=dot color=black l=1 i=join;
symbol2 v=square color=black l=2 i=join;
symbol3 v=triangle color=black l=3 i=join;
symbol4 v=circle color=black l=4 i=join;
run;
```