SERUM MINERAL CONCENTRATIONS IN WEANED MONTANA RAM LAMBS AND EFFECTS OF DIETARY ZINC SOURCE AND CONCENTRATION ON DEVELOPING TARGHEE RAMS

by

Chad McBride Page

A thesis submitted in partial fulfillment
of the requirements for the degree

of

Master of Science

in

Animal and Range Sciences

MONTANA STATE UNIVERSITY
Bozeman, Montana

November 2017

DEDICATION

To Chantell, my wife and the love of my life. Our dreams are larger together, than they would have ever been alone.

ACKNOWLEDGMENTS

I would like to sincerely thank my wife, Chantell, who helped every step of the way. My Children George, Lorena, and Evelyn who kept me motivated and on my toes with their fun personalities. From a young age my parents George and Bonnie taught me to work hard and have confidence in myself. My grandparents who loved me and taught me to respect my education and animals, thank you. It would be impractical to think that I could thank all the people that have helped me to arrive where I am at today, but know that I extend my deepest appreciation for you.

I would like to thank my committee members, Drs. Whit Stewart, Tom Murphy, Megan Van Emon, and James Berardinelli. Each of you has given advice, guidance, support, and encouragement which enabled my success. In one way or another you have all inspired me to be a better scientist, student, and person. The long and sometimes unexpected days of work will be looked at as fun times and moments that I cherish.

I would also like to thank the support from the Zinpro Corporation especially Connie and Jason who gave valuable words of advice. Thanks should also be given to The National Sheep Industry Improvement Center and Montana State Agriculture Experiment Station who also supported the completion of this work. I would also like to thank my fellow graduate student who assisted with my projects and were my friends throughout this process; you sure did make graduate school fun.

TABLE OF CONTENTS

TABLE OF CONTENTS CONTINUED

LIST OF TABLES

LIST OF TABLES CONTINUED

Table Page

LIST OF FIGURES

Figure Page

ABSTRACT

Trace mineral deficiencies exist in livestock operations and can contribute to decreased productivity and profitability. The objective of the first study was to quantify serum trace mineral concentrations in weaned ram lambs, with particular emphasis on Se and Zn. Serum samples (n = 221) were collected from ram lambs at 21 ranches throughout Montana and analyzed for Co, Cu, Fe, Mn, Mo, Se, and Zn concentrations. Additionally, water samples were analyzed for pertinent characteristics. Of ranches surveyed, only 67% provided a complete mineral supplement. Sheep that were provided supplementary trace mineral had greater serum Se concentrations ($P < 0.001$). Based on serum trace mineral concentration reference ranges, the two most commonly deficient and marginally deficient minerals across Montana were Se (19 and 23.8% of ranches, respectively) and Zn (9.5 and 57.1%, respectively). Of ranches sampled, 40 and 35% of water samples exceeded excessive concentrations in Na and sulfates, respectively. This regional knowledge of serum trace mineral concentrations in a sample of ram lambs can provide information for ranches to evaluate current and future mineral supplementation needs, as well as aid the feed industry in designing formulations for mineral premixes. Based on these results, a second study was designed with the objective to evaluate the effects of dietary Zn source and concentration on serum Zn status, growth performance, wool traits, and reproductive characteristics of developing yearling rams. Forty-four Targhee rams were used in an 84-d completely randomized design and were fed one of three pelleted dietary treatments: 1) a control diet without fortified Zn; 2) a diet fortified with a Zn amino acid complex (ZnAA); and 3) a diet fortified with $ZnSO_4$. Serum samples were collected from each ram at four time periods to quantify serum Zn and testosterone concentrations. $ZnSO_4$ had greater ($P \leq 0.03$) serum Zn concentrations compared to other treatments. Rams consuming ZnAA had greater ($P \leq 0.03$) average daily gain than rams in the other treatments. Wool regrowth was greater ($P < 0.001$) in the $ZnSO_4$ treatment group compared to control. These results indicate that source and concentration of a Zn supplement appears to affect ram development.

CHAPTER ONE

INTRODUCTION

Sheep production in the western U.S. relies heavily on rangelands as a primary feed source. This reliance on rangeland plant communities could lead to clinical or subclinical trace mineral deficiencies, which may limit productivity of livestock operations. Minerals perform essential functions in animals including structural, physiological, catalytic, and regulatory roles (Suttle, 2010). However, forage trace mineral concentrations are highly variable across rangelands as they are largely influenced by soil geochemistry and stage of plant maturity (Mathis et al., 2004; Smith et al., 2014; Jones and Tracy, 2013). Trace minerals are generally obtained through feed components, but may be obtained from water as well (NRC, 2007).

Montana consists of over 380,832 km^2 of diverse geography, which may lead to variability of trace mineral concentration in forage. Additionally, Montana has an estimated 200,000 breeding sheep which was the 5th largest inventory in the U.S. (USDA-NASS, 2017). Previous studies related to trace minerals indicated that Se and Zn concentrations in forages across the western U.S. were less than adequate for animal health and performance (Dargatz and Ross, 1996; Mathis et al., 2004). Mineral deficiencies in breeding sheep, particularly Se and Zn, may have negative effects on reproductive performance and longevity, presenting a financial burden when investing in breeding rams (Suttle, 2010). Despite a large sheep population and general knowledge of

trace mineral deficiencies, specific research attempting to quantify trace mineral status in Montana sheep populations has not been examined.

Since feed Zn concentrations are generally below recommended levels (>30 ppm; NRC, 2007) for growing rams, the fourth chapter of this thesis focuses primarily on the effect of supplementing it. This is justified, considering Zn is the second most abundant trace mineral in the body with important functions involved in reproduction (Kumar et al., 2006), gene expression (Berg, 1990), immune function (Spears and Weiss, 2008), and wool growth in sheep (White et al., 1994). Furthermore, subclinical deficiencies of Zn could be more prevalent than other trace minerals because sheep do not sequester large amounts of it in any one organ (NRC, 2007; Herdt and Hoff, 2011). Optimal concentrations of dietary Zn are not well understood and, with such a high tolerance to Zn toxicity, there is potential to study its effects in sheep when fed at greater levels than those commonly recommended (NRC, 2007). Therefore, one objective of this thesis was to quantify the effects that dietary Zn source and concentration have on Zn status, growth performance, and wool characteristics of developing Targhee rams.

CHAPTER TWO

LITERATURE REVIEW

Importance of Minerals in Livestock Production Systems

Feed costs to meet nutritional requirements have significant impacts on both operational costs and returned revenue in sheep production (LMIC, 2016). Mineral composition is an important aspect of nutritional value in feedstuffs. Within the last century, minerals have proven to play critical roles in biological processes, generally in one of four important functions: structural, physiological, catalytic, and regulatory (Suttle, 2010). The small ruminant nutrient requirements suggested by the National Research Council (2007) indicate that mineral nutrition should be a priority because production, reproduction, immunity, and survival may all be altered when a mineral is outside its adequate range within a feedstuff. Further research quantifying optimal dietary concentrations and sources of mineral are warranted as sheep continue to be selected for greater production standards (i.e., greater lambing percentages, rapid growth, and greater wool production).

Minerals are typically divided into macro and micro subclasses. Macro minerals are required in a larger amount within the body than micro minerals and are usually measured in percentage of feed or in g per head per day. The identified essential macro minerals for livestock include: Ca, P, S, Na, Mg, Cl, and K (Underwood, 1981). Micro or trace minerals are needed in smaller quantities, which are usually expressed in ppm or mg per kg. Nevertheless, trace minerals still play a significant role in the growth rate,

reproductive capacity, and immune system function of animals. The trace minerals that have been identified as essential for the health and productivity of livestock include: As, Co, Cr, Cu, F, Fe, I, Mo, Mn, Mo, Ni, Se, Si, Sn, V, and Zn (Underwood, 1981).

Forage and Soil Mineral Variability

Forages or roughages are generally utilized by ruminants as a primary feed source, due to the fermentation of insoluble carbohydrates (e.g., cellulose and hemicelullose) which occurs in the rumen and supplies energy to the animal (Pond et al., 2005). Concentrations of minerals in forage plants are determined by four factors: 1) the genus, species, or strain of the plant; 2) the type of soil in which the plant grows; 3) the climatic or seasonal conditions during the plant's growth; and 4) the stage of maturity of the plant (Underwood, 1981). Forage mineral concentrations have been quantified across the world to assess whether or not they meet livestock recommendations. This section outlines studies conducted in recent years throughout the U.S.

Mortimer et al. (1999) reported forage mineral concentrations collected in 23 U.S. states. In total, 709 forage samples were taken from 687 operations. Each sample was analyzed for dry matter content, crude protein, and acid detergent fiber. Additionally, forage Cu, Fe, Mn, Mo, Se, Zn, and Vitamin E concentrations were quantified. The authors considered Zn, Se, and Cu adequate for cattle within the ranges of 30 – 500, 200 – 2000, and 10 – 100 ppm, respectively, which are within sheep recommendations (NRC, 2007). By these standards, the trace minerals of most concern were Zn, Se, and Cu which were estimated to be marginally deficient or deficient in 77%, 69.5%, and 66.7% of the samples, respectively. However, this report did not take into account the number of

samples that were indicated to have high levels of antagonistic minerals, which could lead to a lower bioavailability for grazing livestock (Mortimer et al., 1999).

Mathis and Sawyer (2004) conducted a forage mineral survey in 42 locations across New Mexico in 2001 and 2002. Selected sites were representative of New Mexico's 9 different land resource areas. Each site was sampled once in the fall and once in winter for two consecutive years and Co, Cu, Fe, Mn, Mo, Se, and Zn concentrations were quantified. There was a large variation in mineral concentration, even among forages collected in the same resource area. Selenium was of most concern with 92% of samples below the NRC recommendations for beef cattle (0.1 ppm; NRC 2016). Additionally, 77% of samples fell below the requirement for Zn (30 ppm NRC, 2016).These studies indicate that variability of trace minerals exist among forages and geographical regions in the U.S., which is of economic importance to livestock production since many have a direct effect on fertility and muscle growth.

Trace mineral nutrition is dependent upon soil and plant levels and animal age and stage of production (Judson and MacFarlane, 1998). This interaction can be complicated because soil pH, soil type, and amount of precipitation all contribute to forage mineral concentration (Underwood, 1981). In general soil Zn concentrations typically range from 10 – 300 ppm, but on average are 50 ppm and do not vary much from the parent rock (McDowell, 2017). Results from geological mineral surveys can be used to determine if a geographic area is, on average, deficient in one or more soil minerals (Smith et al., 2014). However, Judson and MacFarlane (1998) suggested that

soil and plant mineral content are not accurate predictors of mineral disorders or status of grazing livestock.

Animal mineral status could be adequate despite soil and forage deficiencies because sheep: ingest a certain amount of soil which is amplified during high stocking rates, have the ability to store minerals to utilize during times of deficiency, and often select the most nutritous part of the plant for consumption (Judson and MacFarlane, 1998). Nevertheless, animals with high physiological demands, such as young rapidly growing rams or ewes during pregnancy and lactation, are the most susceptable to trace mineral deficiencies (Judson and MacFarlane, 1998). These studies stress the importance for feedstuff analyses on operations, since range forage Zn concentrations are often below recommended levels for sheep production systems in the U.S. (Grings et al., 1996; Mathis and Sawyer, 2004).

Zinc Sources for Sheep

On average, sheep production systems in the western U.S. have lower variable costs ($92.16 ewe/yr) than other regions, which is in part due to the decreased dependence of grain and hay as feedstuffs and greater use of federal range land and pasture (LMIC, 2016). While this generally reduces production costs, it could lead to mineral deficiencies. Grings et al. (1996) quantified trace mineral concentrations in Montana range forages and their physiological states. Live warm season grasses and forbs were the only samples that contained sufficient Zn for cattle (30 ppm; NRC, 2016). However, this level does not meet the recommendation for growing lambs (38 ppm Zn; NRC, 2007). Additionally, Zn concentrations in rangeland forages are frequently below

recommendations for grazing livestock (Grings et al., 1996; Jones and Tracy, 2013; Rauzi et al., 1969). Generally, forage Zn concentration declines with plant maturity, including Blue Grama (*Bouteloua gracilis*) and Western Wheatgrass (*Pascopyrum smithii*; Jones and Tracy, 2013; Rauzi et al., 1969), both native to Montana.

Successive cuttings of hay crops within a season seem to be associated with diminishing Zn concentrations. A study conducted by Reid et al. (1987) reported the effects of foliar and soil Zn fertilization on alfalfa Zn concentrations. Zinc absorption from non-treated alfalfa ranged from 6.7 – 11.5% between cuttings, however there was no statistical difference in Zn absorption by cutting in these control plants. The soil fertilized treatment declined in alfalfa Zn concentration, up to 6 ppm DM, with each successive cutting in the year. Foliar application of Zn had a larger effect, and alfalfa Zn concentration increased (24 – 69 ppm DM) with each successive cutting (Reid et al., 1987).

Sheep operations that feed high percentages of grain and concentrates should understand that Zn concentrations differ little between plant species, and are influenced mainly by soil Zn content (Suttle, 2010; McDowell, 2017). Common grains such as wheat, oats, barley, and millet contain, on average, 26-35 ppm Zn, while corn is slightly lower with an average content of 25 ppm (Suttle, 2010; McDowell, 2017). If soil Zn content is low, application of a fertilizer with appropriate concentrations of Zn, as illustrated earlier, could be a possible solution to increase Zn concentrations in forages (Reid et al., 1987). However, there may not be a direct economic incentive for the grain or hay producer to do so. Therefore, if forage or concentrate trace mineral content is

below recommended levels for a particular class of sheep, supplementation may be necessary.

Zinc Supplementation Strategies

The goal of mineral supplementation is to meet recommended levels if the basal diet is not sufficient. Zinc and other trace minerals can be offered free-choice or delivered by injection, oral drench, and rumen bolus. Free choice mineral supplements are common on sheep operations and are usually provided in block or loose granulized form. Mineral supplements provided in a block form are convenient, but it is difficult for animals to consume the recommended daily amount. McDowell (1996) estimated that animals will consume, on average, 10% less mineral in block form than in loose form. Loose supplement should be kept out of the elements to prevent caking, which decreases palatability and increases molding (McDowell 1996).

The greatest challenge with supplementing mineral in a free choice manner is the unavoidable variation in intake within a flock. Ragen et al. (2015) reported that ewes in similar production stages (non-pregnant and non-lactating) had a large variation in individual consumption of mineral supplement offered *ad libitum*. Ewes in confinement whose consumption was low or none, adequate, or above recommended levels ranged from 17-60%, 33-66%, and 6-17%, respectively, across years. Ewes grazing that consumed low or none, adequate, or above recommended levels of mineral supplement ranged from 0-7%, 69-72%, and 24-28%, respectively, across years. The authors also reported on average a greater proportion of ewes consuming granulized mineral supplement *ad libitum* in a pasture setting compared to ewes in confinement (71% vs.

50%, respectively). However, greater mineral intake by animals with lower stocking densities seems to contradict findings from Ducker et al. (1981), who reported decreased intake with increased grazing area per ewe. Ragen et al, (2015) presented the argument that ewes grazing smaller pastures more frequently passed by the mineral supplement, which could have increased consumption compared to ewes fed in confinement.

McDowell (1996) identified factors that contributed to variation in free choice mineral intake including: soil fertility, forage type, season, available energy and protein, individual requirements, salt content of water, palatability of mineral mixture, and physical forms of minerals. In cases when Na concentrations in water are excessive, animals may refuse a salt-mineral mix offered *ad libitum* (Petersen et al., 2015), which is the driving factor in mineral supplement consumption in ruminants further complicating intake variation within flocks. Additionally, Bowman and Sowell (1997) listed several animal factors contributing to variability in supplementation intake including previous experience and social interactions. For example, Lobato et al. (1980) reported greater supplement intake in lambs after weaning if they were exposed before weaning.

Zinc Source Bioavailability

The most common forms of Zn for supplementation are ZnO and $ZnSO_4$ (McDowell, 2017). However, in recent years, chelated or organic forms have become commercially available. Chelation refers to a special type of complex formed between a ligand and a metal ion (Kratzer and Vohra, 1986). To be classified as a chelate, the ligand or chelating agent must contain a minimum of two functional groups (oxygen, nitrogen, amino acid, hydroxyl) each capable of donating a pair of electrons to a metal and forming

a heterocyclic ring structure with it (Kratzer and Vohra, 1986). Commercially available organic or chelated forms of Zn include Zn methionine (Zn Meth), Zn lysine (Zn Lys), Zn protienate, and Zn amino acid complexes (ZnAA).

The form that Zn or other trace minerals is offered in is important because they can vary in their bioavailability. Bioavailability refers to the fraction of the ingested nutrient that is absorbed and available for utilization in normal physiological functions (Fairweather-Tait and Hurrell, 1996). It is generally reported that organic sources of Zn are more bioavailable than inorganic sources (Kincaid et al., 1997; Spears, 2003). Cao et al. (2000) investigated the bioavailability of eight different commercially available Zn sources. Zinc bioavailability was determined using tissue Zn concentrations and metallothionine, which is up-regulated with increasing Zn levels. The authors reported the organic sources Zn proteinate, ZnAA, and Zn Meth were 130, 110, and 113% more bioavailable, respectively, than $ZnSO_4$ (Cao et al., 2000).

However, Spears (1989) conducted several lamb metabolism studies that evaluated the bioavailability and retention of Zn Meth and ZnO. It was concluded that the two Zn sources resulted in a similar bioavailability based on plasma alkaline phosphatase activity (Zn Meth = 109 U/liter and ZnO = 91 U/liter) and ADG (276 and 315 g/d, respectively). However, the authors reported a greater retention of Zn Meth than ZnO because of a slower rate of decline in plasma Zn, indicating that sources can be metabolized differently post absorption. In a later paper, Spears (1996) expounded on a theory that the bioavailability of organic mineral sources could be greater because reactions with other dietary components is less likely to form complexes that inhibit

absorption. Under this theory, a Zn Meth complex would be more stable in the rumen allowing greater absorbance compared to inorganic sources of Zn.

Rojas et al. (1995) compared the bioavailability of two inorganic (ZnO and $ZnSO_4$) and two organic forms (Zn Meth and Zn lys) of Zn. Lambs were restricted to 1 kg of a basal diet containing 16 – 20 ppm Zn and supplemented 360 mg/d of their respective Zn sources. By d 55, serum Zn concentrations were greater in Zn Lys (1.58 μg/mL) than Zn Meth (0.78 μg/mL), but not $ZnSO_4$ (0.87 μg/mL). Therefore, the organic forms (Zn Meth and Zn Lys) had similar or greater bioavailability than the most bioavailable inorganic form ($ZnSO_4$). Additionally, it was concluded that Zn Lys was the most bioavailable to sheep as it was found in greater concentration in the kidneys, liver, and pancreas following treatment than Zn Meth, $ZnSO_4$, and ZnO (Rojas et al., 1995).

Antagonists to Zn bioavailability can be acquired from many different sources. Drinking water is a common source of antagonistic minerals, which may limit the bioavailability of Zn (Socha, 2003). Antagonists include competitive inhibitors, which share the same high affinities for biological carriers. For example, Zn and Fe both share a high affinity for transferrin, a plasma protein that transports Fe in blood to organs (Sandstrom and Lonnderdal, 1989). When present in high levels, Ca, P, and Fe can decrease Zn absorbance and overall bioavailability. Zinc's ionic configuration with its 10 outer electrons in d orbital forms a tetrahedral sp^3 chelated configuration. This structure prefers to interact with configurations that are identical to Cu^+ and Cd^{2+} which have a coordination number of four. Sulfur interacts with several minerals including Cu, Mo, Se, and Zn and high concentrations of sulfates in water can act as antagonists to these

minerals, possibly causing clinical or subclinical deficiencies. Petersen et al. (2015) estimated the variability of water mineral content across location, year, and water source at the Fort Keogh Livestock and Range Research Laboratory (Miles City, MT). Water characteristics that exceeded maximum tolerable concentrations and could act antagonistically to Zn included Fe and SO_4 in 66% and 37% of samples, respectively. A combination of more bioavailable Zn sources and managing for possible antagonists could increase productivity in operations where animals are experiencing clinical or subclinical Zn deficiencies.

Importance of Zinc in Ruminant Diets

Zinc is the second most abundant trace mineral in the body behind Fe and is involved in the function of over 300 enzymes (Valle and Falchuk, 1993; Suttle, 2010). The major functions of Zn in livestock are gene expression, appetite control, fat absorption, and antioxidant defense (Suttle, 2010). The first evidence of Zn as an essential nutrient was confirmed in laboratory animals. Todd et al. (1933) fed rats basal diets with very little Zn (1.6 ppm), with special care to provide concurrently known essential vitamins and amino acids. Rats supplemented 5 mg of either ZnO or $ZnCl_2$ had superior growth compared to those on the deficient diet (Todd et al., 1933). Ott et al. (1964) described the role of Zn in lambs by feeding diets with low (2.7 ppm) and high levels of Zn (100 ppm). They reported that lambs fed the Zn deficient diet were slower growing, less feed efficient, and developed parakeratotic lesions. Since then, Zn

deficiencies have been shown to impair growth and reproductive characteristics in ruminants and will be reviewed in future sections.

Zinc Recommendations and Tolerances

Dietary Zn should be present in adequate levels on a continual basis because it is only stored in small amounts that are readily available in the body (McDowell, 2017). Although Zn is relatively nontoxic, it is less tolerable at high levels in ruminants than in monogastric animals (NRC, 2007). As discussed previously, Zn dietary recommendations can be challenging because of low concentrations commonly found in forages during certain times of the year and the variability in animal feed stuffs across locations.

The small ruminant NRC (1985; 2007) recommends dietary concentrations of Zn between 20-39 ppm DM for adult sheep and gestating and lactating ewes. Zinc requirements are especially important in wool producing sheep (White et al., 1994), and these requirements may vary by genotype (blackface vs. Merino). These recommendations are based on a factorial approach and reflect the minimum concentrations necessary to prevent clinical symptoms. Cattle recommendations for dietary Zn are less specific than sheep, only giving the recommendation of 30 ppm Zn regardless of the physiological state of the animal (NRC, 2007; 2016).

Zinc toxicity is relatively rare but has been reported in sheep (Davies et al., 1977). Both naturally occurring and induced cases of Zn toxicity have similar effects including: loss of appetite and condition, diarrhea, dehydration, subcutaneous edema, profound weakness, jaundice, and anemia (Allen et al., 1983). Pathological changes also occur in the pancreas, kidney, liver, rumen, abomasum, small intestine, and adrenal gland.

However, the greatest contributor to the deterioration of animal health due to Zn toxicity are lesions in the kidney and abomasum (Allen et al., 1983).

Ott et al. (1966) conducted four experiments to determine the maximum level at which Zn could be fed to lambs without having detrimental effects on performance. They concluded that Zn consumption above 1.5 g/kg caused depressed feed consumption, compared to 1 g/kg which decreased gains and feed efficiency, but increased overall mineral consumption. A separate experiment reported lambs fed 0.5 and 1 g Zn/kg feed had greater DMI, ADG, and feed efficiency than lambs consuming 2 and 4 g Zn/kg feed (Ott et al., 1966). This suggests that optimal concentrations of Zn could increase profitability in lamb production systems.

Zinc Deficiency Associated Disorders

Zinc associated disorders are most commonly associated with deficiency since toxicity is relatively rare. Rumen microbes have the ability to digest phytate, a compound that reduces the availability of dietary Zn (Suttle, 2010). This attribute of ruminal microbes could decrease the severity of Zn deficiency in ruminants compared to monogastric animals. Zinc deficiency is classified in clinical or subclinical states (Underwood and Somers, 1969). It is thought that subclinical or marginal Zn deficiencies are more common because of the lack of visual symptoms exhibited by the animal. Nevertheless, subclinical deficiencies impact animal growth and fertility (Underwood and Suttle, 1999). For example, if dietary Zn concentration was 20 ppm, signs of growth reduction would likely not be apparent, but testicular development may be impaired in prepubertal rams (Underwood and Somers, 1969).

Commonly observed Zn deficiency disorders are indicated by anorexia, abnormalities of the skin and appendages, skeletal abnormalities, and reproductive disorders (Suttle, 2010). Blackmon et al. (1967) compiled a list of clinical signs of Zn deficiency which included: inflammation of the nose and mouth with sub mucous hemorrhages, decreased wool quality, joint stiffness, bowing of the legs, and cracks in the skin around the hooves, ears, nostrils, and scrotum, to name a few. Zinc deficiency causes a sheep's fleece to become loose and reduces fiber strength, both of which have detrimental effects on wool quality (McDowell, 2017). Zinc deficiencies and their negative effects could possibly be curtailed in livestock operations if Zn animal status was easily assessed and reconciled.

Determining Zinc Status in Ruminants

The mineral status of an animal is generally determined by their concentration in the body tissues that store them. For example, liver biopsies are recommended to accurately quantify concentrations of Cu and Mn since this organ acts as a storage site (Suttle, 2010). However, the majority of literature concludes that there is no organ or tissue that is utilized as a major storage pool for Zn (Herdt and Hoff, 2011; Suttle, 2010; NRC 2007; Salgueiro, 2000). Therefore, individual animal or population Zn status is commonly quantified through its concentration in serum or plasma (Herdt and Hoff, 2011; Salgueiro, 2000). Due to the lack of Zn storage in organs, serum and plasma concentrations are indicative of dietary concentration at the time of collection. However, age, stress, infection, and feed restriction can all have impacts on serum and plasma Zn concentration (Kincaid and Hodgson, 1989). For example, plasma Zn can be initially

reduced by acute phase response to infection (Wellinghausen and Rink, 1998). Additionally, serum Zn can be decreased by hyper thermal stress and ketosis in dairy cattle (Wegner et al., 1973).

Other tissues, proteins, and enzymes have been explored as indicators of Zn status. Kincaid (1999) described several of these in a review of quantifying trace mineral status in ruminants. Metallothionine concentrations in serum and erythrocytes can be used as indicators of Zn status, which is useful because they are less affected by infection (Kincaid, 1999). Liver Zn concentration is particularly useful when the primary interest is the Zn to Cu ratio, because of the antagonistic relationship between these two metals (Hatfield et al., 2001). A list of Zn status indicators is presented in Table 1.

Table 1. Zinc status indicators

Plasma Zn concentration
Serum Zn concentration
Plasma metallothionein
Erythrocyte metallothionein
Erythrocyte Zn concentration
Leukocyte Zn concentration
Neutrophil Zn concentration
5’ Nucleotidase activity
Alkaline phosphatase activity
Hair/hoof Zn concentration
Liver Zn concentration

Adapted from (Kincaid, 1999; Salgueiro et al., 2000; Suttle, 2010; Herdt and Hoff, 2011)

Suttle (2010) reported that serum and plasma Zn concentrations are normal within a range of 0.8 - 1.2 mg or 12.3 - 18.5 μmol l^{-1}. However, the lower end of this range may be too low for healthy livestock and they suggest 0.6 – 1.2 μg/mL as an adequate serum concentration to prevent over diagnosing Zn deficiencies in populations. With these

levels, serum Zn concentrations are classified as deficient (<0.4 mg l^{-1}), marginally deficient (0.4 - 0.6 mg l^{-1}), adequate/normal (0.6 - 1.2 mg l^{-1}), and excessive (>1.2 mg l^{-1}). Adequate Zn status helps to ensure that sheep are reaching their full potential in a production setting, since Zn deficiencies may reduce lamb growth, wool growth, and reproductive characteristics.

The Role of Zinc in Livestock Production

Growth

The largest factors affecting economic returns to the average sheep enterprise are lambing percentage and market lamb prices, indicating greater profit margins from a greater total weight of lamb marketed (LMIC, 2016). This is similar to most livestock production systems, and because Zn has been shown to have an effect on growth, the majority of research concerning Zn supplementation has focused on these effects.

Underwood and Somers (1969) reported that growth rates were similar between ram lambs fed 17 and 32 ppm Zn, but a diet of 2.4 ppm resulted in a 35% lower BW at the end of the trial. This was in part due to rams on the lowest Zn diet having 37% lower feed intake than the other treatments. Conclusions can be made that Zn deficiency decreases overall nutrient intake, therefore decreasing the growth of young sheep. The effect of Zn on feed intake and growth may stem from its role in appetite. A study conducted in Australia by Martin and White (1992) summarized effects found from supplemental $ZnSO_4$ were attributed to an increase in appetite and, therefore, a greater energy consumption. The authors reported that *ad libitum* feed intake was greatest (1532

g/d) in rams offered the highest dietary Zn diet (27 ppm) compared to the lowest Zn diet (4 ppm Zn) which consumed 693 g/d. Moreover, the live weight of rams fed diets of 4 ppm Zn was 60% that of the rams consuming 10, 17, and 27 ppm Zn, all of which did not differ (Martin and White, 1992).

Garg et al. (2008) conducted a 150 d feeding trial to quantify the effects that organic Zn supplementation had on growth, nutrient utilization, and mineral profiles in lambs. The basal diet consisted of concentrate mix and wheat straw in a 60:40 ratio, with a Zn concentration of 34 ppm. The control treatment was fed the basal diet, while the experimental treatments were fed an additional 20 ppm Zn in the form of either $ZnSO_4$ or Zn Meth. Average daily gain in Zn Meth supplemented lambs was 5.46 g/day greater and feed to gain ratio was 1.29 lower compared to lambs in the control and $ZnSO_4$ groups, which did not differ from one another. Authors did report these findings as statistically different, but are likely biologically and economically insignificant in sheep production with less than 0.5 kg gain difference over the course of a month. Digestibility coefficients of ADF were greater by 0.07 in treatment groups supplemented 20 ppm Zn Meth compared to the $ZnSO_4$ group (Garg et al., 2008). Cellulose digestion coefficients were also 0.09 greater in the Zn Meth treatment compared to the control, but the $ZnSO_4$ treatment did not differ from either group (Garg et al., 2008).

The effects of Zn on growth and intake performance may be in part modulated by its relationship with cholecystokinin (CCK). Farningham et al. (1993) infused CCK and propionate both separately and in combination into the portal vein of ewes fed *ad libitum.* Infusion of CCK and propionate together decreased feed intake within the first two hours,

whereas CCK infusion alone did not. However, feed intake over a 24 hr period was not different between treatments. Frequency of reticular contractions were reduced by 35% when infused with CCK alone and by 41% when CCK and propionate were infused in combination compared to the control diet. Digestive motility may be reduced in Zn-deficient animals because of increased CCK secretion in intestinal tissues, but evidence from Farningham et al. (1993) indicates that CCK does not reduce feed intake through changes in motility alone. Cholecystokinin may induce satiety by other mechanisms with several additional roles in endocrine and neurocrine functions such as regulating gall bladder contraction, pancreatic secretion, gastric emptying, and satiety mechanisms (Blanchard and Cousins, 1996). While growth traits are one of the most economically important traits to a sheep producer, Zn also participates in biological processes that are involved in other profitable traits.

Wool Production

From 2010 to 2015 the sale of wool accounted for 6 – 12% of the total income on the average U.S. sheep operation (LMIC, 2016). Therefore, understanding how Zn contributes to wool growth could increase the economic impact its production has in a flock. Wool fibers consist of three layers: the epidermis, cortex, and medulla. The epidermis is the thin outermost layer providing protection with overlapping flattened cells. The cortex provides strength and elasticity, with long and thin cortical cells. The innermost layer of the wool fiber is the medulla, usually absent in fine wool breeds (Ensminger, 1970), and is thought to be due to incomplete keratinization of coarse fibers.

Wool consists mainly of keratin, which is also the primary constituent of hair, nails, hooves, and horns (Ensminger, 1970). The keratinization process is a cellular differentiation that transforms living, functional epidermal cells into cornified, dead, and structurally stable cells with no metabolic activity (Mulling, 2000). Wool characteristics that may be influenced by available nutrients include mean fiber diameter, staple length, and fiber strength (Reis and Sahlu, 1994), all of which contribute to the overall quality of a wool clip. The major nutrients that are limiting in wool growth are essential amino acids (AA), sulfur containing AA, Cu, Zn, folic acid, and pyridoxine (Reis and Sahlu, 1994). The most important functions Zn plays in keratinization is through catalytic and metalloenzymes needed for differentiation of keratinocytes, structural protein formation dependent on Zn-finger proteins, and its regulatory function finalizing the keratinization process (Tomlinson et al., 2004).

White et al. (1994) conducted a study quantifying the effects that varying levels of dietary Zn has on wool growth and characteristics. A pair fed study over the course of 96 d was conducted utilizing twenty 16 wk old male merino rams. Ram lambs fed 10 mg Zn/kg DM exhibited reduced wool growth compared to rams fed levels of 17 and 27 ppm DM feed. Zinc deficiency in these rams seemed to have negatively affected the keratinization process, with no negative effect on mitotic activity per follicle bulb. However, the Zn content of the wool was not reflective of dietary Zn intake or plasma concentrations. Zn concentrations in wool only declined after dietary Zn concentrations were low enough to induce clinical signs or major histological changes in the wool fiber (White et al., 1994).

Reproduction

Zinc deficiencies have been determined to delay puberty, decrease testicular size, and decrease libido in ruminants (Underwood and Somers, 1969; Martin and White, 1992). Additionally, Zn deficiencies are speculated to have a more profound effect in males than in females (Mortimer et al., 1999). The impairment that Zn has on male fertility appears to be associated with the role of Zn as an activator of enzymes involved in the steroidogenesis that results in the secretion of testosterone and related hormones (Martin and White, 1992; Martin et al., 1994).

Underwood and Somers (1969) conducted a study to evaluate the effect of level of dietary Zn concentration on reproductive characteristics in ram lambs. Twenty Merino cross rams (16 wk old) were utilized in a two-phase study. The first phase divided the rams into four treatments: 1) the basal diet (2.4 ppm Zn) *ad libitum*; 2) pair fed with treatment 1, and supplemented an additional 30 ppm Zn; 3) the basal diet *ad libitum* and supplemented an additional 15 ppm Zn; and 4) the basal diet *ad libitum* and supplemented an additional 30 ppm Zn. The Zn supplementation source was $ZnSO_4$. This phase continued for approximately 24 weeks until lambs in the deficient group showed clinical signs and needed restorative action. At the end of the first phase, a testicle was removed from each ram.

Average testicle weights for treatments 1, 2, 3, and 4 were 17.6, 40.8, 37.5, and 77.1 g, respectively (Underwood and Somers, 1969). Testicle weights were statistically different between treatments 3 and 4, however, these groups did not differ in DMI. This indicates that Zn concentrations greater than 17.4 ppm can be beneficial for optimal

testicular growth. Rams in treatment 1 had no measureable sperm cells in urine or ejaculate samples at the end of phase 1. Rams in treatment 4 had greater (2.291 billion) daily sperm production than both treatment 2 and 3 (1.047 and 1.174 billion, respectively). Decreased dietary Zn intake in treatment 3 was associated with increased abnormal sperm (25.5%) compared to treatment 2 (6.8%) that had similar Zn intake as treatment 4. During the second phase, all animals received the same treatment as group 4 and all clinical signs of deficiency were reversed within 20 wk.

Martin and White (1992) evaluated the effects that increasing concentrations of dietary Zn (4, 10, 17, and 27 ppm) had on gonadotropin secretion and testicular growth in young male sheep. An additional pair-fed group accounted for the effect Zn has on feed intake, and was limited to the same amount of feed (27 ppm Zn) that the 4 ppm Zn ate the day prior. Feed intake was approximately 47% less in the treatment receiving 4 ppm Zn compared to the other treatments, resulting in a 18 kg difference in BW at the end of the study. Similar to Underwood and Somers (1969), these authors attributed differences between treatment groups to variation in feed intake and overall nutrient intake.

Testicular growth was greatest in rams receiving 17 and 27 ppm Zn compared to all other treatments (Martin and White, 1992). The authors reported that LH pulse frequency fell among all treatments throughout the study, but remained most similar to pretreatment values in animals fed 10 or 17 ppm Zn (Martin and White, 1992), most likely due to increased age and pubertal development. Follicle Stimulating Hormone concentrations were greatest in animals fed 17 or 27 ppm Zn compared to the other treatments. The authors also stated that by omitting the pair-fed control there was a

significant positive relationship between dietary Zn concentration and plasma FSH concentration, which may indicate that increased concentrations of dietary Zn may be beneficial to increased plasma FSH concentrations.

Later, Martin et al. (1994) quantified the effects that varying concentrations of dietary Zn treatments from Martin and White (1992) study had specifically on testicular growth, testosterone secretion, and plasma inhibin. Further inspection of testicular development and evaluation of microscopic images of seminiferous tubule development between rams of in these treatments indicated that rams fed *ad libitum* had the greatest development, while the pair-fed treatment was markedly reduced but not to the extent shown in rams given 4 ppm Zn. This is understandable, considering animals fed 4 ppm Zn produced less testosterone compared to all other treatments. Plasma inhibin concentrations of the 17 and 27 ppm Zn treatments had the largest reductions throughout their course of the study.

Kumar et al. (2006) conducted a study with 16 crossbred bulls (*Bos indicus* × *Bos taurus*) where semen and sperm characteristics before and after 6 mo of Zn supplementation were quantified. Treatments 1, 2 and 3 were supplemented 0, 35, and 70 ppm $ZnSO_4$, respectively, and treatment 4 was supplemented 35 ppm Zn propionate. After 6 mo of Zn supplementation, bulls in treatments 3 and 4 showed no difference in ejaculate volume (5.86 to 6.38 mL), sperm concentration (1,410 to 1,472 million ml^{-1}), percentages of live sperm (86.6 to 87.3%), or serum testosterone (3.17 to 3.52 ng ml^{-1}). The reported reproductive characteristics were all greater in bulls of treatments 3 and 4 compared to bulls in treatments 1 and 2. However, bulls in treatment 4 had greater sperm

number per ejaculate (9,392 x 10^6/ejaculate), mass motility (4.33 on a 0 to 5 scale), and bovine cervical mucous penetration test (29.6 mm). These findings indicated that 35 ppm Zn propionate supplemented to bulls was as or more beneficial than 70 ppm $ZnSO_4$ in most male reproductive traits. Moreover, in almost all sperm characteristics, bulls in treatments 3 and 4 had higher quality than bulls that received 35 ppm $ZnSO_4$ or no supplementation (Kumar et al., 2006).

A recent study by Geary et al. (2016) used 50 peripubertal bulls to quantify the effects of varying Zn concentrations and sources on semen quality, endocrine status, scrotal circumference, and age at puberty. Bulls were stratified by age and scrotal circumference, then divided into one of five treatments and individually fed the same basal diet (7.1 ppm Zn). Diets were based on concentration and source of supplemental trace minerals and included: 1) 360 mg $ZnSO_4$; 2) 360 mg ZnAA; 3) 360 mg $ZnSO_4$ and 360 mg ZnAA; 4) 360 mg $ZnSO_4$ and 720 mg ZnAA; and 5) 1,080 mg $ZnSO_4$. Semen was collected via electro-ejaculation and scrotal circumference and BW was recorded on d -14, 14, 42, 70, and 98 from the start of treatment. No differences were reported between treatments for sperm concentration, sperm motility, or semen Zn concentrations. Also, there was no significant difference in scrotal circumference or testosterone concentration between bulls of these treatments. No differences in age at puberty were observed among treatments.

Summary

The reviewed literature indicated that trace mineral deficiencies exist worldwide and animals may be deficient or marginally deficient because of low forage concentrations of trace minerals. One of these trace minerals is Zn, which is found in marginal concentrations throughout most of the year in rangeland forages. The large contribution Zn has on biological functions and activities warrants research to quantify the prevalence of clinical and subclinical deficient animals in local regions and its associated impacts on livestock production. Difficulties arise when evaluating Zn status in animals because of the relatively small storage pools in the body. Furthermore, subclinical Zn deficiencies, such as reduced wool production, body growth, immune system function, and reproductive characteristics, are often difficult to assess in sheep.

Given the production impacts of Zn, its source and concentration in supplemental feedstuffs has been the focus of many studies. It would be beneficial to continue Zn research in sheep related to differences in biological and production effects among Zn sources and concentrations. Many studies have focused on the effects of dietary Zn deficiencies compared to adequate concentrations, but few have investigated the effects that increased concentration, i.e. above NRC recommendations and source (inorganic vs. organic) have on growth, wool production, and reproduction characteristics of sheep. This is especially true in regard to investigating the potential effects of supranutritional supplementation of different Zn sources on developing rams, which is one focus of the present research.

Literature Cited

Allen, J. G., H. G. Masters, R. L. Peet, K. R. Mullins, R. D. Lewis, S. Z. Skirrow, and J. Fry. 1983. Zinc toxicity in ruminants. J. Comp. Path. 93:363-377

Blackmon, D. M., W. J. Miller, and J. D. Morton. 1967. Zinc deficiency in ruminants. Vet. Med. 62:265.

Blanchard, R. K. and R. J. Cousins. 1996. Differential display of intestinal mRNAs regulated by dietary zinc. Proc. Natl. Acad. Sci. USA 93:6863-6868.

Bowman, J. G. P., and B. F. Sowell. 1997. Delivery method and supplement consumption by grazing ruminants: A review. J. Anim. Sci. 75:543-550.

Cao, J., P. R. Henry, R. Guo, R. A. Howlwerda, J. P. Toth, R. C. Littell, R. D. Miles, and C. B. Ammerman. 2000. Chemical characteristics and relative bioavailability of supplemental organic zinc sources for poultry and ruminants. J. Anim. Sci. 78:2039-2054.

Davies, N. T., H. S. Soliman, W. Corrigall, and A. Flett. 1977. The susceptibility of suckling lambs to zinc toxicity. Br. J. Nutr. 38:153-156.

Ducker, M. J., P. T. Kendall, R. G. Hemingway, and T. H. McClelland. 1981. An evaluation of feedblocks as a means of providing supplementary nutrient to ewes grazing upland/hill pastures. Anim. Prod. 33:51-57.

Ensminer, M. E. 1970. Sheep and Wool Science. Animal Agriculture series. The interstate printers and publishers, inc. Danville, Illinois.

Fairweather, S., and R. F. Hurrel. 1996. Bioavailability of minerals and trace elements. Nutrition Research Reviews. 9:295-324.

Farningham, D. A. H., J. G. Mercer, and C. B. Lawrence. 1993. Satiety signal in sheep: Involvement of CCK, propionate, and vagal CCK binding sites. Physiol. Behav. 54:437.

Garg, A. K., Vishal Mudgal, and R. S. Dass. 2008. Effect of organic zinc supplementation on growth, nutrient utilization and mineral profile in lambs. Anim. Feed Sci. Technol. 144:82-96.

Geary, T. W., W. L. Kelly, D. S. Spickard, C. K. Larson, E. E. Grings, and R. P. Ansotegui. 2016. Effect of supplemental trace mineral level and form on peripubertal bulls. Ani. Repro. Sci. 168:1-9.

Grings, E. E., M. R. Haferkamp, R. K. Heitschmidt, and M. G. Karl. 1996. Mineral dynamics in forages of the Northern Great Plains. J. Range Manage. 49:234-240. doi:10.2307/4002884.

Hatfield, P. G., C. K. Swenson, R. W. Kott, R. P. Ansotegui, N. J. Roth, and B. L. Robinson. 2001. Zinc and copper status in ewes supplemented with sulfate- and amino acid-complexed forms of zinc and copper. J. Anim. Sci. 79:261-266.

Herdt, T. H., and B. Hoff. 2011. The use of blood analysis to evaluate trace mineral status in ruminant livestock. Vet. Clin. North Am. Food Anim. Pract. 27:255-283.

Jones, G. B., and B. F. Tracy. 2013 Evaluating seasonal variation in mineral concentration of cool-season pasture herbage. Grass Forage Sci. 70:94-101.

Judson, G. J., and J. D. McFarlane. 1998. Mineral disorders in grazing livestock and the usefulness of soil and plant analysis in the assessment of these disorders. Aust. J. Exp. Agric. 38:707-723.

Kincaid, R. L. 1999. Assessment of trace mineral status of ruminats: A review. Proc. Amer. Soc. Anim. Sci. 77:1-10.

Kincaid, R. L., B. P. Chew, and J. D. Cronrath. 1997. Zinc oxide and amino acids as sources of dietary zinc for calves: Effects on uptake and immunity. J. Dairy Sci. 80:1381-1388.

Kincaid, R. L., and A. S. Hodgson. 1989. Relationship of Selenium concentrations in blood of calves to blood selenium of the dam and supplemental selenium. J. Dairy Sci. 72:259-263.

Kratzer, F. H., and P. Vohra. 1986. Chelates in nutrition. CRC Press.

Kumar, N., R. P. Verma, L. P. Singh, V. P. Varshney, and R. S. Dass. 2006. Effect of different levels and sources of zinc supplementation on quantitative and qualitative semen attributes and serum testosterone level in crossbred cattle (Bos indicus × Bos taurus) bulls. Reprod. Nutr. Dev. 46:663-675.

Livestock Marketing Information Center (LMIC). 2016. U.S. baseline lamb cost of production model. https://d1cqrq366w3ike.cloud-front.net/http/DOCUMENT/SheepUSA/COP%20Project%20%20Summary%20Low.pdf. (Accessed 4 October 2017).

Lobato, J. F. P., G. R. Pearce, and R. G. Beilharz. 1980. Effect of early familiarization with dietary supplements on the subsequent ingestion of molasses-urea blocks by sheep. Appl. Anim. Ethol. 6:149-161.

Martin, G. B., and C. L. White. 1992. Effects of dietary zinc deficiency on gonadotrophin secretion and testicular growth in young male sheep. J. Reprod. Fertil. 96:497-507.

Martin, G. B., C. L. White, C. M. Markey, and M. A. Blackberry. 1994. Effects of dietary zinc deficiency on the reproductive system of young male sheep: testicular growth and the secretion of inhibin and testosterone. Journal of Reproduction and Fertility. 101:87-96.

Mathis, C., and J. Sawyer. 2004. New Mexico forage mineral survey. Proc. West. Sec. Amer. Soc. Anim. Sci. 55.

McDowell. L. R. 1996. Feeding minerals to cattle on pasture. Anim. Feed. Sci. Technol. 60:247-271.

McDowell, L. R. 2017. Mineral Nutrition History, The Early Years. First Edition Design Pubishing, Inc, Sarasota, FL. DOI 10.21899/978-1506904580.

Mortimer, R. G., D. Dargatz, and L. R. Corah. 1999. Forage analyses from cow/calf herds in 23 states. University of Tennesse.

Mulling, C. 2000. Three-dimensional appearance of bovine epidermal keratinocytes in different stages of differentiation revealed by cell maceration and scanning electron microscope investigation. Folia Morphol. 59:239-246.

NRC. 1985. Nutrient requirements of sheep. 5th ed. Natl. Acad. Press, Washington, DC.

NRC. 2007. Nutrient Requirements of sheep. 7th ed. Natl. Acad. Press, Washington, DC.

NRC. 2016. Nutrient Requirements of beef cattle. 8th ed. Natl. Acad. Press, Washington, DC. Doi:10.17226/19014.

Ott, E. A., W. H. Smith, R. B. Harrington, and W. M. Beeson. 1966. Zinc toxicity in ruminants. I. Effect of high levels of dietary zinc on gains, feed consumption and feed efficiency of lambs. J. Anim. Sci. 25:414-418.

Ott, E. A., W. H. Smith, M. Stob, and W. M. Beeson. 1964. Zinc deficiency syndrome in the young lamb. J. Nutr. 82:41-50.

Paterson, J., C. Swenson, B. Johnson, and R. Ansotequi. 1999. Assessing the role of copper and zinc in the cow-calf production cycle. Proc. Mid-south Ruminant Nutrition Conference.

Petersen, M. K., J. M. Muscha, J. T. Mulliniks, R. C. Waterman, A. J. Roberts, and M. J. Rinella. 2015. Sources of variability in livestock water quality over 5 years in the Northern Great Plains. J. Anim. Sci. 93. doi:10.2527/jas2014-8028.

Pond, W. G., D. C. Church, K. R. Pond, and P. A. Schoknecht. 2005. Basic animal nutrition and feeding. John Wiley & Sons. Hoboken, NJ.

Rauzi, F., L. I. Painter, and A. K. Dobrenz. 1969. Mineral and protein contents of Blue Grama and Western Wheatgrass (contenido de minerals y proteinase n el Navajita Azul (Bouteloua gracilis) y Western Wheatgrass (Agropyron smithii)). J. Range Manage. 1:47-49.

Ragen, D. L., E. E. Nix, R. L. Endecott, P. G. Hatfield, M. K. Petersen, and J. G. P. Bowman. 2015. Individual mineral supplement intake by ewes swath grazing or confinement fed pea-barley forage. Anim. Feed. Sci. Technol. 200:107-111.

Reid, R. L., G. A. Jung, W. L. Stout, and T. S. Ranney. 1987. Effects of varying zinc concentrations on quality of alfalfa for lambs. J. Anim. Sci. 64:1735-1742.

Reis, P. J., and T. Sahlu. 1994. The nutritional control of the growth and properties of mohair and wool fibers: a comparative review. J. Anim. Sci. 72:1899-1907.

Ricketts, M. J., J. Bodner, J. W. Stuth, and D. Tolleson. 2002. Montana rangeland and livestock mineral study. http://www.nrcs.usda.gov/wps/portal/nrcs/detail/mt/technical/landuse/pasture/?cid=nrcs144p2_057072 (Accessed 15 September 2015).

Rojas, L., L. McDowell, R. Cousins, F. Martin, N. Wilkinson, A. B. Johnson, J. Velasquez. 1995. Relative bioavailability of two organic and two inorganic zinc sources fed to sheep. J. Anim. Sci. 73:1202-1207.

Salgueiro, M. J., M. Zubillaga, A. Lysionek, M. I. Sarabia, R. Caro, T. Paoli, A. Hager, R. Weill, and J. Boccio. 2000. Zinc as an essential micronutrient: a review. Nutrition Research 20. 5:747-755.

Sandstrom, B., and B. Lonnerdal. 1989. Promoters and antagonists of zinc absorption. In: Zinc in Human Biology (Mills, C. F., ed.), pp. 57-78, Springer-Verlag, London

Smith, D. B., W. F. Cannon, L. G. Woodruff, Solano, Federico, J. E. Killburn, and D. L. Fey. 2014. Geochemical and mineralogical data for soils of the conterminous United States. U.S. Geological Survey. doi:10.3133/ofr20141082

Socha, M. T., S. M. Ensley, D. J. Tomlinson, and A. B. Johnson. 2003 Variability of water composition and potential impact on animal performance. In: Proc. Of Intermt. Nutr. Conf., Salt Lake City, UT. Utah State Univ., Logan. P. 85-96.

Spears, J. W. 1989. Zinc methionine for ruminants: Relative bioavailability of zinc in lambs and effects on growth and performance of growing heifers. J. Anim. Sci. 67:835-843.

Spears, J. W. 1996. Organic trace minerals in ruminant nutrition. Anim. Feed Sci. Technol. 58:151-163.

Spears, J. W. 2003. Trace mineral bioavailability in ruminants. J. Nutr. 133:1506S-1509S.

Suttle, N. F. 2010. Mineral nutrition of livestock. 4th ed. CABI International. Cambridge, MA.

Todd, W. R., C. A. Elvehjem, and E. B. Hart. 1933. Zinc in the nutrition of the rat. The Am. J. Physiol. 107:146-156.

Tomlinson, D. J., C. H. Mulling, and T. M. Fakler. 2004. Invited Review: Formation of Keratins in the Bovine Claw: Roles of Hormones, Minerals, and Vitamins in Functional Claw Integrity. J. Dairy Sci. 87:797-809.

Underwood, E. J. 1981. The Mineral Nutrition of Livestock. 2nd ed. Commonw. Agric. Bur., Slough, UK.

Underwood, E. J., and M. Somers. 1969. Sudies of zinc nutrition in sheep. I. The relation of zinc to growth, testicular development, and spermatogenesis in young rams. Aust. J. Agric. Res. 20:889-897.

Underwood, E. J., and N. F. Suttle. 1999. The Mineral Nutrition of Livestock. 3rd ed. CABI International. Cambridge, MA.

Vallee, B. L., and K. H. Falchuk. 1993. The biochemical basis of zinc physiology. Physiol. Rev. 73:79-118.

Ward, M., and G. Lardy. 2009. Beef cattle mineral nutrition.

Wegner, T. N., D. E. Ray, C. D. Lox, and G. H. Stott. 1973. Effect of stress on serum zinc and plasma corticoids in dairy cattle. J. Dairy Sci. 56:748-752.

Wellinghausen, N., and L. Rink. 1998. The significance of zinc for leukocyte biology. J. Leukoc. Biol. 64:571-577.

White, C., Martin, G., Hynd, P., & Chapman, R. 1994. The effect of zinc deficiency on wool growth and skin and wool follicle histology of male Merino lambs. Br. J. Nutr. 71:425-435.

CHAPTER THREE

SURVEY OF SERUM TRACE MINERAL CONCENTRATION IN WEANED MONTANA RAM LAMBS: IMPLICATIONS FOR TRACE MINERAL SUPPLEMENTATION IN WEANED RAMS

Contribution of Authors and Co-Authors

Author: C. M. Page

Contributions: Main person for data collection, data analysis, interpretation of article, and drafting of the paper.

Co-Author: T. W. Murphy

Contributions: Critical in statistical analysis, data interpretation, and revisions.

Co-Author: M. L. Van Emon

Contributions: Aided in experimental design, data collection, data interpretation, and revisions.

Co-Author: J. G. P. Bowman

Contributions: Aided in data analysis and paper content.

Co-Author: S. A. Wyffels

Contributions: Aided in data analysis, paper content, and revisions.

Co-Author: W. C. Stewart

Contributions: Principal Investigator, critical to obtaining funding, experimental design, data collection, data analysis, data interpretation, and critical revision of paper.

Manuscript Information Page

C. M. Page, T. W. Murphy, M. L. Van Emon, J. G. P. Bowman, S. A. Wyffels, and W. C. Stewart

Journal of Animal Science

Status of Manuscript: (Put an x in one of the options below)
_X__ Prepared for submission to a peer-reviewed journal
____ Officially submitted to a peer-review journal
____ Accepted by a peer-reviewed journal
____ Published in a peer-reviewed journal

ABSTRACT

Clinical and subclinical trace mineral deficiencies can limit productivity in western sheep production systems. The objective of this research was to determine the proportion of ranches that supplemented with trace minerals and to quantify serum trace mineral concentrations in ram lambs post-weaning across Montana with particular emphasis on Se and Zn. Serum samples (n = 221) were collected from ram lambs 8 to 10 mo of age (BW 52.8 ± 16 kg) at 21 ranches throughout Montana and analyzed for Co, Cu, Fe, Mn, Mo, Se, and Zn. Ranches were classified as deficient, marginally deficient, adequate, or excessive by flock mean mineral serum concentrations. Additionally water samples were analyzed for pertinent characteristics. The average and range of concentrations for each trace mineral across ranches were: Co (0.93 ± 0.07 ng/mL; 0.10-6.22 ng/mL), Cu (0.83 ± 0.02 µg/mL; 0.30-1.61 µg/mL), Fe (156.47 ± 3.49 µg/dL; 26-350 µg/dL), Mn (2.51 ± 0.21 ng/mL; 0.7-31.3 ng/mL), Mo (37.92 ± 4.57 ng/mL; 2.8-456.5 ng/mL), Se (103.67 ± 3.51 ng/mL; 16-197 ng/mL), and Zn (0.73 ± 0.01 µg/mL, 0.30-1.74 µg/mL). Of ranches surveyed, only 67% provided a complete mineral supplement. Sheep that were provided supplementary trace mineral had greater serum Se concentrations ($P < 0.001$), though mineral supplementation resulted in greater numerical serum concentrations of every trace mineral except Cu. Based off serum trace mineral concentration reference ranges, the two most commonly deficient and marginally deficient minerals across Montana were Se (19% of ranches deficient; 23.8% of ranches marginally deficient) and Zn (9.5% of ranches deficient; 57.1% of ranches marginally deficient). Of ranches sampled, 40 and 35% of water samples exceeded excessive

concentrations in Na and sulfates, respectively. This regional knowledge of serum trace mineral concentrations in a sub-population of ram lambs can provide information for ranches to evaluate current and future mineral supplementation needs, as well as aid the feed industry in designing formulations for mineral premixes.

Key words: Montana, ram lambs, selenium, sheep, trace minerals, zinc

[1]Support for this study was provided by the National Sheep Industry Improvement Center and Montana Agricultural Experiment Station.
[2]Authors wish to expresses gratitude to Sarah Spear and Monica Ebert for their assistance.
[3]Corresponding author: Whit.Stewart@uwyo.edu

INTRODUCTION

Sheep operations in the western U.S. rely heavily on rangelands as their primary feed source which could lead to clinical or subclinical trace mineral deficiencies and limit animal productivity. Minerals perform essential functions including structural, physiological, catalytic, and regulatory roles (Suttle, 2010). Forage trace mineral concentrations are highly variable across rangelands as they are largely influenced by soil geochemistry and plant stage of maturity (Mathis et al., 2004; Smith et al., 2013; Jones and Tracy, 2015). Generally, trace minerals are obtained through feed components, with the exception of excessive mineral concentrations in water (NRC, 2007).

Montana consists of over 380,832 km^2 of diverse geography, resulting in a high potential for variability of trace mineral concentration of Montana's forage. Additionally, Montana has an estimated 200,000 breeding sheep which was the 5th largest inventory in the U.S. (USDA-NASS, 2017). Previous research reported Se and Zn concentrations in forages across the western U.S. were less than adequate for animal health and

performance (Dargatz and Ross, 1996; Mathis et al., 2004). Mineral deficiencies in breeding sheep, particularly Se and Zn, have negative effects on reproductive performance and longevity (Suttle, 2010), which may present potential production losses in breeding sheep.

No previous study has quantified trace mineral status in Western breeding sheep populations. Therefore, the objective of this research was to quantify serum trace mineral concentrations in Montana ram lambs post-weaning with particular emphasis on Se and Zn. It was hypothesized that mineral supplementation strategies and trace mineral serum concentrations would vary within and across flocks sampled, and Se and Zn serum concentrations would be lower than adequate.

MATERIALS AND METHODS

The experimental protocol for this study was approved by the Institutional Animal Care and Use Committee of Montana State University (2016-AA04). This study was conducted from September 24 to November 23, 2015. Twenty-one seedstock operations located across 15 counties and a wide range of production environments in Montana were sampled. Locations spanned from Dillon (45.2158˚ N, 112.6342˚ W) to Wolf Point, MT (48.0914˚ N, 105.6425˚ W), representing a distance of approximately 805 km (Figure 1).

Participating ranches were selected for sampling based on their intent for developing and marketing rams to commercial operations. A homogeneous group of 8-10 mo old rams (52.8 ± 16 kg) were sampled within two months post weaning, to broadly assess trace mineral status across the state. This sub-population of ram lambs was

sampled due to similar developmental stage at a time of year when dietary trace mineral consumed came from late season forages or harvested feedstuffs. Breed composition of the rams included Targhee (n = 95), Rambouillet (n = 47), Columbia (n = 20), South African Meat Merino (n = 1), Suffolk (n = 12), Hampshire (n = 15), Debouillet (n = 2), Merino (n = 2), and various crosses (n = 27). A total of 221 rams were randomly sampled across ranches for analysis. Within each ranch, at least 15% of the ram lamb population was sampled following recommendations for adequate sample size by Herdt and Hoff (2011).

All blood samples were collected via jugular venipuncture into 13 × 100 mm trace mineral royal blue top vacutainer tubes (Covidien, Mansfield, MA) without any additives. Blood was centrifuged at 2800 × *g* for 15 min, and serum was decanted into two aliquots in 2 mL tubes and stored at -20˚C for later analyses. Serum samples were shipped on ice overnight for trace mineral analysis at a commercial laboratory (Michigan State University Diagnostic Center for Population and Animal Health, East Lansing). Cobalt, Cu, Fe, Mn, Mo, Se, and Zn concentrations in serum were quantified using an ionized coupled plasma mass spectrometry method (Wahlen et al., 2005).

Each operation was surveyed on whether ram lambs were offered a mineral and vitamin package to evaluate supplementation effects on serum trace mineral concentrations. Instances where ranches only provided a source of NaCl and not a complete trace mineral package were classified as un-supplemented. If supplementation occurred, there was no attempt to distinguish consumption or supplementation levels. Due to logistical and financial limitations, basal dietary trace mineral concentrations were

not collected or analyzed. Serum trace mineral concentrations were classified as deficient, marginally deficient, adequate, and excessive according to the values in Table 1.

Samples (500 mL) were collected from the water source utilized by ram lambs at each ranch and analyzed by a commercial laboratory (Midwest laboratories, Inc; Omaha, NE) for livestock suitability. Water characteristics (package W1 livestock suitability) included Ca, Cu, Fe, Mg, Na, chloride, nitrate-nitrogen, sulfate, and total dissolved solid content as well as pH and conductivity. Water characteristics were quantified using a light emission technique where prepared samples are injected into a high energy plasma that forces the elements in the injected sample to emit light wavelengths that are specific to each metal present. Ions of aqueous samples are separated and measured for conductivity.

Statistical analyses

Descriptive statistics of serum trace mineral concentrations were estimated within and across ranch using the MEANS procedure of SAS (version 9.4; SAS Inst. Inc., Cary, NC). To determine the effect of supplementation on serum trace mineral concentration, ranch was considered the experimental unit and serum trace mineral concentration was analyzed in the GLM procedure with the fixed effect of mineral supplementation (supplemented or un-supplemented). Pearson's correlation coefficients among individual ram serum trace mineral concentrations were estimated using the CORR procedure. Additionally, Pearson's correlation coefficients among ranch mean serum Se and Zn

concentrations and water quality characteristics were also estimated. Differences were considered significant between supplemented and un-supplemented ranches at $P \leq 0.05$, and a tendency to be different at $P \leq 0.10$.

RESULTS AND DISCUSSION

Mean concentrations and CV of Co, Cu, Fe, Mn, Mo, Se, and Zn within ranch are reported in Table 2. Minimum, maximum, and mean serum concentrations from all 221 samples are reported in Table 3. The Serum trace minerals with the greatest variation across ranches were Co, Mn, and Mo with CVs of 42.7, 75.8, and 52 respectively. Of Montana ranches surveyed, only 67% provided a complete mineral supplement. Supplemental minerals can have positive effects on animal reproduction, immunity, and feed intake (McDowell, 1996; Stewart et al., 2012; Keady et al., 2017). However, inadequate intake may result in subclinical deficiencies, which are difficult to detect because there are no specific symptoms (Paterson and Engle, 2005). Inadequate mineral intake can decrease forage consumption and feed efficiency, reproductive efficiency, disease resistance, and ADG (Paterson and Engle, 2005). Variation in free choice mineral intake is caused by a wide variety of factors such as supplemental type and delivery method, as well as animal factors including experience, fear of feeding apparatus, and breed (Bowman and Sowell, 1997). Anecdotally, producers might not supplement with a trace mineral package because of cost or return on investment, weatherization and subsequent inedibility, and difficulty of supplementing in rotational and extensive grazing systems. The effect of supplementation on trace mineral concentration in ram

lamb blood serum is reported in Table 4. Serum concentrations of individual trace minerals are discussed in further detail below.

Cooperative field studies help producers and scientists alike to understand in-field management practices, specifically, mineral supplementation strategies, trace mineral concentrations in feedstuffs, mineral deficiencies in the animal, and the effects of supplementation on animal status (Dargatz and Ross, 1996; Menzies et al., 2003; Ademi et al., 2017; Keady et al., 2017). Selenium and Zn are trace minerals commonly found to be in inadequate concentrations in both rangeland and harvested forages across the western U.S. (Dargatz and Ross, 1996; Mathis et al., 2004), including plants native to Montana range lands such as Blue Grama *(Bouteloua gracilis*) and Western Wheatgrass (*Pascopyrum smithii*; Rauzi et al., 1969; Jones and Tracy, 2013). Serum trace mineral concentrations have been used when investigating population trace mineral status as a practical and less invasive method, because there are difficulties in assessing mineral status from an evaluation of the diet (Herdt and Hoff, 2011). When feasible, mineral status should be evaluated based on specific mineral concentrations of tissues that have been identified as storage pools for a particular mineral.

Serum Co

Provision of a mineral supplement across ranches sampled had no effect (P = 0.29) on serum Co concentrations of weaned ram lambs. Cobalt blood serum concentrations ranged from 0.10 – 6.22 ng/mL with a mean of 0.93 ± 0.07 ng/mL. Across Montana, ranches were classified as adequate in Co with mean serum concentrations

meeting or exceeding 0.10 ng/mL. Liver tissue and blood have been used to quantify Co status in sheep, generally with blood being more responsive to nutritional changes in Co concentrations (Suttle, 2010; Keady et al., 2017).

Mammalian tissues are not known to have specific requirements for Co, but it is required by rumen microorganisms for synthesis of vitamin B_{12} (NRC, 2007), which catalyzes activity of methylmalonyl-CoA mutase and methionine synthase (Kennedy et al., 1992). Methionine synthase is necessary for rumen microbes to produce propionate, a metabolite that is a major determinant of the host's Co responsiveness (Suttle, 2010) and the only gluconeogenic volatile fatty acid. Keady et al. (2017) concluded that Co supplementation decreased days to slaughter in lambs. Authors reported increased response to Co supplementation as the grazing season progressed (Keady et al., 2017). Recommended dietary concentrations of Co are between 0.10 and 0.20 ppm for sheep, with consideration that sheep are more susceptible to Co deficiencies than cattle and goats (NRC, 2007). Diets containing legumes generally have higher concentrations of Co than grasses, followed by grains which are usually poor sources (NRC, 2007).

Serum Cu

Provision of a mineral supplement across ranches sampled had no effect (P = 1.00) on serum Cu concentrations of weaned ram lambs, which was expected as sheep mineral rarely contains added copper. Copper serum concentrations ranged from 0.30 – 1.61 µg/mL with a mean of 0.83 ± 0.02 µg/mL. However, interpretation of serum Cu concentrations is of little utility as it shows minimal fluctuation. Nevertheless, the mean

serum Cu concentrations were within an adequate range (Table 1). There were no ranches sampled that were classified as deficient in serum Cu concentrations, but 19% were classified as marginally deficient, 71.4% adequate, and 9.5% excessive. Authors acknowledge that liver biopsies would have been a superior indicator of Cu status (Kincaid, 2000), but due to the collection of samples from privately-owned stud prospects, time constraints, and resources, liver biopsies were not feasible.

Copper is an essential trace mineral involved in many enzyme activities which functions include ATP production within mitochondria, collagen and bone formation, and optimal nervous system function (McDowell, 2017). Regional areas around the world have been identified as deficient in Cu, and deficiencies in gestating ewes can cause ataxia in lambs (Bennetts and Chapman, 1937). Serum Cu concentrations from the current study indicate that Cu deficiencies are not widespread, however this interpretation should be interpreted with caution based on limitations of data collected in the current study. Historically, dietary Cu recommendations were 7 - 11 mg/kg DM (NRC, 1985), but current dietary recommendations are based off of a factorial method and a set of equations that take into account physiological status *(i.e.* growing, gestating, or lactating) of the animal and factors that affect Cu absorption (NRC, 2007). Sheep are generally more susceptible to Cu toxicity and, therefore, mineral supplement packages fortified for cattle should be avoided. Nutritional management of Cu should also include consideration of Mo, S, and Fe as interaction and subsequent availability can result. For example, rams housed in confinement with high levels of Cu and inadequate amounts of

Mo and S were more susceptible to Cu toxicity than animals on pasture (Buck and Sharma, 1969).

Serum Fe

Provision of a mineral supplement across ranches sampled had no effect (P = 0.55) on serum Fe concentrations among weaned ram lambs, and all ranches were classified as either adequate or excessive with serum Fe concentrations. Serum Fe concentrations ranged from 26.00 – 350.00 µg/dL with a mean of 156.47 ± 3.49 µg/dL. Many ranches were actually within the excessive range for serum Fe concnetration, which may indicate a potential for antagonism with Cu and S. However, Fe toxicity in ruminants is rarely experienced because of limited absorption when levels are high in diets (Herdt and Hoff, 2011). Both liver and serum concentrations are normally used as indicators of Fe status in animals since depletion of serum concentrations occurs just prior to anemia in deficient animals.

Iron is the most abundant trace mineral in the body and approximately 60% of it is found in hemoglobin, which is essential to O_2 and CO_2 transportation (NRC, 2007). Forages show marked seasonality in Fe concentrations with peaks in spring and autumn, although most livestock feeds contain high concentrations of Fe, resulting in few cases of deficiencies (Suttle, 2010). Suggested NRC (2007) dietary recommendations for Fe are 55 mg/kg for growing sheep. However, sheep grazing irrigated pastures that have a greater likelihood of internal parasites could possibly require higher concentrations of Fe.

Serum Mn

Ranches providing a mineral supplement across ranches sampled had no effect (*P* = 0.21) on serum Mn concentrations compared to ranches that did not supplement. Serum Mn concentrations from ranches ranged from 0.70 – 31.30 ng/mL with a mean of 2.51 ng/mL. The average serum Mn concentration across sampled Montana ram lambs is higher than the adequate range. However, Mn toxicity is rare in ruminants, even at high dietary concentrations (NRC, 2007).

Liver, whole blood, and serum are the most frequently sampled to quantify Mn concentrations (Kincaid, 2000). Plasma Mn concentrations are maintained within a narrow range (5 – 10 µg/l) in cattle consuming diets with a wide range of Mn concentrations (40 – 1000 mg/kg), in part due to the liver's ability to remove excess quantities of Mn (Gibbons et al., 1976). Functions of Mn include involvement in bone development, protection against oxidative tissue damage, and carbohydrate, fat, and protein biochemical processes (Herdt and Hoff, 2011). Testicular growth is believed to be optimized with 19–30 ppm (Masters et al., 1988), a range that is similar for obtaining adequate growth in sheep (20 – 25 ppm; NRC, 2007).

Serum Mo

Additional supplementation of mineral to basal diet had no effect (*P* = 0.71) on serum Mo concentrations of weaned ram lambs. Serum Mo concentrations ranged from 2.80 – 456.50 ng/mL with a mean of 37.92 ± 4.57 ng/mL. A large variation in serum Mo concentrations was observed within and across ranches (Table 2). Conclusions for the

cause of this large variation is hard to verify without dietary mineral concentrations. However, U.S. geological survey soil mineral data indicated ranches that were greatest in serum Mo concentration were located in areas with high soil Mo concentrations (Smith et al., 2014). Serum Mo concentrations reflect Mo dietary concentrations and assessment is usually in cases where Mo toxicity or Cu deficiency is a concern (Kincaid, 2000).

Molybdenum is an essential trace mineral because of its requirement in the reduction of nitrate to nitrite in bacteria (Williams and Da Silva, 2002), although essential requirements are low and clear signs of deficiencies have been reported in few species (McDowell, 2017). Toxicity of Mo in ruminants varies by species, chemical form, type of diet, S concentration in diet, and Cu status of the animal (NRC, 2007). Molybdenum is also recognized for its antagonistic effect on Cu availability in ruminants (Mason, 1986). Dietary concentrations of Mo are recommended at 0.5 ppm for sheep (NRC, 2007).

Serum Se

Ranches that provided a complete mineral had greater ($P < 0.01$) serum Se concentrations compared to ranches that offered no additional Se supplementation post weaning. Serum Se concentrations were greater on ranches that supplemented mineral (133.14 ± 9.37 ng/mL) than those that did not (68.49 ± 13.23 ng/mL). Ranches surveyed were organized into those who supplemented mineral or not, and their mean serum concentrations and Se status are reported in Table 5. Of ranches surveyed, 19% were deficient (19% un-supplemented), 23.8% marginally deficient (14.3% supplemented and 9.5% un-supplemented), 42.9% adequate, and 14.3% excessive (9.5% supplemented and

4.8% un-supplemented). Regional Se deficiencies were observed, with serum Se concentrations lower in Montana ranches near the eastern front of the Rocky Mountains, apparently in association with soil mineral concentrations reported in USGS mineral maps (Smith et al., 2014). Globally, incidences of regional Se deficiencies have also been reported both in sheep populations and forage samples (Dargatz and Ross, 1996; Ademi et al., 2017). Sheep whole blood Se concentrations in Kosovo were positively affected by supplementation independent of supplementation type including injectable, mineral block, mineral premixes, and feed compounds (Ademi et al., 2017).

Results suggest the mean serum Se concentrations are within adequate reference ranges (Table 3) but approach marginal status. Clinical signs of Se deficiency are often manifested as nutritional myopathy (*i.e.* white muscle disease), but can also result in production losses in subclinical instances. Marginal (subclinical) Se deficiencies can result in decreased growth performance, loss of milk yield, decreased reproductive performance, and loss of wool production but can be remedied with Se supplementation (Slen et al, 1961; Gabbedy, 1971; McDonald, 1975; Suttle, 2010). Selenium dietary recommendations are 0.5 mg/kg for growth in sheep (NRC, 2007), but other studies indicate and recommend that additional supplementation could be beneficial to ewe and lamb performance (Stewart et al., 2012; Ademi et al., 2017).

Serum Zn

Additional supplementation of mineral to the basal diet had no effect (P = 0.24) on serum Zn concentrations of weaned ram lambs. Serum Zn concentrations ranged from

0.30 – 1.74 µg/mL with a mean of 0.73 ± 0.01 µg/mL. Mean serum Zn concentrations indicated 9.5% of ranches were deficient, 57.1% marginally deficient, 33.3% adequate, and none were excessive. In general, production losses from animals classified as marginally deficient are difficult to quantify because subclinical signs such as mild hypophagia and subsequent growth performance, reduced wool growth, decreased fertility (Underwood and Somers, 1969; Martin and White, 1992; White et al., 1994) are not generally quantified and prescriptively remedied at the ranch-level. Production losses due to clinical or subclinical trace mineral deficiencies were not quantified in the current study and can only be speculated. However, considering 57% of ranches across Montana were classified as marginally deficient, production losses are possible.

The results of the current study agree with findings from Ademi et al. (2017), who reported no effect of supplementation on whole blood Zn concentrations in Eastern Europe. Considerations should be made of Zn source bioavailability in mineral supplements, because organic sources of mineral are generally identified as more bioavailable than inorganic sources (Rojas et al., 1995; Spears, 2003).

Zinc is the second most abundant trace mineral in the body with important functions involved in reproduction (Kumar et al., 2006), gene expression (Berg, 1990), immune function (Spears and Weiss, 2008), and wool growth in sheep (White et al., 1994). Subclinical deficiencies in Zn could be more frequent than other trace minerals because the body does not sequester large amounts of available Zn in any one organ, hence the large percentage of ranches that are classified as marginally deficient (NRC, 2007; Herdt and Hoff, 2011). Cool season grasses decrease in Zn concentration as the

grazing season progresses and plants go into a state of dormancy (Rauzi et al., 1969; Jones and Tracy, 2013). This coincides with optimal timing for efforts to increase body condition of ewes before the breeding season, weaning and marketing lambs. Considering the major production periods of weaning, breeding, and lambing occur when dietary Zn components are limited to dormant rangelands or harvested forages the provision of supplemental Zn is especially warranted.

Optimal concentrations of dietary Zn are not well understood, but high tolerance to dietary Zn in most mammals indicates potential for higher supplementation levels than the broad range of 24 - 51 mg/kg DM for growing sheep (NRC, 2007). Furthermore supra-nutritional levels might be warranted to match requirements with the desired level of performance. Page et al. (2017) reported increased production in wool growth and ADG in rams consuming supranutritional concentrations of dietary Zn (>90 mg/kg DM). Testes are tissues in the body that contain high concentrations of Zn and are impacted by low Zn levels, which can reduce male reproductive efficiency (Apgar, 1992). The effect of Zn deficiency on reproductive function appears to be more prominent in males than females, possibly because enzymes involved in steroidogenesis (Mortimer et al., 1999).

Relationships among serum trace mineral concentrations

Estimated Pearson correlation coefficients between serum trace mineral concentrations using individual ram lamb records are reported in Table 6. Serum Se was moderately and positively correlated (0.33; $P < 0.001$) with serum Co. Additionally, serum Se was positively correlated ($P < 0.05$) with serum Mn, however, the magnitude of

the correlation coefficient was low (0.14). Grace and Lee (1990) reported increased tissue Se and Mn at approximately the same rate as % increase in diet. Serum Zn was moderately and positively correlated (0.31; $P < 0.001$) with serum Fe. However, Zn concentration has been reported to increase as Fe concentration decreases in pancreas tissue (Grace and Lee, 1990). Significant correlations ($P < 0.07$) were also found between serum Zn and serum Co (-0.14), Cu (0.12), Mn (0.16), and Se (0.16), however, their absolute values were all low. While serum Cu concentration may not be the most accurate indicator of status, others have reported similar results in that Zn did not negatively impact Cu concentrations despite their antagonistic relationship (Hatfield et al., 2001). Pearson correlation coefficients between all other pairs of serum trace minerals were not significantly different from zero ($P > 0.10$).

Water characteristics

Water samples were analyzed for livestock suitability from 20 of the participating ranches to identify and characterize any water quality concerns. Water quality characteristics across the 20 ranches sampled are reported in Table 7. Extremes in water quality traits can affect the biological availability of certain trace minerals, by acting as a medium for excess or antagonistic minerals and toxic substances (NRC, 2005). However, interpreting antagonistic relationships from water quality is challenging in the current field survey study. Furthermore, water quality can be difficult to define because it is influenced by taste, smell, turbidity, or electrical conductivity (Socha et al., 2003).

In the current study, Na, sulfate, and pH water characteristics exceeded maximum tolerable concentrations in 40, 35, and 20% of sampled ranches, respectively. Petersen et al. (2015) reported a similar percentage of water sources that had characteristics exceeding maximum tolerable concentrations included Na, sulfate, and pH in 42, 37, and 36% of the samples, respectively. Water sources in the study by Petersen et al. (2015) were sampled throughout the year at the Fort Keogh Livestock and Range Research Laboratory located near Miles City, MT. In cases when Na concentrations in water are excessive, animals may refuse a salt-mineral mix offered *ad libitum* (Petersen et al., 215), which is the driving factor in mineral supplement consumption in ruminants. This potentially adds to intake variation within flocks. A greater concentration and intake of S in water and feed may reduce Se bioavailability, since these two minerals have similar physical and chemical properties (Spears, 2003). Sulfur interacts with several other minerals including Cu, Mo, and Zn and high concentrations of sulfates in water sources could act as an antagonist, possibly causing deficiencies in the current study. An acceptable pH range for livestock water is 6 to 8.5 (Socha et al., 2003). Additionally, high levels of Ca, P, Mg, and S dissolved in drinking water can limit water intake, therefore limiting DMI (NRC, 2007). Total dissolved solid (TDS) concentrations of 2,000 to 4,900 ppm may cause temporary water refusal, and this is more common in younger animals (NRC, 2007). TDS concentrations between 4,900 and 7,000 ppm should be assessed with caution, and any water sources with levels greater should be avoided. Results from the present study outline the importance of regularly testing drinking water to account for characteristics that could influence mineral supplementation management programs.

Relationships among water characteristics and serum trace mineral concentrations

Estimated Pearson correlation coefficients between ranch mean serum Se and Zn concentrations and water characteristics are displayed in Table 8. Serum Se was positively and moderately correlated ($P < 0.08$) with water Na, sulfate, and TDS range of correlations. This relationship with sulfate disagrees with others that reported diets with increased S content limit the bioavailability of Se (Ivancic & Weiss, 2001; Spears, 2003). All other Pearson correlation coefficients were not significantly different from zero ($P > 0.10$). This could be due to the fact that serum mineral status may be less affected by water characteristics, or that serum is not the appropriate indicator for detection of its influences in some minerals.

CONCLUSIONS

Results from the current study provide insight on serum trace mineral concentrations in a sub population of developing ram lambs in addition to the broader concern that 33% of ranches were not providing a mineral supplement. Supplementation of trace minerals had an influence on Se serum concentrations among Montana ram lambs, especially near the eastern Rocky Mountain front. On average, serum Se concentrations were lower in animals located in western Montana, likely due to soil and forage Se deficiencies. Selenium and Zn were the two most deficient and marginally deficient minerals across Montana ram lamb populations, and additional supplementation of these trace minerals is recommended. This regional knowledge of serum trace mineral

concentrations can provide information for ranches to evaluate current and future mineral supplementation programs, as well as aid private industry in meeting precision formulation for mineral premixes. Variation in trace mineral status is multi-facetted, yet basal dietary concentrations are the basis from which ranch-level supplementation decisions should be generated. Soil geochemistry maps may also help estimate regional differences. Additional considerations include individual intake variation, forage species maturity, season, bioavailability of trace mineral chemical form, and mineral antagonists.

LITERATURE CITED

Ademi, A., A. Bernhoft, E. Govasmark, H. Bytyqi, T. Sivertsen, and B. R. Singh. 2017. Selenium and other mineral concentrations in feed and sheep's blood in Kosovo. Transl. Anim. Sci. 1:97-107.

Apgar, J. 1992. Zinc and reproduction: an update. J. Nutr. Biochem. 3:266-278.

Bennets, H., and F. E. Chapman. 1937. Copper deficiency in sheep in Western Australia: A preliminary account of the aetiology of enzootic ataxia of lambs and an anaemia of ewes. Austr. Vet. J. 13:138-149.

Berg, J. M. 1990. Zinc fingers and other metal-binding domains. Elements for interactions between macromolecules. J. Biol. Chem. 265:6513-6516.

Bowman, J. G. P., and B. F. Sowell. 1997. Delivery method and supplement consumption by grazing ruminants: a review. J. Anim. Sci. 75:543-550.

Buck, W. B., and R. M. Sharma. 1969 Copper toxicity in sheep. Iowa State University Veterinarian. 31:1.

Dargatz, D. A., and P. F. Ross. 1996. Blood selenium concentrations in cows and heifers on 253 cow-calf operations in 18 states. J. Anim. Sci. 74;2891-2895.

Gabbedy, B. 1971. Effect of selenium on wool production, body weight and mortality of young sheep in Western Australia. Austr. Vet. J. 47:318-322.

Gibbons, R. A., S. N. Dixon, K. Hallis, A. M. Russell, B. F. Sansom, and H. W. Symonds. 1976. Manganese metabolism in cows and goats. 1976. Biochim. Biophys. Acta. 444:1-10.

Grace, N. D., and J. Lee. Effect of Co, Cu, Fe, Mn, Mo, Se, and Zn supplementation on the elemental content of soft tissues and bone in sheep grazing ryegrass/whit clover pasture. New Zealand Journal of Agricultural Science. 33:635-647.

Grings, E. E., M. R. Haferkamp, R. K. Heitschmidt, and M. G. Karl. 1996. Mineral dynamics in forages of the Northern Great Plains. J. Range Manage. 49:234-240. doi:10.2307/4002884.

Hatfield, P. G., C. K. Swenson, R. W. Kott, R. P. Ansotegui, N. J. Roth, and B. L. Robinson. 2001. Zinc and copper status in ewes supplemented with sulfate- and amino acid-complexed forms of zinc and copper. J. Anim Sci. 79:261-266.

Herdt, T. H., and B. Hoff. 2011. The use of blood analysis to evaluate trace mineral status in ruminant livestock. Vet. Clin. North Am. Food Anim. Pract. 27:255-283.

Ivancic, J., and W. P. Weiss. 2001. Effect of dietary sulfur and selenium concentrations on selenium balance of lactating Holstein cows. J. Dairy Sci. 84:225-232.

Jones, G. B., and B. F. Tracy. 2015 Evaluating seasonal variation in mineral concentration of cool-season pasture herbage. Grass Forage Sci. 70:94-101.

Keady, T. W. J., J. P. Hanrahan, and S. P. Fagan. 2017. Cobalt supplementation, alone or in combination with vitamin B_{12} and selenium: Effects on lamb performance and mineral status. J. Anim. Sci. 95:379-386.

Kincaid, R. 2000. Assessment of trace mineral status of ruminants: a review. J. Anim. Sci. 77:1-10.

Kumar, N., R. P. Verma, L. P. Singh, V. P. Varshney, and R. S. Dass. 2006. Effect of different levels and sources of zinc supplementation on quantitative and qualitative semen attributes and serum testosterone level in crossbred cattle (Bos indicus × Bos taurus) bulls. Reprod. Nutr. Dev. 46:663-675.

Martin, G. B., and C. L. White. 1992. Effects of dietary zinc deficiency on gonadotrophin secretion and testicular growth in young male sheep. J. Reprod. Fertil. 96:497-507.

Mason, J. 1986. Thiomolybdates: Mediators of molybdenum toxicity and enzyme inhibitors. Toxicology 42:99.

Masters, D. G., D. I. Paynter, J. Briegel, S. K. Baker, and D. B. Purser. 1988. Influence of manganese intake on body, wool and testicular growth of young rams and the concentration of manganese and activity of manganese enzymes in tissues. Aust. J. Agric. Res. 39:517-524.

Mathis, C., and J. Sawyer. 2004. New Mexico forage mineral survey. Proc. West. Sec. Amer. Soc. Anim. Sci. 55:182-185.

McDonald, J. 1975. Selenium-response unthriftiness of young Merino sheep in central victoria. Austr. Vet. J. 51:433-435.

McDowell, L. R. 1996. Feeding minerals to cattle on pasture. Anim. Feed. Sci. Technol. 60:247-271.

McDowel, L. R. 2017. Mineral Nutrition History, The Early Years. First Edition Design Pubishing, Inc, Sarasota, FL. DOI 10.21899/978-1506904580.

Menzies, P. I., H. Boermans, B. Hoff, T. Durzi, and L. Langs. 2003. Survey of the status of copper, interacting minerals, and vitamin E levels in the livers of sheep in Ontario. Can. Vet. J. 44:898-906.

NRC. 1985. Nutrient Requirements of Sheep. 6th rev. ed. Natl. Acad. Press, Washington, DC.

NRC. 2005. Mineral Tolerance of Domestic Animals. Washington, DC: National Academies Press.

NRC. 2007. Nutrient Requirements of Small Ruminants: Sheep, Goats, Cervids, and New World Camelids. Natl. Acad. Press, Washington, DC.

Page, C. P., I. McGregor, M. L. Van Emon, T. W. Murphy, C. K. Larson, J. G. Berardinelli, and W. C. Stewart. 2017. Effects of zinc source and dietary concentration on zinc status, growth performance, and wool characteristics in developing rams. Proc. West. Sec. Amer. Soc. Anim. Sci. 68.

Petersen, M. K., J. M. Muscha, J. T. Mulliniks, R. C. Waterman, A. J. Roberts, and M. J. Rinella. 2015. Sources of variability in livestock water quality over 5 years in Northern Great Plains. J. Anim. Sci. 93:1792-1801.

Ragen, D. L., E. E. Nix, R. L. Endecott, P. G. Hatfield, M. K. Petersen, J. G. Bowman. 2015. Individual mineral supplement intake by ewes swath grazing or confinement fed pea-barley forage. Anim. Feed. Sci. 200:107-111.

Slen, S., A. Demiruren, and A. Smith. 1961. Note on the effects of selenium on wool growth and body gains in sheep. Can. J. Anim. Sci. 41:263-265.

Smith, D. B., W. F. Cannon, L. G. Woodruff, Solano, Federico, J. E. Killburn, and D. L. Fey. 2014. Geochemical and mineralogical data for soils of the conterminous United States. U.S. Geological Survey. doi:10.3133/ofr20141082.

Socha, M. T., S. M. Ensley, D. J. Tomlinson, and A. B. Johnson. 2003 Variability of water composition and potential impact on animal performance. In: Proc. Of Intermt. Nutr. Conf., Salt Lake City, UT. Utah State Univ., Logan. P. 85-96.

Spears, J. W. 2003. Trace mineral bioavailability in ruminants. J. Nutr. 133:1506S-1509S.

Spears, J. W., and W. P. Weiss. 2008. Role of antioxidants and trace elements in health and immunity of transition dairy cows. Vet. J. 176:70-76.

Stewart, W. C., G. Bobe, W. R. Vorachek, G. J. Pirelli, W. D. Mosher, T. Nichols, R. J. Van Saun, N. E. Forsberg, and J. A. Hall. 2012. Organic and inorganic selenium: II. Transfer efficiency from ewes to lambs. J. Anim. Sci. 90:577-584.

Suttle, N. F. 2010. Mineral nutrition of livestock. 4th ed. CAB Int., Wallingford, UK. doi:10.1079/9781845934729.0000.

Suttle, N. F., and A. C. Field. 1968. Effect of intake of copper, molybdenum, and sulphate on copper metabolism in sheep. 1. Clinical condition and distribution of copper in blood of the pregnant ewes. J. Comparative Pathol. 93:379-389.

Underwood, E. J., and M. Somers. 1969. Sudies of zinc nutrition in sheep. I. The relation of zinc to growth, testicular development, and spermatogenesis in young rams. Aust. J. Agric. Res. 20:889-897.

USDA-NASS. 2017. U.S. Sheep and Goat Report. http://usda.mannlib.cornell.edu/usda/current/SheeGoat/SheeGoat-01-31-2017.pdf (Accessed 10 March 2016).

Wahlen, R., L. Evans, J. Turner, and R. Hearn. 2005. The use of collision/reaction cell ICP-MS for the determination of 18 elements in blood and serum samples. Spectroscopy 20:12.

White, C., G. Martin, P. Hynd, and R. Chapman. 1994. The effect of zinc deficiency on wool growth and skin and wool follicle histology of male Merino lambs. Br. J. Nutr. 71:425-435.

Williams, R. J. P., J. J. R. F. Da Silva. 2002. The involvement of molybdenum in life. Biochem. Biophys. Res. Commun. 292:293-299.

Table 1. Criteria for classification of ranches based on blood serum concentrations[1]

	Blood serum concentration						
Classification	Co, ng/mL	Cu, µg/mL	Fe, µg/dL	Mn, ng/mL	Mo, ng/mL	Se, ng/mL	Zn, µg/mL
Deficient	-	<0.50	<77.0	-	-	<50.0	<0.60
Marginally Deficient	-	0.50 – 0.70	77.0 – 116.0	-	-	50.0 – 110.0	0.60 – 0.80
Adequate	>0.10	0.70 – 1.00	116.0 – 122.0	0.50 – 2.00	12.0 – 30.0	110.0 – 160.0	0.80 – 1.20
Excessive	-	>1.00	>122.0	-	-	>160.0	>1.20

[1]Reference ranges were adapted from Herdt and Hoff (2011) and Michigan State University Diagnostic Center for Population and Animal Health.

Table 2. Means and coefficient of variations (CV, %) of serum trace mineral concentrations of ram lambs within each sampled ranch[1]

		Mineral													
		Co, ng/mL		Cu, µg/mL		Fe, µg/dL		Mn, ng/mL		Mo, ng/mL		Se, ng/mL		Zn, µg/mL	
Ranch	*n*	Mean	CV	Mean	CV	Mean	CV	Mean	CV	Mean	CV	Mean	CV	Mean	CV
1	30	0.27	28.4	0.84	21.3	179.5	24.9	1.88	42.1	6.0	23.3	25.2	21.1	0.71	13.4
2	10	0.55	28.8	0.67	14.9	155.8	17.1	2.94	163.6	12.0	29.7	49.0	14.3	0.60	11.7
3	10	2.71	40.7	0.99	10.3	165.6	16.0	1.52	47.4	20.0	111.5	89.2	36.4	0.62	19.9
4	10	1.01	42.7	0.75	22.3	135.9	22.7	2.56	112.8	277.8	43.9	90.5	17.4	0.80	30.8
5	10	1.18	105.4	0.59	11.9	138.0	11.2	2.87	124.6	76.5	128.6	159.10	4.56	0.63	20.9
6	10	0.35	37.7	1.18	22.6	139.9	29.9	3.52	144.4	18.8	42.9	127.2	7.97	0.92	17.8
7	10	0.34	23.3	0.83	20.3	120.0	57.5	2.24	46.8	39.4	41.5	25.9	18.2	0.69	22.6
8	9	0.90	68.4	0.91	18.7	202.3	30.3	5.16	94.9	8.17	68.2	154.4	13.8	1.17	29.0
9	10	2.07	49.1	0.94	28.7	119.9	33.0	2.20	26.2	20.4	48.6	102.2	13.1	0.67	11.7
10	10	0.29	29.7	0.51	32.3	143.8	10.7	1.70	35.1	20.2	32.6	45.7	14.1	0.71	14.3
11	7	0.14	50.7	0.76	18.7	296.4	10.8	1.73	30.9	29.7	13.5	84.0	13.3	1.01	7.39
12	10	2.30	20.5	0.67	14.8	158.8	20.4	3.70	50.1	8.52	23.5	146.1	6.76	0.67	11.6
13	10	0.95	44.5	0.86	14.6	146.0	38.3	5.14	180.0	10.8	38.8	123.1	7.32	0.54	14.0
14	10	3.84	33.6	0.71	14.9	134.5	14.4	2.41	110.9	8.31	28.2	160.6	9.96	0.67	8.50
15	10	1.16	34.7	0.77	22.2	140.4	20.0	1.96	27.2	51.1	122.8	135.8	13.9	0.64	25.1
16	10	0.37	21.5	0.92	11.1	136.3	31.5	1.66	44.6	10.8	24.4	166.9	11.4	0.89	23.9
17	10	0.39	22.8	0.99	26.9	148.4	18.9	1.94	30.4	51.0	74.7	154.5	6.11	0.54	16.6
18	5	1.40	49.1	0.85	16.3	171.8	30.6	1.66	39.9	10.1	35.9	124.2	17.6	0.88	9.1
19	10	0.32	83.2	1.01	25.2	146.3	56.9	2.36	78.8	104.4	90.1	163.7	10.4	0.81	17.3
20	10	0.21	34.4	0.76	10.4	178.6	19.0	2.04	52.7	16.1	29.2	134.9	9.24	0.78	14.6
21	10	0.21	48.2	0.98	25.1	135.7	24.1	2.36	108.5	10.7	39.3	81.2	20.0	0.65	29.1
Average CV:			42.7		19.2		25.6		75.8		52.0		13.7		17.6

[1]Ranch location referenced on Figure 1.

$n \geq 15\%$ of ram lamb population at each ranch.

Table 3. Minimum, maximum, mean, and coefficient of variation (CV) of serum trace mineral concentrations from Montana ram lambs (*n* = 221) measured across ranches

Trace mineral	Minimum	Maximum	Mean	CV, %	Adequate range[1]
Co, ng/mL	0.10	6.22	0.93	117.1	≥ 0.10
Cu, µg/mL	0.30	1.61	0.83	27.0	0.70 - 2.00
Fe, µg/dL	26.0	350.0	156.5	33.2	116.0 – 222.0
Mn, ng/mL	0.70	31.3	2.51	121.8	12.0 – 30.0
Mo, ng/mL	2.80	465.5	37.9	179.2	0.50 – 2.00
Se, ng/mL	16.0	197.0	103.7	50.3	110.0 – 160.0
Zn, µg/mL	0.30	1.74	0.73	27.4	0.80 – 1.20

[1] Adequate ranges were adapted from Herdt and Hoff (2011) and Michigan State University Diagnostic Center for Population and Animal Health

Table 4. Effect of mineral supplementation on ranch serum trace mineral concentrations

Trace mineral	Mineral Supplement				*P* - value
	Supplemented, *n* = 14		Un-supplemented, *n* = 7		
	LS Mean	SEM	LS Mean	SEM	
Co, ng/mL	1.16	0.26	0.67	0.37	0.29
Cu, µg/mL	0.83	0.04	0.83	0.06	1.00
Fe, µg/dL	160.5	10.3	149.5	14.6	0.55
Mn, ng/mL	2.75	0.27	2.14	0.39	0.21
Mo, ng/mL	42.7	16.4	31.8	23.2	0.71
Se, ng/mL	133.1	9.36	68.6	13.2	< 0.001
Zn, µg/mL	0.77	0.04	0.68	0.06	0.24

Table 5. Distribution of Se status across 21 Montana sheep operations and whether supplementation occurred

	Classification[1]			
% Ranches	Deficient <50 ng/mL	Marginally deficient 50-110 ng/mL	Adequate 110-160 ng/mL	Excessive >160 ng/mL
Supplemented	0.0	14.3	42.9	9.5
Un-supplemented	19.0	9.5	0.0	4.8

[1]Reference ranges were adapted from Herdt and Hoff (2011) and Michigan State University Diagnostic Center for Population and Animal Health.

Table 6. Estimated Pearson correlation coefficients between serum trace mineral concentrations measured on ram lambs (n = 221) measured across ranches

	Co	**Cu**	**Fe**	**Mn**	**Mo**	**Se**	**Zn**
Co	1.00	-0.10	-0.11	0.03	-0.06	0.33**	-0.14*
Cu	-	1.00	-0.08	0.05	0.02	0.09	0.12*
Fe	-	-	1.00	-0.03	-0.09	-0.09	0.31**
Mn	-	-	-	1.00	-0.03	0.14**	0.16**
Mo	-	-	-	-	1.00	0.05	0.02
Se	-	-	-	-	-	1.00	0.16**
Zn	-	-	-	-	-	-	1.00

*Estimated Pearson correlation coefficient is different from zero ($P < 0.10$).
**Estimated Pearson correlation coefficient is different from zero ($P < 0.05$).

Table 7. Average and range of concentrations, percentage of samples exceeding maximum upper level, and concentration at maximum upper limit for water minerals, compounds, total dissolved solids (TDS), and pH evaluated from 20 ranches

Variable	Average	Range	Samples exceeding maximum upper limit for livestock, %	Maximum upper limit[1]
Ca, mg/kg	58.42	1.21 to 153.00	0	200
Cu, mg/kg	0.01	n.d. to 0.08	0	0.50
Cl, mg/kg	27.55	1.00 to 205.00	0	300
Fe, mg/kg	0.12	n.d. to 1.11	10	0.40
Mg, mg/kg	35.76	0.35 to 177.00	5	100
Na, mg/kg	238.83	4.16 to 1070.00	40	300
Nitrate, mg/kg	6.88	n.d. to 93.00	0	100
Sulfate, mg/kg	373.26	n.d. to 2720.00	35	300
TDS, mg/kg	1007.17	164.00 to 3520.00	5	3,000
pH	7.78	7.00 to 8.75	20	8.50

[1]Maximum upper levels are from Socha et al. (2003).
n.d. = not detectable in laboratory analysis

Table 8. Estimated Pearson correlation coefficients between mean serum Se and Zn concentration and water minerals, compounds, total dissolved solids (TDS), and pH evaluated from 20 ranches

	Trace mineral	
Water characteristic	Se	Zn
Ca	0.18	-0.26
Cu	0.11	0.01
Cl	0.37	0.02
Fe	0.20	-0.18
Mg	0.35	-0.16
Na	0.59**	-0.20
Nitrate	0.24	0.06
Sulfate	0.40*	-0.23
TDS	0.61**	-0.26
pH	-0.07	0.33

*Estimated Pearson correlation coefficient is different from zero ($P < 0.10$).

**Estimated Pearson correlation coefficient is different from zero ($P < 0.05$).

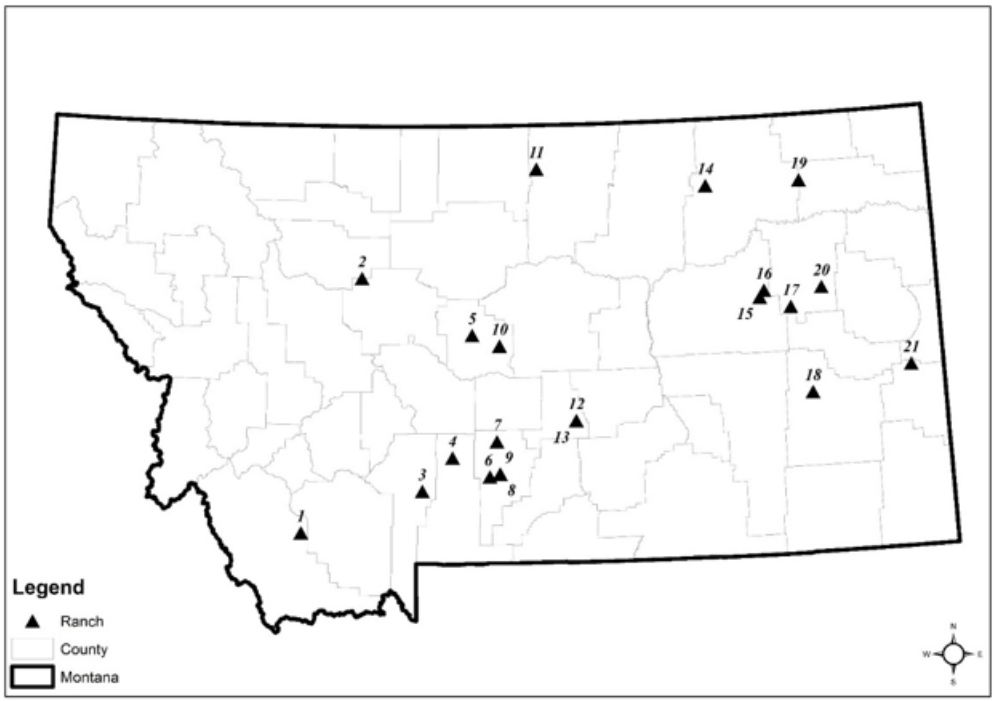

Figure 1. Map of sampling locations across Montana.

CHAPTER FOUR

EFFECTS OF ZINC SOURCE AND DIETARY CONCENTRATION ON ZINC STATUS, GROWTH PERFORMANCE, WOOL TRAITS, AND REPRODUCTIVE CHARACTERISTICS IN DEVELOPING RAMS

Contribution of Authors and Co-Authors

Author: C. M. Page

Contributions: Main author, data collection, data analysis, interpretation of article, and drafting of the paper.

Co-Author: M. L. Van Emon

Contributions: Critical in experimental design, data collection, data interpretation, and revisions.

Co-Author: T. W. Murphy

Contributions: Critical in statistical analysis, data collection, data interpretation, and revisions.

Co-Author: C. K. Larson

Contributions: Critical in experimental design and revisions.

Co-Author: J. G. Berardinelli

Contributions: Critical in experimental design, data collection, and revisions.

Co-Author: I. R. McGregor

Contributions: Aided in data collection and revisions.

Co-Author: W. C. Stewart

Contributions: Prinicipal investigator, critical in obtaining funding, experimental design, data collection, data interpretation, data analysis, and revisions.

Manuscript Information Page

C. M. Page, M. L. Van Emon, T. W. Murphy, C. K. Larson, J. G. Berardinelli, I. R. McGregor, and W. C. Stewart

Journal of Animal Science

Status of Manuscript: (Put an x in one of the options below)
_X__ Prepared for submission to a peer-reviewed journal
____ Officially submitted to a peer-review journal
____ Accepted by a peer-reviewed journal
____ Published in a peer-reviewed journal

ABSTRACT

The objectives of this study were to evaluate the effects of dietary Zn source and concentration on Zn status, growth performance, wool traits, and reproductive characteristics in developing rams. Forty-four Targhee rams (14 mo; 68 ± 18 kg BW) were used in an 84-d completely randomized design, and were fed one of three pelleted dietary treatments: 1) a control without fortified Zn (CON; n = 15); 2) a diet fortified with a Zn amino acid complex (ZnAA, Zinpro Corp; n = 14); and 3) a diet fortified with $ZnSO_4$ ($ZnSO_4$; n = 15). Growth and wool traits measured throughout the course of the study were ADG, DMI, G:F, BW, loin muscle depth (LMD), back fat (BF), wool staple length (SL), and average fiber diameter (AFD). Blood was collected from each ram at four time periods to quantify serum Zn and testosterone concentrations. Semen was collected 1-2 d after the trial was completed. There were no differences in DMI (P = 0.18), BW (P = 0.45), LMD (P = 0.48), BF (P = 0.47), and AFD (P = 0.9) among treatment groups. $ZnSO_4$ had greater ($P \leq 0.03$) serum Zn concentrations compared to ZnAA and CON treatments. Rams consuming ZnAA had greater ($P \leq 0.03$) ADG than $ZnSO_4$ and CON. There tended to be differences among groups for G:F (P = 0.06), with ZnAA being numerically greater than $ZnSO_4$ and CON. SL was greater ($P < 0.001$) in $ZnSO_4$ and tended to be longer (P = 0.06) in ZnAA treatment group compared to CON. No differences were observed among treatments in scrotal circumference (P = 0.59), testosterone (P = 0.59), semen concentration (P = 0.24), % motility (P = 0.37), % live sperm (P = 0.90), and % sperm abnormalities (P = 0.23). These results indicate that the source and concentration of a Zn supplement appeared to improve ram development,

specifically ADG, serum Zn concentrations, SL, and there was a tendency to improve G:F. These results may be used to make sound management decisions when accounting for minerals with developing rams in Montana and other northern range lands.

Key words: Bioavailability, ram, testosterone, trace minerals, zinc

[1]Authors wish to thank the Montana Agricultural Station, Zinpro Corporation, Kim Hager and David Miller from CHS, and the National Sheep Industry Improvement Center for partial funding of this research.
[2]Corresponding author: Whit.Stewart@uwyo.edu

INTRODUCTION

Western sheep production systems rely largely on rangeland plant communities as the primary feed source. This reliance on the rangeland plant community could lead to mineral deficiencies, which may limit the productivity of livestock operations. Mineral concentrations in forages are highly variable across rangelands (Mathis et al., 2004) with influential factors such as soil geochemistry (Smith et al., 2013) and forage stage of maturity (Jones and Tracy, 2015). Studies have suggested that the chemical form of a mineral source plays an important role in bioavailability; generally with organic sources being more bioavailable than inorganic sources (Rojas et al., 1995; Cao et al., 2000; Spears, 2003). A survey conducted to quantify serum Zn concentrations in Montana ram lamb populations indicated that approximately 14% of ranches sampled were categorized as being deficient and 52% marginally deficient in Zn (Page et al., 2016).

Zinc is the second most abundant trace mineral in the body with important functions involved in reproduction (Kumar et al., 2006), gene expression (Berg, 1990), immune function (Spears and Weiss, 2008), and wool growth in sheep (White et al.,

1994). Subclinical deficiencies in Zn could be more frequent than other trace minerals because the body does not sequester large amounts of Zn in any one organ. (NRC, 2007; Herdt and Hoff, 2011). Optimal concentrations of dietary Zn are not well understood, and with such high tolerance to dietary Zn in most mammals, there is potential for greater supplementation levels than the recommended concentrations for sheep (NRC, 2007). The objective of the present study was to quantify the effects of dietary Zn source and concentrations on developing ram Zn status, growth performance, wool traits, and reproductive characteristics.

MATERIALS AND METHODS

Animals and Diets

Experimental procedures described herein were approved by the Agriculture Animal Care and Use Committee of Montana State University (#2016-AA09). All animals used in this study were provided by the Montana Agricultural Experiment Station, and the study was conducted at the Fort Ellis Research Station at Montana State University (Bozeman, MT).

Forty-four purebred Targhee rams (14 mo; 68 ± 18 kg BW) were utilized in an 84 d completely randomized design. Rams were stratified by BW and initial serum Zn concentration then allocated to one of three pelleted dietary treatments: 1) control diet without fortified Zn (**CON**; n = 15; Table 1); 2) a diet fortified with a Zn amino acid complex (**ZnAA**, Zinpro Corp; n = 14); and 3) a diet fortified with $ZnSO_4$ (**$ZnSO_4$**; n = 15). The basal diet was formulated to meet 100% of nutrient and Zn requirements of

developing rams (24 mg of Zn/kg; NRC, 2007). Zinc dietary treatments were formulated to provide 300% of Zn (72 mg Zn/kg) and 100% of nutrient requirements. Ram treatment groups were randomly assigned to drylot pens (6.4 × 21.9 m) with approximately one third of the pen covered by a three-sided barn. Rams were fitted with an electronic identification tag and each pen was equipped with two GrowSafe bunks (GrowSafe Systems Ltd., Airdrie, AB, Canada) to monitor individual intake. Rams had *ad libitum* access to NaCl, feed, and water. Rams were denied access to a complete free choice granulized mineral premix for 50 d prior to the start of the study to normalize trace mineral status. Rams were fed a dry hay (14 ppm Zn) diet from -16 to 0 d before the trial to deplete circulating Zn concentration in the blood.

Feed Intake, growth, ultrasound, and wool data

Rams were equipped with an electronic identification ear tag and adapted to the GrowSafe system for 16 d prior to the start of the study. Individual intake data was recorded by the GrowSafe system. Elevated platforms were used to modify GrowSafe beef cattle stanchions and feed bunks (0.79 × 1 m) for sheep. Rams were weighed on consecutive days on d -1 and 0, 27 and 28, 55 and 56, and 83 and 84. For the consecutive days at the beginning and end of the study, rams were fasted 12 h before BW was measured.

Ultrasonic measurements of loin muscle depth (**LMD**) and back fat (**BF**) were collected on d 0, 28, 56, and 84 by the same National Sheep Improvement Program certified ultrasound technician using a real-time ultrasound device (ibex-pro, E.I. Medical

Imaging, Loveland, CO). Before each ultrasound image was captured, the area was shorn and vegetable oil was used as a conductive medium. Images were captured between the 12^{th} and 13^{th} rib with an 8-5 MHz 66 mm linear array transducer.

Wool mid-side samples were collected from rams on d 0 and 84, and wool staple length (**SL**) regrowth over the 84-d study was measured at 5 locations and averaged for each ram. Wool mid-side samples were prepared and analyzed for average fiber diameter (**AFD**) and other metrology traits by the Montana State Wool Lab utilizing the Optical-based Fiber Diameter Analyzer 2000 (ASTM, 1990).

Blood collection and serum assays

Blood samples were collected via jugular venipuncture into 13 × 100 mm royal blue top vacutainer tubes (Covidien, Mansfield, MA) without additives for later serum trace mineral analysis. The first blood sample for trace mineral analysis was obtained on d -16 of the study for the purpose of stratifying groups by serum Zn status, and additional samples were collected on d 28, 56, and 84 of the study. Blood tubes were kept on ice and allowed to clot for approximately 4 h (Herdt and Hoff, 2011) and then centrifuged at 2700 × *g* for 30 min at 4 ºC. Serum was decanted into two aliquots in 12 × 75 mm plastic culture tubes and stored at -20 ˚C for later analysis. Serum Zn concentrations were determined by a commercial laboratory (Michigan State University Diagnostic Center for Population and Animal Health, East Lansing) using an ionized coupled plasma mass spectrometry method (Wahlen et al., 2005).

Blood for serum testosterone assay was collected into 10 mL red top serum vacutainer tubes (BD Vacutainer, Franklin Lakes, NJ) and serum was extracted in the same manner as given previously. These blood collections took place on d 0, 28, 56, and 84 (d 0 = start of supplementation). Testosterone concentrations were assayed using enzyme-linked immunoassay kits (ENZO Life Sciences©, Farmingdale, NY, USA) validated for sheep serum. Intra- and inter-assay CVs for a pooled sample that contained 4.27 ng/mL of testosterone were 0.13 % and 3.55 %, respectively. The sensitivity of this assay was 54.26 pg/mL.

Semen evaluation and scrotal circumference

Scrotal circumference (**SC**) was recorded immediately prior to BW on d 0, 28, 56, and 84 by the same technician. Scrotal circumference was measured at the widest portion of the testes with a manual metal tape (Hammerstedt, 1996). Ten days prior to semen collection, rams were given 1 h fence-line exposure to ewes. Ten multiparous ewes of varying ages went through an estrous synchronization protocol in order to show signs of heat at the time of semen collection. A controlled internal drug releasing devise (**CIDR**) and PGF_{2a} protocol was utilized, where each ewe received a CIDR for 7 d. On d 7, the CIDR was removed and each ewe received an intramuscular injection of 12.5 mg of PGF_2 (Dinoprost tromethamine; ProstaMate® , Vedico, Inc., St Joseph, MO, USA). The ewes were divided into two groups, five utilized on d 85 and five on d 86. Only ewes showing signs of heat were used during semen collection. On the day of collection, rams were given 1 h fence-line interaction with ewes prior to semen collection. Rams were

brought into a pen where each ram was allowed 5 min to mount a restrained ewe. Before mounting and ejaculation occured, rams were secured into a ground level head catch. Semen samples were collected by electro-ejaculation (Standard Precision Electronics, Littleton, CO). Semen concentration was determined using a NucleoCounter SP-100 according to the manufacturer's protocol (ChemoMetic A/S, Allerod, Denmark). Semen was diluted 10:3990 with a phosphate-buffered saline (pH = 7.4) and placed on a warmed hemocytometer (Iimproved Neubauer hemocytometer; GmbH an Co., Hamburg, Germany) under a microscope at 400x magnification to quantify percentages of progressively motilite sperm. Slides were prepared by staining 10 μL of diluted semen with 10 μL trypan blue for counting live and dead cells and morphology counts. Spermatozoa live/dead counts and morphology was evaluated by counting 100 random cells at 1000x magnification, and abnormalities were estimated in each ejaculate using standard procedures outlined in Hafez and Hafez (2000). A sample of undiluted semen from each ram that had successful ejaculates and chilled to 4^{o} C until centrifugation. Semen was centrifuged 12,000 × *g* for 30 min at 4o C, and seminal plasma was decanted to assay trace mineral concentrations as described previously.

Statistical Analyses

Data were analyzed as a completely randomized design with individual ram as the experimental unit. Growth performance, intake data, and testosterone concentrations were analyzed as repeated measures using the MIXED procedure of SAS (version 9.4; SAS Inst. Inc., Cary, NC). The model included the fixed effects of treatment, day, and their

interaction, and the random effect of ram. An autoregressive covariance structure with heterogeneous variance across day was assumed, which was found to be the most parsimonious using Akaike's information criteria. Wool traits measured at the end of the trial were analyzed using the GLIMMIX procedure with the fixed effect of treatment and the wool measurement at the start of the trial was fit as a linear covariate. Semen and sperm characteristics were analyzed using the GLM procedure of SAS. Fixed effects included treatment, collection day, and their interaction. Data are presented as least squares means of main effects and differences were considered significant at $P \leq 0.05$ and as a tendency at $P \leq 0.10$.

RESULTS AND DISCUSSION

Effects of Zn source and dietary concentration on ADG, DMI, G:F, and BW are presented in Tables 2 and 3. There was no difference ($P = 0.18$) among treatments for DMI. There was an observed d by treatment interaction ($P = 0.05$; Table 8) for BW. Overall, there was no difference ($P = 0.45$) among treatments in BW. However, rams consuming ZnAA had greater ($P \leq 0.03$) ADG than $ZnSO_4$ and CON rams. There was no observed d by treatment interaction ($P = 0.44$) for ADG. Similar results were found in lambs supplemented with Zn-methionine and ZnO, with a tendency of Zn-methionine to increase growth performance (Spears, 1989). There tended to be differences among groups for G:F ($P = 0.06$) with ZnAA being greater than $ZnSO_4$ and CON. Although Zn deficiency is less of a clinical problem in ruminant animals (Herdt and Hoff, 2011), some of the initial signs of deficiency include reduced feed intake and growth rates (Herdt and

Hoff, 2011). The effects of Zn on growth and intake performance may in part, be modulated by its relationship with cholecystokinin. Cholecystokinin secretion is increased in Zn-deficient intestinal tissues and serves roles in endocrine and neurocrine functions regulating gall bladder contraction, pancreatic secretion, gastric emptying, and satiety mechanisms (Blanchard and Cousins, 1996). Effects of Zn on ultrasound measurements of LMD and BF are presented in Table 3. There were no differences in LMD or BF ($P \geq 0.47$) among treatments. There was no d by treatment interaction observed in LMD or BF ($P = 0.71$; $P = 0.54$ respectively).

Wool traits for rams measured in the study are presented in Table 3. Average wool fiber diameter did not differ ($P = 0.96$) among treatments regardless of Zn source or concentration. Staple length was greatest ($P < 0.001$) in rams consuming fortified $ZnSO_4$ diets compared to CON; whereas ZnAA tended ($P = 0.06$) to have longer staple length over the 84-d study than CON. It is well known that Zn is a major constituent in wool (NRC, 2007) and plays a critical role in the keratinization process through structural and regulatory factors and processes (Tomlinson et al., 2004). This could provide a reasonable explanation for rams consuming greater concentrations of Zn tending to have longer SL than rams consuming lesser concentrations of dietary Zn. Zinc deficiencies have been observed to reduce wool growth and impair its keratinization (White et al., 1994).

Serum Zn concentrations were greatest ($P \leq 0.03$) in $ZnSO_4$ (Table 3; Figure 1); whereas, serum Zn concentration did not differ ($P = 0.12$) between ZnAA and CON. There was no d by treatment interaction observed ($P = 0.22$; Table 8) for serum Zn

concentration. Zinc homeostasis is tightly regulated in the body (Herdt and Hoff, 2011), and resultant Zn tissue concentrations remain relatively constant over a wide range of Zn intakes. There is no clear site of accumulation of Zn throughout the body and its absorption is reduced under conditions of ample intake (NRC, 2007; Herdt and Hoff, 2011). This may be a reason for explaining the observation that serum Zn concentrations in ZnAA were not different than CON in the present study. In a similar study, Zn absorption did not differ in sheep that consumed Zn in multiple forms and similar dietary concentrations, but retention was greater in lambs treated with Zn Methionine than with a ZnO source, indicating difference in metabolism post-absorption or tissue retention (Spears, 1989).

Effects of dietary treatments on SC and system testosterone concentration throughout the course of the study are presented in Table 5. Scrotal circumference did not differ ($P = 0.59$) among rams in these treatments but increased ($P < 0.001$) from d 0 to d 84 of the study. There tended to be a d by treatment interaction ($P = 0.08$; Table 8) for SC. This was expected both as these rams increase in age and as hours of daylight decreased during the study. Similarly testosterone concentrations did not differ ($P = 0.59$) between treatment groups and increased throughout the trial. There was a d by treatment interaction observed ($P = 0.03$) for serum testosterone concentration. This is due to treatment groups having different serum testosterone concentrations at d 28 of the study, while at d 0, 56, and 84 treatments did not differ from each other (Table 8). These findings are similar to those of Geary et al. (2016), in that they observed no differences in SC among bulls supplemented with varying concentrations and sources of trace minerals.

However, in the study by Geary et al. (2016) the supplements did not supply additional dietary Zn alone, therefore any conclusions delineating effects of Zn may not be appropriate. Many studies evaluating the effects of Zn on reproduction characteristics or processes of rams were primarily focused on clinically deficiency signs and symptoms (Underwood and Somers, 1969; Martin and White, 1992; Martin et al., 1994). These studies established the current dietary Zn recommendations (30 ppm; Suttle 2010) for adequate testicular growth in developing rams. Inadequate Zn concentrations in ram diets may limit development of the seminiferous tubules, the primary site for spermatogenesis (Martin et al., 1994). Our failure to observe differences among rams in the treatments in the present study may be due to feeding greater than recommended dietary concentrations of Zn to rams in all treatment groups including CON.

Effects of dietary treatment on post-trial semen and sperm characteristics are reported in Table 6. Semen concentrations (sperm cells/mL) did not differ (P = 0.24) among treatments. Rams supplemented with ZnAA numerically had greater semen concentration (2,818 × 10^6/mL) compared to rams in the CON and $ZnSO_4$ treatments (1,148 × 10^6/mL; 1,380 × 10^6/mL respectively). Percentages of progressively motile sperm (P = 0.37), percentage of live sperm (P = 0.90), and percentage of abnormalities (P = 0.23) in ejaculates did not differ among treatment groups. Kumar et al. (2016) conducted a study evaluating the effects of varying concentrations of dietary Zn and source have on semen qualities in crossbred bulls (*Bos indicus* × *Bos taurus*). They reported a greater semen concentration from bulls supplemented with 70 ppm $ZnSO_4$ and 35 ppm Zn propionate compared to bulls that recieved 35 ppm $ZnSO_4$ or no

supplemented Zn to a basal diet that contained 33 ppm. However, Kumar et al. (2016) carried out the study over the course of 3 cycles of spermatogenesis or (blank) d, which could have allowed a more sufficient time to produce differences in relatively non-seasonal breeding cattle. In the present study we evaluating the effects of supranutritional dietary Zn concentrations and alternate sources of Zn in sheep. To our knowledge, this is the first study to do this. However, the effects that Zn deficiency has on semen attributes has been evaluated by Underwood and Somers (1969), who reported decreased production of sperm in rams receiving diets below 30 ppm Zn. Furthermore they reported that there were greater number of abnormalities in sperm cells from rams fed dietary Zn concentrations below 30 ppm.

Treatment effects on seminal plasma trace mineral concentrations are reported in Table 7. Seminal plasma Mn concentrations were greater ($P = 0.01$) in ZnAA and $ZnSO_4$ than in CON rams. Grace and Lee (1990) reported a similar observation of a positively correlated relationship between pancreatic Zn and Mn concentrations. Manganese deficiencies can have negative effects on testicular growth relative to body growth (Masters et al., 1998), and may also decrease conception rates (Underwood and Suttle, 1999). No differences were observed in seminal plasma concentrations of any other trace minerals that were assayed among treatment groups. Zinc concentration in seminal plasma has reported to be correlated positively with sperm concentration and other semen parameters in fertile vs. infertile men (Colagar et al., 2009). In the present, Zn concentrations were not elevated in ram seminal plasma. One might hypothesize that lack of differences in seminal Zn concentration may be due to the fact that rams were fed

adequate levels necessary for proper testicular and reproductive function for the ram in all treatments including rams in the CON.

CONCLUSIONS

Overall, Zn source and concentration affected ADG, serum Zn concentration, staple length, and tended to increase feed efficiency. However, reproductive characteristics such as scrotal circumference, testosterone production, and semen characteristics seemed to be unaffected by the current treatments. This could be because of dietary Zn concentrations above recommended levels in all treatments. Results indicate the beneficial effects of supranutritional Zn concentrations beyond basal dietary concentrations. Although Zn retention and metabolic pathways of Zn metabolism were not investigated, results indicate that greater dietary Zn concentrations can enhance nutritional strategies in ram development. These findings might be directly applicable to producers developing white-face type rams for fall ram sales in the mountain west and northern plains regions. Futhermore the results of this may be applicable to other aspects of sheep production, such as replacement ewes and slaughter lambs to increase ADG and wool growth.

LITERATURE CITED

American Society for Testing and Materials (ASTM). 1990. Annual book of ASTM standards. Standard tests method D584. Wool content of raw wool – Laboratory scale. Sec. 7, Vol. 07.01:193-197. ASTM, Philadelphia, PA.

Berg, J. M. 1990. Zinc fingers and other metal-binding domains. Elements for interactions between macromolecules. J. Biol. Chem. 265:6513-6516.

Blanchard, R. K. and R. J. Cousins. 1996. Differential display of intestinal mRNAs regulated by dietary zinc. Proc. Natl. Acad. Sci. USA 93:6863-6868.

Colagar, A. H., E. T. Marzony, and M. J. Chaichi. 2009. Zinc levels in seminal plasma are associated with sperm quality in fertile and infertile men. Nutrition Research. 29:82-88.

Geary, T. W., W. L. Kelly, D. S. Spickard, C. K. Larson, E. E. Grings, and R. P. Ansotegui. 2016. Effect of supplemental trace mineral level and form on peripubertal bulls. Ani. Repro. Sci. 168:1-9.

Grace, N. D., and J. Lee. Effect of Co, Cu, Fe, Mn, Mo, Se, and Zn supplementation on the elemental content of soft tissues and bone in sheep grazing ryegrass/whit clover pasture. New Zealand Journal of Agricultural Science. 33:635-647.

Grings, E. E., M. R. Haferkamp, R. K. Heitschmidt, and M. G. Karl. 1996. Mineral dynamics in forages of the Northern Great Plains. J. Range Manage. 49:234-240. doi:10.2307/4002884.

Hafez, E. S. E., and B. hafez, editors. 2000. Reproduction in farm animals. 7th ed. Lippincott Williams and Wilkins, Philadelphia, PA. doi:10.1002/9781119265306

Herdt, T. H., and B. Hoff. 2011. The use of blood analysis to evaluate trace mineral status in ruminant livestock. Vet. Clin. North Am. Food Anim. Pract. 27:255-283.

Jones, G. B., and B. F. Tracy. 2015 Evaluating seasonal variation in mineral concentration of cool-season pasture herbage. Grass Forage Sci. 70:94-101.

Kumar, N., Verma, R. P., Singh, L. P., Varshney, V. P., & Dass, R. S. 2006. Effect of different levels and sources of zinc supplementation on quantitative and qualitative semen attributes and serum testosterone level in crossbred cattle (Bos indicus × Bos taurus) bulls. Reprod. Nutr. Dev. 46:663-675.

Martin, G. B., and C. L. White. 1992. Effects of dietary zinc deficiency on gonadotrophin secretion and testicular growth in young male sheep. J. Reprod. Fertil. 96:497-507.

Martin, G. B., C. L. White, C. M. Markey, and M. A. Blackberry. 1994. Effects of dietary zinc deficiency on the reproductive system of young male sheep: testicular growth and the secretion of inhibin and testosterone. Journal of Reproduction and Fertility. 101:87-96.

Masters, D. G., D. I. Paynter, J. Briegel, S. K. Baker, and D. B. Purser. 1998. Influence of manganese intake on body, wool and testicular growth of young rams and on the concentration of manganese and the activity of manganese enzymes in tissues. Australian Journal of Agricultural Research. 39:517-524.

Mathis, C., and J. Sawyer. 2004. New Mexico forage mineral survey. Proc. West. Sec. Amer. Soc. Anim. Sci. 55

NRC. 2007. Nutrient Requirements of sheep. 7th ed. Natl. Acad. Press, Washington, DC.

Page, C. M., M. L. Van Emon, S. Spear, T. W. Murphy, J. G. Bowman, and W. C. Stewart. 2016. Survey of serum trace mineral concentrations in weaned Montana ram lambs. Proc. West. Sec. Amer. Soc. Anim. Sci. 67

Rojas, L., L. McDowell, R. Cousins, F. Martin, N. Wilkinson, A. B. Johnson, J. Velasquez. 1995. Relative bioavailability of two organic and two inorganic zinc sources fed to sheep. J. Anim. Sci. 73:1202-1207.

Smith, D. B., W. F. Cannon, L. G. Woodruff, Solano, Federico, J. E. Killburn, and D. L. Fey. 2014. Geochemical and mineralogical data for soils of the conterminous United States. U.S. Geological Survey. doi:10.3133/ofr20141082

Spears, J. W. 1989. Zinc methionine for ruminants: Relative bioavailability of zinc in lambs and effects on growth and performance of growing heifers. J. Anim. Sci. 67:835-843

Spears, J. W. 2003. Trace mineral bioavailability in ruminants. J. Nutr. 133:1506S-1509S.

Spears J. W., and W. P. Weiss. 2008. Role of antioxidants and trace elements in health and immunity of transition dairy cows. Vet. J. 176:70-76

Suttle, N. F. 2010. Mineral nutrition of livestock. 4th ed. CABI Publishing, New York.

Tomlinson, D. J., C. H. Mulling, and T. M. Fakler. 2004. Invited Review: Formation of Keratins in the Bovine Claw: Roles of Hormones, Minerals, and Vitamins in Functional Claw Integrity. J. Dairy Sci. 87:797-809.

Underwood, E. J., and M. Somers. 1969. Studies of zinc nutrition in sheep. I. The relation of zinc to growth, testicular development, and spermatogenesis in young rams. Aust. J. Agric. Res. 20:889-897.

Underwood, E. J., and F. Suttle. 1999. The Mineral Nutrition of Livestock. 3rd ed. CAB International, Wallinford, UK.

Wahlen, R., L. Evans, J. Turner, and R. Hearn. 2005. The use of collision/reaction cell ICP-MS for the determination of 18 elements in blood and serum samples. Spectroscopy 20:12.

White, C., Martin, G., Hynd, P., & Chapman, R. 1994. The effect of zinc deficiency on wool growth and skin and wool follicle histology of male Merino lambs. Br. J. Nutr. 71:425-435.

Table 1. Chemical and nutrient composition of dietary treatments

	Dietary Treatments[1]		
Item	CON	ZnAA	$ZnSO_4$
Ingredient, %			
Alfalfa, DHY-17	37.47	37.43	37.46
Corn, ground	30	30	30
Soybean hulls	15	15	15
Malt sprouts	10	10	10
Molasses, cane	4	4	4
Calcium carbonate	1.35	1.35	1.35
Ammonium chloride	1	1	1
Mineral premix	1.18	1.18	1.18
Nutrient Composition[2]			
DM, %	90.41	90.41	90.41
CP, %[3]	17.6	16.8	17.4
NDF, %	30.72	30.71	30.72
ADF, %	20.72	20.71	20.72
Ash, %	7.65	7.65	7.67
Mineral Composition[2]			
Ca, %	1.24	1.24	1.24
P, %	0.35	0.35	0.35
K, %	1.5	1.5	1.5
S, %	0.19	0.19	0.19
Mg, %	0.21	0.21	0.21
Na, %	0.39	0.39	0.39
Fe, mg/kg	237.37	237.2	237.31
Mn, mg/kg	89.14	89.13	89.14
Cu, mg/kg	7.87	7.86	7.87
Zn, mg/kg[3]	47.5	95.5	91.5
I, mg/kg	0.9	0.9	0.9

[1]Dietary treatments: 1) control diet without fortified zinc (CON); 2) a diet fortified with a Zn amino acid complex (ZnAA); and 3) a diet fortified with $ZnSO_4$.
[2]Calculated concentration in diets.
[3]Analyzed concentration in diets.

Table 2. Least squares means for the main effects of dietary Zn source and period on the ADG, DMI, and feed efficiency of yearling Targhee rams.

	Treatment[1]					Period				
Item	CON	ZnAA	$ZnSO_4$	SEM[2]	*P* – value	d 0 to 28	d 29 to 56	d 57 to 84	SEM[2]	*P* – value
ADG, kg/d	0.33[b]	0.40[a]	0.34[b]	0.18	0.03	0.44[a]	0.40[a]	0.23[b]	0.20	<0.001
DMI, kg/d	3.11	3.32	3.18	0.81	0.18	2.81[a]	3.43[b]	3.37[b]	0.57	<0.001
G:F	0.109	0.124	0.109	0.005	0.06	0.158[a]	0.115[b]	0.068[c]	0.005	<0.001

[1]Dietary treatments: 1) control diet without fortified zinc (CON; Table 1); 2) a diet fortified with a Zn amino acid complex (ZnAA, Zinpro Corp); and 3) a diet fortified with $ZnSO_4$.

[2]Greatest SEM presented (n = 15).

[a-c]Means within a row and main effect with no common superscripts are different ($P < 0.05$).

Table 3. Least squares means for the main effects of dietary Zn source and day on ultrasound and wool traits of yearling Targhee rams.

	Treatment[1]					Day					
Item[3]	CON	ZnAA	$ZnSO_4$	SEM[2]	*P* – value	0	28	56	84	SEM[2]	*P* – value
BW, kg	83.7	87.0	84.1	2.02	0.45	68.4[a]	80.8[b]	92.1[c]	98.4[d]	1.30	<0.001
LMD, mm	30.02	29.80	29.13	0.55	0.48	25.30[a]	28.34[b]	30.56[c]	34.41[d]	0.50	<0.001
BF, mm	4.45	4.70	4.70	0.17	0.47	2.98[a]	4.28[b]	5.25[c]	5.96[d]	0.15	<0.001
SL, mm	23.37[b]	25.91[b]	26.67[a]	1.02	< 0.01	—	—	—	—	—	—
AFD, µm	22.1	22.1	22.0	0.34	0.96	—	—	—	—	—	—

[1]Dietary treatments: 1) control diet without fortified zinc (CON; Table 1); 2) a diet fortified with a Zn amino acid complex (ZnAA, Zinpro Corp); and 3) a diet fortified with $ZnSO_4$.
[2]Greatest SEM presented (n = 15).
[3]LMD: loin muscle depth; BF: back fat; SL: wool staple length; AFD: average fiber diameter.
[a-d]Means within a row and main effect with no common superscripts are different (P < 0.05).

Table 4. Least squares means for the main effects of dietary Zn source and day on serum trace mineral concentrations of yearling Targhee rams.

	Treatment[1]					Day					
Mineral	CON	ZnAA	$ZnSO_4$	SEM[2]	*P* – value	0[4]	28	56	84	SEM[2]	*P* – value
Co, ng/mL	8.02[a]	5.91[b]	8.01[a]	0.39	< 0.001	0.35[a]	12.47[b]	10.35[c]	6.08[d]	0.59	<0.001
Cu, µg/mL	3.20	0.92	0.92	1.43	0.41	0.69	0.96	4.03	1.03	3.21	<0.001
Fe, µg/dL	154.63	158.62	167.23	8.54	0.55	132.75[b]	161.89[a]	162.48[a]	183.53[a]	8.18	<0.001
Mn, ng/mL	2.31	2.49	2.21	0.12	0.29	3.02	0.87	3.28	2.17	0.14	<0.001
Mo, ng/mL	37.41	37.24	38.29	2.31	0.94	28.11	35.58	35.80	51.09	2.28	<0.001
Se, ng/mL	145.63	142.43	149.08	2.13	0.09	110.02	144.81	146.89	181.14	2.78	<0.001
Zn, µg/mL	0.63[b]	0.66[b]	0.71[a]	0.16	< 0.01	0.54[a]	0.53[a]	0.71[b]	0.89[c]	0.02	<0.001

[1]Dietary treatments: 1) control diet without fortified zinc (CON; Table 1); 2) a diet fortified with a Zn amino acid complex (ZnAA, Zinpro Corp); and 3) a diet fortified with $ZnSO_4$.
[2]Greatest SEM presented (n = 15).
[4]d 0 measurements were collected d -16.
[a-d]Means within a row and main effect with no common superscripts are different (P < 0.05).

Table 5. Least squares means for the main effects of dietary Zn source and day on scrotal circumference (SC) and serum testosterone concentrations of yearling Targhee rams.

	Treatment[1]					Day					
	CON	ZnAA	$ZnSO_4$	SEM[2]	*P* – value	0	28	56	84	SEM[2]	*P* – value
SC[3], cm	38.71	38.19	38.92	0.52	0.59	33.81^{c}	38.17^{b}	41.29^{a}	41.17^{a}	0.39	< 0.001
Testosterone, ng/mL	5.34	5.54	6.33	0.77	0.59	4.56	5.52	6.17	6.69	0.91	0.06

[1]Dietary treatments: 1) control diet without fortified zinc (CON; Table 1); 2) a diet fortified with a Zn amino acid complex (ZnAA, Zinpro Corp); and 3) a diet fortified with $ZnSO_4$.
[2]Greatest SEM presented (n = 15).
[3]SC: Scrotal circumference
[a-d]Means within a row and main effect with no common superscripts are different ($P < 0.05$).

Table 6. Least squares means (± SE) for the main effect of dietary Zn source on semen and sperm characteristics of yearling Targhee rams.

	Treatment[1]			
Semen Characteristics	CON	ZnAA	$ZnSO_4$	*P* - value
cells ml^{-1}, log_{10}	9.06 ± 0.14	9.45 ± 0.19	9.14 ± 0.14	0.24
Motility, %	47.31 ± 4.02	39.81 ± 5.42	40.20 ± 3.81	0.37
Sperm Characteristics				
Live, %	70.93 ± 3.68	68.01 ± 4.96	69.84 ± 3.68	0.90
Normal, %	80.47 ± 4.25	93.74 ± 5.73	86.7 8 ± 4.25	0.23

[1]Dietary treatments: 1) control diet without fortified zinc (CON; Table 1); 2) a diet fortified with a Zn amino acid complex (ZnAA, Zinpro Corp); and 3) a diet fortified with $ZnSO_4$.

Table 7. Least squares means (± SE) for the main effect of dietary Zn source on seminal plasma trace mineral concentrations of yearling Targhee rams.

	Treatment[1]			
Mineral	CON	ZnAA	$ZnSO_4$	*P* - value
Co, ng/mL	2.19 ± 0.24	2.57 ± 0.68	3.69 ±1.01	0.34
Cu, µg/mL	0.06 ± 0.01	0.07 ± 0.01	0.07 ± 0.01	0.91
Fe, µg/dL	0.30 ± 0.04	0.33 ± 0.06	0.38 ± 0.04	0.46
Mn, ng/mL	66.89^{b} ± 10.16	188.55^{a} ± 50.24	131.97^{a} ± 27.19	0.01
Mo, ng/mL	6.86 ± 0.64	5.88 ± 0.58	7.48 ± 1.08	0.31
Se, ng/mL	141.31 ± 16.61	154.08 ± 17.41	145.85 ± 16.05	0.86
Zn, µg/mL	2.38 ± 0.37	1.87 ± 0.25	2.10 ± 0.42	0.50

[1]Dietary treatments: 1) control diet without fortified zinc (CON; Table 1); 2) a diet fortified with a Zn amino acid complex (ZnAA, Zinpro Corp); and 3) a diet fortified with $ZnSO_4$.

[a,b]Means within a row with no common superscripts are different ($P < 0.05$).

Table 8. Least-squares means (± SE) for the interaction of treatment and day on BW, scrotal circumference (SC), serum testosterone concentration, and serum Zn concentration.

		Variable			
Day	Treatment[1]	BW, kg	SC, cm	Testosterone, ng/mL	Zn, µg/mL
0	CON	68.30 ± 2.17	33.79 ± 0.62	4.40 ± 0.67	0.54 ± 0.02
	ZnAA	68.80 ± 2.25	33.03 ± 0.67	5.30 ± 0.72	0.53 ± 0.02
	$ZnSO_4$	68.10 ± 2.17	34.61 ± 0.62	3.99 ± 0.67	0.55 ± 0.02
28	CON	80.10 ± 2.22	38.21 ± 0.66	4.30 ± 0.83^{c}	0.49 ± 0.03^{b}
	ZnAA	82.19 ± 2.30	37.82 ± 0.71	4.72 ± 0.90^{b}	0.53 ± 0.03^{ab}
	$ZnSO_4$	80.15 ± 2.22	38.54 ± 0.66	7.56 ± 0.83^{a}	0.58 ± 0.03^{a}
56	CON	89.86 ± 1.98	41.41 ± 0.52	5.69 ± 1.19	0.65 ± 0.02^{b}
	ZnAA	94.75 ± 2.05	40.32 ± 0.56	6.17 ± 1.27	0.71 ± 0.03^{ab}
	$ZnSO_4$	91.56 ± 1.98	41.69 ± 0.52	6.66 ± 1.19	0.76 ± 0.02^{a}
84	CON	96.37 ± 1.80^{b}	41.42 ± 0.50	6.99 ± 1.54	0.84 ± 0.03^{b}
	ZnAA	102.12 ± 1.86^{a}	40.95 ± 0.54	5.95 ± 1.66	0.87 ± 0.03^{ab}
	$ZnSO_4$	96.71 ± 1.80^{b}	40.84 ± 0.50	7.13 ± 1.54	0.96 ± 0.03^{a}

[1]Dietary treatments: 1) control diet without fortified zinc (CON; Table 1); 2) a diet fortified with a Zn amino acid complex (ZnAA, Zinpro Corp); and 3) a diet fortified with $ZnSO_4$.

[a-c]Treatment means within a day with no common superscripts are different ($P < 0.05$).

CHAPTER FIVE

CONCLUSIONS

Results from the serum trace mineral survey in weaned Montana ram lambs provide insight on serum trace mineral concentrations in a sub population of developing ram lambs in addition to the broader concern that 33% of ranches were not providing a mineral supplement. Supplementation of trace minerals had an influence on Se serum concentrations among Montana ram lambs, and is especially warranted along the eastern Rocky Mountain. Selenium and Zn were the two most deficient and marginally deficient minerals across Montana ram lamb populations, and additional supplementation of these trace minerals is recommended. This regional knowledge of serum trace mineral concentrations can provide information for ranches to evaluate current and future mineral supplementation programs, as well as aid private industry in meeting precision formulation for mineral premixes. Variation in trace mineral status is multi-facetted, yet basal dietary concentrations are the basis from which ranch-level supplementation decisions should be generated. Soil geochemistry maps may also help estimate regional differences. Additional considerations include individual intake variation, forage species maturity, season, bioavailability of trace mineral chemical form and mineral antagonists.

Observations from our Zn feeding trial with rams indicated that Zn source and concentration affected ADG, serum Zn concentration, staple length, and tended to increase feed efficiency. However, reproductive characteristics such as scrotal circumference, testosterone production, and semen characteristics seemed to be

unaffected by the current treatments. This could be because of dietary Zn concentrations above recommended levels in all treatments. Results indicate the beneficial effects of supranutritional Zn concentrations beyond basal dietary concentrations. Although Zn retention and metabolic pathways of Zn metabolism were not investigated, results indicate that greater dietary Zn concentrations can enhance nutritional strategies in ram development. These findings might be directly applicable to producers developing white-face type rams for fall ram sales in the mountain west and northern plains regions. Futhermore the results of this may be applicable to other aspects of sheep production, such as replacement ewes and slaughter lambs to increase ADG and wool growth.

CUMULATIVE LITERATURE CITED

Ademi, A., A. Bernhoft, E. Govasmark, H. Bytyqi, T. Sivertsen, and B. R. Singh. 2017. Selenium and other mineral concentrations in feed and sheep's blood in Kosovo. Transl. Anim. Sci. 1:97-107.

Allen, J. G., H. G. Masters, R. L. Peet, K. R. Mullins, R. D. Lewis, S. Z. Skirrow, and J. Fry. 1983. Zinc toxicity in ruminants. J. Comp. Path. 93:363-377

Apgar, J. 1992. Zinc and reproduction: an update. J. Nutr. Biochem. 3:266-278.

Bennets, H., and F. E. Chapman. 1937. Copper deficiency in sheep in Western Australia: A preliminary account of the aetiology of enzootic ataxia of lambs and an anaemia of ewes. Austr. Vet. J. 13:138-149.

Berg, J. M. 1990. Zinc fingers and other metal-binding domains. Elements for interactions between macromolecules. J. Biol. Chem. 265:6513-6516.

Blackmon, D. M., W. J. Miller, and J. D. Morton. 1967. Zinc deficiency in ruminants. Vet. Med. 62:265.

Blanchard, R. K. and R. J. Cousins. 1996. Differential display of intestinal mRNAs regulated by dietary zinc. Proc. Natl. Acad. Sci. USA 93:6863-6868.

Bowman, J. G. P., and B. F. Sowell. 1997. Delivery method and supplement consumption by grazing ruminants: A review. J. Anim. Sci. 75:543-550.

Buck, W. B., and R. M. Sharma. 1969 Copper toxicity in sheep. Iowa State University Veterinarian. 31:1.

Cao, J., P. R. Henry, R. Guo, R. A. Howlwerda, J. P. Toth, R. C. Littell, R. D. Miles, and C. B. Ammerman. 2000. Chemical characteristics and relative bioavailability of supplemental organic zinc sources for poultry and ruminants. J. Anim. Sci. 78:2039-2054.

Colagar, A. H., E. T. Marzony, and M. J. Chaichi. 2009. Zinc levels in seminal plasma are associated with sperm quality in fertile and infertile men. Nutrition Research. 29:82-88.

Dargatz, D. A., and P. F. Ross. 1996. Blood selenium concentrations in cows and heifers on 253 cow-calf operations in 18 states. J. Anim. Sci. 74;2891-2895.

Davies, N. T., H. S. Soliman, W. Corrigall, and A. Flett. 1977. The susceptibility of suckling lambs to zinc toxicity. Br. J. Nutr. 38:153-156.

Ducker, M. J., P. T. Kendall, R. G. Hemingway, and T. H. McClelland. 1981. An evaluation of feedblocks as a means of providing supplementary nutrient to ewes grazing upland/hill pastures. Anim. Prod. 33:51-57.

Ensminer, M. E. 1970. Sheep and Wool Science. Animal Agriculture series. The interstate printers and publishers, inc. Danville, Illinois.

Fairweather, S., and R. F. Hurrel. 1996. Bioavailability of minerals and trace elements. Nutrition Research Reviews. 9:295-324.

Farningham, D. A. H., J. G. Mercer, and C. B. Lawrence. 1993. Satiety signal in sheep: Involvement of CCK, propionate, and vagal CCK binding sites. Physiol. Behav. 54:437.

Gabbedy, B. 1971. Effect of selenium on wool production, body weight and mortality of young sheep in Western Australia. Austr. Vet. J. 47:318-322.

Garg, A. K., Vishal Mudgal, and R. S. Dass. 2008. Effect of organic zinc supplementation on growth, nutrient utilization and mineral profile in lambs. Anim. Feed Sci. Technol. 144:82-96.

Geary, T. W., W. L. Kelly, D. S. Spickard, C. K. Larson, E. E. Grings, and R. P. Ansotegui. 2016. Effect of supplemental trace mineral level and form on peripubertal bulls. Ani. Repro. Sci. 168:1-9.

Gibbons, R. A., S. N. Dixon, K. Hallis, A. M. Russell, B. F. Sansom, and H. W. Symonds. 1976. Manganese metabolism in cows and goats. 1976. Biochim. Biophys. Acta. 444:1-10.

Grace, N. D., and J. Lee. Effect of Co, Cu, Fe, Mn, Mo, Se, and Zn supplementation on the elemental content of soft tissues and bone in sheep grazing ryegrass/whit clover pasture. New Zealand Journal of Agricultural Science. 33:635-647.

Grings, E. E., M. R. Haferkamp, R. K. Heitschmidt, and M. G. Karl. 1996. Mineral dynamics in forages of the Northern Great Plains. J. Range Manage. 49:234-240. doi:10.2307/4002884.

Hafez, E. S. E., and B. hafez, editors. 2000. Reproduction in farm animals. 7th ed. Lippincott Williams and Wilkins, Philadelphia, PA. doi:10.1002/9781119265306

Hatfield, P. G., C. K. Swenson, R. W. Kott, R. P. Ansotegui, N. J. Roth, and B. L. Robinson. 2001. Zinc and copper status in ewes supplemented with sulfate- and amino acid-complexed forms of zinc and copper. J. Anim. Sci. 79:261-266.

Herdt, T. H., and B. Hoff. 2011. The use of blood analysis to evaluate trace mineral status in ruminant livestock. Vet. Clin. North Am. Food Anim. Pract. 27:255-283.

Ivancic, J., and W. P. Weiss. 2001. Effect of dietary sulfur and selenium concentrations on selenium balance of lactating Holstein cows. J. Dairy Sci. 84:225-232.

Jones, G. B., and B. F. Tracy. 2013 Evaluating seasonal variation in mineral concentration of cool-season pasture herbage. Grass Forage Sci. 70:94-101.

Judson, G. J., and J. D. McFarlane. 1998. Mineral disorders in grazing livestock and the usefulness of soil and plant analysis in the assessment of these disorders. Aust. J. Exp. Agric. 38:707-723.

Keady, T. W. J., J. P. Hanrahan, and S. P. Fagan. 2017. Cobalt supplementation, alone or in combination with vitamin B_{12} and selenium: Effects on lamb performance and mineral status. J. Anim. Sci. 95:379-386.

Kincaid, R. L. 1999. Assessment of trace mineral status of ruminats: A review. Proc. Amer. Soc. Anim. Sci. 77:1-10.

Kincaid, R. L., B. P. Chew, and J. D. Cronrath. 1997. Zinc oxide and amino acids as sources of dietary zinc for calves: Effects on uptake and immunity. J. Dairy Sci. 80:1381-1388.

Kincaid, R. L., and A. S. Hodgson. 1989. Relationship of Selenium concentrations in blood of calves to blood selenium of the dam and supplemental selenium. J. Dairy Sci. 72:259-263.

Kratzer, F. H., and P. Vohra. 1986. Chelates in nutrition. CRC Press.

Kumar, N., R. P. Verma, L. P. Singh, V. P. Varshney, and R. S. Dass. 2006. Effect of different levels and sources of zinc supplementation on quantitative and qualitative semen attributes and serum testosterone level in crossbred cattle (Bos indicus × Bos taurus) bulls. Reprod. Nutr. Dev. 46:663-675.

Livestock Marketing Information Center (LMIC). 2016. U.S. baseline lamb cost of production model. https://d1cqrq366w3ike.cloud-front.net/http/DOCUMENT/SheepUSA/COP%20Project%20%20Summary%20Low.pdf. (Accessed 4 October 2017).

Lobato, J. F. P., G. R. Pearce, and R. G. Beilharz. 1980. Effect of early familiarization with dietary supplements on the subsequent ingestion of molasses-urea blocks by sheep. Appl. Anim. Ethol. 6:149-161.

Martin, G. B., and C. L. White. 1992. Effects of dietary zinc deficiency on gonadotrophin secretion and testicular growth in young male sheep. J. Reprod. Fertil. 96:497-507.

Martin, G. B., C. L. White, C. M. Markey, and M. A. Blackberry. 1994. Effects of dietary zinc deficiency on the reproductive system of young male sheep: testicular growth and the secretion of inhibin and testosterone. Journal of Reproduction and Fertility. 101:87-96.

Mason, J. 1986. Thiomolybdates: Mediators of molybdenum toxicity and enzyme inhibitors. Toxicology 42:99.

Masters, D. G., D. I. Paynter, J. Briegel, S. K. Baker, and D. B. Purser. 1988. Influence of manganese intake on body, wool and testicular growth of young rams and the concentration of manganese and activity of manganese enzymes in tissues. Aust. J. Agric. Res. 39:517-524.

Mathis, C., and J. Sawyer. 2004. New Mexico forage mineral survey. Proc. West. Sec. Amer. Soc. Anim. Sci. 55.

McDonald, J. 1975. Selenium-response unthriftiness of young Merino sheep in central victoria. Austr. Vet. J. 51:433-435.

McDowell. L. R. 1996. Feeding minerals to cattle on pasture. Anim. Feed. Sci. Technol. 60:247-271.

McDowell, L. R. 2017. Mineral Nutrition History, The Early Years. First Edition Design Pubishing, Inc, Sarasota, FL. DOI 10.21899/978-1506904580.

Menzies, P. I., H. Boermans, B. Hoff, T. Durzi, and L. Langs. 2003. Survey of the status of copper, interacting minerals, and vitamin E levels in the livers of sheep in Ontario. Can. Vet. J. 44:898-906.

Mortimer, R. G., D. Dargatz, and L. R. Corah. 1999. Forage analyses from cow/calf herds in 23 states. University of Tennesse.

Mulling, C. 2000. Three-dimensional appearance of bovine epidermal keratinocytes in different stages of differentiation revealed by cell maceration and scanning electron microscope investigation. Folia Morphol. 59:239-246.

NRC. 1985. Nutrient requirements of sheep. 5th ed. Natl. Acad. Press, Washington, DC.

NRC. 2005. Mineral Tolerance of Domestic Animals. Washinton, DC: National Academies Press.

NRC. 2007. Nutrient Requirements of sheep. 7th ed. Natl. Acad. Press, Washington, DC.

NRC. 2016. Nutrient Requirements of beef cattle. 8th ed. Natl. Acad. Press, Washington, DC. Doi:10.17226/19014.

Ott, E. A., W. H. Smith, R. B. Harrington, and W. M. Beeson. 1966. Zinc toxicity in ruminants. I. Effect of high levels of dietary zinc on gains, feed consumption and feed efficiency of lambs. J. Anim. Sci. 25:414-418.

Ott, E. A., W. H. Smith, M. Stob, and W. M. Beeson. 1964. Zinc deficiency syndrome in the young lamb. J. Nutr. 82:41-50.

Page, C. P., I. McGregor, M. L. Van Emon, T. W. Murphy, C. K. Larson, J. G. Berardinelli, and W. C. Stewart. 2017. Effects of zinc source and dietary concentration on zinc status, growth performance, and wool characteristics in developing rams. Proc. West. Sec. Amer. Soc. Anim. Sci. 68.

Paterson, J., C. Swenson, B. Johnson, and R. Ansotequi. 1999. Assessing the role of copper and zinc in the cow-calf production cycle. Proc. Mid-south Ruminant Nutrition Conference.

Petersen, M. K., J. M. Muscha, J. T. Mulliniks, R. C. Waterman, A. J. Roberts, and M. J. Rinella. 2015. Sources of variability in livestock water quality over 5 years in the Northern Great Plains. J. Anim. Sci. 93. doi:10.2527/jas2014-8028.

Pond, W. G., D. C. Church, K. R. Pond, and P. A. Schoknecht. 2005. Basic animal nutrition and feeding. John Wiley & Sons. Hoboken, NJ.

Rauzi, F., L. I. Painter, and A. K. Dobrenz. 1969. Mineral and protein contents of Blue Grama and Western Wheatgrass (contenido de minerals y proteinase n el Navajita Azul (Bouteloua gracilis) y Western Wheatgrass (Agropyron smithii)). J. Range Manage. 1:47-49.

Ragen, D. L., E. E. Nix, R. L. Endecott, P. G. Hatfield, M. K. Petersen, and J. G. P. Bowman. 2015. Individual mineral supplement intake by ewes swath grazing or confinement fed pea-barley forage. Anim. Feed. Sci. Technol. 200:107-111.

Reid, R. L., G. A. Jung, W. L. Stout, and T. S. Ranney. 1987. Effects of varying zinc concentrations on quality of alfalfa for lambs. J. Anim. Sci. 64:1735-1742.

Reis, P. J., and T. Sahlu. 1994. The nutritional control of the growth and properties of mohair and wool fibers: a comparative review. J. Anim. Sci. 72:1899-1907.

Ricketts, M. J., J. Bodner, J. W. Stuth, and D. Tolleson. 2002. Montana rangeland and livestock mineral study. http://www.nrcs.usda.gov/wps/portal/nrcs/detail/mt/technical/landuse/pasture/?cid=nrcs144p2_057072 (Accessed 15 September 2015).

Rojas, L., L. McDowell, R. Cousins, F. Martin, N. Wilkinson, A. B. Johnson, J. Velasquez. 1995. Relative bioavailability of two organic and two inorganic zinc sources fed to sheep. J. Anim. Sci. 73:1202-1207.

Salgueiro, M. J., M. Zubillaga, A. Lysionek, M. I. Sarabia, R. Caro, T. Paoli, A. Hager, R. Weill, and J. Boccio. 2000. Zinc as an essential micronutrient : a review. Nutrition Research 20. 5:747-755.

Sandstrom, B., and B. Lonnerdal. 1989. Promoters and antagonists of zinc absorption. In: Zinc in Human Biology (Mills, C. F., ed.), pp. 57-78, Springer-Verlag, London

Slen, S., A. Demiruren, and A. Smith. 1961. Note on the effects of selenium on wool growth and body gains in sheep. Can. J. Anim. Sci. 41:263-265.

Smith, D. B., W. F. Cannon, L. G. Woodruff, Solano, Federico, J. E. Killburn, and D. L. Fey. 2014. Geochemical and mineralogical data for soils of the conterminous United States. U.S. Geological Survey. doi:10.3133/ofr20141082

Socha, M. T., S. M. Ensley, D. J. Tomlinson, and A. B. Johnson. 2003 Variability of water composition and potential impact on animal performance. In: Proc. Of Intermt. Nutr. Conf., Salt Lake City, UT. Utah State Univ., Logan. P. 85-96.

Spears, J. W. 1989. Zinc methionine for ruminants: Relative bioavailability of zinc in lambs and effects on growth and performance of growing heifers. J. Anim. Sci. 67:835-843.

Spears, J. W. 1996. Organic trace minerals in ruminant nutrition. Anim. Feed Sci. Technol. 58:151-163.

Spears, J. W. 2003. Trace mineral bioavailability in ruminants. J. Nutr. 133:1506S-1509S.

Spears, J. W., and W. P. Weiss. 2008. Role of antioxidants and trace elements in health and immunity of transition dairy cows. Vet. J. 176:70-76.

Stewart, W. C., G. Bobe, W. R. Vorachek, G. J. Pirelli, W. D. Mosher, T. Nichols, R. J. Van Saun, N. E. Forsberg, and J. A. Hall. 2012. Organic and inorganic selenium: II. Transfer efficiency from ewes to lambs. J. Anim. Sci. 90:577-584.

Suttle, N. F. 2010. Mineral nutrition of livestock. 4th ed. CABI International. Cambridge, MA.

Suttle, N. F., and A. C. Field. 1968. Effect of intake of copper, molybdenum, and sulphate on copper metabolism in sheep. 1. Clinical condition and distribution of copper in blood of the pregnant ewes. J. Comparative Pathol. 93:379-389.

Todd, W. R., C. A. Elvehjem, and E. B. Hart. 1933. Zinc in the nutrition of the rat. The Am. J. Physiol. 107:146-156.

Tomlinson, D. J., C. H. Mulling, and T. M. Fakler. 2004. Invited Review: Formation of Keratins in the Bovine Claw: Roles of Hormones, Minerals, and Vitamins in Functional Claw Integrity. J. Dairy Sci. 87:797-809.

Underwood, E. J. 1981. The Mineral Nutrition of Livestock. 2nd ed. Commonw. Agric. Bur., Slough, UK.

Underwood, E. J., and M. Somers. 1969. Sudies of zinc nutrition in sheep. I. The relation of zinc to growth, testicular development, and spermatogenesis in young rams. Aust. J. Agric. Res. 20:889-897.

Underwood, E. J., and N. F. Suttle. 1999. The Mineral Nutrition of Livestock. 3rd ed. CABI International. Cambridge, MA.

USDA-NASS. 2017. U.S. Sheep and Goat Report. http://usda.mannlib.cornell.edu/usda/current/SheeGoat/SheeGoat-01-31-2017.pdf (Accessed 10 March 2016).

Vallee, B. L., and K. H. Falchuk. 1993. The biochemical basis of zinc physiology. Physiol. Rev. 73:79-118.

Wahlen, R., L. Evans, J. Turner, and R. Hearn. 2005. The use of collision/reaction cell ICP-MS for the determination of 18 elements in blood and serum samples. Spectroscopy 20:12.

Ward, M., and G. Lardy. 2009. Beef cattle mineral nutrition.

Wegner, T. N., D. E. Ray, C. D. Lox, and G. H. Stott. 1973. Effect of stress on serum zinc and plasma corticoids in dairy cattle. J. Dairy Sci. 56:748-752.

Wellinghausen, N., and L. Rink. 1998. The significance of zinc for leukocyte biology. J. Leukoc. Biol. 64:571-577.

White, C., Martin, G., Hynd, P., & Chapman, R. 1994. The effect of zinc deficiency on wool growth and skin and wool follicle histology of male Merino lambs. Br. J. Nutr. 71:425-435.

Williams, R. J. P., J. J. R. F. Da Silva. 2002. The involvement of molybdenum in life. Biochem. Biophys. Res. Commun. 292:293-299.