The effects of organic zinc supplementation on production performance and health of high producing dairy cows

by

María J. Oconitrillo Hidalgo

A thesis submitted to the graduate faculty

in partial fulfillment of the requirements for the degree of

MASTER OF SCIENCE

Major: Animal Science

Program of Study Committee:
Ranga Jayasooriya-Arachchige, Major Professor
Lance H. Baumgard
James E. Koltes
Bill Mahanna

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

Iowa State University
Ames, Iowa

2021

DEDICATION

This work is dedicated to all the women in science that have previously and are still paving the way for other girls and women.

TABLE OF CONTENTS

Page

LIST OF FIGURES v

LIST OF TABLES vi

ACKNOWLEDGMENTS vii

ABSTRACT viii

CHAPTER 1. LITERATURE REVIEW 1
- Zinc, a Metal and an Essential Nutrient 1
- Biochemical and Physiological Importance of Zinc in Animals 2
- Role of Zinc in the Immune Response 3
- The Role of Zinc in Maintaining Redox Balance 5
  - Non-Enzymatic Antioxidants 6
  - Enzymatic Antioxidants 7
- Dietary Zinc Sources and Forms 8
- Zinc-Amino Acid Complexes 10
- The Absorption of Zinc 12
- Factors Affecting Zinc Absorption 13
- Distribution of Zinc in the Body 14
- Effects of Zinc Deficiency in Ruminants 15
- Zinc and Udder Health 16
- Organic Zinc Supplements in Modern Dairy Cows 17
- The Effects of Organic vs. Inorganic Zinc in Lactating Dairy Cows: A Meta-Analysis of Controlled Trials 19
  - Literature Search and Data 19
  - Statistical Analysis 21
  - Overall Effect Size and Factors Explaining the Heterogeneity 22
- Literature Cited 25

CHAPTER 2. EFFECTS OF ORGANIC ZINC SUPPLEMENTATION ON IMMUNOGLOBULIN AND ANTIOXIDANT CONCENTRATIONS IN BLOOD, SOMATIC CELL COUNTS, AND MILK PRODUCTION OF HIGH PRODUCING DAIRY COWS
- Abstract 39
- Introduction 40
- Materials and Methods 43
  - Animals and Treatments 43
  - Feeding and Sample Collection 43
  - Analysis of Antioxidant Concentrations in Blood 45
  - Analysis of Zinc Concentration in Milk and Serum 45

Calculations and Statistical Analyses 46
Results and Discussion 46
Feed Intake and Production Performance 47
Zinc Concentrations in Blood Serum and Milk 50
Immunoglobulin and Antioxidant Marker Concentrations in Blood 51
Acknowledgements 54
References 54
Tables and Figures 59

CHAPTER 3. GENERAL CONCLUSION 66
Literature Cited 69

LIST OF FIGURES

Page

Figure 1.1. Weekly mean milk yield (**A**) and dry matter intake (**B**) of cows receiving Zn-Met at 76 mg/kg DM (CTL) and cows receiving Zn-Met at 96 mg/kg DM (+Zn-Met)..... 64

Figure 1.2. The averages at baseline and least squares means and associated standard errors of somatic cell count in milk (SCC, **A**) and Zn concentration in milk (**B**) 35 and 70 d after increasing added Zn in the diet from 76 mg/kg DM (CTL) to 96 mg/kg DM using a Zn-methionine chelate (+Zn-Met) 65

LIST OF TABLES

Page

Table 1.1 Zn concentrations (mg/kg of DM) in commonly used feed ingredients (source: www.feedipedia.org)..... 9

Table 1.2. A summary of changes in the performances of dairy cows for replacing inorganic Zn with organic Zn in the diet 18

Table 1.3. A summary of study characteristics 20

Table 1.4. The effects of replacing completely inorganic zinc in dairy cow diet with organic zinc and heterogeneity of those effects 23

Table 1.5. Effect size estimates from mixed-effect models and the heterogeneity explained by the model..... 24

Table 2.1. Ingredient and nutrient composition of the basal TMR1 59

Table 2.2. Ingredients of the mineral mix for the diets 60

Table 2.3. Dry matter intake, milk production, and feed efficiency (milk yield: DMI) for increasing added Zn in the diet from 76 mg/kg DM (CTL) to 96 mg/kg DM using a new Zn-methionine chelate (+Zn-Met) 61

Table 2.4. Hematology parameters of cows 35 and 70 d after increasing added Zn in the diet from 76 mg/kg DM (CTL) to 96 mg/kg DM using a Zn-methionine chelate (+Zn-Met) 62

Table 2.5. Serum Zn, immunoglobulin, and oxidative markers in blood of cows 35 and 70 d after increasing added Zn in the diet from 76 mg/kg DM (CTL) to 96 mg/kg DM using a Zn-methionine chelate (+Zn-Met)..... 63

ACKNOWLEDGMENTS

I would like to express my most sincere gratitude to Dr. Ranga Appuhamy Jayasooriya, my major professor, for his guidance and patience, but primarily for welcoming me and extending me a hand in times of need. I would also like to extend my appreciation to my committee members: Dr. Lance H. Baumgard, Dr. James E. Koltes, and Dr. Bill. Mahanna, for their efforts and contributions to my research and my development as a scientist.

To my fellow graduate students and lab managers: Handagala Wickramasinghe, Nadiia Stepanchenko, Sonia Rodríguez Jiménez, Johana Mayorga, Megan Abeyta, Julie Opgenorth, Brady Goetz, and Samantha Lei, thank you for your support and contribution to this work. You truly are a great community of scientists.

I would also like to extend my gratitude to the staff and students that I had the pleasure to work with during all my time spent at the Iowa State Dairy Farm: Miguel Rangel, Katie England, David Sullivan, Melissa Kosht, Mary Healey, and Charlie. To my hard-working and bold undergraduate students: Jacob Reichert, Shaundra Shepherd, Taylor Flemming, Hallie Mentzer, and Brynnen Gardner. Thank you for making me a better professional.

To my beloved family. My mom, dad, and brother. Thank you for the endless and unconditional support. For the encouragement throughout my decisions and for being my rock in the hard times away from home. A huge thank you to the other side of my support system: Arely Quirós, Paula Mora, María Paula Vargas, Diego Morales, Adriana Benavides, Shaundra Shepherd, McKenzie Beals, and Bubbles Beals Weber. Thank you for your friendship and love. I could have never made it without my friends and family. Lastly, I want to thank my abuelito en el cielo, my abuelita Isabel, and Mamá for you are the reason I became a first-generation graduate student.

ABSTRACT

Sustainable dairy production equally demands improvements in milk production as well as the well-being of dairy cows. In that context, zinc (**Zn**), an essential trace mineral, is hypothesized to improve both of those aspects as it serves as an activator of a large number of enzymes and protein factors required for nutrient metabolism, immune defense, and antioxidant systems. In recent decades, supplementation of organic Zn over inorganic Zn in the diet for lactating dairy cows has emerged as a result of the greater bioavailability of organic Zn compared to its inorganic counterparts. Among organic Zn, Zn-amino acid complexes (**Zn-AA**) are widely used in the livestock industry. There have been several studies comparing Zn-AA vs. inorganic Zn (ZnO or $ZnSO_4$) related to dry matter intake (**DMI**) and milk production of dairy cows published in the literature. As a part of the literature review in this thesis, we performed a meta-analysis using data from nine such studies and the *metafor* package of the R statistical software. The mean difference (MD, MD = mean response for organic Zn – mean response for inorganic Zn) was used as the effect size of interest. Random effect models were employed to summarize the overall effect size and estimate the heterogeneity of the effect size between the studies. Mixed-effect models were employed then to explore the factors responsible for the heterogeneity. The random-effect model analysis revealed 0.42 kg/d, 0.57 kg/d, 18 g/d, 0.04%, and 23 g/d increases ($P < 0.10$) in DMI, milk yield, milk protein yield, milk protein %, and milk fat yield for complete replacement of inorganic Zn in the diet with Zn-AA. The added Zn concentration, stage of lactation, and duration of feeding organic Zn vs. inorganic Zn explained 24 to 72% of the heterogeneity of those effect sizes. The effect sizes of somatic cell count in milk (**SCC**) was significantly heterogeneous ($P < 0.01$) but the amount of inorganic Zn (mg/kg DM) being replaced with Zn-AA explained 97% of the heterogeneity of the effect size and had a

positive relationship with it. Based on this relationship and the literature demonstrating positive relationships of SCC with oxidative stress of dairy cows, we hypothesized that Zn-AA supplementation would improve the immune response including immunoglobulin production and antioxidant capacity of high-producing dairy cows. To test this hypothesis, we determined the effects of increasing added Zn concentration in the diet from 76 to 96 mg/kg DM by using a Zn-methionine complex (**Zn-Met**) on SCC and immunoglobulin, antioxidant enzymes, and oxidative stress marker concentrations in the blood beside the effects on DMI and milk production of high producing dairy cows. In this experiment, 12 Holstein dairy cows (67 ± 2.5 days in milk) were randomly assigned to 1) a total mixed ration (**CTL**) containing 76 mg/kg of dry matter (kg DM) of Zn-Met (ZINPRO®, Zinpro Corporation, Eden Prairie, MN, n = 6) and 2) CTL top-dressed with extra 20 mg/kg DM of a new Zn-Met (**+Zn-Met**; Amipro Zn®, Debon Agri-tech Group, Shanghai, China, n = 6) for 70 d. Dry matter intake and milk yield were recorded daily and milk component concentrations and SCC were determined biweekly. Concentrations of Zn in blood and milk, and concentrations of reduced (**GSH**) and oxidized (**GSSG**) glutathione, malondialdehyde (**MDA**), catalase (**CAT**), superoxide dismutase (**SOD**), and immunoglobulins A (**IgA**), G (**IgG**), and M (**IgM**) in the blood were analyzed on d 0, 35, and 70. Treatment effects on all response variables except SCC were analyzed using the MIXED procedure of SAS with fixed effects of treatment, time, parity, treatment × time interaction, covariate effects of baseline measurements, and random effect of the cow. The SCC were analyzed using the GLIMMIX procedure of SAS with the Poisson distribution. Dry matter intake decreased by 1.2 kg/d for +Zn-Met compared to CTL throughout the study. The CTL had a greater milk yield (2.0 kg/d) than +Zn-Met only during the first 35 d, whereas the milk yield of +Zn-Met was 1.25 kg/d greater than CTL during the last 35 d. Milk protein and fat percentages,

and fat yield were not affected by the Zn supplement. There was an interaction between treatment and time on milk protein yield as +Zn-Met had lower and greater milk protein yield than CTL during the first and last 35 d of the study, respectively. Zinc concentration in milk tended to be greater for +Zn-Met relative to CTL (4.48 vs. 4.06 ppm) and the Zn supplement tended to decrease SCC throughout the study. Serum Zn was similar between treatments on d 35 but tended to be higher for +Zn-Met on d 70 (1.06 vs. 0.81 ppm). The +Zn-Met was associated with lower plasma GSH: GSSG on d 35 and lower serum SOD on d 70 relative to CTL. The +Zn-Met did not affect the concentrations of IgA, IgG, IgM, MDA, and CAT. In summary, increasing added Zn content in the diet (76 to 96 mg/kg DM) using Zn-Met improved milk yield and SCC of dairy cows even though DMI decreased and some blood markers indicated increased whole-body oxidative stress. Future experiments designed to address the circadian rhythm of oxidative stress and day-to-day variability of oxidative stress markers in the blood would help understand the true effects of Zn supplementation on antioxidant capacity and oxidative stress of dairy cows. Measuring antioxidant enzyme activity beside the concentrations would also help capture those effects correctly. The Zn-Met product, Aminopro Zn® used in the present study seems to negatively affect the palatability. Further investigations with greater sample sizes is warranted to draw robust conclusions about the effects of Aminopro Zn® on DMI and the production performance of lactating dairy cows.

CHAPTER 1. LITERATURE REVIEW

This review of literature will introduce the importance of Zinc (Zn) in the body related to immunity and antioxidant capacity. It will then discuss the role of dietary Zn in dairy cows related to production, health, and wellbeing. The review will be concluded with a meta-analysis summarizing the effects of organic Zn replacing inorganic Zn in the diet on dry matter intake (DMI) and milk production of dairy cows; all providing the scientific background for my hypothesis, objectives, and methodology of the animal experiment described in Chapter 2.

Zinc, a Metal and an Essential Nutrient

Zinc (Zn) was extracted as a metal for the first time in India during the early seventh century BC (Beckman, 1846). It was not until the eleventh century when China started using Zn. Europeans seem to begin using Zn much later (Hanley, 1933). However, a Swiss physician and alchemist, Paracelsus, gave Zn its modern name using the German word “Zinke”, meaning pointed, which referred to its crystal structure’s sharp edges (Hanley, 1933; Barak and Helmke, 1993). Zinc is a metallic element with the atomic number 30 and an average atomic mass of 65.38 u, and it is the 23rd most abundant element in Earth’s crust (Skalny et al., 2021). Zinc reacts with acids and alkaline solutions to produce Zn (II) cations (Zn^{2+}) and H_2 gas. Several Zn (II) types such as sulfides, sulfates, oxides, carbonates, phosphates, and silicates are found in nature (Barak and Helmke, 1993). The chief Zn (II) is sulfide sphalerite constituting nearly all of the world’s Zn ore. Even though Zn is usually stable in dry air, oxidation, commonly known as corrosion, is accelerated under hot and humid conditions. The corrosion of Zn produces Zn oxide, which forms a light gray film that prevents further corrosion (Dorton, 2005). As a result, Zn is widely used to protect other metals, including iron, from corrosion (Abd El-Lateef et al., 2015).

Even though Zn is required in small quantities and thus called a micronutrient, Zn is essential for the life of both plants and animals. In plants, Zn activates enzymes required to synthesize certain proteins and carbohydrates. Zinc is essential for synthesizing auxins, the hormones stimulating plant growth (Nielsen, 2012). Moreover, Zn plays a critical role in plants' defense mechanisms against pathogens and pests (Cabot et al., 2019). In animals, Zn plays key roles in cell division, nutrient metabolism, immune function, antioxidants system, and reproduction by serving as an important part of the structure of biomolecules or by regulating enzyme activity (Vallee and Falchuk, 1993; Prasad, 1998; Bae et al., 2010; Hara et al., 2017).

Biochemical and Physiological Importance of Zinc in Animals

Zinc is a structural component of approximately 3000 proteins representing nearly 10% of the total proteome in humans (Kambe et al., 2015). In 1940, Zn was established as an enzyme cofactor with the discovery of Zn in the active site of the carbonic anhydrase (Keilin and Mann, 1940). Since then, more than 300 enzymes have been found to contain Zn in their active sites and are collectively known as Zn enzymes. The Zn enzymes are members of multiple enzyme classes including lyases, isomerases, ligases, oxidoreductases, transferases, and hydrolases. More than 50% of Zn enzymes are members of the hydrolases family (Andreini and Bertini, 2012). The mechanisms by which Zn enhances enzyme activity vary depending on the enzyme class (Andreini and Bertini, 2012). The common mechanisms include facilitating the enzyme binding to the substrate and making the substrate receptive to the enzymes (Cooper, 2000). Moreover, Hanas et al. (1983) discovered that Zn was an essential component of a group of transcription factors called Zn finger transcription factors (ZF-TF). Besides regulating gene transcription, ZF-TF are also part of cellular signaling pathways that regulate proliferation and apoptosis of epithelia including the mammary epithelium (McCall et al., 2000; Cassandri et al., 2017).

Mammary glands import twice as much Zn that is transferred through the placenta during gestation in humans (Kelleher et al., 2009). Intracellular concentrations of Zn have been found to stimulate signaling pathways such as JAK2/STAT5, and MAPK that enhance proliferation, differentiation, and survival of mammary epithelial cells (Kelleher et al., 2009). The abundance and activity of Zn transporters in the cell membrane and organelle membranes control the intracellular Zn concentrations and thus the signaling pathways. Interestingly, the expression and localization of Zn transporters in mammary cells respond significantly to pro-inflammatory cytokines such as TNF-α (Kelleher et al., 2009). This suggests an involvement of Zn in developing the immune response by the mammary glands (Ip et al., 1992; Kelleher et al., 2009).

Role of Zinc in the Immune Response

The roles of Zn in the immune defense and anti-inflammatory responses have been studied well (Shankar and Prasad, 1998; Overbeck et al., 2008; Prasad, 2008; Hojyo and Fukada, 2016). The ability of the body to identify and combat harmful micro-organisms relies on innate and adaptive immune cells, both of which utilize Zn for various purposes (John et al., 2010). The innate immune cells that immediately act against pathogens include macrophages, neutrophils, and natural killer (NK) cells. These cells recognize pathogens by detecting pathogen-associated molecular patterns (PAMP) via pattern recognition receptors (PRR). The binding of a PAMP to a PRR results in activation of signaling pathways leading to cytokine production, neutrophil degranulation, phagocytosis, and antigen presentation to adaptive immune cells (Suresh and Mosser, 2013; Maywald et al., 2017). Zinc deficiencies can impair the innate immune response by compromising NK cell activity, and phagocytosis in macrophages and neutrophils (Keen and Gershwin; 1990; Ibs and Rink, 2003; Haase and Rink, 2009). Additionally, NK cell receptors

require Zn to maintain the immune memory and thus prevent the nonspecific pathogen killing (Rajagopalan et al., 1995). In essence, Zn is required for optimal innate immune function.

The adaptive immune system relies upon Zn for ideal potency. Adaptive immune cells produce specific and lasting responses against pathogens and include predominantly two types of lymphocytes: T and B cells (Medzhitov and Janeway, 2000; Turvey and Broide, 2010). The T cells are classified into $CD4^+$ (T helper) and $CD8^+$ (cytotoxic T cells) which undergo maturation in the thymus (Germain, 2002; Hojyo and Fukada, 2016). The $CD4^+$ cells assist B cells to produce antibodies while $CD8^+$ induces cell death by interacting with pathogen-infected cells. Zinc deficiency contributes to thymic atrophy (DePasquale-Jardieu and Fraker, 1979; Quarterman and Humpries, 1979), decreasing mature T-cell counts in rats (Nodera et al., 2001; Fraker, 2005). Zinc deficiency can also impair thymulin activity, a Zn-dependent hormone promoting T-cell maturation in the thymus (Dardenne et al., 1984; Prasad et al., 1988). Moreover, Zn deficiency can compromise B cell maturation in the bone marrow (Fraker et al., 1995; Shankar and Prasad, 1998; Ibs and Rink, 2003), decreasing antibody production, as previously shown in calves (Perryman et al., 1989).

Antibodies, also known as immunoglobulins, are proteins found on the surface of B cells. There are five classes of immunoglobulins namely IgM, IgG, IgA, IgD, and IgE (Hoffman et al., 2016). The IgM initiates inflammatory reactions against pathogens through the complementary pathway which is an important part of the immune system that complements the clearance of pathogenic microbes by antibodies and phagocytic cells (Boes, 2000). The IgG is the most abundant immunoglobulin found in the blood and extracellular fluid. It consists of four subtypes (Panda and Ding, 2015) that bind to many types of pathogens such as bacteria, virus and fungi, and protect the body from infections (Schroeder and Cavacini, 2010). The IgA is the most

abundant antibody isotype found in mucosal immune system and acts as an important first line of defense (Mak and Saunders, 2006). Despite the very low concentrations in blood, IgD plays a significant role in the immune response activating basophils and mast cells (Schroeder and Cavacini, 2010). Production of IgE by B cells is linked to allergic reactions. However, IgE has the shortest half-life and is present at the lowest concentration in the blood among all immunoglobulins (Lawrence et al., 2017). Related primarily to its ability to enhance B cell maturation, Zn deficiency has been shown to decrease immunoglobulin production (Perryman et al., 1989). For instance, dietary supplementation of Zn has been shown to increase serum concentrations of immunoglobulins in Zn-deficient calves (Dresler et al., 2016), and broilers (Bartlett and Smith, 2003).

The Role of Zinc in Maintaining Redox Balance

Maintaining the homeostatic balance of reduction and oxidation molecules becomes a challenge during immune-activating events as inflammation can increase production of free radicals including reactive oxygen species (ROS; Sullivan et al., 1980; Bruno et al., 2007; Prasad et al., 2007; Eide, 2011; Colitti et al., 2019). Free radicals, a component of ROS, are produced during nutrient metabolism (Fang et al., 2002). Therefore, high-producing animals including modern dairy cows with high metabolic demands and an increased likelihood to develop health disorders exhibit a significant production of free radicals. The most critical free radicals are derived from ROS, including superoxide anion (O_2^-), hydroxyl radical (•OH) and hydrogen peroxide (H_2O_2), and reactive nitrogen species (RNS) including nitrogen dioxide (NO_2) and nitric oxide ($NO^{\bullet}$). Mammals have evolved with a controlled production of ROS and RNS as part of the immune defense to kill pathogens. The production of free radicals can damage biomolecules such as proteins, amino acids (AA), lipids, and DNA, causing cell injury or oxidative stress

(Weidinger and Kozlov, 2015). However, mammals have also evolved with an antioxidant system that scavenges free radicals to counterbalance the deleterious effect of ROS and mitigate the oxidative stress (Sies, 1997; Birben et al., 2012). According to Sies (1997), the antioxidants can be classified into two groups, enzymatic and non-enzymatic. They are highly variable in solubility; some antioxidants are soluble in fat, while others are soluble in water. Moreover, some antioxidants are synthesized in the body, but the others must be obtained from the diet (Surai, 2007).

Non-Enzymatic Antioxidants

Vitamin C and E, beta-carotene, glutathione, and selenium (Se) are well known non-enzymatic antioxidants (Burk, 2002; Birben et al., 2012). Vitamin C, also known as ascorbic acid, is a water-soluble vitamin synthesized in plants and mammals, excluding primates and guinea pigs (Drouin et al., 2011). Ascorbic acid is a reducing agent that donates electrons and thus neutralizes free radicals (Kabel, 2014). Vitamin E is a lipid-soluble vitamin that protects the cell membrane from oxidant-induced membrane injury by reacting with lipid radicals generated from lipid peroxidation. α-Tocopherol is the most common and biologically active form of vitamin E in the body (Engin, 2009; Birben et al., 2012). Carotenoids are pigments synthesized by plants and micro-organisms. β-Carotene is a principal antioxidant carotenoid known for inhibiting the initiation of the lipid peroxidation chain reactions (El-Agamey et al., 2004; McDowell et al., 2007). Glutathione is a tripeptide (cysteine, glutamate, and glycine) and a predominant antioxidant present in many cell types. Glutathione works as a substrate or co-substrate of the glutathione peroxidase enzyme (Briviba and Sies, 1994). Under normal cellular conditions, up to 98% of glutathione found in the cells (e.g., nucleus, endoplasmic reticulum, and mitochondria) is present in its reduced form (GSH) which can serve as an antioxidant in several

ways (Masella et al., 2005). Under excessive production of free radicals, GSH contributes to the reduction of H_2O_2 into H_2O and O_2 by glutathione peroxidase. In this reaction, GSH is converted into its oxidized form namely glutathione disulfide (GSSG). The glutathione reductase enzyme will then reduce GSSG back to GSH by using NADPH as an electron donor (Bansal and Bilaspuri, 2008; Birben et al., 2012). Plasma concentrations of GSH and GSSG are often used to describe whole-body glutathione status (Dalle-Donne et al., 2006). In that regard, the ratio between reduced and oxidized forms of glutathione (GSH: GSSG) is determined to assess the degree of oxidative stress with small values indicating elevated oxidative stress (Lykkesfeldt and Svendsen, 2007). One limitation of determining GSH: GSSG is that GSH can be autoxidized during laboratory analysis, leading to incorrectly low ratios (Asensi et al., 1994; Jones, 2006). Selenium is a trace-mineral that is a part of the AA selenocysteine. Therefore, Se is found bound to proteins called selenoproteins in the body. Some selenoproteins are powerful enzymes in the body's antioxidant system including glutathione peroxidase and thioredoxin reductase (Burk, 2002; Zoidis et al., 2018).

Enzymatic Antioxidants

Superoxide dismutase (SOD), catalase (CAT), and glutathione peroxidase (GSH-Px) are major enzymes involved in the body's antioxidant system. The SOD is a family of metal-containing enzymes that catalyzes the conversion of superoxide anion (O_2^-) into less reactive H_2O_2 and O_2. Two isoforms of SOD: mitochondrial manganese SOD (MnSOD) and cytoplasmic copper (Cu), Zn SOD (Cu, Zn SOD) are found in eukaryotic cells (Fridovich, 1995; Davies, 2000). Although SOD can attenuate O_2^-, the resulting H_2O_2 is still a potent ROS. Therefore, further reduction of H_2O_2 is required and those reactions are catalyzed by the other antioxidant enzymes such as GSH-Px and CAT (Davies, 2000). As the name implies, Zn is an integral

component of Cu, Zn SOD and is required to achieve maximum catalytic action of the enzyme (Coudray et al., 1992). Moreover, Zn deficiency can increase the reduced status of Cu in the active sites by turning Cu, Zn SOD into a pro-oxidant (Roberts et al., 2007). Glutathione peroxidase is a selenium-containing antioxidant enzyme which is a tetrameric protein in charge of reducing H_2O_2 and lipid hydroperoxides by utilizing GSH as an electron donor (Higuchi, 2014). According to Tüzün et al. (2002), plasma concentrations of GSH-Px increase as ROS increase, thus increased GSH-Px abundance can also be used as an indicator of oxidative stress. Catalase is a well-known H_2O_2 scavenger composed of 4 identical monomers, and each monomer contains a heme group at the active site. Catalase breaks down two molecules of H_2O_2 into one molecule of O_2 and two molecules of H_2O. In addition, CAT is equipped with a mechanism that prevents it from being inactivated by H_2O_2 (Von Ossowski et al., 1993; Kirkman and Gaetani, 2007). Although CAT does not have a defined Zn requirement, reduced CAT activity in the liver, kidney and, blood have been observed in Zn-deficient mice (Day and Skidmore, 1947). In summary, the antioxidant pool relies on Zn to maintain optimum redox balance and prevent oxidative stress. Evidence related to dairy cows is, however, very limited in the scientific literature even though oxidative stress seems to be inevitable during lactation of high-producing cows (Kelleher et al., 2009; Pal et al., 2010; Dresler et al., 2016).

Dietary Zinc Sources and Forms

Zinc concentration of common feeds for livestock are presented in Table 1.1. Those values were obtained from the Food and Agriculture Organization's Animal Feed Resources Information System (www.feedpedia.org). The Zn concentrations vary widely as reported previously (INRAE et al., 2009). With respect to feeding management in North America, a lactating cow diet containing 55% corn silage, 25% corn grain, 12% soybean meal, and 8% corn

distiller's grain would provide only 32 mg of Zn in each kg of dietary DM; which is lower than the concentration recommended to meet the requirements of the animal (e.g., 60 mg/kg of DM by NRC, 2001). The situation of other trace minerals is the same, and therefore, adding trace mineral supplements (TMS) to the diet is a common practice in animal agriculture (Ledoux and Shannon, 2005).

Table 1.1. Zn concentrations (mg/kg of DM) in commonly used feed ingredients (source: www.feedipedia.org)

Feed ingredient	Average	Range
Alfalfa hay	27	20-30
Triticale	27	15-50
Barley grain	30	24-44
Wheat grain	31	19-46
Citrus pulp, dried	14	6-57
Wheat straw	17	8-28
Cotton seeds	35	29-42
Bermuda grass	44	40-47
Soybean hulls	48	15-90
Soybean meal	57	45-77
Corn silage	27	14-79
Corn distillers grains	62	43-105
Corn gluten feed	63	46-79

Organic and inorganic supplements are two major categories of TMS used in diets for cattle. Inorganic TMS can be defined as minerals that are not bound to carbon and commonly provided to animals in the form of inorganic salts including sulfates, oxides, chlorides, and carbonates. The principal forms of inorganic Zn fed to cattle are Zn oxide (ZnO) and Zn sulfate ($ZnSO_4$). These inorganic salts have been utilized widely over many decades because of their high rumen solubility and low cost (Ammerman and Goodrich, 1983; Nollet et al., 2007). However, some inorganic salts can interact with other minerals in the rumen and form insoluble or indigestible compounds in the rumen, decreasing absorption in the small intestine. For

example, sulfates can interact with Cu and produce insoluble Cu sulfide in the rumen (McDonald et al., 1996; Ward et al., 1996; Spears, 2003; Goff, 2018).

Over the last few decades, organic forms of minerals have become more popular because of their high bioavailability (e.g., high intestinal absorption) and the forms being similar to what found in the body (Spears, 1996). Organic trace minerals (OTM) are identified as a promising way to increase the utilization in the body and decrease the fecal losses that can contribute to heavy metal pollution in soil and water (Wenk, 1998). Feeding OTM to dairy cattle has been shown to improve reproductive performance, hoof health, and milk production (Kellogg et al., 2004; Overton and Yasui, 2014). The most common organic mineral forms utilized in the industry are: 1) metal amino acid complexes (MAAC) produced by complexing a soluble metal salt and an amino acid, 2) metal proteinates which are chelates of a soluble salt and a partially hydrolyzed protein, and 3) metal polysaccharide complexes which are combinations of a soluble salt and a polysaccharide (Spears, 1996; Ledoux and Shannon, 2005).

Zinc-Amino Acid Complexes

The pharmaceutical industry has pioneered manufacturing organic Zn derivatives such as Zn-ricinoleate and Zn-pyrithione which are commonly used for treating skin conditions (Abendrot et al., 2020). Despite their improved bioavailability, those derivatives seem to be less safe for feeding livestock due to their strong antimicrobial properties (Abendrot et al., 2020). Alternatively, Zn complexed with AA has shown satisfactory safety profiles, high bioavailability, and potential antioxidant activities (Schlegel and Windisch, 2006; Abendrot et al., 2020). Chelation is a widely used method to produce Zn-amino acid complexes (Zn-AA). However, it should be understood that not all Zn-AA are chelates as some Zn-AA are made with other methods, such as capping Zn nanoparticles in AA (Mofokeng et al., 2018). The Zn-AA

complexes are developed using several different AA such as aspartate (Zn-Asp), glutamate (Zn-Glu), lysine (Zn-Lys), and methionine (Zn-Met). Those complexes have been shown to improve Zn bioavailability in both humans and animals. Sauer et al. (2017) showed that Zn-Glu, Zn-Lys, and Zn-Met had similar absorption rates in the human intestine *in vitro*. Moreover, they concluded that the advantage of Zn-AA lies in their ability to utilize non-saturable transport mechanisms for absorption in the small intestine. Among Zn-AA, Zn-Met is widely used as a dietary Zn supplement for cattle. The Zn-Met approved to feed animals contain pure Met, methionine sulfate, or a hydroxyl Met analog (Rychen et al., 2017). In early studies testing Zn-Met relative to $ZnSO_4$ or ZnO in ruminants, Greene et al. (1988) did not observe any change on plasma Zn concentration but noted improved carcass quality and marbling in steers. Spears (1989) reported that Zn retention was greater in lambs fed Zn-Met than ZnO, indicating that Zn-Met has greater bioavailability. Research at Washington State University (Pullman, WA) showed a 1.3 kg/d increase in milk production of dairy cows consuming Zn-Met in the 1980s (Kincaid et al., 1984; Kellogg et al., 2004). A meta-analysis by Kellogg et al. (2004) summarized the results of twelve trials where Zn-Met was supplemented to a diet containing only inorganic Zn. The Zn-Met supplementation varied from 180 to 400 mg/d of Zn per head across the studies. Results showed increased milk yields for the Zn-Met supplementation. Additionally, the authors highlighted more noticeable reductions in somatic cell count (SCC) when Zn-Met supplementation was around 400 vs. 180 mg/cow/d. The meta-analysis performed by Kellogg et al (2004) was semi-quantitative. More systematic meta-analysis performed to summarize the effects of organic Zn vs. inorganic Zn on production performance of dairy cows is described later in this chapter.

The Absorption of Zinc

The majority of Zn in the diet is absorbed by transcellular transport in the small intestine of ruminants (Miller, 1970; Cousins, 2010). The ZIP4 is a Zn transporter specifically expressed in the apical membrane of the enterocytes and is primarily responsible for the absorption of Zn from the lumen into the enterocytes (Wang and Zhou, 2010). The abundance of ZIP4 in the apical membrane increases as the diet becomes Zn deficient (Mao et al., 2007). Other Zn transporters such as ZIP11 and ZIP14 are also expressed in the apical membrane of the enterocytes and contribute to Zn absorption to some extent (Cousins, 2010). As a result of the involvement of transporter proteins, the absorption of Zn in the small intestine exhibits saturation kinetics such that the percentage of dietary Zn that is absorbed decreases as Zn concentration in the diet increases (Spears, 2003). The role of energy in Zn absorption is poorly understood. However, there have been some evidence supporting the notion that those saturable processes transporting Zn can be energy-dependent (Moltedo et al., 2000). Once Zn is absorbed into the enterocytes, it is utilized within the enterocytes or moved across the cytoplasm toward the basolateral membrane (Goff, 2018). Certain carrier proteins such as ZnT2 and ZnT4-7 are in charge of moving Zn across vesicles, endosomes, and the Golgi apparatus towards the basolateral membrane. The Zn efflux transporter, ZnT1 residing in the basolateral membrane, is exclusively responsible for transporting Zn from the enterocytes to the interstitial space from where Zn is eventually released to the portal blood (Cousins, 2010). Zinc released to the blood is carried away from the small intestine bound to albumin (Evans and Winter, 1975; NRC, 2001). When intracellular Zn^{2+} concentration is high in the enterocytes, metallothionein, a cellular Zn-binding protein, binds Zn^{2+} and prevents the accumulation of free Zn^{2+} that could interfere with the Zn^{2+} gradient and thus, impair the absorption (Goff, 2018). When animals experience a

depletion of plasma Zn, Zn bound to metallothionein is released to the portal blood for systemic distribution (Krezel and Maret, 2017; Goff, 2018). Moreover, Zn bound to metallothionein is excreted in the feces when the enterocytes die and are sloughed off (NRC, 2001). Hansard et al. (1968) reported that heifers consuming 100 ± 20 mg of Zn/d absorbed only about 22% of the dietary Zn intake. Also considering the findings of Miller and Cragle (1965) demonstrating an average Zn absorption of 12% in mature cows, the NRC (2001) suggests a fixed absorption coefficient of 15% for dairy cattle at any age or production level. Nonetheless, the absorption of Zn can vary markedly depending on multiple factors.

Factors Affecting Zinc Absorption

The requirement is a major factor associated positively with the absorption of Zn in both monogastric and ruminant species (Suttle, 2010). Age is another major factor. Miller and Cragle (1965) report a notable discrepancy in Zn absorption between neonatal calves and calves at 12 months of age (55 and 20%, respectively). The other minerals and organic chelates in the diets can also alter Zn absorption. Copper and Zn are antagonistic to one another at the absorption in the small intestine (Goff, 2018). Copper usually decreases Zn absorption when the dietary Cu: Zn ratio is greater than 50:1, which is, however, uncommon in diets formulated for cattle (Van Campen, 1969). According to the NRC (2001), organic chelators in feeds can affect Zn absorption by forming insoluble complexes with it. Phytates in plants are such chelators that can bind to Zn and decrease the absorption in nonruminant animals. Owing to the phytase enzyme produced by rumen bacteria, phytate-bound Zn is liberated in the rumen and thus available for absorption in the small intestine in ruminants. Overall, the current understanding of the Zn absorption in ruminants (e.g., the efficiencies and mechanisms) is primarily about Zn^{2+} in organic forms such as ZnO and $ZnSO_4$. More research is needed to comprehend the absorption of

Zn-AA becoming increasingly popular. The improved understanding will help determine the true supply of Zn to the animal.

Distribution of Zinc in the Body

Once absorbed, Zn is widely distributed in the body but found at very high concentrations in the liver, kidneys, and pancreas because of high concentrations of metallothionein in those organs (Feaster et al., 1954; Kincaid et al., 1976). Muscle tissue, bone, and skin represent minor storages and Zn in bone is, however, not available for metabolic use (McDowell, 2003). In sheep, wool represents a greater proportion of the total Zn reserve compared to both muscle and bone pools (Ott et al., 1966; Suttle, 2010). The plasma Zn pool represents about 20% of the total blood Zn pool and the majority of Zn in plasma (75-80%) is loosely bound to albumin (Van de Top, 2005). The remaining Zn in plasma is found firmly bound to alpha-2-macroglobulin (Evans and Winter, 1975; Hosnedlová et al., 2007; Handing et al., 2016). The non-plasma Zn in the blood is found predominantly in erythrocytes bound to carbonic anhydrase (85%) and Cu, Zn-SOD (5%) (Pond et al., 2004; Suttle, 2010). Plasma and liver have the highest turnover rates, whereas muscle and bone are often associated with the lowest turnover rates (Miller, 1970). The body maintains Zn homeostasis by adjusting the absorption, tissue deposition, secretion into milk, and fecal excretion of endogenous Zn (Pekas, 1966; Miller, 1969).

Blood serum and liver Zn concentrations of dairy cows vary between 0.7 and 1.3 µg/mL and 100 and 400 mg/kg dry weight, respectively (NRC, 2001). Dairy cows with serum Zn under 0.4 µg/ml, and liver Zn below 100 mg/kg dry weight are considered Zn-deficient (NRC, 2001). However, in evaluating serum and liver concentrations of Zn, the physiological status and environmental factors have to be taken into consideration. For example, environmental stresses can affect the distribution of Zn across tissues and extracellular fluid compartments, independent

of dietary Zn level (Goff and Stabel, 1990). Nonetheless, diets deficient in Zn are usually related to low serum Zn concentrations. Mills et al. (1967) concluded that if serum Zn concentrations drop below 0.4 µg/ml and stay below this value for about a week, the growth can be negatively affected in lambs and calves. In addition, Adema disease also known as hereditary thymic hypoplasia or hereditary parakeratosis has been recognized to impair Zn absorption in cattle. When calves and heifers having this genetic disorder consume Zn-deficient diets, the serum Zn concentration declines markedly leading to a low abundance of lymphocytes and thus a high susceptibility to infections (Perryman et al., 1989; Machen et al., 1996). In dairy cows, milk Zn concentration varies between 3.4 and 5.8 µg/ml with an average of 4.0 µg/ml (Archibald, 1944). The majority of Zn in milk is associated with casein. Milk Zn concentration is greater in primiparous than multiparous cows partly as a result of the increased dilution of milk components by higher milk volumes of multiparous compared to primiparous cows (Miller et al., 1989). Milk Zn does not respond as significantly as plasma Zn to high Zn diets suggesting that the mammary glands discriminate against Zn at higher dietary and plasma concentrations (Miller, 1970). Even though plasma Zn concentration alone is often used to assess the availability of Zn in cattle, it is recommended to consider Zn levels in other tissues such as the liver and hair for better assessment of the Zn status (Spolders et al., 2010).

Effects of Zinc Deficiency in Ruminants

Trace minerals including Zn serve as cofactors of enzymes involved in the metabolism of carbohydrates, protein, and fat. Therefore, trace mineral deficiencies can negatively affect the production efficiencies of farm animals. For instance, Zn deficiencies are associated with reduced average daily gain in cattle (Miller et al., 1964; NRC, 2001). Because Zn is required for the keratinization process (Fuchs, 1995; Smart and Cymbaluk, 1997; Tomlinson et al., 2004), Zn

deficiency in sheep and goats has been studied comprehensively. Zinc deficient goats can develop hair loss, dull hair coats, fissures on feet, and shorter horns (Miller et al., 1964; Neathery et al., 1973). Zinc deficiency in adult sheep has been shown to cause wool shedding, wrinkled skin, and skin lesions of hyperparakeratosis (Ott et al., 1964; Suliman et al., 1988). In the case of lambs, growth retardation and hypogonadism are major manifestations of Zn deficiency (Miller and Miller, 1962; Mills et al., 1967; Spears, 1989). Among the early research in of cattle, Legg and Sears (1960) showed that cows and calves on Zn-deficient pasture developed skin parakeratosis over about 40% of their body and, in some cases, had reduced weight gain. Those authors reported that oral or intravenous Zn supplementation could alleviate parakeratosis and hair loss. Miller and Miller (1962) observed that Holstein dairy calves, after being fed a low-Zn diet for 8.5 weeks, had lower DMI and developed inflamed nose and mouth, swollen feet, and skin parakeratosis. Zinc supplementation to the diet improved those symptoms and increased feed intake. Further research in cattle indicated some negative consequences of Zn deficiency on male reproduction, for instance, small testicle size and increased concentration of abnormal spermatozoa in the semen of bulls (Miller and Miller, 1962; Pitts et al., 1966). Zinc deficiency can also decrease conception rates (Masters and Fels, 1980; Kumar et al., 2006; Kundu et al., 2014) and negatively affect the udder health of dairy cows.

Zinc and Udder Health

The high prevalence of mastitis is a major concern in modern dairy farms as jeopardizes farm profitability, animal wellbeing, and the processing quality of milk (Ruegg, 2017). Moreover, treating mastitis is a major reason for antibiotic use in dairy farms which is disapproved by some consumer communities. Therefore, antibiotics-free management strategies aimed at minimizing mastitis incidences are of great interest (Machado et al., 2013). Any such

strategy is supposed to prevent pathogens from entering the udder, enhance immune cell function in the udder, or both. The teat canal itself represents the first line of defense against pathogens as it has a unique and specialized mechanism to prevents milk leakage and acts as a barrier against pathogen entry as it can regenerate quickly the sealing of the teat canal lining which is mainly made of keratin (Paulrud, 2005; Smolenski, 2018).

The second line of defense against pathogens in the teat canal consists of somatic cells, including epithelial cells and leukocytes. Leukocytes increase during mastitis infection or injury and have phagocytic properties and combat invading organisms (Sordillo et al., 1997; Alhussien and Dang, 2018). Despite the absence of evidence for an effect on leukocytes, Zn is found to enhance the integrity and health of the mammary epithelium (Sordillo et al., 1997; Ho and Ames, 2002; Roohani et al., 2013). Moreover, Zn plays catalytic, structural, and regulatory roles in the synthesis of keratin (Mülling et al., 1999). The catalytic role of Zn is related to its capacity to act as an activator of Zn metalloenzymes that catalyze the differentiation of keratinocytes producing keratin. Zinc also plays a key role in the production of structural proteins during the keratinization and regulates calmodulin, a protein required for differentiation of keratinocytes (Tomlinson et al., 2004). Therefore, Zn supplementation is linked with reduced milk SCC and the incidence of mastitis in dairy cows (Cope et al., 2009; Bakhshizadeh et al., 2019). Some studies have shown lowered SCC of dairy cows when inorganic Zn in the diet was replaced with organic Zn (Sobhanirad et al. 2010; Bakhshizadeh et al., 2019).

Organic Zinc Supplements in Modern Dairy Cows

As mentioned previously, organic trace minerals have become more popular in recent years as a result of research indicating greater bioavailability compared to their inorganic counterparts. Nonetheless, the effects of organic Zn vs. inorganic Zn on the performance of dairy

cows in the literature appear to be mixed and inconclusive in terms of statistical inference. Some findings of studies are presented in Table 1.2.

Table 1.2. A summary of changes in the performances of dairy cows for replacing inorganic Zn with organic Zn in the diet

Study	DMI (kg/d)	Milk yield (kg/d)	Milk fat (%)	Milk protein (%)	SCC ($\times 10^3$ cells/mL)
Ballantine et al. (2002)	+1.3	+1.2*	0.0	0.0	NA
Cope et al. (2009)	+0.9	+2.4	-0.1	-0.1	-2.1
Faulkner et al. (2017)	+0.2	-3.0	+0.2	+0.1	NA
Toni et al. (2007)	NA[1]	+1.0	-0.1	+0.1	-22.5
Yasui et al. (2019)	+0.2	+0.4	-0.1	0.0	-6.6
Sobhanirad et al. (2010)	+0.2	+1.5	-0.1	+0.1	-266.5*
Del Valle et al. (2015)	+0.8	+0.7	+0.2	+0.1	NA
Shaffer et al. (2016)	-0.14*	+0.5	+0.1*	0.0	NA
Bakhshizadeh et al. (2019)	+0.4	+0.8*	0.0	+0.13*	-11.2*

[1] NA = Data unavailable

*Statistically significant ($P < 0.10$)

The majority of studies observed numerical increases in DMI of lactating cows ranging from 0.2 to 1.3 kg/d when inorganic Zn was replaced with organic Zn. On the contrary, Shaffer et al. (2016) reported a statistically significant decrease in DMI. Moreover, the majority reports improvements in milk yield relative to controls but only those in Ballantine et al. (2002) and Bakhshizadeh et al. (2019) were statistically significant. The changes in milk fat and protein concentrations were numerical different and results were mixed. Only Bakhshizadeh et al. (2019) reported a statistically significant increase in milk protein concentration. Of the studies reporting SCC, Sobhanirad et al. (2010) and Bakhshizadeh et al. (2019) observed a significant decrease in SCC. As a part of this literature review, a meta-analysis was performed to compare and combine those results to draw robust conclusions about the effects of organic Zn compared to inorganic Zn in the diet while exploring the factors responsible for the heterogeneity of the effect sizes among individual studies.

The Effects of Organic vs. Inorganic Zinc in Lactating Dairy Cows: A Meta-Analysis of Controlled Trials

Literature Search and Data

Literature searches were performed using the journal of dairy science (https://www.journalofdairyscience.org/) and the science direct (https://www.sciencedirect.com/) databases using keywords: “Zinc”, “dietary Zinc”, “Zinc supplementation”, and “dairy cows” in the title. Once the duplicates were removed, both searches resulted in 93 articles. Suitable articles were chosen by evaluating the abstracts for specific selection criteria. For instance, only the articles published after 2000 were chosen to have a representative sample of modern dairy cows. Moreover, the studies had to be in vivo using lactating dairy cows. The articles had to report treatment means of production performance including milk yield and milk component yields along with corresponding standard errors of the treatment means (SEM) and the sample size (n). Of 93 articles, 73 were excluded as they were not animal trials using lactating dairy cows, did not include production performance parameters or did not report SEM. From the remainder 20 articles, only 9 corresponded to studies testing the effects of complete replacement of inorganic Zn in the diet with organic Zn and were thus included in the present meta-analysis. The excluded studies involved partial replacements of inorganic Zn or focused on exploring the effects of only inorganic Zn supplements.

The data retrieved from those 9 articles included the treatment means of DMI, milk yield, milk protein percentage and yield, milk fat percentage and yield, and SCC and associated n and SEM of control (inorganic Zn) and treatment (organic Zn) groups. Additionally, the form of Zn and concentration of Zn (mg/kg DM) in the diet, duration of the trial, DIM of cows at the beginning the study, and neutral detergent fiber (NDF) and crude protein (CP) concentrations in

the diet were retrieved. Three studies (Cope et al., 2009; Faulkner et al., 2017; Yasui et al., 2019) had compared two concentrations of organic Zn in each study providing two observations (treatment means) of some response variables per study. Therefore, the final dataset included 12 observations for most of the response variables. A summary of the characteristics of studies included in the meta-analysis is given in Table 1.3. The sample size (n) varied widely across the studies so did the dietary Zn content (42 to 542 mg/kg DM) and duration of feeding organic Zn vs. inorganic Zn (21 to 125 d). The cows were in early or mid-lactation and the majority of organic Zn was Zn-AA. Five studies had used a mixture of organic chelates including not only Zn but also other trace minerals such as Cu and Mn. A binary variable vas created to distinguish the chelates containing only Zn (Zn-AA) and those containing Zn plus the other trace minerals (TM-AA).

Table 1.3. A summary of study characteristics

Author(s) and Year	# Treatment means (N)	Sample size (n)	Lactation stage	Zn content (mg/kg DM)	Organic Zn form[1]	Duration (d)[2]
Ballantine et al. (2002)	1	150	Early	155	TM-AA	125
Toni et al. (2007)	1	90	Early	130	TM-AA	100
Cope et al. (2009)	2	11	Early	42 - 63	Zn-AA	49
Sobhanirad et al. (2010)	1	6	Early	542	Zn-AA	63
Del Valle et al. (2015)	1	10	Mid	62	TM-AA	21
Shaffer et al. (2016)	1	12	Mid	115	Zn-AA	42
Faulkner et al. (2017)	2	8	Mid	80	TM-AA	7
Yasui et al. (2019)	2	12	Mid	55 - 76	TM-AA	21
Bakhshizadeh et al. (2019)	1	6	Early	94	Zn-AA	84

[1]TM-AA = a mixture of Zn plus few other trace minerals such as Cu and Mn
[2]Duration of the trial

Statistical Analysis

The mean difference (MD), which is the difference between mean response of the treatment (organic Zn) and that of control (inorganic Zn) was used as the measure of the effect size.

$$MD = Mean(Treatment) - Mean\ (Control)$$

Separate meta-analyses were conducted to quantify the summarized or overall effect size of replacing inorganic Zn with organic Zn in the diet for DMI, milk yield, milk protein percentage and yield, milk fat percentage and yield, and SCC using the *metafor* package in R (version 2.12.2, R Foundation for Statistical Computing, Vienna, Austria) as described in Viechtbauer (2010) and Appuhamy et al. (2013). The overall effect size was estimated using the following random effect model assuming that:

$$y_i = \mu + u_i + e_i$$

where y_i = the observed effect size or MD in i^{th} study; μ = the overall effect size, u_i = the random deviation from μ specific to i^{th} study, which was unknown but estimated using the data, and e_i = the sampling error that was assumed to be known and calculated using SEM and sample size. The sampling error remained fixed during estimation and, hence, served to weight the individual studies. The u_i was assumed to be normally distributed with mean equal to zero and variance equal to τ^2 (between-study variability or heterogeneity).

The random-effect models were extended to mixed-effect models including fixed effects of variables having a potential to explain the heterogeneity. These analyses are also called meta-regression analyses. The mixed-effect models were given by:

$$y_i = \mu + \beta_1 x_{i1} + \cdots\cdots\cdots + \beta_p x_{ip} + u_i + e_i$$

where x_{ij} = the value of j^{th} explanatory variable (j = 1, 2, …, p) of i^{th} study; and β_j = change in the effect size for unit increase in j^{th} explanatory variable and u_i is the remaining or unexplained random deviation from μ in i^{th} study. We used the form of organic Zn (Zn-AA or TM-AA), dietary Zn concentration, duration of feeding organic Zn replacing inorganic Zn, DIM, and CP and NDF concentrations in the diet as explanatory variables. The continuous variables were centered on the mean before included in the model. That way, the intercept of the meta-regression model becomes equivalent to μ. All explanatory variables were first regressed together, and non-significant ($P > 0.10$) variables were then eliminated in a step-wise manner using a backward variable selection procedure to obtain the final model including variables explaining a considerable proportion of the heterogeneity.

Overall Effect Size and Factors Explaining the Heterogeneity

The overall effects of using organic Zn vs. inorganic Zn in the diet on DMI and production performance, and the heterogeneity of those effects are presented in Table 1.4. The overall effects correspond to the average or the middle value of a given explanatory variable, for instance, the median of 94 mg/DM kg for inorganic Zn being replaced with organic Zn. Factors explaining a considerable percentage of the heterogeneity are presented in Table 1.5. Complete replacement of dietary inorganic Zn (94 mg/DM kg) with organic Zn increased DMI of lactating dairy cows by 0.42 kg/d ($P < 0.01$). The change in DMI was negatively related to the Zn concentration in the diet. For each unit (mg/DM kg) increase in the Zn concentration above 94 mg/DM kg, there was a 0.01 kg/d decline in DMI improvement. The dietary Zn concentration explained 72% of the heterogeneity of the effect on DMI (Table 1.5). The replacement of inorganic Zn with organic Zn tended to increase milk yield by 0.57 kg/d ($P = 0.08$). Days in milk

was negatively associated with the improvement of milk yield ($P = 0.02$) suggesting more benefits of the replacement in early vs. mid-lactation.

Table 1.4. The effects of replacing completely inorganic zinc in dairy cow diet with organic zinc and heterogeneity of those effects

			Effect size (μ)		Heterogeneity (τ^2)	
Response variable	N*	Mean (CTL)†	Estimate	*P*-value	Estimate	*P*-value
DMI, kg/d	10	24.40	0.42±0.12	<0.01	0.09	<0.01
Milk yield, kg/d	12	38.19	0.57±0.32	0.08	0.97	<0.01
Milk protein yield, g	12	1155	18.2±9.2	0.04	576	<0.01
Milk protein, %	12	3.04	0.04±0.02	0.02	0.002	<0.01
Milk fat yield, g	12	1356	24.3±13.3	0.07	1344	<0.01
Milk fat, %	12	3.60	0.06±0.04	0.13	0.02	<0.01
SCC, ×1000 cells/mL	5	119	-27±18	0.153	1733	<0.01

*Number of observations (treatment means)
†Average response of the control group

Milk protein percentage and milk protein yield increased for the replacement of inorganic Zn with organic Zn. The increment in milk protein yield was positively related to the duration of feeding organic Zn. For each extra day of feeding organic Zn instead of inorganic Zn, milk protein yield tended to increase 0.32 g/d ($P = 0.09$; Table 1.5). Milk fat percentage increased by 0.06% for organic Zn in the diet and that increment was positively related to DIM, suggesting greater improvements in milk fat concentration for organic Zn in the diet as the lactation progresses. The positive relationship between the milk fat percentage improvement and DIM could be at least partially attributed to the milk yield improvement that decreases with DIM. The Zn concentration in the diet explained 97% of the heterogeneity of the response of SCC for organic Zn replacing inorganic Zn in the diet. Once heterogeneity was explained, replacing inorganic Zn with organic Zn decreased SCC by 16 $\times 10^3$/mL ($P < 0.01$; Table 1.5). It is noteworthy that the type of organic Zn (Zn-AA vs. TM-AA) did not affect the heterogeneity of any response variable indicating the replacement with organic Zn alone or organic Zn plus the

other trace minerals had similar effects on the performances of dairy cows studied in this meta-analysis.

Table 1.5. Effect size estimates from mixed-effect models and the heterogeneity explained by the model

Response	N*	Effect size estimates			% of heterogeneity explained
		Variable†	Estimate	*P*-value	
DMI, kg/d	10	Intercept	0.42±0.12	<0.01	72
		Zn-Conc.	-0.01±0.00	<0.01	
Milk yield, kg/d	12	Intercept	0.48±0.29	0.10	24
		DIM	-0.01±0.00	0.02	
Milk protein yield, g/d	12	Intercept	17.4±8.1	0.03	32
		Duration	0.32±0.19	0.09	
Milk fat, %	12	Intercept	0.065±0.020	0.02	57
		DIM	0.002±0.001	<0.01	
SCC, ×1000 cells/mL	5	Intercept	-16.0±3.2	<0.01	97
		Zn-Conc.	-0.20±0.02	<0.01	

*Number of observations (treatment means)
†Zn-Conc. = added Zn in the diet (mg/kg DM); DIM = days in milk at the beginning of the study; Duration (d) = duration of feeding before measuring the responses

In summary, the present meta-analysis showed that Zn-AA could improve DMI, milk production performance, and udder health of lactating dairy cows compared to inorganic Zn in the diet. However, it is necessary to interpret and extrapolate those effects by taking multiple factors such as the Zn concentration in the diet and the stage of lactation into consideration. Even though Zn has been shown to play important roles in developing the immune response and contribute to the body's antioxidant system as described comprehensively in this literature review, the literature provides little information for such roles of Zn in dairy cows. The experiment described in the next chapter attempts to fill that knowledge gap to some extent.

Literature Cited

Abd El-Lateef, H. M., A. R. El-Sayed, and H. S. Mohran. 2015. Role of Ni content in improvement of corrosion resistance of Zn–Ni alloy in 3.5% NaCl solution. Part I: Polarization and impedance studies. Trans. Nonferrous Met. Soc. China. 25:2807-2816. https://doi.org/10.1016/S1003-6326(15)63906-1.

Abendrot, M., L. Chęcińska, J. Kusz, K. Lisowska, K. Zawadzka, A. Felczak, and U. Kalinowska-Lis. 2020. Zinc (II) complexes with amino acids for potential use in dermatology: synthesis, crystal structures, and antibacterial activity. Molecules. 25:951. https://doi.org/10.3390/molecules25040951.

Alhussien, M. N., and A. K. Dang. 2018. Milk somatic cells, factors influencing their release, future prospects, and practical utility in dairy animals: an overview. Vet. World. 11:562-577. https://doi.org/10.14202/vetworld.2018.562-577.

Ammerman, C. B., and R. D. Goodrich. 1983. Advances in mineral nutrition in ruminants. J. Anim. Sci. 57:519-533. https://doi.org/10.2527/animalsci1983.57Supplement_2519x.

Andreini, C., and I. Bertini. 2012. A bioinformatics view of Zn enzymes. J. Inorg. Biochem. 111:150-156. https://doi.org/10.1016/j.jinorgbio.2011.11.020.

Appuhamy, J. A., A. B. Strathe, S. Jayasundara, C. Wagner-Riddle, J. Dijkstra, J. France, and E. Kebreab. 2013. Anti-methanogenic effects of monensin in dairy and beef cattle: a meta-analysis. J. Dairy Sci. 96:5161-5173. https://doi.org/10.3168/jds.2012-5923.

Archibald, J. G. 1944. Zinc in cows' milk*. J. Dairy Sci. 27:257-261. https://doi.org/10.3168/jds.S0022-0302(44)92592-0.

Asensi, M., J. Sastre, F. V. Pallardo, J. M. Estrela, and J. Viña. 1994. Determination of oxidized glutathione in blood: high-performance liquid chromatography. Methods Enzymol. 234:367-371. https://doi.org/10.1016/0076-6879(94)34106-0.

Bae Y. S., N. D. Hill, Y. Bibi, J. Dreiher, and A. D. Cohen. 2010. Innovative uses for zinc in dermatology. Dermatol. Clin. 28:587-597. https://doi.org/10.1016/j.det.2010.03.006.

Bakhshizadeh, S., F. M. Aghjehgheshlagh, A. Taghizadeh, J. Seifdavati, and B. Navidshad. 2019. Effect of zinc sources on milk yield, milk composition and plasma concentration of metabolites in dairy cows. S. Afr. J. Anim. Sci. 49:884-891. http://dx.doi.org/10.4314/sajas.v49i5.11.

Ballantine H. T., M. T. Socha, D. J. Tomlinson, A.B. Johnson, A. S. Fielding, J. K. Shearer, and S. R. Van Amstel. 2002. Effects of feeding complexed zinc, manganese, copper, and cobalt to late gestation and lactating dairy cows on claw integrity, reproduction, and lactation performance. Prof. Anim. Sci. 18:211-218. https://doi.org/10.15232/S1080-7446(15)31524-2.

Bansal, A. K., and G. S. Bilaspuri. 2008. Oxidative stress alters membrane sulfhydryl status, lipid and phospholipid contents of crossbred cattle bull spermatozoa. Anim. Reprod. Sci. 104:398-404. https://doi.org/10.1016/j.anireprosci.2007.06.017.

Barak, P., and P. A. Helmke. 1993. The chemistry of zinc. Pages 1-13 in Zinc in Soils and Plants. A. D. Robson. Springer Science & Business Media, Berlin, DE.

Bartlett, J. R., and M. O. Smith. 2003. Effects of different levels of zinc on the performance and immunocompetence of broilers under heat stress. Poult. Sci. 82:1580-1588. https://doi.org/10.1093/ps/82.10.1580.

Beckmann, J. 1846. Zinc. Page 31 in A History of Inventions, Discoveries and Origins. Fourth edition. W. Francis and J. W. Griffith. Public Domain.

Birben, E., U. M. Sahiner, C. Sackesen, S. Erzurum, and O. Kalayci. 2012. Oxidative stress and antioxidant defense. World Allergy Organ. J. 5:9-19. https://doi.org/10.1097/WOX.0b013e3182439613.

Boes, M. 2000. Role of natural and immune IgM antibodies in immune responses. Mol. Immunol. 37:1141-1149. https://doi.org/10.1016/s0161-5890(01)00025-6.

Briviba, K., and H. Sies. 1994. Nonenzymatic antioxidants defense systems. Pages 107-128 in Natural Antioxidants in Human Health and Disease. B. Frei. Academic Press Limited, Oval Road, London.

Bruno, R. S., Y. Song, S. W. Leonard, D. J. Mustacich, A. W. Taylor, M. G. Traber, and E. Ho. 2007. Dietary zinc restriction in rats alters antioxidant status and increases plasma F2 isoprostanes. J. Nutr. Biochem. 18:509-518. https://doi.org/10.1016/j.jnutbio.2006.09.001.

Burk, R. F. 2002. Selenium, an antioxidant nutrient. Nutr. Clin. Care. 5:75-79. https://doi.org/10.1046/j.1523-5408.2002.00006.x.

Cabot, C., S. Martos, M. Llugany, B. Gallego, R. Tolrà, and C. Poschenrieder. 2019. A role for zinc in plant defense against pathogens and herbivores. Front. Plant Sci. 10:1-15. https://doi.org/10.3389/fpls.2019.01171.

Cassandri M., A. Smirnov, F. Novelli, C. Pitolli, M. Agostini, M. Malewicz, G. Melino, and G. Raschellà. 2017. Zn-finger proteins in health and disease. Cell Death Disc. 3:1-12. https://doi.org/10.1038/cddiscovery.2017.71.

Colitti, M., B. Stefanon, G. Gabai, M. E. Gelain, and F. Bonsembiante. 2019. Oxidative stress and nutraceuticals in the modulation of the immune function: current knowledge in animals of veterinary interest. Antioxidants (Basel). 8:28. https://doi.org/10.3390/antiox8010028.

Cooper, G. M. 2000. The Central Role of Enzymes as Biological Catalysts. In the Cell, A Molecular Approach. 2nd edition. Oxford University Press, Oxford, ENG.

Cope, C. M., A. M. Mackenzie, D. Wilde, and L. A. Sinclair. 2009. Effects of level and form of dietary zinc on dairy cow performance and health. J. Dairy Sci. 92:2128-2135. https://doi.org/10.3168/jds.2008-1232.

Coudray, C., M. J. Richard, F. Laporte, P. Faure, A. M. Roussel, and A. Favier. 1992. Superoxide dismutase activity and zinc status: a study in animals and man. J. Nutr. Environ. Med. 3:13-26. https://doi.org/10.3109/13590849208997956.

Cousins, R. J. 2010. Gastrointestinal factors influencing Zn absorption and homeostasis. Int. J. Vitam. Nutr. Res. 80:243-248. https://doi.org/10.1024/0300-9831/a000030.

Dalle-Donne, I., R. Rossi, R. Colombo, D. Giustarini, and A. Milzani. 2006. Biomarkers of oxidative damage in human disease. Clin. Chem. 52:601-623. https://doi.org/10.1373/clinchem.2005.061408.

Dardenne, M., W. Savino, S. Wade, D. Kaiserlian, D. Lemonnier, and J. F. Bach. 1984. In vivo and in vitro studies of thymulin in marginally Zn-deficient mice. Eur. J. Immunol. 14:454-458. https://doi.org/10.1002/eji.1830140513.

Davies, K. J. 2000. Oxidative stress, antioxidant defenses, and damage removal, repair, and replacement systems. IUBMB Life. 50:279-289. https://doi.org/10.1080/713803728.

Day, H. G., and B. E. Skidmore. 1947. Some effects of dietary zinc deficiency in the mouse. J. Nutr. 33:27-38. https://doi.org/10.1093/jn/33.1.27.

Del Valle, T. A., E. F. de Jesus, P. G. de Paiva, V. P. Bettero, F. Zanferari, T. S. Acedo, L. F. M. Tamassia, and F. P. Rennó. 2015. Effect of organic sources of minerals on fat-corrected milk yield of dairy cows in confinement. R. Bras. Zootec. 44:103-108. https://doi.org/10.1590/S1806-92902015000300004.

DePasquale-Jardieu, P., and P. J. Fraker. 1979. The role of corticosterone in the loss in immune function in the Zn-deficient A/J mouse. J. Nutr. 109:1847-1855. https://doi.org/10.1093/jn/109.11.1847.

Dorton, K. L. 2005. Effects of trace mineral supplementation, trace mineral source, growth implants, and induced morbidity on performance, trace mineral status, immune function, carcass characteristics, and lipid metabolism in steers. PhD thesis. Department of Animal Science, Colorado State University, Fort Collins.

Dresler, S., J. Illek, and L. Zeman. 2016. Effects of organic zinc supplementation in weaned calves. Acta Vet. Brno. 85:49-54. https://doi.org/10.2754/avb201685010049.

Drouin, G., J. R. Godin, and B. Pagé. 2011. The genetics of vitamin C loss in vertebrates. Curr. Genomics. 12:371-378. https://doi.org/10.2174/138920211796429736.

Eide, D. J. 2011. The oxidative stress of zinc deficiency. Metallomics. 3:1124-1129. https://doi.org/10.1039/c1mt00064k.

El-Agamey, A., G. M. Lowe, D. J. McGarvey, A. Mortensen, D. M. Phillip, T. G. Truscott, and A. J. Young. 2004. Carotenoid radical chemistry and antioxidant/pro-oxidant properties. Arch. Biochem. Biophys. 430:37-48. https://doi.org/10.1016/j.abb.2004.03.007.

Engin, K. N. 2009. Alpha-tocopherol: looking beyond an antioxidant. Mol. Vis. 15:855-860.

Evans, G. W., and T. Winter. 1975. Zn transport by transferrin in rat portal blood plasma. Biochem. Biophys. Res. Commun. 66:1218–1224. https://doi.org/10.1016/0006-291X(75)90488-X.

Fang, Y. Z., S. Yang, and G. Wu. 2002. Free radicals, antioxidants, and nutrition. Nutrition. 18:872-879. https://doi.org/10.1016/S0899-9007(02)00916-4.

Faulkner, M. J., B. A. Wenner, L. M. Solden, and W. P. Weiss. 2017. Source of supplemental dietary copper, zinc, and manganese affects fecal microbial relative abundance in lactating dairy cows. J. Dairy Sci.199:1037-1044. https://doi.org/10.3168/jds.2016-11680.

Feaster, J. P., S. L. Hansard, J. T. McCall, F. H. Skipper, and G. K. Davis. 1954. Absorption and tissue distribution of radiozinc in steers fed high-Zn rations. J. Anim. Sci. 13:781-788. https://doi.org/10.2527/jas1954.134781x.

Fraker, P. J. 2005. Roles for cell death in Zn deficiency. J. Nutr. 135:359-362. https://doi.org/10.1093/jn/135.3.359.

Fraker, P. J., F. Osati-Ashtiani, M. A. Wagner, and L. E. King. 1995. Possible roles for glucocorticoids and apoptosis in the suppression of lymphopoiesis during Zn deficiency: a review. J. AM. Coll. Nutr. 14:11-17. https://doi.org/10.1080/07315724.1995.10718467.

Fridovich, I. 1995. Superoxide radical and superoxide dismutases. Annu. Rev. Biochem. 64:97-112. https://doi.org/10.1146/annurev.bi.64.070195.000525.

Fuchs, E. 1995. Keratins and the skin. Annu. Rev. Cell Dev. Biol. 11:123-154. https://doi.org/10.1146/annurev.cb.11.110195.001011.

Germain, R. N. 2002. T-cell development and the CD4-CD8 lineage decision. Nat. Rev. Immunol. 2:309-322. https://doi.org/10.1038/nri798.

Goff, J. P. 2018. Invited review: Mineral absorption mechanisms, mineral interactions that affect acid-base and antioxidant status, and diet considerations to improve mineral status. J. Dairy Sci. 101:2763-2813. https://doi.org/10.3168/jds.2017-13112.

Goff, J. P., and J. R. Stabel. 1990. Decreased plasma retinol, alpha-tocopherol, and Zn concentration during the periparturient period: effect of milk fever. J. Dairy Sci. 73:3195-3199. https://doi.org/10.3168/jds.S0022-0302(90)79010-8.

Greene, L. W., D. K. Lunt, F. M. Byers, N. K. Chirase, C. E. Richmond, R. E. Knutson, and G. T. Schelling. 1988. Performance and carcass quality of steers supplemented with zinc oxide or zinc methionine. J. Anim. Sci. 66:1818-1823. https://doi.org/10.2527/jas1988.6671818x.

Haase, H., and L. Rink. 2009. The immune system and the impact of zinc during aging. Immun. Ageing. 6:9. https://doi.org/10.1186/1742-4933-6-9.

Hanas, J. S., D. J. Hazuda, D. F. Bogenhagen, F. Y. Wu, and C. W. Wu. 1983. Xenopus transcription factor A requires Zn for binding to the 5 S RNA gene. J. Biol. Chem. 258:14120-14125. https://doi.org/10.1016/S0021-9258(17)43831-2.

Handing, K. B., I. G. Shabalin, O. Kassaar, S. Khazaipoul, C. A. Blindauer, A. J. Stewart, M. Chruszcz, and W. Minor. 2016. Circulatory zinc transport is controlled by distinct interdomain sites on mammalian albumins. Chem Sci. 7:6635-6648. https://doi.org/10.1039/C6SC02267G.

Hanley, H. R. 1933. The story of zinc I. J. Chem. Edu. 10:600-604. https://doi.org/10.1021/ed010p600.

Hansard, S. L., A. S. Mohammed, and J. W. Turner. 1968. Gestation age effects upon maternal-fetal Zn utilization in the bovine. J. Anim. Sci. 27: 1097-1102. https://doi.org/10.2527/jas1968.2741097x.

Hara, T., T. A. Takeda, T. Takagishi, K. Fukue, T. Kambe, and T. Fukada. 2017. Physiological roles of zinc transporters: molecular and genetic importance in zinc homeostasis. J. Physiol. Sci. 67:283-301. https://doi.org/10.1007/s12576-017-0521-4.

Higuchi, M. 2014. Antioxidant properties of wheat bran against oxidative stress. Pages 181-199 in Wheat and Rice in Disease Prevention and Health. R. R. Watson, V. R. Preedy, and S. Zibadi. Academic Press, Cambridge, MA.

Ho, E., and B. N. Ames. 2002. Low intracellular zinc induces oxidative DNA damage, disrupts p53, NFkappa B, and AP1 DNA binding, and affects DNA repair in a rat glioma cell line. Proc. Natl. Acad. Sci. U S A. 99:16770-16775. https://doi.org/10.1073/pnas.222679399.

Hoffman, W., F. G. Lakkis, and G. B. Chalasani. 2016. Cells, antibodies, and more. Clin. J. Am. Soc. Nephrol. 11:137-154. https://doi.org/10.2215/cjn.09430915.

Hojyo, S., and T. Fukada. 2016. Roles of Zn signaling in the immune system. J. Immunol. Res. 2016:1-21. https://doi.org/10.1155/2016/6762343.

Hosnedlová B., J. Trávníček, and M. Šoch. 2007. Current view of the significance of zinc for ruminants: a review. ATS. 40:57-64.

Ibs, K. H., and L. Rink. 2003. Zn-altered immune function. J. Nutr. 133:1452S-1456S. https://doi.org/10.1093/jn/133.5.1452s.

INRAE, CIRAD, AFZ, and FAO. 2009. Feedipedia. Accessed June 28, 2021. https://www.feedipedia.org/.

Ip, M. M., S. F. Shoemaker, and K. M. Darcy. 1992. Regulation of rat mammary epithelial cell proliferation and differentiation by tumor necrosis factor-alpha. Endocrinology. 130:2833-2844. https://doi.org/10.1210/endo.130.5.1572296.

John. E., T. C. Laskow, W. J. Buchser, B. R. Pitt, P. H. Basse, L. H. Butterfield, P. Kalinski, and M. T. Lotze. 2010. Zn in innate and adaptive tumor immunity. J. Transl. Med. 8:118. https://doi.org/10.1186/1479-5876-8-118.

Jones, D. P. 2006. Redefining oxidative stress. Antioxid. Redox Signal. 8:1865-1879. https://doi.org/10.1089/ars.2006.8.1865.

Kabel, A. M. 2014. Free radicals and antioxidants: role of enzymes and nutrition. World J. Nutr. Health. 2:35-38. https://doi.org/10.12691/jnh-2-3-2.

Kambe, T., T. Tsuji, A. Hashimoto, and N. Itsumura. 2015. The physiological, biochemical, and molecular roles of Zn transporters in Zn homeostasis and metabolism. Physiol. Rev. 95:749-784. https://doi.org/10.1152/physrev.00035.2014.

Keen, C. L., and M. E. Gershwin. 1990. Zn deficiency and immune function. Annu. Rev. Nutr. 10:415-430. https://doi.org/10.1146/annurev.nu.10.070190.002215.

Keilin, D., and T. Mann. 1940. Carbonic anhydrase. Purification and nature of the enzyme. Biochem J. 34:1163-1176. https://doi.org/10.1042/bj0341163.

Kelleher, S. L., Y. A. Seo, and V. Lopez. 2009. Mammary gland zinc metabolism: regulation and dysregulation. Genes. Nutr. 4:83-94. https://doi.org/10.1007/s12263-009-0119-4.

Kellogg, D. W., D. J. Tomlinson, M. T. Socha, and A. B. Johnson. 2004. Review: effects of Zn methionine complex on milk production and somatic cell count of dairy cows: twelve-trial summary. Prof. Anim. Sci. 20:295-301. https://doi.org/10.15232/S1080-7446(15)31318-8.

Kincaid, R. L., A. S. Hodgson, R. E. Riley, Jr., and J. D. Cronrath. 1984. Supplementation of diets for lactating cows with zinc oxide and zinc methionine. J. Dairy Sci. 67:103.

Kincaid, R. L., W. J. Miller, R. P. Gentry, M.W. Neathery, and D.L. Hampton. 1976. Intracellular distribution of Zn and Zn-65 in calves receiving high but nontoxic amounts of Zn. J. Anim. Sci. 59:552-555. https://doi.org/10.3168/jds.s0022-0302(76)84239-7.

Kirkman, H. N., and G. F. Gaetani. 2007. Mammalian catalase: a venerable enzyme with new mysteries. Trends Biochem. Sci. 32:44-50. https://doi.org/10.1016/j.tibs.2006.11.003.

Krezel, A., and W. Maret. 2017. The functions of metamorphic metallothioneins in Zn and copper metabolism. Int. J. Mol. Sci. 18: 1237. https://doi.org/10.3390/ijms18061237.

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 x Bos taurus) bulls. Reprod Nutr Dev. 46:663-675. https://doi.org/10.1051/rnd:2006041.

Kundu, M. S., A. K. De, S. Jeyakumar, J. Sunder, A. Kundu, and T. Sujatha. 2014. Effect of Zn supplementation on reproductive performance of Teressa goat. Vet. World. 7:380-383. https://doi.org/10.14202/vetworld.2014.380-383.

Lawrence, M. G., J. A. Woodfolk, A. J. Schuyler, L. C. Stillman, M. D. Chapman, and T. A. Platts-Mills. 2017. Half-life of IgE in serum and skin: consequences for anti-IgE therapy in patients with allergic disease. J. Allergy Clin. Immunol. 139:422-428.e4. https://doi.org/10.1016/j.jaci.2016.04.056.

Ledoux, D. R., and M. C. Shannon. 2005. Bioavailability and antagonists of trace minerals in ruminant metabolism. Pages 23-37 in Proc. Proceeding of Florida Ruminant Nutrition Symposium. Florida, USA. Citeseer.

Legg, S. P., and L. Sears. 1960. Zn sulphate treatment of parakeratosis in cattle. Nature. 186:1061-1062. https://doi.org/10.1038/1861061a0.

Lykkesfeldt, J., and O. Svendsen, 2007. Oxidants and antioxidants in disease: oxidative stress in farm animals. Vet. J. 173:502-511. https://doi.org/10.1016/j.tvjl.2006.06.005.

Machado, V. S., M. L. Bicalho, R. V. Pereira, L. S. Caixeta, W. A. Knauer, G. Oikonomou, R. O. Gilbert, and R. C. Bicalho. 2013. Effect of an injectable trace mineral supplement containing selenium, copper, zinc, and manganese on the health and production of lactating Holstein cows. Vet. J. 197:451-456. https://doi.org/10.1016/j.tvjl.2013.02.022.

Machen, M., T. Montgomery, R. Holland, E. Braselton, R. Dunstan, G. Brewer, and V. Yuzbasiyan-Gurkan. 1996. Bovine hereditary Zn deficiency: lethal trait A 46. J. Vet. Diagn. Invest. 8:219-227. https://doi.org/10.1177/104063879600800212.

Mak, T. W., and M. E. Saunders. 2006. Chapter 20 - mucosal and cutaneous immunity. Pages 583-609 in The Immune Response: Basic and Clinical Principles. Academic Press, Cambridge, MA.

Mao, X., B. E. Kim, F. Wang, D. J. Eide, and M. J. Petris. 2007. A histidine-rich cluster mediates the ubiquitination and degradation of the human Zn transporter, hZIP4, and protects against Zn cytotoxicity. J. Biol. Chem. 282:6992–7000. https://doi.org/10.1074/jbc.M610552200.

Masella, R., R. Di Benedetto, R. Varì, C. Filesi, and C. Giovannini. 2005. Novel mechanisms of natural antioxidant compounds in biological systems: involvement of glutathione and glutathione-related enzymes. J. Nutr. Biochem. 16:577-586. https://doi.org/10.1016/j.jnutbio.2005.05.013.

Masters, D. G., and H. E. Fels. 1980. Effect of Zn supplementation on the reproductive performance of grazing Merino ewes. Biol. Trace Elem. Res. 2:281-290. https://doi.org/10.1007/BF02783826.

Maywald, M., I. Wessels, and L. Rink. 2017. Zinc signals and immunity. Int. J. Mol. Sci. 18:2222. https://doi.org/10.3390/ijms18102222.

McCall, K. A., C. Huang, and C. A. Fierke. 2000. Function and mechanism of Zn metalloenzymes. J. Nutr. 130:1437S-1446S. https://doi.org/10.1093/jn/130.5.1437S.

McDonald, M., I. Mila, and A. Scalbert. 1996. Precipitation of metal ions by plant polyphenols: optimal conditions and origin of precipitation. J. Agric. Food Chem. 44:599-606. https://doi.org/10.1021/jf950459q.

McDowell, L. R. 2003. Chapter 12 – Zn. Pages 357 – 395 in Minerals in Animal and Human Nutrition. Second edition. Elsevier Science B.V., Amsterdam, NL.

McDowell, L. R., N. Wilkinson, R. Madison, and T. Felix. 2007. Vitamins and minerals functioning as antioxidants with supplementation considerations. Pages 30-31 in Proc. Florida Ruminant Nutrition Symposium; Best Western Gateway Grand: Gainesville, FL, USA. Citeseer.

Medzhitov, R., and C. Janeway Jr. 2000. Innate immune recognition: mechanisms and pathways. Immunol. Rev. 173:89-97. https://doi.org/10.1034/j.1600-065x.2000.917309.x.

Miller, J. K., and R. G. Cragle. 1965. Gastrointestinal sites of absorption and endogenous secretion of zinc in dairy cattle. J. Dairy. Sci. 48:370-373. https://doi.org/10.3168/jds.s0022-0302(65)88231-5.

Miller, J. K., and W. J. Miller. 1962. Experimental Zn deficiency and recovery of calves. J. Nutr. 76:467-474. https://doi.org/10.1093/jn/76.4.467.

Miller, W. J. 1969. Absorption, tissue distribution, endogenous excretion, and homeostatic control of Zn in ruminants. Am. J. Clin. Nutr. 22:1323-1331. https://doi.org/10.1093/ajcn/22.10.1323.

Miller, W. J. 1970. Zn nutrition of cattle: a review. J. Dairy Sci. 53:1123-1135. https://doi.org/10.3168/jds.S0022-0302(70)86355-X.

Miller, W. J., H. E. Amos, R. P. Gentry, D. M. Blackmon, R. M. Durrance, C. T. Crowe, A. S. Fielding, and M. W. Neathery. 1989. Long-term feeding of high zinc sulfate diets to lactating and gestating dairy cows. J Dairy Sci. 72:1499-1508. https://doi.org/10.3168/jds.s0022-0302(89)79260-2.

Miller, W. J., W. J. Pitts, C. M. Clifton, and S. C. Schmittle. 1964. Experimentally produced Zn deficiency in the goat. J. Dairy Sci. 47:556-559. https://doi.org/10.3168/jds.S0022-0302(64)88713-0.

Mills, C. F., A. C. Dalgarno, R. B. Williams, and J. Quarterman. 1967. Zn deficiency and the Zn requirements of calves and lambs. Br. J. Nutr. 21: 751-768. https://doi.org/10.1079/bjn19670076.

Mofokeng, T. P., M. J. Moloto, P. M. Shumbula, P. Nyamukamba, P. K. Mubiayi, S. Takaidza, and L. Marais. 2018. Antimicrobial activity of amino acid-capped zinc and copper sulphide nanoparticles. J. Nanotechnol. 2018:1-9. https://doi.org/10.1155/2018/4902675.

Moltedo, O., C. Verde, A. Capasso, E. Parisi, P. Remondelli, S. Bonatti, X. Alvarez-Hernandez, J. Glass, C. G. Alvino, and Leone. 2000. Zinc transport and metallothionein secretion in the intestinal human cell line Caco-2. J. Biol. Chem. 275:31819-31825. https://doi.org/10.1074/jbc.M002907200.

Mülling, C. K., H. H. Bragulla, S. Reese, K. D. Budras, and W. Steinberg. 1999. How structures in bovine hoof epidermis are influenced by nutritional factors. Anat. Histol. Embryol. 28:103-108. https://doi.org/10.1046/j.1439-0264.1999.00180.x.

National Research Council. 2001. Nutrient Requirements of Dairy Cattle. 7th rev. ed. Natl. Acad. Press, Washington, DC.

Neathery, M. W., W. J. Miller, D. M. Blackmon, F. M. Pate, and R. P. Gentry. 1973. Effects of long-term Zn deficiency on feed utilization, reproductive characteristics, and hair growth in the sexually mature male goat. J. Dairy Sci. 56:98-105. https://doi.org/10.3168/jds.S0022-0302(73)85121-5.

Nielsen, F. H. 2012. History of zinc in agriculture. Adv. Nutr. 3:783-789. https://doi.org/10.3945/an.112.002881.

Nodera, M., H. Yanagisawa, and O. Wada. 2001. Increased apoptosis in a variety of tissues of Zn-deficient rats. Life Sci. 69:1639-1649. https://doi.org/10.1016/s0024-3205(01)01252-8.

Nollet, L., J. D. van der Klis, M. Lensing, and P. Spring. 2007. The effect of replacing inorganic with organic trace minerals in broiler diets on productive performance and mineral excretion. J. Appl. Poult. Res. 16:592-597. https://doi.org/10.3382/japr.2006-00115.

Ott, E. A., W. H. Smith, M. Stob, and W. M. Beeson. 1964. Zn deficiency syndrome in the young lamb. J. Nutr. 82:41-50. https://doi.org/10.1093/jn/82.1.41.

Ott, E. A., W. H. Smith, R. B. Harrington, H. E. Parker, and W. M. Beeson. 1966. Zn toxicity in ruminants. iv. physiological changes in tissues of beef cattle. J. Anim. Sci. 25:432-438. https://doi.org/10.2527/jas1966.252432x.

Overbeck, S., L. Rink, and H. Haase. 2008. Modulating the immune response by oral Zn supplementation: a single approach for multiple diseases. Arch. Immunol. Ther. Exp. (Warsz). 56:15-30. https://doi.org/10.1007/s00005-008-0003-8.

Overton, T. R., and T. Yasui. 2014. Practical applications of trace minerals for dairy cattle. J. Anim. Sci. 92:416-426. https://doi.org/10.2527/jas.2013-7145.

Pal, D. T., N. K. S. Gowda, C. S. Prasad, R. Amarnath, U. Bharadwaj, G. S. Babu, and K. T. Sampath. 2010. Effect of copper- and zinc-methionine supplementation on bioavailability, mineral status and tissue concentrations of copper and zinc in ewes. J. Trace Elem. Med. Biol. 24:89-94. https://doi.org/10.1016/j.jtemb.2009.11.007.

Panda, S., and J. L. Ding. 2015. Natural antibodies bridge innate and adaptive immunity. J. Immunol. 194:13-20. https://doi.org/10.4049/jimmunol.1400844.

Paulrud, C. O. 2005. Basic concepts of the bovine teat canal. Vet. Res. Commun. 29:215-245. https://doi.org/10.1023/B:VERC.0000047496.47571.41.

Pekas, J. C. 1966. Zn 65 metabolism: gastrointestinal secretion by the pig. Am. J. Physiol. 211:407-413. https://doi.org/10.1152/ajplegacy.1966.211.2.407.

Perryman, L. E., D. R. Leach, W. C. Davis, W. D. Mickelsen, S. R. Heller, H. D. Ochs, J. A. Ellis, and E. Brummerstedt. 1989. Lymphocyte alterations in Zn-deficient calves with lethal trait A 46. Vet. Immunol. Immunopathol. 21:239–248. https://doi.org/10.1016/0165-2427(89)90034-2.

Pitts, W. J., W. J. Miller, O. T. Fosgate, J. D. Morton, and C. M. Clifton. 1966. Effect of Zn deficiency and restricted feeding from two to five months of age on reproduction in Holstein bulls. J. Dairy Sci. 49:995-1000. https://doi.org/10.3168/jds.S0022-0302(66)87997-3.

Pond W. G., D. C. Church, K. R. Pond, and P. A. Schoknecht. 2004. Micro- (Trace) Mineral Elements. Pages 192-196 in Basic Animal Nutrition and Feeding. 5th edition. John Wiley & Sons, New Jersey, USA.

Prasad, A. S. 1998. Zinc in human health: An update. J. trace elem. exp. med. 11:63-87. https://doi.org/10.1002/(SICI)1520-670X(1998)11:2/3<63::AID-JTRA2>3.0.CO;2-5.

Prasad, A. S. 2008. Zn in human health: effect of Zn on immune cells. Mol. Med. 14:353-357. https://doi.org/10.2119/2008-00033.Prasad.

Prasad, A. S., F. W. Beck, B. Bao, J. T. Fitzgerald, D. C. Snell, J. D. Steinberg, and L. J. Cardozo. 2007. Zinc supplementation decreases incidence of infections in the elderly: effect of zinc on generation of cytokines and oxidative stress. Am. J. Clin. Nutr. 85:837-844. https://doi.org/10.1093/ajcn/85.3.837.

Prasad, A. S., S. Meftah, J. Abdallah, J. Kaplan, G. J. Brewer, J. F. Bach, and M. Dardenne. 1988. Serum thymulin in human Zn deficiency. J. Clin. Invest. 82:1202-1210. https://doi.org/10.1172/jci113717.

Quarterman, J., and W. R. Humphries. 1979. Effect of Zn deficiency and Zn supplementation on adrenals, plasma steroids and thymus in rats. Life Sci. 24:177-183. https://doi.org/10.1016/0024-3205(79)90128-0.

Rajagopalan, S., C. C. Winter, N. Wagtmann, and E. O. Long. 1995. The Ig-related killer cell inhibitory receptor binds zinc and requires zinc for recognition of HLA-C on target cells. J. Immunol. 155:4143-4146.

Roberts, B. R., J. A. Tainer, E. D. Getzoff, D. A. Malencik, S. R. Anderson, V. C. Bomben, K. R. Meyers, P. A. Karplus, and J. S. Beckman. 2007. Structural characterization of zinc-deficient human superoxide dismutase and implications for ALS. J. Mol. Biol. 373:877-890. https://doi.org/10.1016/j.jmb.2007.07.043.

Roohani, N., R. Hurrell, R. Kelishadi, and R. Schulin. 2013. Zinc and its importance for human health: An integrative review. J. Res. Med. Sci. 18:144-157. http://www.ncbi.nlm.nih.gov/pmc/articles/pmc3724376/.

Ruegg, P. L. 2017. A 100-Year Review: Mastitis detection, management, and prevention. J. Dairy Sci. 100:10381-10397. https://doi.org/10.3168/jds.2017-13023.

Rychen, G., G. Aquilina, G. Azimonti, V. Bampidis, M. L. Bastos, G. Bories, A. Chesson, P. S. Cocconcelli, G. Flachowsky, J. Gropp, B. Kolar, M. Kouba, S. López-Puente, A. M. López, B. Mayo, F. Ramos, M. Saarela, R. E. Villa, R. J. Wallace, P. Wester, A. Pechova, G. López-Gálvez, and A. Mantovani. 2017. Safety and efficacy of zinc chelate of methionine sulfate for all animal species. EFSA J. 15:e04859. https://doi.org/10.2903/j.efsa.2017.4859.

Sauer, A. K., S. Pfaender, S. Hagmeyer, L. Tarana, A. K. Mattes, F. Briel, S. Küry, T. M. Boeckers, and A. M. Grabrucker. 2017. Characterization of zinc amino acid complexes for zinc delivery in vitro using Caco-2 cells and enterocytes from hiPSC. Biometals. 30:643-661. https://doi.org/10.1007/s10534-017-0033-y.

Schlegel, P., and W. Windisch. 2006. Bioavailability of zinc glycinate in comparison with zinc sulphate in the presence of dietary phytate in an animal model with Zn labelled rats. J. Anim. Physiol. Anim. Nutr (Berl). 90:216-22. https://doi.org/10.1111/j.1439-0396.2005.00583.x.

Schroeder, H. W. Jr., and L. Cavacini. 2010. Structure and function of immunoglobulins. J. Allergy Clin. Immunol. 125:S41-S52. https://doi.org/10.1016/j.jaci.2009.09.046.

Shaffer, J., K. Pandalaneni, L. Mamedova, J. DeFrain, J. Amamcharla, and B. Bradford. 2016. Effects of dietary zinc source and level on mammary epithelia and dairy food chemistry. Kans. Agric. Exp. Stn. res. rep. 2:1-6. https://doi.org/10.4148/2378-5977.1329.

Shankar, A. H., and A. S. Prasad. 1998. Zn and immune function: the biological basis of altered resistance to infection. Am. J. Clin. Nutr. 68:447S-463S. https://doi.org/10.1093/ajcn/68.2.447s.

Sies, H. 1997. Oxidative stress: oxidants and antioxidants. Exp. Physiol. 82:291-295. https://doi.org/10.1113/expphysiol.1997.sp004024.

Skalny A. V., M. Aschner, and A. A. Tinkov. 2021. Zinc. Adv. Food Nutr. Res. 96:251-310. https://doi.org/10.1016/bs.afnr.2021.01.003.

Smart, M., and N. F. Cymbaluk. 1997. Role of nutritional supplements in bovine lameness-review of nutritional toxicities. In Lameness in Cattle. Third edition. P. R. Greenough, A.D. Weaver, ed. W.B. Saunders, Philadelphia, USA.

Smolenski, G. A. 2018. The bovine teat canal: Its role in pathogen recognition and defense of the mammary gland. PhD Thesis. The University of Waikato, Hamilton, New Zealand.

Sobhanirad, S., D. Carlson, and R. Bahari Kashani. 2010. Effect of zinc methionine or zinc sulfate supplementation on milk production and composition of milk in lactating dairy cows. Biol. Trace Elem. Res. 136:48-54. https://doi.org/10.1007/s12011-009-8526-3.

Sordillo, L. M., K. Shafer-Weaver, and D. DeRosa. 1997. Immunobiology of the mammary gland. J. Dairy Sci. 80:1851-1865. https://doi.org/10.3168/jds.s0022-0302(97)76121-6.

Spears, J. W. 1989. Zinc methionine for ruminants: relative bioavailability of zinc in lambs and effects of growth and performance of growing heifers. J. Anim. Sci. 67:835-843. https://doi.org/10.2527/jas1989.673835x.

Spears, J. W. 1996. Organic trace minerals in ruminant nutrition. Anim. Feed Sci. Technol. 58:151-163. https://doi.org/10.1016/0377-8401(95)00881-0.

Spears, J. W. 2003. Trace mineral bioavailability in ruminants. J. Nutr. 133:1506S-1509S. https://doi.org/10.1093/jn/133.5.1506s.

Spolders, M., M. Höltershinken, U. Meyer, J. Rehage, and G. Flachowsky. 2010. Assessment of reference values for copper and zinc in blood serum of first and second lactating dairy cows. Vet. Med. Int. 2010:1-8. https://doi.org/10.4061/2010/194656.

Suliman, H. B., A. I. Abdelrahim, A. M. Zakia, and A. M. Shommein. 1988. Zn deficiency in sheep: field cases. Trop. Anim. Health Prod. 20:47-51. https://doi.org/10.1007/BF02239646.

Sullivan, J. F., M. M. Jetton, H. K. Hahn, and R. E. Burch. 1980. Enhanced lipid peroxidation in liver microsomes of zinc-deficient rats. Am. J. Clin. Nutr. 33:51-56. https://doi.org/10.1093/ajcn/33.1.51.

Surai, P. F. 2007. Natural antioxidants in poultry nutrition: new developments. Pages 26-30 in Proc. Proceedings of the 16th European symposium on poultry nutrition. World Poultry Science Association.

Suresh, R., and D. M. Mosser. 2013. Pattern recognition receptors in innate immunity, host defense, and immunopathology. Adv. Physiol. Educ. 37:284-291. https://doi.org/10.1152/advan.00058.2013.

Suttle, N. F. 2010. Zinc. Pages 426-458 in Mineral Nutrition of Livestock. 4th edition. S. Hulbert and K. Hill. CAB International, Wallingford, UK.

Tomlinson, D. J., C. H. Mülling, 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. https://doi.org/10.3168/jds.s0022-0302(04)73223-3.

Toni, F., L. Grigoletto, C. J. Rapp, M. T. Socha, and D. J. Tomlinson. 2007. Effect of replacing dietary inorganic forms of zinc, manganese, and copper with complexed sources on lactation and reproductive performance of dairy cows. Prof. Anim. Sci. 23:409-416. https://doi.org/10.15232/S1080-7446(15)30996-7.

Turvey, S. E., and D. H. Broide. 2010. Innate immunity. J. Allergy Clin. Immunol. 125:S24-32. https://doi.org/10.1016/j.jaci.2009.07.016.

Tüzün, A., A. Erdil, V. Inal, A. Aydin, S. Bağci, Z. Yeşilova, A. Sayal, N. Karaeren, and K. Dağalp. 2002. Oxidative stress and antioxidant capacity in patients with inflammatory bowel disease. Clin. Biochem. 35:569-572. https://doi.org/10.1016/S0009-9120(02)00361-2.

Vallee, B. L., and Falchuk, K. H. 1993. The biochemical basis of zinc physiology. 1:79-118. https://doi.org/10.1152/physrev.1993.73.1.79.

Van Campen, D. R. 1969. Copper interference with the intestinal absorption of Zn-65 by rats. J. Nutr. 97:104-108. https://doi.org/10.1093/jn/97.1.104.

Van de Top, M. A. 2005. Reviews on the mineral provision in ruminants (XII): zinc metabolism and requirements in ruminants. Pages 5-6 in Handleiding mineralenvoorziening rundvee, schapen en geiten. Centraal Veevoederbureau, Lelystad, NL.

Viechtbauer, W. 2010. Conducting meta-analysis in R with the metafor package. J. Stat. Softw. 36:1–48. http://dx.doi.org/10.18637/jss.v036.i03.

Von Ossowski, I., G. Hausner, and P. C. Loewen. 1993. Molecular evolutionary analysis based on the amino acid sequence of catalase. J. Mol. Evol. 37:71-76. https://doi.org/10.1007/BF00170464.

Wang, X., and B. Zhou. 2010. Dietary Zn absorption: a play of zips and znts in the gut. IUBMB Life. 62:176-182. https://doi.org/10.1002/iub.291.

Ward, J. D., J. W. Spears, and E. B. Kegley. 1996. Bioavailability of copper proteinate and copper carbonate relative to copper sulfate in cattle. J. Dairy Sci. 79:127-132. https://doi.org/10.3168/jds.S0022-0302(96)76343-9.

Weidinger, A., and A. V. Kozlov. 2015. Biological activities of reactive oxygen and nitrogen species: oxidative stress versus signal transduction. Biomolecules. 5:472-484. https://doi.org/10.3390/biom5020472.

Wenk, C. 1998. Recent advances in animal feed additives such as metabolic modifiers, antimicrobial agents, probiotics, enzymes and highly available minerals - review -. Asian-Australas. J. Anim. Sci. 13:86-95. https://doi.org/10.5713/ajas.2000.86.

Yasui, T., R. M. Ehrhardt, G. R. Bowman, M. Vázquez-Añon, J. D. Richards, C. A. Atwell, and T. R. Overton. 2019. Effects of trace mineral amount and source on aspects of oxidative metabolism and responses to intramammary lipopolysaccharide challenge in midlactation dairy cows. Animal. 13:1000-1008. https://doi.org/10.1017/S1751731118002525.

Zoidis, E., I. Seremelis, N. Kontopoulos, and G. P. Danezis. 2018. Selenium-dependent antioxidant enzymes: actions and properties of selenoproteins. Antioxidants (Basel). 7:66-91. https://doi.org/10.3390/antiox7050066.

CHAPTER 2. EFFECTS OF ORGANIC ZINC SUPPLEMENTATION ON IMMUNOGLOBULIN AND ANTIOXIDANT CONCENTRATIONS IN BLOOD, SOMATIC CELL COUNTS, AND MILK PRODUCTION OF HIGH PRODUCING DAIRY COWS

M. J. Oconitrillo, H. K. J. P. Wickramasinghe, and J. A. D. R. N. Appuhamy

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

Modified from a manuscript to be submitted to *Journal of Dairy Science*

Abstract

Zinc (**Zn**) supplements have been shown to enhance antibody production and alleviate oxidative stress. The evidences are, however, sparse for dairy cows. Zinc-methionine complexes (**Zn-Met**) provide Zn with enhanced bioavailability. The objective of this study was to examine the effects of increasing added Zn concentration in the diet from 76 to 96 mg/kg DM by using Zn-Met on serum and plasma immunoglobulin and antioxidant concentrations, and somatic cell count in milk (**SCC**) besides the effects on DMI and milk production of high producing dairy cows. Twelve Holstein dairy cows (67 ± 2.5 days in milk) were randomly assigned to 1) a total mixed ration (**CTL**) containing 76 mg/kg DM of Zn-Met (ZINPRO®, Zinpro Corporation, Eden Prairie, MN, n = 6) and 2) CTL top-dressed with extra 20 mg/kg DM of a new Zn-Met (**+Zn-Met**; Amipro Zn®, Debon Agri-tech Group, Shanghai, China, n = 6) for 70 d. Dry matter intake and milk yield were recorded daily and milk component concentrations and SCC were determined biweekly. Concentrations of Zn in blood and milk, and concentrations of reduced (**GSH**) and oxidized (**GSSG**) glutathione, malondialdehyde (**MDA**), catalase (**CAT**), superoxide dismutase (**SOD**), and immunoglobulins A (**IgA**), G (**IgG**), and M (**IgM**) in the blood were analyzed on d 0, 35, and 70. Treatment effects on all response variables except SCC were

analyzed using the MIXED procedure of SAS with fixed effects of treatment, time, parity, treatment × time, covariate effects of baseline measurements, and random effect of the cow. The SCC were analyzed using the GLIMMIX procedure of SAS with the Poisson distribution. Dry matter intake decreased by 1.2 kg/d for +Zn-Met than CTL throughout the study. The CTL had a greater milk yield (2.0 kg/d) than +Zn-Met during the first 35 d, whereas milk yield of +Zn-Met was 1.25 kg/d greater than CTL during the last 35 d. Milk protein and fat percentages, and fat yield were not affected by the Zn supplement. There was a significant interaction between treatment and time on milk protein yield as +Zn-Met had lower and greater milk protein yield than CTL during the first and last 35 d of the study, respectively. Zinc concentration in milk tended to be greater for +Zn-Met relative to CTL (4.48 vs. 4.06 ppm) and the Zn supplement tended to decrease SCC throughout the study. Blood serum Zn was similar between treatments on d 35 but tended to be higher for +Zn-Met on d 70 (1.06 vs. 0.81 ppm). The +Zn-Met was associated with decreased GSH: GSSG in blood plasma on d 35 and decreased blood serum concentrations of IgG and SOD on d 70. In summary, increasing added Zn content in the diet (76 to 96 mg/kg DM) using Zn-Met improved milk yield and SCC even though DMI and plasma IgG decreased and some blood markers, such as GSH: GSSG, indicated a positive association between the Zn supplementation and the degree of whole-body oxidative stress.

Introduction

Zinc (**Zn**) is an essential trace mineral that plays a critical role in regulating enzymes involved in nutrient metabolism and numerous biochemical reactions in the body (Mills et al., 1967; Maret, 2013). The significance of Zn in the innate and adaptive immune responses has also been recognized (Fraker et al., 1977). Zinc enhances NK cell activity and phagocytosis of macrophages and neutrophils (Rink and Gabriel, 2000). Moreover, Zn is required to maintain the

thymus, where T cells undergo maturation (DePasquale-Jardieu and Fraker, 1979; Quarterman and Humpries, 1979). Mature T cells induce the production of antibodies such as immunoglobulin A (**IgA**), G (**IgG**), and M (**IgM**). Zinc deficiency can lead to immunoglobulin deficiency that is associated with increased incidences of mastitis in dairy cows (Nansen, 1972). Zinc can also decrease the rate of intramammary infections of dairy cows by enhancing teat canal keratinization (Capuco et al., 1992; Paulrud, 2005). As a result of the health disorders and high metabolic demands, high-producing dairy cows can experience significant oxidative stress (Abuelo et al., 2015). Zinc is an integral component of the body's antioxidant system that alleviates oxidative stress by neutralizing reactive oxygen species such as superoxide anions (**O_2^-**), and hydrogen peroxide (**H_2O_2**). For instance, Zn increases the catalytic activity of superoxide dismutase (**SOD**) that converts O_2^- into less potent H_2O_2 (Davies, 2000). Moreover, Zn enhances the activity of glutathione peroxidase that catalyzes the oxidation of glutathione (**GSH**) to glutathione disulfide (**GSSG**) which is required to neutralize H_2O_2 into H_2O and O_2 (Bansal and Bilaspuri, 2008; Birben et al., 2012). Nonetheless, the role of Zn in the immunoglobulin production and antioxidative system of dairy cows is yet to be elucidated.

Because of inadequate concentrations and low bioavailability of Zn in regular feed ingredients, adding Zn supplements to the diet is a common practice in animal agriculture (Ledoux and Shannon, 2005). Forms of inorganic Zn including sulfates, oxides, chlorides, and carbonates have been widely used as added Zn sources over several decades. As a result of greater bioavailability and the resemblance to the forms in the body, organic forms of Zn garnered significant interest in the last two decades. By exploiting the ability of Zn^{2+} to create chelates with amino acids (**AA**), Zn-AA complexes (**Zn-AA**) are manufactured and have become the most popular form of organic Zn in several industries. Among Zn-AA, Zn-methionine

complexes (**Zn-Met**) are widely used as added organic Zn in dairy cow diets. The current nutrient requirement models of dairy cows do not account for differences in Zn bioavailability among different Zn sources and offer a general estimate of the Zn requirement. The National Research Council model (NRC, 2001) predicts the added Zn^{2+} concentration at 63 mg/kg of DM for an average cow weighing 650 kg and producing 40 kg of milk/d. This prediction covers only the maintenance and lactation requirements and does not account for potential requirements of the immune response (e.g., plasma Zn concentration). A meta-analysis by Kellogg et al. (2004) highlighted significant improvements in SCC when the added Zn in the diet was increased above the current recommendations. Producers often feed Zn more than what is recommended by the NRC (2001) model as a recently conducted dairy farm survey (Duplessis et al., 2021) reports an average added Zn concentration of 76 mg/kg DM in TMR. Increasing added Zn up to 90 mg/kg DM with Zn-Met improved the growth and plasma haptoglobin in beef cattle fed ractopamine hydrochloride (Genther-Schroeder et al., 2016), and increased DMI and deceased rectal temperature of steers challenged with bovine rhinotracheitis virus (Chirase et al., 1991). Moreover, Zhao et al. (2015) showed that increasing organic Zn in the diet from 50 to 100 mg/kg DM increased serum concentrations of Zn, GSH and SOD in lactating dairy cows. Based on previous research we hypothesized that increasing Zn-Met in the diet above 76 mg/kg DM would improve health, antioxidant capacity, and production performances of high producing dairy cows. The objective of this study was to examine the effects of increasing added Zn concentration in the diet from 76 to 96 mg/kg DM by using Zn-Met on blood serum and plasma immunoglobulin and antioxidant concentrations and milk SCC besides the effects on DMI and milk production of high producing dairy cows.

Materials and Methods

Animals and Treatments

All animal procedures were approved by the Institutional Animal Care and Use Committee at Iowa State University (IACUC-20-104). The study was conducted from August to October 2020 at the Dairy Research and Teaching Farm at Iowa State University. Twelve lactating Holstein dairy cows (628.0 ± 19.0 kg of BW; 67.0 ± 2.5 DIM) were matched by parity and DIM, and randomly assigned to one of two dietary treatments: 1) a total mixed ration (**CTL**) containing 76 mg/kg DM of Zn-Met (ZINPRO®, Zinpro Corporation, Eden Prairie, MN, n = 6) and 2) CTL top-dressed with extra 20 mg/kg DM of a new Zn-Met (**+Zn-Met**; Amipro Zn®, Debon Agri-tech Group, Shanghai, China, n = 6) for 70 d. The +Zn-Met received 20 mg/kg DM more Zn-Met (AmiproZn®, DeBon Agri-Tech Group, Shanghai, China) in the basal TMR. AmiproZn® is standardized to contain 17% of Zn^{2+} in the form of ZnO compared to 18% of Zn^{2+} in the form of $ZnSO_4$ in ZINPRO®. Individual cows in +Zn-Met received 3.0 g/d AmiproZn® providing 516 mg/d of Zn^{2+}, which was equivalent to 20 mg/kg DM of Zn^{2+}. The Zn-Met supplement, AmiproZn® was delivered mixing in 25g of ground corn top-dressed onto the basal TMR. The CTL received 25 g of top-dressed ground corn without the Zn-Met supplement, AmiproZn®. The sample size (n = 6) was determined with a power analysis (power = 0.80 and $\alpha = 0.05$) for SCC [$\sigma = 20 \times 10^3$/mL (Al-Qaisi et al., 2020)], IgG [$\sigma = 1.2$ mg/mL (Burton et al., 1991)], and SOD [$\sigma = 1.1$ U/mL (Cope et al., 2009)].

Feeding and Sample Collection

Animals were housed in a free-stall barn and had access to clean drinking water throughout the study period. Cows were individually fed ad libitum (110% of previous day intake) twice daily (0600 and 1500 h) using the Calan Broadbent Feeding System (American

Calan Inc., Northwood, NH). Cows were milked twice (1100 and 2300 h), and milk yield was recorded daily. Cows were allowed to acclimate to housing and the feeding conditions 6 d prior to the start of the study. The acclimation was followed by a 4 d baseline measurement period where baseline blood, DMI, BW, BCS, and milk production parameters were recorded. Then, feeding the Zn-Met supplement, was begun and continued over the next 70 d. The Zn-Met supplementation was fed only at the morning feeding. Samples of the basal diet were collected six times a week and composited into weekly samples for nutrient analysis (Dairyland Laboratories Inc., Arcadia, WI; Table 2.1). Orts samples were collected six times a week and composited into weekly samples for dry matter content analysis by oven-drying (60^{o}C for 48 h). Milk samples were collected from both milking sessions once every two weeks and stored at 4°C with a preservative (Bronopol tablet; D & F Control Systems Inc., San Ramon, CA) until being analyzed for milk composition. Milk samples were analyzed for true protein, fat, lactose, MUN, and SSC in Dairy Lab Services, Dubuque, IA. Separate milk samples were collected on d 0, 35, and 70, composited proportionate to the milk weights at each session within the day, and kept frozen at -20°C until being analyzed for Zn concentration (described below). Blood was drawn by jugular venipuncture into vacutainer tubes (Becton, Dickinson and Company, Franklin Lakes, NJ) for obtaining plasma and serum at 16:00 h on d 0, 35, and 70. Blood collected for serum was allowed to clot at room temperature for about an hour before centrifugation. After centrifugation at 1,500 × g and 4°C for 15 min, serum and plasma were harvested and stored at -20°C until further analysis. An extra blood sample was obtained into 5 mL vacutainer tube (K2EDTA; Becton, Dickinson and Company, Franklin Lakes, NJ), stored at 4°C, and sent to the Veterinary Diagnostic Laboratory at Iowa State University (Ames, IA) for analysis of the hematology within 24 after blood had been collected.

Analysis of Antioxidant Concentrations in Blood

Plasma concentrations of reduced (**GSH**) and oxidized (**GSSG**) glutathione, malondialdehyde (**MDA**), and serum concentrations of catalase (**CAT**), superoxide dismutase (**SOD**), and immunoglobulins including IgG, IgA, and IgM were analyzed using commercial kits according to the instructions provided by the manufacturer (GSH and GSSG: Arbor Assays, Ann Arbor, MI; MDA: Cayman Chemical, Ann Arbor, MI; CAT: Invitrogen, Carlsbad, CA; SOD: Cayman Chemical, Ann Arbor, MI; IgG, IgA, IgM: Bethyl Laboratories, Montgomery, TX). Each analysis included 36 samples representing 12 cows at d 0, 35, and 70 of the study. Each sample was analyzed in duplicates, and all the samples were analyzed in a single assay (one 96 well plate). The analysis was, however, repeated for a given sample when the coefficient of variation across the duplicates exceeded 10%.

Analysis of Zinc Concentration in Milk and Serum

Milk and the serum samples were analyzed using inductively coupled plasma mass spectrometry (ICP-MS) in the Veterinary Diagnostic Laboratory at Iowa State University (Ames, IA).

Milk. A 0.25 mL portion of each milk sample was transferred to a 15 mL tube, and 0.25 mL of 70% nitric acid was added into it. Samples were digested at 60^{o}C for 1.0 h and then brought up to 5.0 mL using 1.0% nitric acid. Samples were then vortexed, centrifuged, and finally filtered through a 0.45 µm filter disc. Filtered samples were injected into the ICP-MS (PlasmaQuant MS Elite®, Analytik Jena, Germany).

Serum. The samples were diluted at 1:20 by mixing a 0.25 mL portion of each serum sample with 4.75 mL of 1.0% nitric acid in a 15 mL tube. The diluted samples were vortexed and injected into the ICP-MS (PlasmaQuant MS Elite®, Analytik Jena, Germany).

Calculations and Statistical Analyses

Protein, fat, and lactose yields were calculated by multiplying the milk yield by the corresponding component concentration. The gross feed efficiency **(FE**) was calculated taking the ratio between milk yield (kg/d) and DMI (kg/d). Milk yield and milk fat and protein yields were used to calculate energy corrected milk (**ECM**, kg/d) with the following formula (Tyrrell and Reid, 1965):

ECM = [(0.327 × Milk yield, kg/d) + (12.95 × fat yield, kg/d) + (7.65 × protein yield, kg/d)]

The ratio between reduced and oxidized glutathione (**GSH: GSSG**) was calculated to describe the degree of whole-body oxidative stress (Lykkesfeldt and Svendsen, 2007).

Statistical analysis. All the data except SCC were analyzed using the MIXED procedure of SAS (version 9.4, SAS Institute Inc., Cary, NC). The statistical model included fixed effects of treatment (CTL or +Zn-Met), study period (d 0 to 35 or d 35 to 70) or sampling time point (d 35 or d 70), parity of the cows (primiparous or multiparous), treatment × time, and covariate effects of baseline measurements. The random effect was the cow nested in treatment. The same model was applied using PROC GLIMMIX with Poisson distribution for the analysis of SCC. All data are expressed as least squares means and statistical significance of the effects was declared at $P \leq 0.05$. The tendencies were discussed at $0.05 < P < 0.10$.

Results and Discussion

Sustainable dairy production equally demands improvements in milk production as well as the well-being of dairy cows. In that context, trace minerals and vitamins are fundamental for enhancing the immune defense and alleviating stresses besides their role in improving milk production (Rabiee et al., 2010; Overton and Yasui, 2014). Zinc is a trace mineral that performs structural, catalytic, and regulatory functions in the body. Zinc serves an activator of a large

number of enzymes required for nutrient metabolism, antibody production, and neutralizing reactive oxygen species. Improving the bioavailability, and determining the supply for optimum immune function and oxidative metabolism are principal goals of trace mineral nutrition of dairy cattle (Overton and Yasui, 2014). In that regard, Zinc-AA are shown to be associated with greater bioavailability compared to their inorganic counterparts. Zinc-Met has become the most popular form of organic Zn in dairy cow rations. Determining the supply of Zn for optimum immune function and the redox status has been difficult as relevant data is limited for dairy cows. Therefore, DMI and production performance, in the present study, we measured some components of the immune defense and antioxidant system of dairy cows in response to increasing Zn-Met from 76 mg/kg DM, the average concentration in TMR to 96 mg/kg DM. The results are discussed below.

Feed Intake and Production Performance

Milk yield and DMI over the 70 d study period is presented in Figure 2.1. Milk yield, ECM, milk components, DMI, and the feed efficiency (milk yield: DMI) during the first (0 to 35 d) and the second half (35 to 70 d) of the study are presented in Table 2.3. Those periods correspond to 67 to 102, and 102 to 137 DIM, respectively. Dairy cows supplemented with Zn-Met had 1.20 kg/d lower DMI than CTL throughout the study periods, when the difference in baseline (d 0) DMI was taken into consideration ($P < 0.01$, Figure 2.1). In line with the present observation, the meta-analysis discussed in previous chapter showed a decline in the improvements of DMI for replacing inorganic Zn with organic Zn as added Zn concentration in the diet increased. Allahyari et al. (2019) observed significantly increased plasma concentrations of anorexigenic hormones such as leptin and insulin in Holstein cows when added Zn in the diet was increased from 110 to 250 mg/kg DM. In Allahyari et al. (2019), 25% of the added Zn was

organic (Zn-Met). More investigations are needed, however, to determine if those anorexigenic effects stem from the source of Zn, the dose of Zn, or the interaction between those two factors. In the present study, the new Zn-Met product itself can decrease the palatability of cows. In that regard, it is noteworthy that Amipro Zn® in +Zn-Met is different from ZINPRO® in CTL in terms of the Met content (78% in Amipro Zn® vs. 21% in ZINPRO®), the form of Zn^{2+} (ZnO in Amipro Zn® vs. $ZnSO_4$ in ZINPRO®), and the structure (double ring structure of Amipro Zn® vs. single ring structure of ZINPRO®).

The Zn supplementation affected milk yield in a time-dependent manner as indicated by the treatment × time interaction in Figure 2.1 ($P = 0.04$) and Table 2.3 ($P < 0.01$). Milk yield of +Zn-Met was 2.00 kg less than CTL in the first half of the study ($P < 0.01$) but increased and was 1.25 kg greater than CTL in the second half of the study ($P = 0.02$). Lactose yield, the major drive of milk volume was tended to be affected by a similar interaction between treatment and period ($P = 0.07$). The DMI decline being more prominent at the beginning than the end of the study could be at least partly responsible for the significant decrease in milk yield during the first half of the study (Figure 2.1). The second half of the study (35-70) overlaps with DIM (102-137) where milk yield gradually decreases after achieving the peak lactation as a result of decreasing secretory cell numbers in the mammary glands. The increased milk yield for +Zn-Met could be a result of the mammary glands being able to sustain the secretory cell numbers in mid-lactation because Zn can stimulate cellular signaling pathways (e.g., JAK2/STAT5, and MAPK) that enhance the proliferation and survival of mammary epithelial cells (Kelleher et al., 2009). Moreover, Zn can increase or decrease lactose synthesis and thereby regulates milk volume depending on the concentrations of Mn^{2+} in mammary epithelial cells (Permyakov and Berliner, 2000). The time × +Zn-Met interaction on milk and lactose yields could be related to a potential

interaction between Zn^{2+} and Mn^{2+} in the mammary glands that change with the stage of lactation. Milk protein and fat concentrations and milk fat yield were not affected by +Zn-Met ($P > 0.60$). Similar to what was observed for milk and lactose yields, +Zn-Met tended to affect milk protein yield differently in the first and second half of the study ($P = 0.07$). During the first 35 d of experiment, milk protein yield of CTL was 50g greater than that of +Zn-Met, whereas, during the next 35 d, milk protein yield of +Zn-Met was 40g greater than that of CTL. The present result agrees with our meta-analysis from chapter 2, showing improved protein yield response for organic Zn supplements as lactation progresses. Energy corrected milk ($P = 0.55$) or feed efficiency (milk yield: DMI, $P = 0.19$) were not affected by +Zn-Met.

Figure 2.2.A presents SCC of CTL and +Zn-Met that were adjusted for baseline SCC also presented in the same figure. Dairy cows supplemented with Zn-Met tended to have lower SCC than CTL throughout the study ($P = 0.07$). However, the reduction was more prominent during the last 5 weeks of the study (252 vs. 69×10^3 cells/mL, $P = 0.08$) than during the first 5 weeks (457 vs. 251×10^3 cells/mL, $P = 0.30$). Even though SCC may not be as sensitive as bacterial culture in identifying udder infections (Middleton et al., 2004), its relationship with total viable bacterial counts in milk makes SCC an effective udder health indicator (Kaşikçi et al., 2012). Nyman et al. (2016) demonstrated that SCC as low as 74×10^3 cells/mL was related to intramammary infections even though SCC equal to 200×10^3 cells/mL is usually used as the cutoff to determine subclinical mastitis in dairy cows. Somatic cells in milk contain primarily white blood cells and cells sloughed from the mammary epithelium. Zinc can potentially decrease contribution of epithelial cells to SCC as it has been shown to improve the integrity of the mammary epithelium (Cook-Mills and Fraker, 1993; Weng et al., 2018). The insignificant treatment differences in white blood cell counts of the hematology analysis (Table 2.4) also

indicates that the decreased SCC for +Zn-Met could be attributed to a decreased contribution of the epithelial cells to SCC.

Zinc Concentrations in Blood Serum and Milk

Zinc concentrations in blood serum and milk are presented in Table 2.5 and Figure 2.2.B, respectively. Zinc concentration in the milk of +Zn-Met tended to be greater than that of CTL throughout the study (4.48 vs. 4.06 ppm, $P = 0.08$). The milk Zn concentrations across treatments were within the reference ranges in the literature (Parkash and Jenness, 1967; Miller, 1970). Spolders et al. (2010) reports serum Zn concentrations of lactating dairy from several studies. A summary of those data gives a reference range of 0.71 to 1.35 ppm and the serum concentrations of Zn in the present study are within that range. There was a significant interaction between treatment and time for serum Zn concentration ($P = 0.04$, Table 2.5). When measured on d 35, CTL and +Zn-Met had similar ($P = 0.47$) serum Zn concentrations at 0.76 ppm. Zinc absorbed from the gastrointestinal tract and Zn excreted in milk are key determinants of serum Zn in lactating cows. Similar serum concentrations between the groups even though +Zn-Met had greater dietary Zn concentration reflect similar amounts of Zn absorbed in both groups or milk in +Zn-Met drawing a greater amount of Zn from the serum relative to CTL on d 35. The reduced DMI of +Zn-Met can make the absorbed Zn in +Zn-Met similar to CTL. However, the increased milk yield of CTL would draw more Zn from the blood and thus make the serum Zn concentration of CTL still lower than +Zn-Met on d 35. Overall, it appears that the serum Zn concentrations could not be fully explained with the absorbed and milk Zn flows alone. Perhaps, extramammary tissues had significant Zn requirement and the supplemented Zn-Met was more bioavailable for those tissues on d 35.

When measured on d 70, serum Zn concentration of +Zn-Met had increased relative to the concentration at d 35 (1.06 vs. 0.71 ppm, $P < 0.01$) and was greater than that of CTL (1.06 vs. 0.80 ppm, $P = 0.05$). Considering the DMI difference that faded away with time (Figure 2.1.B) and the increased milk production of +Zn-Met toward d 70, the greater serum Zn of +Zn-Met vs. that of CTL on d 70 suggests a decreased Zn requirement by extramammary tissues and thus the Zn supplementation was in excess compared to whole-body Zn requirement as lactation progressed.

Immunoglobulin and Antioxidant Marker Concentrations in Blood

Plasma concentrations of antibodies such as IgA, IgM, and IgG have been shown to decrease in response to Zn deficiency in several animal species (DePasquale-Jardieu and Fraker, 1984, Fraker et al., 1977). Zinc deficiency can impair B cell development and thereby decrease the production of antibodies (Keen and Gershwin, 1990; Ibs and Rink, 2003; Kurosaki et al., 2015). Dietary Zn supplements have been shown to increase the blood immunoglobulin concentrations of calves, broilers, and piglets (Bartlett and Smith, 2003; Dresler et al., 2016; Wang et al., 2020). In the present study, we hypothesized that +Zn-Met would be associated with increased concentrations of immunoglobulin in the blood. The results (Table 2.5), however, disagree with our hypothesis as +Zn-Met did not affect the concentration of any immunoglobulin (IgA, IgG, and IgM) measured in this study ($P = 0.25$). A few studies in the literature report the immunoglobulin responses for Zn supplementation of dairy cows. Chen et al. (2020) showed that supplementation of Zn at 20, 40, or 60 mg/kg DM in the form of Zn-Met did not affect blood concentration of IgA, IgG, or IgM in dairy cows. On the other hand, Chandra et al. (2014) observed greater plasma IgG concentrations in pre and post-partum cows for Zn supplemented at 110 mg/kg DM compared to a 50 mg/kg DM supplement. Perhaps, a greater Zn-Met supplement

than the supplement of the present study could have resulted in improved immunoglobulin responses in high producing dairy cows.

Oxidative stress is a metabolic dysfunction that favors the oxidation of biomolecules such as DNA, protein, and lipids, contributing to the oxidative damage of cells and tissues (Marreiro et al., 2017). The literature implicates Zn plays a role in protecting cells and tissues from oxidative damage in humans (Wilking et al., 2013). Such evidence is sparse for dairy cows even though they can experience significant oxidative stress as a result of high metabolic demands during lactation (Castillo et al., 2006). In the present study, we examined the effect of Zn supplementation on antioxidant capacity and the degree of oxidative stress in dairy cows by evaluating concentrations of antioxidant enzymes including CAT and SOD, the concentration of MDA, a degradation product of lipid peroxidation, and GSH: GSSG the blood. The results (Table 2.5) showed no effect of +Zn-Met on CAT, and MDA concentrations ($P > 0.170$). The serum concentrations of SOD were similar between CTL and +Zn-Met at d 35 ($P = 0.86$) but increased for CTL as of d 70 ($P = 0.05$) while that of +Zn-Met remained unchanged. Consequently, the serum SOD concentration of CTL was greater than +Zn-Met on d 70 ($P = 0.04$). Superoxide dismutase is a family of metal-containing enzymes that catalyzes the conversion of superoxide into less reactive products (Davies, 2000). Zinc is required to achieve maximum catalytic action of SOD. In Pal et al. (2010) both concentration and activity of plasma SOD in ewes increased while a Cu and Zn supplement was fed, suggesting plasma Zn concentration could also give an idea about the activity of SOD. On the other hand, Cope et al. (2009) showed no difference in plasma SOD concentrations among cows fed different doses and sources of added Zn. The reason behind the increased SOD concentration in CTL relative to +Zn-Met toward the end of the present study is unclear to us. The concentration of GSH

decreased ($P = 0.02$) and the concentration of GSSG tended to increase ($P = 0.09$) for +Zn-Met compared to CTL on d 35. Consequently, GSH: GSSG decreased for +Zn-Met relative to CTL ($P < 0.01$) on d 35. Plasma GSH: GSSG is often used to describe the degree of whole-body oxidative stress with small values indicating elevated oxidative stress (Lykkesfeldt and Svendsen, 2007). Therefore, the lower GSH: GSSG on d 35 demonstrate elevated oxidative stress for +Zn-Met, which disagree with our hypothesis. It is noteworthy that Zn has dual action as either an anti-oxidant or a pro-oxidant. It has been shown that both zinc deficiency and zinc surplus can cause cellular oxidative stress (Lee et al., 2018). However, the serum or milk Zn concentrations did not indicate a deficiency or an excess of Zn on d 35.

In summary, the results of the present study highlight that increasing added Zn concentration in the diet (76 to 96 mg/kg DM) using Zn-Met improved milk yield and SCC of high producing dairy cows even though DMI and blood markers indicating whole-body antioxidant capacity decreased. Since oxidative stress has a circadian rhythm (Wilking et al., 2013) and the blood markers of oxidative stress exhibit a high day-to-day variability (Goldfarb et al., 2014) future experiments designed to address those variabilities would help capture the true effects of Zn supplementation on antioxidant capacity and oxidative stress of dairy cows. Measuring antioxidant enzyme activity besides the concentrations would also help capture those effects correctly. The Zn-Met product, Aminopro Zn® used for the Zn supplement in the present study could negatively affect the palatability of cows. Further investigations with greater sample sizes can be warranted to draw robust conclusions about the effects of the Zn-Met product on feed intake and milk production of dairy cows.

Acknowledgements

The authors gratefully acknowledge DeBon Agri-Tech Group (Shanghai, China) for the funding support. The support received from Mrs. Cori Cooper and the staff of Dairy Research and Teaching Farm at Iowa State University is also greatly appreciated.

References

Abuelo, A., J. Hernández, J. L. Benedito, and C. Castillo. 2015. The importance of the oxidative status of dairy cattle in the periparturient period: revisiting antioxidant supplementation. J. Anim. Physiol. Anim. Nutr. (Berl). 99:1003-1016. https://doi.org/10.1111/jpn.12273.

Allahyari, S., M. Chaji, and M. Mamuie. 2019. Investigation changes in production, some blood hormones, and metabolites, serum and colostrum IgG of calves of Holstein cows fed with two levels of zinc supplement in transitional period. J. Appl. Anim. Res. 47:440-448. https://doi.org/10.1080/09712119.2019.1653301.

Al-Qaisi M., E. A. Horst, E. J. Mayorga, B. M. Goetz, M. A. Abeyta, I. Yoon, L. L. Timms, J. A. Appuhamy, and L. H. Baumgard. 2020. Effects of a Saccharomyces cerevisiae fermentation product on heat-stressed dairy cows. J. Dairy Sci. 103:9634-9645. https://doi.org/10.3168/jds.2020-18721.

Bansal, A. K., and G. S. Bilaspuri. 2008. Oxidative stress alters membrane sulfhydryl status, lipid and phospholipid contents of crossbred cattle bull spermatozoa. Anim. Reprod. Sci. 104:398-404. https://doi.org/10.1016/j.anireprosci.2007.06.017.

Bartlett, J. R., and M. O. Smith. 2003. Effects of different levels of zinc on the performance and immunocompetence of broilers under heat stress. Poult. Sci. 82:1580-1588. https://doi.org/10.1093/ps/82.10.1580.

Birben, E., U. M. Sahiner, C. Sackesen, S. Erzurum, and O. Kalayci. 2012. Oxidative stress and antioxidant defense. World Allergy Organ. J. 5:9-19. https://doi.org/10.1097/WOX.0b013e3182439613.

Burton, J. L., B. W. McBride, B. W. Kennedy, J. H. Burton, T. H. Elsasser, and B. Woodward. 1991. Serum immunoglobulin profiles of dairy cows chronically treated with recombinant bovine somatotropin. J. Dairy Sci. 74:1589-1598. https://doi.org/10.3168/jds.S0022-0302(91)78321-5.

Capuco, A.V., S. A. Bright, J. W. Pankey, D. L. Wood, R. H. Miller, and J. Bitman. 1992. Increased susceptibility to intramammary infection following removal of teat canal keratin. J. Dairy Sci. 75:2126-2130. https://doi.org/10.3168/jds.S0022-0302(92)77972-7.

Castillo, C., J. Hernández, I. Valverde, V. Pereira, J. Sotillo, M. L. Alonso, and J. L. Benedito. 2006. Plasma malonaldehyde (MDA) and total antioxidant status (TAS) during lactation in dairy cows. Res. Vet. Sci. 80:133-139. https://doi.org/10.1016/j.rvsc.2005.06.003.

Chandra, G., A. Aggarwal, M. Kumar, A. K. Singh, V. K. Sharma, and R. C. Upadhyay. 2014. Effect of additional vitamin E and zinc supplementation on immunological changes in peripartum Sahiwal cows. J. Anim. Physiol. Anim. Nutr. 98:1166-1176. https://doi.org/10.1111/jpn.12190.

Chen, F., Y. Li, Y. Shen, Y. Guo, X. Zhao, Q. Li, Y. Cao, X. Zhang, Y. Li, Z. Wang, Y. Gao, and J. Li. 2020. Effects of prepartum zinc-methionine supplementation on feed digestibility, rumen fermentation patterns, immunity status, and passive transfer of immunity in dairy cows. J. Dairy Sci. 103:8976-8985. https://doi.org/10.3168/jds.2019-17991.

Chirase, N. K., D. P. Hutcheson, and G. B. Thompson. 1991. Feed intake, rectal temperature, and serum mineral concentrations of feedlot cattle fed zinc oxide or zinc methionine and challenged with infectious bovine rhinotracheitis virus. J. Anim. Sci. 69:4137-4145. https://doi.org/10.2527/1991.69104137x.

Cook-Mills, J. M., and P. J. Fraker. 1993. Functional capacity of the residual lymphocytes from zinc-deficient adult mice. Br. J. Nutr. 69:835-848. https://doi.org/10.1079/BJN19930084.

Cope, C. M., A. M. Mackenzie, D. Wilde, and L. A. Sinclair. 2009. Effects of level and form of dietary zinc on dairy cow performance and health. J. Dairy Sci. 92:2128-2135. https://doi.org/10.3168/jds.2008-1232.

Davies, K. J. 2000. Oxidative stress, antioxidant defenses, and damage removal, repair, and replacement systems. IUBMB Life. 50:279-289. https://doi.org/10.1080/713803728.

DePasquale-Jardieu, P., and P. J. Fraker. 1979. The role of corticosterone in the loss in immune function in the Zn-deficient A/J mouse. J. Nutr. 109:1847-1855. https://doi.org/10.1093/jn/109.11.1847.

DePasquale-Jardieu, P., and P. J. Fraker. 1984. Interference in the development of a secondary immune response in mice by zinc deprivation: persistence of effects. J. Nutr. 114:1762-1769. https://doi.org/10.1093/jn/114.10.1762.

Dresler, S., J. Illek, and L. Zeman. 2016. Effects of organic zinc supplementation in weaned calves. Acta Vet. Brno. 85:49-54. https://doi.org/10.2754/avb201685010049.

Duplessis, M., L. Fadul-Pacheco, D. E. Santschi, and D. Pellerin. 2021. Toward precision feeding regarding minerals: what is the current practice in commercial dairy herds in Québec, Canada?. Animals (Basel). 11:1320. https://doi.org/10.3390/ani11051320

Fraker, P. J., S. M. Haas, and R. W. Luecke. 1977. Effect of zinc deficiency on the immune response of the young adult A/J mouse. J. Nutr. 107:1889-1895. https://doi.org/10.1093/jn/107.10.1889.

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

Goldfarb, A. H, R. S. Garten, J. Waller, and J. D. Labban. 2014. Day to day variability and reliability of blood oxidative stress markers within a four-week period in healthy young men. J Biomark. 2014:248313. https://doi.org/10.1155/2014/248313.

Ibs, K. H., and L. Rink. 2003. Zinc-altered immune function. J. Nutr. 133:1452S-1456S. https://doi.org/10.1093/jn/133.5.1452s.

Kaşikçi, G., O. Çetin, E. B. Bingöl, and M. Can Gündüz. 2012. Relations between electrical conductivity, somatic cell count, California Mastitis Test and some quality parameters in the diagnosis of subclinical mastitis in dairy cows. Turk. J. Vet. Anim. Sci. 36:49-55.

Keen, C. L., and M. E. Gershwin. 1990. Zinc deficiency and immune function. Annu. Rev. Nutr. 10:415-430. https://doi.org/10.1146/annurev.nu.10.070190.002215.

Kelleher, S. L., Y. A. Seo, and V. Lopez. 2009. Mammary gland zinc metabolism: regulation and dysregulation. Genes. Nutr. 4:83-94. https://doi.org/10.1007/s12263-009-0119-4.

Kellogg, D. W., D. J. Tomlinson, M. T. Socha, and A. B. Johnson. 2004. Review: effects of zinc methionine complex on milk production and somatic cell count of dairy cows: twelve-trial summary. Prof. Anim. Sci. 20:295-301. https://doi.org/10.15232/S1080-7446(15)31318-8.

Kurosaki, T., K. Kometani, and W. Ise. 2015. Memory B cells. Nat. Rev. Immunol. 15:149-159. https://doi.org/10.1038/nri3802.

Ledoux, D. R., and M. C. Shannon. 2005. Bioavailability and antagonists of trace minerals in ruminant metabolism. Pages 23-37 in Proc. Proceeding of Florida Ruminant Nutrition Symposium. Florida, USA. Citeseer.

Lee, S. R. 2018. Critical role of zinc as either an antioxidant or a prooxidant in cellular systems. Oxid. Med. Cell. Longev. 2018:1-11. https://doi.org/10.1155/2018/9156285.

Lykkesfeldt, J., and O. Svendsen, 2007. Oxidants and antioxidants in disease: oxidative stress in farm animals. Vet. J. 173:502-511. https://doi.org/10.1016/j.tvjl.2006.06.005.

Maret, W. 2013. Zinc biochemistry: from a single zinc enzyme to a key element of life. Adv. Nutr. 4:82-91. https://doi.org/10.3945/an.112.003038.

Marreiro, D. D., K. J. Cruz, J. B. Morais, J. B. Beserra, J. S. Severo, and A. R. de Oliveira. 2017. Zinc and oxidative stress: current mechanisms. Antioxidants (Basel). 6:24. https://doi.org/10.3390/antiox6020024.

Middleton, J. R., D. Hardin, B. Steevens, R. Randle, and J. W. Tyler. 2004. Use of somatic cell counts and California Mastitis Test results from individual quarter milk samples to detect subclinical intramammary infection in dairy cattle from a herd with a high bulk tank somatic cell count. J. Am. Vet. Med. Assoc. 224:419-423. https://doi.org/10.2460/javma.2004.224.419.

Miller, W. J. 1970. Zn nutrition of cattle: a review. J. Dairy Sci. 53:1123-1135. https://doi.org/10.3168/jds.S0022-0302(70)86355-X.

Mills, C. F., J. Quarterman, R. B. Williams, and A. C. Dalgarno. 1967. The effects of zinc deficiency on pancreatic carboxypeptidase activity and protein digestion and absorption in the rat. Biochem J. 102:712-718. https://doi.org/10.1042/bj1020712.

Nansen, P. 1972. Selective immunoglobulin deficiency in cattle and susceptibility to infection. Acta Pathol. Microbiol. Scand. B. Microbiol. Immunol. 80:49-54. https://doi.org/10.1111/j.1699-0463.1972.tb00129.x.

National Research Council. 2001. Nutrient Requirements of Dairy Cattle. 7th rev. ed. Natl. Acad. Press, Washington, DC.

Nyman, A. K., U. Emanuelson, and K. P. Waller. 2016. Diagnostic test performance of somatic cell count, lactate dehydrogenase, and N-acetyl-beta-D-glucosaminidase for detecting dairy cows with intramammary infection. J. Dairy Sci. 99:1440–1448. https://doi.org/10.3168/jds.2015-9808.

Overton, T. R., and T. Yasui. 2014. Practical applications of trace minerals for dairy cattle. J. Anim Sci. 92:416-426. https://doi.org/10.2527/jas.2013-7145.

Pal, D. T., N. K. S. Gowda, C. S. Prasad, R. Amarnath, U. Bharadwaj, G. S. Babu, and K. T. Sampath. 2010. Effect of copper- and zinc-methionine supplementation on bioavailability, mineral status and tissue concentrations of copper and zinc in ewes. J. Trace Elem. Med. Biol. 24:89-94. https://doi.org/10.1016/j.jtemb.2009.11.007.

Parkash, S., and R. Jenness. 1967. Status of zinc in cow's milk. J. Dairy Sci. 50:127-134. https://doi.org/10.3168/jds.s0022-0302(67)87376-4.

Paulrud, C. O. 2005. Basic concepts of the bovine teat canal. Vet. Res. Commun. 29:215-245. https://doi.org/10.1023/B:VERC.0000047496.47571.41.

Permyakov, E. A., and L. J. Berliner. 2000. α-Lactalbumin: structure and function. FEBS Lett. 473:269-274. https://doi.org/10.1016/S0014-5793(00)01546-5.

Quarterman, J., and W. R. Humphries. 1979. Effect of Zn deficiency and Zn supplementation on adrenals, plasma steroids and thymus in rats. Life Sci. 24:177-183. https://doi.org/10.1016/0024-3205(79)90128-0.

Rabiee, A. R., I. J. Lean, M. A. Stevenson, and M. T. Socha. 2010. Effects of feeding organic trace minerals on milk production and reproductive performance in lactating dairy cows: a meta-analysis. J. Dairy Sci. 93:4239-4251. https://doi.org/10.3168/jds.2010-3058.

Rink, L., and P, Gabriel. 2000. Zinc and the immune system. Proc. Nutr. Soc. 59:541-552. https://doi.org/10.1017/S0029665100000781.

Spolders, M., M. Höltershinken, U. Meyer, J. Rehage, and G. Flachowsky. 2010. Assessment of reference values for copper and zinc in blood serum of first and second lactating dairy cows. Vet. Med. Int. 2010:1-8. https://doi.org/10.4061/2010/194656.

Tyrrell, H. F., and J. T. Reid. 1965. Prediction of the energy value of cow's milk. J. Dairy Sci. 48:1215–1223. https://doi.org/10.3168/jds.S0022-0302(65)88430-2.

Wang, Q., J. Ying, P. Zou, Y. Zhou, B. Wang, D. Yu, W. Li, and X Zhan. 2020. Effects of dietary supplementation of humic acid sodium and zinc oxide on growth performance, immune status and antioxidant capacity of weaned piglets. Animals (Basel). 10:2104. https://doi.org/10.3390/ani10112104.

Weng, X., A. P. A. Monteiro, J. Guo, C. Li, R. M. Orellana, T. N. Marins, J. K. Bernard, D. J. Tomlinson, J. M. DeFrain, S. E. Wohlgemuth, and S.Tao. 2018. Effects of heat stress and dietary zinc source on performance and mammary epithelial integrity of lactating dairy cows. J. Dairy Sci. 101:2617-2630. https://doi.org/10.3168/jds.2017-13484.

Wilking, M., M. Ndiaye, H. Mukhtar, and N. Ahmad. 2013. Circadian rhythm connections to oxidative stress: implications for human health. Antioxid Redox Signal. 19:192-208. https://doi.org/10.1089/ars.2012.4889.

Zhao, X. J, Z. P. Li, J. H. Wang, X. M. Xing, Z.Y. Wang, L. Wang, and Z. H. Wang. 2015. Effects of chelated Zn/Cu/Mn on redox status, immune responses and hoof health in lactating Holstein cows. J. Vet. Sci. 16:439-46. https://doi.org/10.4142/jvs.2015.16.4.439.

Tables and Figures

Table 2.1. Ingredient and nutrient composition of the basal TMR diet[1]

Item	Value
Ingredient composition, % of DM	
Corn silage	42.73
Alfalfa hay	12.75
Ground corn	17.13
Corn gluten feed	8.65
Expeller soybean	4.59
Soybean meal[2]	4.41
Straw	1.24
Mineral Mix[3]	8.49
Nutrient composition, % of DM	
DM	52.50
Starch	26.97
CP	15.33
NDF	31.23
ADF	20.50
Ether Extract	4.05
NEL (Mcal/kg of DM)	1.65
Ca	0.94
P	0.38
Mg	0.34
K	1.51
Na	0.58
Fe (mg/kg DM)	375.33
Mn (mg/kg DM)	64.00
Zn (mg/kg DM)	76.00
Cu (mg/kg DM)	14.00

[1]Basal diet DM = 52.5%.
[2]Soyplus (Dairy Nutrition Plus, Ralston, IA).
[3]See Table 2 for mineral mix ingredients.

Table 2.2. Ingredients of the mineral mix for the diets

Ingredient	% of DM
Bypass soybean	19.22
Blood meal	13.89
Calcium carbonate	14.42
Palm fat[1]	13.31
Sodium bicarbonate	11.33
Pork meat and bone meal	9.68
Salt	4.74
Soybean meal	3.09
Magnesium oxide	2.66
Dairy trace mineral mix	2.34
Urea	2.45
Choice white grease	1.24
Yeast culture[2]	0.67
Rumen-protected methionine[3]	0.75
Organic chromium[4]	0.10
Monensin[5]	0.07
Biotin 2%	0.04
Organic Zinc[6]	0.02

[1]Palmit 80 (Global Agri-Trade Corporation, Rancho Dominguez, CA).
[2]Nutritek (Diamond V, Cedar Rapids, IA).
[3]Smartamine-M (Adisseo, Alpharetta, GA).
[4]KemTrace (Kemin, Des Moines, IA).
[5]Rumensin 90 (Elanco, Greenfield, IN).
[6]Zinpro 120 (Zinpro Corporation, Eden Prairie, MN).

Table 2.3. Dry matter intake, milk production, and feed efficiency (milk yield: DMI) for increasing added Zn in the diet from 76 mg/kg DM (CTL) to 96 mg/kg DM using a new Zn-methionine chelate (+Zn-Met)

	0 to 35 d		35 to 70 d			*P*- value		
Variable	CTL	+Zn-Met	CTL	+Zn-Met	SEM	Trt	Period	Trt × Period
DMI, kg/d	26.10	24.90	25.80	24.60	0.32	<0.01	0.31	0.96
Milk yield, kg/d	41.90[a]	39.90[b]	39.70[b]	40.90[c]	0.34	0.30	0.12	<0.01
Milk composition								
Protein, %	3.13	3.11	3.24	3.22	0.07	0.83	<0.01	0.92
Fat, %	4.34	4.28	4.47	4.33	0.16	0.63	0.34	0.68
Lactose, %	4.79	4.76	4.69	4.71	0.03	0.92	<0.01	0.24
Protein yield, kg/d	1.30	1.25	1.27	1.31	0.06	0.99	0.64	0.07
Fat yield, kg/d	1.68	1.59	1.67	1.65	0.10	0.66	1.71	0.62
Lactose yield, kg/d	2.00	1.96	1.85	1.97	0.09	0.75	0.11	0.07
ECM, kg/d[1]	46.61	43.04	44.81	44.70	2.52	0.55	0.96	0.27
Milk yield: DMI	1.61	1.64	1.57	1.67	0.04	0.19	0.89	0.46

[1]ECM = [(0.327 × Milk yield, kg/d) + (12.95 × fat yield, kg/d) + (7.65 × protein yield, kg/d)]

Table 2.4. Hematology parameters of cows 35 and 70 d after increasing added Zn in the diet from 76 mg/kg DM (CTL) to 96 mg/kg DM using a Zn-methionine chelate (+Zn-Met)

	35 d		70 d			*P*-value		
Variable	CTL	+Zn-Met	CTL	+Zn-Met	SEM	Trt	Period	Trt × Period
White blood cells, $\times 10^3/\mu L$	9.48	10.05	10.92	11.02	0.92	0.74	0.20	0.80
Red blood cells, $\times 10^3/\mu L$	6.36	6.71	6.60	6.90	0.28	0.40	0.22	0.89
Hemoglobin, g/dL	10.65	11.15	11.25	11.75	0.41	0.40	0.03	0.99
Hematocrit, %	29.19	30.35	30.76	31.87	1.19	0.50	0.07	0.97
MCV, μm^3	46.36	44.82	46.94	45.74	0.69	0.19	<0.01	0.20
Platelets, $\times 10^3/\mu L$	355.40	332.60	358.90	343.20	35.81	0.67	0.78	0.89
Mean platelet volume, μm^3	6.95	5.89	6.48	6.97	0.71	0.69	0.67	0.30
Neutrophils, $\times 10^3/\mu L$	4.69	4.80	6.24	5.40	0.97	0.72	0.28	0.63
Lymphocyte, $\times 10^3/\mu L$	3.58	4.41	3.55	4.66	0.59	0.30	0.59	0.48
Monocyte, $\times 10^3/\mu L$	0.46	0.57	0.50	0.61	0.09	0.29	0.62	0.99
Eosinophils$\times 10^3/\mu L$	0.39	0.32	0.18	0.34	0.11	0.71	0.43	0.33
Basophils, $\times 10^3/\mu L$	0.11	0.13	0.13	0.15	0.02	0.32	0.28	0.99

Table 2.5. Serum Zn, immunoglobulin, and oxidative markers in blood of cows 35 and 70 d after increasing added Zn in the diet from 76 mg/kg DM (CTL) to 96 mg/kg DM using a Zn-methionine chelate (+Zn-Met)

Variable	35 d		70 d			*P*-value		
	CTL	+Zn-Met	CTL	+Zn-Met	SEM	Trt	Period	Trt × Period
Serum Zn, ppm	0.80[b]	0.71[b]	0.82[b]	1.06[a]	0.08	0.38	0.02	0.04
Immunoglobulins								
IgA, mg/mL	0.12	0.12	0.10	0.11	0.01	0.76	0.09	0.11
IgG, mg/mL	27.51	28.88	27.90	22.80	1.75	0.27	0.10	0.11
IgM, mg/mL	1.53	1.53	1.40	1.48	0.19	0.87	0.57	0.80
Antioxidant markers[1]								
CAT, U/mL	3.60	3.95	4.73	3.61	0.69	0.62	0.55	0.28
SOD, U/mL	4.53	4.79	7.70	4.25	1.03	0.18	0.21	0.09
MDA, µM	7.17	9.05	8.64	8.63	1.75	0.68	0.71	0.51
GSH, µM	2.47	2.09	2.37	2.01	0.10	0.02	0.35	0.82
GSSG, µM	0.22[c]	0.40[a]	0.37[ab]	0.34[b]	0.04	0.09	0.15	<0.01
GSH: GSSG	12.00[b]	5.30[a]	6.40[a]	6.40[a]	0.95	<0.01	0.02	<0.01

[1]Reduced (GSH) and oxidized (GSSG) glutathione, malondialdehyde (MDA), catalase (CAT), and superoxide dismutase (SOD)

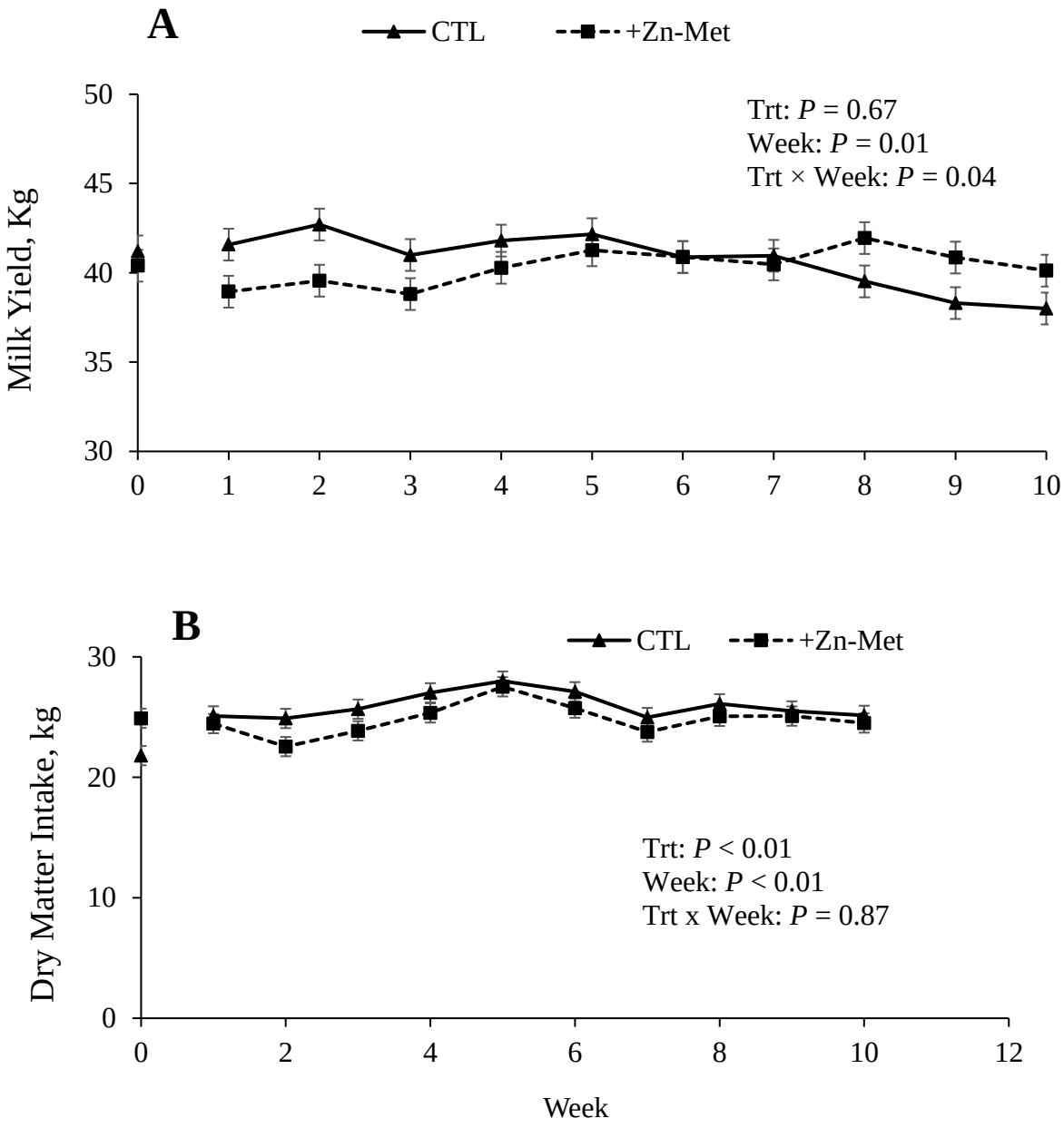

Figure 1.1. Weekly mean milk yield (**A**) and dry matter intake (**B**) of cows receiving Zn-Met at 76 mg/kg DM (CTL) and cows receiving Zn-Met at 96 mg/kg DM (+Zn-Met)

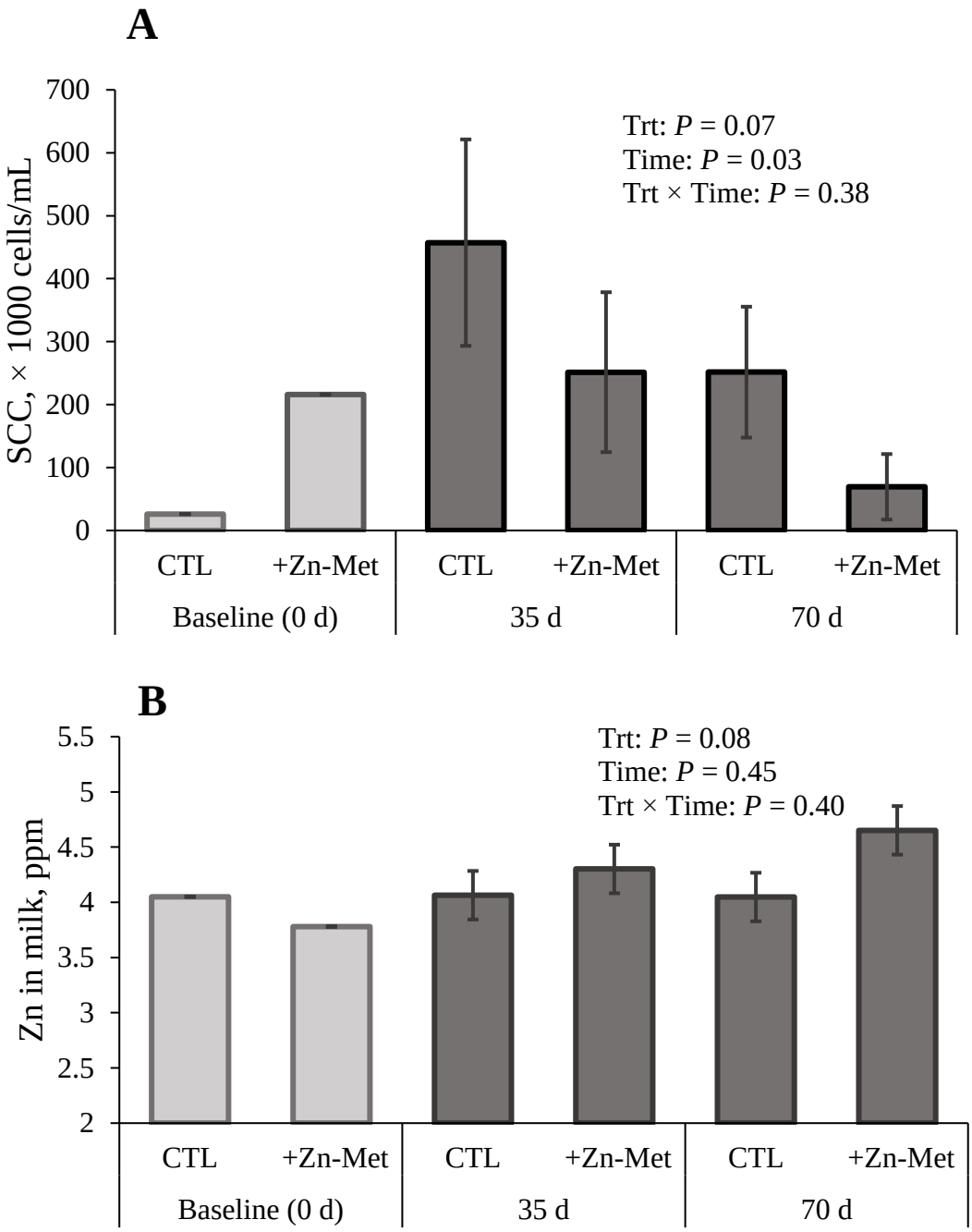

Figure 1.2. The averages at baseline and least squares means and associated standard errors of somatic cell count in milk (SCC, **A**) and Zn concentration in milk (**B**) 35 and 70 d after increasing added Zn in the diet from 76 mg/kg DM (CTL) to 96 mg/kg DM using a Zn-methionine chelate (+Zn-Met)

CHAPTER 3. GENERAL CONCLUSION

This thesis covers a literature review including a meta-analysis and an animal experiment focused on investigating the effects of organic Zinc (Zn) in the diet on milk production, udder health, and immune and antioxidant capacities of lactating dairy cows. There have been several studies focused on the effects of either Zn concentration or the source of Zn (organic vs. inorganic) in the diet on production performance, but little information is available to determine the role of Zn in modulating the immune response (e.g., antibody production) and oxidative stress of dairy cows (Bartlett and Smith, 2003; Birben et al., 2012; Dresler et al., 2016). The present investigations included high-producing dairy cows that are prone to experience oxidative stress and develop health disorders such as mastitis during lactation (Sharma et al., 2011; Turk et al., 2017; Colitti et al., 2019). Increasing emphasis on the sustainability of dairy production demands dietary nutrients with high bioavailability to increase productivity and decrease nutrient excretions to the environment. As a result, inorganic forms of Zn in the diet are recommended to be substituted with the organic forms such as Zn-amino acid complexes (Zn-AA) having greater bioavailability than the inorganic Zn (Rojas et al., 1996; Spears, 1996). Our meta-analysis combine data from nine controlled studies. The findings indicate 0.42 kg/d, 0.57 kg/d, 18 g/d, 0.04%, and 23 g/d increases in dry matter intake (DMI), milk yield, milk protein yield, milk protein %, and milk fat yield respectively for complete replacement of inorganic Zn (ZnO and $ZnSO_4$) in the diet with Zn-AA. The concentration (mg/kg DM) of inorganic Zn being replaced with organic Zn, stage of lactation, and duration of feeding organic Zn vs. inorganic Zn explained 24 to 72% of the heterogeneity

of DMI and milk production responses across individual studies. The studies published in the literature lack the data on fecal and blood concentrations of Zn, and the concentration of other minerals in the diet (e.g., copper) that may have an impact on the bioavailability of Zn. Such data would help further describe the heterogeneity and improve our understanding of the bioavailability and Zn requirement for milk production. Once the heterogeneity of dietary Zn concentration was taken into consideration, complete replacement of dietary inorganic Zn with Zn-AA was related to a 16,000 cells/mL reduction of somatic cell count in milk (SCC). For each unit (mg/kg DM) increase in inorganic Zn that is replaced with Zn-AA, SCC decreased further by 200 cells/mL. In the present meta-analysis, we assumed that the relationships between explanatory variables (e.g., the Zn concentration) and the effect size of inorganic vs. organic Zn were linear, which may not always be the case. Nonlinear meta-analysis using more data would help understand the true relationships. Nonetheless, the observed improvement in SCC postulated a potential to improve the immune responses including antibody production and the antioxidant capacity (Cortinhas et al., 2010) for Zn-AA supplementations.

The objective of the dairy cow trial described in Chapter 2 was to determine the impact of increasing Zn-AA concentration in the diet above the current average (76 mg/kg DM) on Zn, immunoglobulin, and antioxidant concentrations in blood. We used a new Zn-Met chelate (AmiproZn®, DeBon Agri-Tech Group, Shanghai, China) to increase added Zn concentration in TMR from 76 mg/kg DM (ZINPRO®, Zinpro Corporation, Eden Prairie, MN) to 96 mg/kg DM for 10 weeks. The Zn supplement increased Zn concentrations in both blood serum and milk. The increments in both serum and milk Zn were, however, more prominent in the last 5 weeks during mid-lactation than the first 5

weeks during early-lactation, indicating that dietary Zn supply exceeded the requirement in mid-lactation but was likely deficient or barely adequate in the early stage. The milk yield of Zn-supplemented cows was greater than control during the last 5 weeks, whereas it was lower than control in the first 5 weeks. The SCC levels decreased for the Zn supplementation throughout the study but the decrease in SCC was more prominent in the last 5 weeks, suggesting that the Zn supplementation might be adequate to satisfy the requirements of both milk production and udder health in mid-lactation. On the contrary to what has been observed with other species (Bartlett and Smith, 2003; Dresler et al., 2016; Wang et al., 2020), Zn supplementation did not affect plasma immunoglobulin concentrations and decreased the concentration of superoxide dismutase (SOD), and the ratio of reduced to oxidized glutathione (GSH: GSSG) in the serum. The decreased serum concentration of SOD reflects a reduced antioxidant capacity, whereas the decreased serum GSH: GSSG implies increased oxidative stress at the whole-body level. It is noteworthy that the concentration or the abundance of antioxidant enzymes represents just one aspect of the antioxidant capacity. Measurements of the activity of antioxidant enzymes such as SOD would provide better insight into the link between Zn and the antioxidant capacity. Since oxidative stress has a circadian rhythm (Wilking et al., 2013) and the blood markers, particularly GSH: GSSG exhibit high day-to-day variables (Goldfarb et al., 2014), future experiments designed to address those variabilities (e.g., by increasing the blood sampling time points) would help better understand the effects of Zn on antioxidant capacity or oxidative stress of dairy cows. The supplementation of Zn-Met with AmiproZn® (DeBon Agri-Tech Group, Shanghai, China) decreased the DMI of dairy cows by 1.2 kg/d and was associated with 2.0 kg/d lower milk yield in the early stage of

lactation. Further research on this new Zn-Met product, with greater sample sizes, graded levels of supplementations, and additional assays to evaluate oxidative stress are needed to determine its true effects on feed intake and milk production in lactating dairy cattle.

Literature Cited

Bartlett, J. R., and M. O. Smith. 2003. Effects of different levels of zinc on the performance and immunocompetence of broilers under heat stress. Poult. Sci. 82:1580-1588. https://doi.org/10.1093/ps/82.10.1580.

Birben, E., U. M. Sahiner, C. Sackesen, S. Erzurum, and O. Kalayci. 2012. Oxidative stress and antioxidant defense. World Allergy Organ. J. 5:9-19. https://doi.org/10.1097/WOX.0b013e3182439613.

Colitti, M., B. Stefanon, G. Gabai, M. E. Gelain, and F. Bonsembiante. 2019. Oxidative stress and nutraceuticals in the modulation of the immune function: current knowledge in animals of veterinary interest. Antioxidants (Basel). 8:28. https://doi.org/10.3390/antiox8010028.

Cortinhas, C. S., B. G. Botaro, M. C. A. Sucupira, F. P. Rennó, and M. V. D. Santos. 2010. Antioxidant enzymes and somatic cell count in dairy cows fed with organic source of zinc, copper and selenium. Livest. Sci. 127:84–87.

Dresler, S., J. Illek, and L. Zeman. 2016. Effects of organic zinc supplementation in weaned calves. Acta Vet. Brno. 85:49-54. https://doi.org/10.2754/avb201685010049.

Goldfarb, A. H, R. S. Garten, J. Waller, and J. D. Labban. 2014. Day to day variability and reliability of blood oxidative stress markers within a four-week period in healthy young men. J Biomark. 2014:248313. https://doi.org/10.1155/2014/248313.

Rojas, L. X., L.R. McDowell, F.G. Martin, N.S. Wilkinson, A.B. Johnson, and C.A. Njeru. 1996. Relative bioavailability of zinc methionine and two inorganic zinc sources fed to cattle. J. Trace Elem. Med. Biol. 10:205-209. https://doi.org/10.1016/S0946-672X(96)80036-8.

Sharma, N., N. K. Singh, O. P. Singh, V. Pandey, and P. K. Verma. 2011. Oxidative stress and antioxidant status during transition period in dairy cows. Asian-Australas. J. Anim. Sci. 24:479-484. https://doi.org/10.5713/ajas.2011.10220.

Spears, J. W. 1996. Organic trace minerals in ruminant nutrition. Anim. Feed Sci. Technol. 58:151-163. https://doi.org/10.1016/0377-8401(95)00881-0.

Turk, R., M. Koledić, N. Maćešić, M. Benić, V. Dobranić, D. Đuričić, L. Cvetnić, and M. Samardžija. 2017. The role of oxidative stress and inflammatory response in the pathogenesis of mastitis in dairy cows. Mljekarstvo. 67:91-101. https://doi.org/10.15567/mljekarstvo.2017.0201.

Wang, Q., J. Ying, P. Zou, Y. Zhou, B. Wang, D. Yu, W. Li, and X Zhan. 2020. Effects of dietary supplementation of humic acid sodium and zinc oxide on growth performance, immune status and antioxidant capacity of weaned piglets. Animals (Basel). 10:2104. https://doi.org/10.3390/ani10112104.

Wilking, M., M. Ndiaye, H. Mukhtar, and N. Ahmad. 2013. Circadian rhythm connections to oxidative stress: implications for human health. Antioxid Redox Signal. 19:192-208. https://doi.org/10.1089/ars.2012.4889.