5-1995

The Effects of Feeding Chelated Metal Proteinates on Milk Production and Reproductive Performance in Holstein Dairy Cows

Ben James Hardcastle

Utah State University

Follow this and additional works at: https://digitalcommons.usu.edu/etd

Part of the Animal Sciences Commons

Recommended Citation

Hardcastle, Ben James, "The Effects of Feeding Chelated Metal Proteinates on Milk Production and Reproductive Performance in Holstein Dairy Cows" (1995). All Graduate Theses and Dissertations. 3911.
https://digitalcommons.usu.edu/etd/3911

This Thesis is brought to you for free and open access by the Graduate Studies at DigitalCommons@USU. It has been accepted for inclusion in All Graduate Theses and Dissertations by an authorized administrator of DigitalCommons@USU. For more information, please contact dylan.burns@usu.edu.
THE EFFECTS OF FEEDING CHELATED METAL PROTEINATES
ON MILK PRODUCTION AND REPRODUCTIVE
PERFORMANCE IN HOLSTEIN DAIRY COWS

by

Ben James Hardcastle

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

of

MASTER OF SCIENCE

in

Animal Science
(Animal Nutrition)

Approved:

Dr. Randall D. Wiedmeier
Major Professor

Dr. Jeffrey L. Walters
Committee Member

Dr. G. Reed Holyoak
Committee Member

Dr. Robert C. Lamb
Head

Dr. James P. Shaver
Dean of Graduate Studies

UTAH STATE UNIVERSITY
Logan, Utah

1995
ACKNOWLEDGMENTS

A very special thanks to my wife, Julie, for her support and, remarkably, her continued encouragement given throughout my academic pursuit. I want to express my gratitude to my parents, who have always encouraged me, inspired me, and supported my decisions.

Thanks to Dr. Mike Arambel and Dr. Randy Wiedmeier for their confidence in me and providing me the opportunity to continue in my graduate education. I appreciate the help of all my committee members, for their educational advice and expertise in each of their respective fields.

My thanks to Daeyoon Kim and others at the Skaggs Research Laboratory for their friendship, technical skills, and suggestions during my research project.

Finally, I wish to thank Chelated Minerals Corporation for their financial contribution and for supplying the product used to complete this research project.

Ben James Hardcastle
CONTENTS

ACKNOWLEDGMENTS ................................................................. ii
LIST OF TABLES ........................................................................ iv
LIST OF FIGURES ........................................................................ v
ABSTRACT .................................................................................. vi
INTRODUCTION ........................................................................ 1
OBJECTIVES ............................................................................... 5
LITERATURE REVIEW .................................................................. 6
Chelated Minerals ................................................................. 6
Chelate Characteristics ...................................................... 7
AAFCO Definitions ............................................................... 8
The Amino Acid System ......................................................... 9
Trace Mineral Supplementation Effects on Blood Serum Levels ................................................. 10
Solubility, Integrity, and Absorption of Inorganic and Organic Trace Minerals .................................... 11
Feeding Metal Proteinates in Nonruminant Diet ................................................................. 14
Feeding Chelates to Improve Reproductive Performance ......................................................... 15
Organic Mineral Effects on Immune Response ............................................................................. 16
Organic Mineral Effects on Hoof Durability and Integrity ............................................................... 17
MATERIALS AND METHODS .................................................. 18
Experimental Design ............................................................ 18
Statistical Design ................................................................. 20
RESULTS AND DISCUSSION ..................................................... 24
CONCLUSIONS AND IMPLICATIONS ............................................. 34
REFERENCES ............................................................................. 35
<table>
<thead>
<tr>
<th>Table</th>
<th>Description</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Direct mineral involvement in specific enzyme systems</td>
<td>3</td>
</tr>
<tr>
<td>2</td>
<td>Ingredients and nutrient composition of prepartum total mixed ration</td>
<td>19</td>
</tr>
<tr>
<td>3</td>
<td>Ingredients and nutrient composition of postpartum total ration</td>
<td>21</td>
</tr>
<tr>
<td>4</td>
<td>Effect of treatment on milk composition</td>
<td>30</td>
</tr>
<tr>
<td>5</td>
<td>Effect of treatment on total tract apparent nutrient digestibility</td>
<td>31</td>
</tr>
<tr>
<td>6</td>
<td>Effect of treatment on hoof evaluation scores</td>
<td>33</td>
</tr>
<tr>
<td>7</td>
<td>Effect of treatment on reproductive parameters</td>
<td>33</td>
</tr>
</tbody>
</table>
LIST OF FIGURES

<table>
<thead>
<tr>
<th>Figure</th>
<th>Description</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Effect of prepartum treatment on calf birth weight and dry matter intake</td>
<td>25</td>
</tr>
<tr>
<td>2</td>
<td>Effect of treatment on prepartum body weights over time</td>
<td>26</td>
</tr>
<tr>
<td>3</td>
<td>Effect of treatment on postpartum dry matter intake over time</td>
<td>27</td>
</tr>
<tr>
<td>4</td>
<td>Effect of treatment on postpartum body weights over time</td>
<td>28</td>
</tr>
<tr>
<td>5</td>
<td>Effect of treatment on average daily milk yield over time</td>
<td>29</td>
</tr>
<tr>
<td>6</td>
<td>Effect of treatment on mineral blood plasma concentration</td>
<td>31</td>
</tr>
</tbody>
</table>
ABSTRACT

The Effects of Feeding Chelated Metal Proteinates on Milk Production and Reproductive Performance in Holstein Dairy Cows

by

Ben James Hardcastle, Master of Science

Utah State University, 1995

Major Professor: Dr. Randall D. Wiedmeier
Department: Animal, Dairy, and Veterinary Sciences

Twenty-two primiparous Holstein heifers were allocated to one of two treatments. Treatments consisted of: 1) basal ration plus 226.8 g inorganic mineral supplement (control); and 2) basal ration plus 226.8 g inorganic mineral and metal proteinate supplement (50:50). Individual heifer performance was measured during the final 10 weeks of the prepartum period. Feed intake and refusals were recorded daily. Individual heifer body weights recorded weekly did not differ significantly. Blood samples taken at 4-week intervals did not differ in plasma concentration between treatments. Calf birth weights for control heifers were not significantly higher than calves from the treatment heifers.

The same twenty-two Holstein heifers used in the prepartum period were assigned to the same treatment groups for the 20-week postpartum lactation period. Treatments consisted of: 1) basal ration plus 453.6 g inorganic mineral supplement (control); and 2)
basal ration plus 453.6 g inorganic mineral supplement and metal proteinate supplement (50:50). Dry matter intake and milk yield were recorded daily. Milk composition and body weights were recorded weekly. Feed and fecal samples were collected to determine apparent nutrient digestibility, using acid insoluble ash as an internal marker. Blood samples taken at 4-week intervals showed no significant difference (P < .05) in plasma mineral composition. No statistical difference was observed in milk yield between treatments (P < .05). Percent lactose in milk samples from the treatment group was significantly higher (P < .05) than that of the control group. Apparent nutrient digestibilities did not differ between treatment groups. Starting on day 5 postpartum, cows were observed for signs of estrus and bred at first observed estrus after 60 days postpartum. Days to first estrus were significantly lower (P < .05) in the treatment group. The feet of each heifer were critically evaluated and scored according to six separate criteria at the start and at the finish of the trial. The texture category of the hoof evaluation score was significantly lower (P < .05), favoring the treatment group over the control group.
INTRODUCTION

All animal tissues and all feeds they consume contain inorganic or mineral elements in widely varying amounts and proportions. These inorganic elements constitute the ash that remains after ignition. They exist mainly as oxides, carbonates, and sulfates (43). Many mineral elements occur in living tissues in such small amounts that early researchers were unable to measure their precise concentration using the analytical methods then available. They were therefore described as occurring in trace amounts and the term trace elements arose to describe them. This term has remained popular in usage despite the fact that virtually all the trace elements can be estimated in biological materials with accuracy and precision.

Many mineral elements have been shown to be essential for animal growth and performance. Signs of deficiency have been described (48) and are generally divided into two groups: macrominerals and trace minerals. Macrominerals are those required in greater quantities (g/day) and are present in animal tissues at higher concentrations (%). The trace minerals are those that are required in smaller amounts (mg/day) and are generally present in tissues at lower levels (mg/kg). At the present time 26 of the 90 naturally occurring inorganic elements are known to be essential for animal life. These consist of all macro elements, including sulfur, calcium, phosphorus, potassium, sodium, chloride, and magnesium. The trace minerals consist of 15 elements, namely, iron, zinc, copper, manganese, nickel, cobalt, molybdenum, selenium, chromium, iodine, fluorine, tin, silicon, vanadium, and arsenic. Currently, however, several of these elements are not considered to be of practical importance in the feeding of dairy cattle.
The mineral elements exist in the cells and tissue of the animal body in a variety of functional chemical combinations, and in characteristic concentrations that vary with the element and the tissue. Domestic cattle require some of these essential mineral elements as structural components of body organs and tissues or as either components or cofactors of enzyme and hormone systems. Others serve as constituents of body fluids and are involved in the maintenance of osmotic pressure, acid-base balance, membrane permeability, and nerve transmission (50). Table 1 shows a number of major biochemical functions of the three micro elements used in this study.

Trace mineral bioavailability is generally not well understood in ruminant animals. However, as the genetic capability of cattle improves, the relationship between dietary supply and animal requirement will become increasingly important with regard to production and fertility. Because fertility and milk production are partially antagonistic, an understanding of nutritional influence of trace minerals is necessary for maintaining an efficient reproductive record in high-yielding dairy cows over acceptable longevity.

Bioavailability of ingested inorganic minerals may be below 20% due to numerous factors, including reactions with lipids, protein, fiber, oxalic acids, oxides, and vitamins, as well as interactions with other compounds such as phosphates and phytates. Halpin et al. (15) estimated that only 1.7% of the manganese from manganese sulfate in the diet of a chick fed corn-soybean ration was absorbed. In recent years mineral chelates have been introduced to improve absorption and metabolism of essential minerals. All chelates are complexes but not all complexes are chelates. Chelates are unique in that they form a ring structure around the metal ion and have coordinate covalent bonds with the metal. The preference for a
TABLE 1. Direct mineral involvement in specific enzyme systems.

<table>
<thead>
<tr>
<th>Mineral</th>
<th>Component of:</th>
<th>Enzyme Activity</th>
</tr>
</thead>
<tbody>
<tr>
<td>Zinc</td>
<td>DNA Polymerase</td>
<td>Gene function</td>
</tr>
<tr>
<td></td>
<td>Protein Synthesis</td>
<td>Amino acid utilization</td>
</tr>
<tr>
<td></td>
<td>Amino Peptidase</td>
<td>Enzyme activation</td>
</tr>
<tr>
<td></td>
<td>Superoxide Dismutase</td>
<td>Free radical reduction</td>
</tr>
<tr>
<td>Manganese</td>
<td>Pyruvate Decarboxylase</td>
<td>TCA Cycle</td>
</tr>
<tr>
<td></td>
<td>Oxidative Phosphorylation</td>
<td>ATP Synthesis</td>
</tr>
<tr>
<td></td>
<td>Glucose Metabolism</td>
<td>Formation of insulin</td>
</tr>
<tr>
<td></td>
<td>Glycosyltransferase</td>
<td>Prothrombin formation</td>
</tr>
<tr>
<td>Copper</td>
<td>Lysyl Oxidase</td>
<td>Collagen and elastin formation</td>
</tr>
<tr>
<td></td>
<td>Cytochrome C</td>
<td>Oxidative phosphorylation</td>
</tr>
<tr>
<td></td>
<td>Uricase Enzyme</td>
<td>Nitrogen metabolism</td>
</tr>
<tr>
<td></td>
<td>Ceruloplasmin</td>
<td>Iron utilization</td>
</tr>
</tbody>
</table>
chelate over a complex in regard to rumen bypass relates to the strength of the complex/chelate in resisting ruminal degradation. Martel and Calvin (28) stated it has been shown that the stability of chelates is much greater than that of the complexes based on the ring structure found in chelates. Spears et al. (45) fed lambs semipurified diets supplemented with zinc as zinc oxide or zinc methionine. Although animal performance and apparent absorption of the zinc were similar between the two sources of zinc, zinc retention tended to be greater in lambs fed zinc methionine. Buraczewska (7) found that swine absorbed amino acids from peptides faster than when presented in their free forms. Kincaid (24) reported that chelated minerals may alter rumen microflora or may increase the absorption of other nutrients such as vitamin A. Kincaid et al. (25) examined metal proteinate's effect on copper-molybdenum-sulfate interactions. In forages containing molybdenum, copper sulfate was found to be an ineffective source of copper, while copper proteinate provided adequate amounts of copper in the diet. Manspeaker et al. (27) reported increased ovarian activity and increased reproductive performance in 40 first-calf Holstein heifers fed a balanced diet supplemented with amino acid-chelated minerals, which included manganese, copper, and zinc, each bonded to two or more amino acids. With improved genetic capabilities of dairy cattle, the use of chelated mineral supplements requires further investigation.
OBJECTIVES

The objectives of this study were 1) to determine the effect of adding chelated metal proteinates on apparent total tract nutrient digestibility; 2) to determine the effect of chelated metal proteinates on plasma mineral composition; 3) to determine the effect of chelated metal proteinates on hoof integrity; 4) to determine the effect of chelated metal proteinates on reproductive performance; and 5) to determine the effect of chelated metal proteinates on milk yield and milk composition in first-calf Holstein heifers.
LITERATURE REVIEW

Chelated Minerals

During the last decade, concerns have increased with regard to the relationship of mineral nutrition and reproduction in dairy cattle. It is questionable whether the macro and micro mineral bioavailability in most dairy rations is sufficient to meet the cow's maintenance and production needs in view of the improved genetic capability of modern dairy cows. The intake of these bioavailable minerals is especially crucial in the peripartum animal. Chelation is a biochemical process with special requirements. The word is derived from the Greek word Chele', meaning "claw." This describes the effect of the chelating agent to surround the metal, forming certain stable bonds. Proteins or amino acids are examples of chelating agents. Chelated metal proteinates, which carry a neutral charge, can be absorbed as much as 300 to 500 percent more efficiently than their inorganic counterparts (27).

Trace elements are so named because of the very small quantities (100 ppm or less) present in the body and required in the diets of animals (52). Uneven forage quality, variability in mineral concentrations in feeds, environmental stress, and other nutritional deficiency factors have necessitated the use of supplemented mineral sources to meet the demands of high producing livestock and poultry. Cattle forced to subsist on forage inadequate in certain trace elements without supplementation may develop gross deficiency symptoms (47). While the animal's physical makeup contains only small amounts (2-5%) of minerals, they play a vital and important role in nutrition. They furnish structural
materials, constituents of soft tissues and cells, and they regulate many of the vital biological processes (29). Modern dairy cattle fed mixtures of forage with grains and byproducts of milling, oil feeds, brewing, distilling, citrus, sugar, and rendering industries are much less likely to exhibit signs of trace element deficiency. Fertility (20) and immune response (13) may be impaired before clinical symptoms become apparent. Deficiencies of some minerals can cause a negative effect by the lack of thrift, poor gains, inefficient feed utilization, and reduced reproduction performance. All of these factors reduce the productive longevity of animals and result in economic losses.

Bioavailability can be defined as the portion of the mineral that the animal can use to meet its bodily needs. Each mineral is available in certain forms and each form is different in its bioavailability for the animal's feeding purposes. In theory, the use of chelated minerals will increase absorption and utilization of the mineral because of a more favorable binding or stability constant.

**Chelate Characteristics**

Chelated minerals are unique in that they form a ring structure around the metal and have coordinate covalent bonds with the metal. These bonds are shared between the metal and nitrogen (amino) or oxygen (hydroxyl) as donor groups. Fouad (11) explained that lacking nitrogen from amino groups, gluconates, fumarates, and citrates have only metal-to-oxygen bonds and ionic bonding.

At certain pH's, some metal proteinates and amino acid chelates have correct sharing of bonds between metal, and oxygen or nitrogen, and are unique in their neutral charge.
Other ligands with less affinity for metals (citric acid) lack nitrogen but are still commonly found in chelates.

Steps have been taken to formulate a generalistic definition of compounds that consist of metals in association with proteins (metal proteinates). The purpose was to seek official definition status with the Association of American Feed Control Officials (AAFCO), thus allowing mineral supplement manufacturers to more precisely communicate with the end-users in the livestock industry. The development of the definition was hoped to assure livestock producers that they were buying a product that already met a variety of specifications as designated in the official definition. The Metal Proteinate and the Metal Amino Acid Chelate definitions were the result of the following attempts (1).

57.23 **Metal Proteinate** is the product resulting from the chelation of a soluble salt with amino acids or partially hydrolyzed protein. It must be declared as an ingredient as the specific metal proteinate.

57.142 **Metal Amino Acid Chelate** is the product resulting from the reaction of a metal ion from a soluble metal salt with amino acids with a mole ratio of one mole of metal to one to three moles of amino acids to form coordinate covalent bonds. The average weight of the hydrolyzed amino acids must be approximately 150 and the resulting molecular weight of the chelate must not exceed 800. The minimum metal content must be declared. When used as a commercial feed ingredient, it must be declared as a specific metal amino acid chelate.

The amino acids have been shown to be perfectly capable of forming stable chelates under proper conditions. The metal amino acid chelate definition included more defining characteristics of valid amino acid chelates.
The Amino Acid System

It is becoming increasingly clear that the amino acid (AA) profile of ruminally undegraded feed available in the small intestine should be considered. Rumen escape protein is only useful to the extent that it supplies limiting AA (38), or serves in other roles such as a carrier bound to certain essential minerals and nutrients. A ruminant feeding system using AA requirements will have to differentiate between feed nitrogen available in the rumen and AA available in the small intestine. The current NRC and ARC (37, 2) feeding systems differentiate between rumen degradable and rumen escape proteins. However, because of insufficient data, neither system extends to AA. Ruminal degradability of dietary protein and individual amino acids in the protein may differ. Proteins that are insoluble, but available, undergo digestion either in the rumen or postruminally. The associated metabolic value is related to partitioned digestion between the rumen, the small intestine (AA absorbed directly), and the hind gut (19). There are methods by which relative qualities of each fraction can be estimated (21, 9).

In order to properly meet the animal's requirements it is necessary to match nutrient supply with demand. The same amino acids are regarded as essential for ruminant and monogastric animals; however, the amount of each AA required for maintenance, growth, or production is unknown (53). Maximal production stress as seen in the early postpartum cow (within the first 10 weeks of lactation). At this time many cows produce more milk nutrients than they can possibly compensate for through daily intake, and the cow is consequently placed in a negative nutritional phase (27). Hence, the capability of AA to form stable chelates with essential minerals and other vital nutrients may aid in the
bioavailability of these nutrients in dairy rations to more effectively meet the cow's maintenance and production needs.

**Trace Mineral Supplementation Effects on Blood Serum Levels**

Trace minerals are required by animals for a number of biochemical processes. Clinical signs of trace mineral deficiency have been reported in ruminants under practical conditions (39). Bioavailability and apparent absorption of chelated trace minerals suggest an increase in plasma mineral concentrations. Spears (44) fed lambs a diet supplemented with zinc as either zinc oxide or zinc methionine. Although animal performance and apparent absorption of zinc were similar between the two sources, zinc retention tended to be greater in lambs fed zinc methionine. Plasma zinc concentrations tended to remain higher after oral dosing with zinc methionine than zinc oxide. This indicates that following absorption from the digestive tract, zinc methionine is metabolized differently than zinc oxide.

Beeson et al. (5) reported that the effect of supplemental dietary zinc on zinc blood serum levels was inconsistent. Supplemental dietary zinc had virtually no effect on blood serum levels of zinc except when the dietary levels were extremely high (300 or 620 mg/kg). Stake et al. (46) also observed that feeding high levels of zinc would result in increased levels of serum zinc.

Kincaid et al. (25) found bioavailability of dietary copper from copper proteinate was greater than from copper sulfate for calves fed diets containing molybdenum. Copper concentrations in plasma were significantly greater in calves fed copper proteinate than
control calves fed copper sulfate. Liver copper concentrations were also higher in calves fed copper proteinate. Final body weights, plasma zinc, plasma iron, hemoglobin, and hematocrit were not affected by treatment.

Miller (32) in a review of zinc in animal nutrition pointed out that a severe deficiency of zinc will result in depressed serum levels, but because of variability among animals, there may be considerable overlapping in tissue levels between deficient and normal levels.

Henry et al. (18) reported the availability of manganese in manganese oxide and manganese methionine to be 91 and 120%, respectively, relative to manganese sulfate as a standard source. This was based on bone and tissue concentrations of manganese. This suggests that higher availability of manganese methionine may be due to its greater solubility in water or its smaller particle size compared with manganese oxide.

**Solubility, Integrity, and Absorption of Inorganic and Organic Trace Minerals**

Trace minerals that have been complexed or chelated with organic molecules have been reported to be superior to inorganic trace minerals with respect to bioavailability and biological efficiency in numerous species of livestock and poultry. The advantages in bioavailability of minerals from these chelated, complexed, or proteinated supplements are usually attributed to either superior solubility or to the unique chemical structure of the compound.

Heinrich and Conrad (17) studied the stability of iron methionine and zinc methionine by using a bacterial growth curve assay estimate. Growth of the mixed inoculum
from the rumen suggested that iron methionine broke down in simulated ruminal conditions but zinc methionine did not.

Kerley and Ledoux (23) measured the amount of zinc and manganese in ruminal microbial pellets from in vitro incubations of zinc and manganese proteinates and zinc and manganese sulfates. They suggested that the metal proteinates were insoluble and structurally stable under simulated ruminal conditions. It was also estimated that nitrogen from the proteinates could have possibly stimulated microbial proliferation, creating more sites for adsorption of soluble metal ions onto microbial cells, yielding more metal in the microbial pellet.

Brown and Zeringue (6) studied the solubility and stability of fifteen different chelated, proteinated, or complexed mineral products. This study concluded that metals from all products used were very soluble in a buffer at pH 5 and completely soluble in a buffer at pH 2. Gel fraction chromatography indicated that metals solubilized from the products were either no longer bound to the proteinaceous ligand or they were bonded so weakly that they dissociated under gentle gel fraction conditions. This suggests that if metals from chelated, proteinated, or complexed products are solubilized in vivo, they are also uncomplexed in solution. They are also not likely to be absorbed or metabolized differently from soluble metals from inorganic sources.

Until recently, it was widely believed that cattle could absorb only a very small percentage of dietary trace minerals, and this was a fairly fixed value for each element (10, 49). However, recent work has shown that absorption of trace minerals varies tremendously in cattle. Miller and Cragle (30) found numerous absorption sites and great variability in
tissue secretions with regard to zinc fed to dairy cattle. The single most important factor affecting absorption of trace minerals is their content in the diet (32). Miller et al. (33) suggested that as dietary zinc is decreased, the percentage absorbed increased. In an earlier study Miller et al. (34) showed that with high dietary zinc, the absorption percentage is reduced. Another substantial factor affecting zinc absorption is whether or not the animal is clinically zinc deficient. When fed the same diet before and during testing, zinc-deficient animals absorb a higher percentage of administered zinc. Both low dietary zinc and zinc deficiency reduce endogenous fecal zinc excretion (31).

The small intestine is the main site of trace mineral absorption, with the duodenum, or proximal part of the small intestine being a much more active site than the lower intestine (41). Absorption first involves uptake of the mineral by the intestinal mucosa and transport or transfer into the blood (51). Trace mineral excretion is largely by way of the feces. Small amounts are also secreted into the bile, cecum, and colon. Very little trace mineral excretion is by way of the urine (49).

In the high-producing dairy cow, blood supply to the reproductive system is markedly reduced after parturition, which could lead to a mineral deficiency in the reproductive tract. Increasing the amount of inorganic minerals added to a ration to compensate for a deficiency could lead to a toxicity (12), whereas amino acid-chelated minerals have been shown to cause no toxic effects (3).
Feeding Metal Proteinates in Nonruminant Diets

Supplying adequate essential mineral supplementation to the nonruminant serves an equally important role as it does in ruminant nutrition. Nonruminants subjected to deficient levels of trace minerals respond to the deficiency in the same manner as do ruminants. Severe deficiency causes numerous pathological changes, including skin parakeratosis, reduction or cessation of growth, general debility, lethargy, and increased susceptibility to infection (10). These essential mineral elements function largely or entirely in enzyme systems and are involved in protein synthesis, carbohydrate metabolism, and many other biochemical reactions.

The relatively low bioavailability of inorganic minerals used in nonruminant rations has prompted a limited number of studies measuring responses to chelates in monogastric feeding systems. Wedekind et al. (54) summarized the effect of zinc methionine relative to zinc sulfate in chick bioassays. Significant increases in weight gain and tibia Zn content were detected in chicks fed zinc methionine. They concluded, in a multiple linear regression assessment, that Zn methionine bioavailabilities compared to inorganic sources were 124.2% based on growth, and 176.5% based on tibia Zn. Mirando et al. (35) reported significant differences in the reproductive performance of sows supplemented with proteinated trace minerals compared to sows fed inorganic trace minerals. Their results demonstrated that proteinated Zn, Mn, and Cu, provided as a dietary supplement, increased embryo and fetal survival in sows. The duration of estrus was also less in sows fed the proteinated minerals, potentially reducing the boar and labor requirement for mating.
Feeding Chelates to Improve Reproductive Performance

The ruminant animal requires mineral nutrients for many metabolic functions. The amount of these nutrients required increases during times of increased or impending production. The nutritional needs are greater and more critical for fertility, maintaining pregnancy, lactation, and growth. Certain minerals have been shown to be directly related to fertility and reproduction as measured by follicular activity. Manspeaker et al. (27) conducted a study using first-calf Holstein heifers fed a control diet plus an amino acid-chelated mineral supplement. The amino acid-chelated supplement supplied iron, manganese, copper, zinc, potassium and magnesium. This study was conducted from approximately 30 days prepartum until heifers were confirmed pregnant by rectal palpation. Incidence of periglandular fibrosis (a pathologic response in which endometrial tissue does not regenerate properly after parturition) was significantly lower (10 vs 58%) in heifers given chelated minerals. Ovarian activity tended to be higher and embryonic mortality lower for the heifers fed the chelated mineral supplement, although differences were not statistically significant. Kropp (26) evaluated the effects of supplementing amino acid chelates on reproduction in first-calf beef heifers. Beginning at approximately 45 days postcalving, heifers were divided into two groups and supplemented with similar levels of either amino acid chelates or inorganic mineral forms. Estrous was synchronized 70 days postcalving. Percentage of heifers exhibiting estrus and conception rate following synchronization was
significantly higher for heifers fed the chelated mineral mixture. Conception rate for the entire breeding season did not differ but heifers receiving chelated minerals conceived an average of 19 days earlier than those fed the inorganic minerals. This would translate to older, heavier calves at weaning. Hatfield et al. (16) observed an increase in dry matter intake during gestation in ewes fed zinc methionine, but during lactation dry matter intake was not affected. This study also concluded that milk somatic cell count was not influenced by zinc methionine treatment.

**Organic Mineral Effects on Immune Response**

The increased bioavailability of the organic mineral supplements has resulted in positive effects on immune responsiveness and disease resistance in ruminants. Stake et al. (46) studied the effect of zinc oxide and zinc methionine on antibody titer response to viral vaccination in stressed feeder steers. Antibody titers on serum samples measured the immune response to bovine herpesvirus-1 (BHV-1) and parainfluenza vaccination. Steers supplemented with zinc methionine showed antibody titers against BHV-1 that were 31% higher than in steers supplemented with zinc oxide. Chirase et al. (8) observed that calves challenged experimentally with Infectious Bovine Rhinotracheitis (IBR) tended to recover more rapidly from the disease when fed zinc methionine compared to zinc oxide. Johnson et al. (22) examined the effects of zinc methionine on the health and performance of newly
received calves. They found that calves supplemented with zinc methionine gained faster, had decreased morbidity rate, and required fewer medical treatments than calves fed a control diet void of zinc supplementation.

**Organic Mineral Effects on Hoof Durability and Integrity**

Zinc has been shown to be essential for the keratinization of epithelial tissues (32). Reiling et al. (40) studied the effects of supplementing zinc proteinate and zinc sulfate on hoof strength in feedlot heifers. Zinc proteinate-treated heifers showed a trend toward requiring a greater amount of applied force for the shearing of hooves. Hooves from zinc proteinate-treated heifers also appeared to exhibit greater elasticity. Moore et al. (36) reported that dairy cows fed a control diet and a zinc methionine-supplemented diet showed hoof growth and hoof wear that were similar during a one-year period. However, improved hoof scores were observed for texture, heel cracks, and interdigital dermatitis in the zinc methionine-treated cows.
Prepartum Period

Twenty-two prepartum primiparous (10 weeks prior to calving) Holstein heifers (Ave. BW 580 kg) were randomly assigned to one of two treatment groups (11 heifers per treatment). Treatments consisted of 1) basal ration (Table 2) plus 226.8 gm inorganic mineral supplement (control); and 2) basal ration plus 226.8 gm inorganic mineral and metal proteinate supplement (proteinates provided by Chelated Minerals Corporation, Salt Lake City, UT). The treatment supplement contained a 50:50 ratio of inorganic mineral and chelated metal proteinates, including copper proteinate, manganese proteinate, and zinc proteinate (Table 2). The basal ration was balanced according to NRC requirements (37) and individually fed ad libitum twice daily (0600 and 1600 h) with free access to water. Treatments were top dressed with the AM feeding. Five to 10% refusals were allowed with intake adjustments performed daily during the 10-week experimental period. Feed refusals were recorded once daily. Animals were housed in an enclosed freestall barn, utilizing sand as bedding. Heifers were individually fed with the use of Calan gate feeders (American Calan, Inc., Northwood, NH).

Dry matter intake (DMI) was recorded daily. Body weight was monitored weekly. Blood samples were taken at 4-week intervals and analyzed for plasma Cu and Zn levels. Calf birth weights were recorded at parturition.
TABLE 2. Ingredients and nutrient composition of prepartum total mixed ration.

<table>
<thead>
<tr>
<th>Ingredient</th>
<th>DM Basis (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Alfalfa Haylage</td>
<td>50</td>
</tr>
<tr>
<td>Corn Silage</td>
<td>35</td>
</tr>
<tr>
<td>Alfalfa Hay</td>
<td>14</td>
</tr>
<tr>
<td>Vitamin and Mineral Premix(^1,2)</td>
<td>1</td>
</tr>
</tbody>
</table>

**Nutrient Analysis**

<table>
<thead>
<tr>
<th>Nutrient</th>
<th>Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Crude Protein</td>
<td>15.4</td>
</tr>
<tr>
<td>Acid Detergent Fiber</td>
<td>36.1</td>
</tr>
<tr>
<td>Neutral Detergent Fiber</td>
<td>51.3</td>
</tr>
<tr>
<td>Copper (ppm)</td>
<td>12.4</td>
</tr>
<tr>
<td>Manganese (ppm)</td>
<td>28.4</td>
</tr>
<tr>
<td>Zinc (ppm)</td>
<td>27.0</td>
</tr>
</tbody>
</table>

\(^1\) Consisted of 12.0% P; 2.1% Mg; 12.0% Na; .25% K; 5 ppm Co; 20 ppm Se; 1500 ppm Fe; 6000 ppm Zn; 5000 ppm Mn; 800 ppm Cu; 550,400 IU Vitamin A/kg; 165,120 IU Vitamin D/kg; 330 IU Vitamin E/kg.

\(^2\) Treatment and Control Zn, Mn, Cu Composition.

**Treatment:**
- 50% ZnSO\(_4\) - 50% Zn Proteinate
- 50% MnSO\(_4\) - 50% Mn Proteinate
- 50% CuSO\(_4\) - 50% Cu Proteinate

**Control:**
- 100% ZnSO\(_4\)
- 100% MnSO\(_4\)
- 100% CuSO\(_4\)
Postpartum Period

The same twenty-two Holstein heifers (Ave. BW 550 kg) used in the prepartum period were assigned to the same treatment groups for the 20-week postpartum lactation period. Heifers were randomly assigned equally to one of two treatment groups (11 heifers per treatment). Treatments consisted of 1) basal ration (Table 3) plus 453.6 gm inorganic mineral supplement (control); and 2) basal ration plus 453.6 gm inorganic mineral and metal proteinate supplement (proteinates provided by Chelated Minerals Corporation, Salt Lake City, UT). The basal ration was fed as a total mixed ration. The treatment supplement contained a 50:50 ratio of inorganic mineral and chelated metal proteinates including copper proteinate, manganese proteinate, and zinc proteinate (Table 3). The basal ration was balanced according to NRC requirements (39) and individually fed ad libitum twice daily (0530 and 1630 h) with free access to water. Treatments were top dressed with the AM feeding. Five to 10% refusals were allowed with intake adjustments performed daily during the 20-week experimental period. Feed refusals were recorded once daily. Heifers were housed in a freestall barn with sand bedding and individually fed with the use of Calan gate feeders (American Calan, Inc., Northwood, NH).

Dry matter intake (DMI) and milk yield were recorded daily. Body weight was monitored weekly. Representative milk samples from AM and PM milkings were collected from each cow and composited weekly. The milk samples were analyzed at the Utah Dairy Herd Improvement Association (DHIA) laboratory (Logan, UT) for fat, protein, lactose, solid-non-fat (SNF) percent, and somatic cell count (SCC) with the use of a Multispec M Infrared Analyzer (Whedrake, York, England). Blood samples were taken at 4-week
TABLE 3. Ingredients and nutrient composition of postpartum total mixed ration.

<table>
<thead>
<tr>
<th>Ingredient</th>
<th>DM Basis (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Alfalfa Haylage</td>
<td>16.93</td>
</tr>
<tr>
<td>Corn Silage</td>
<td>7.25</td>
</tr>
<tr>
<td>Alfalfa Hay</td>
<td>20.32</td>
</tr>
<tr>
<td>Ground Barley</td>
<td>10.91</td>
</tr>
<tr>
<td>Whole Cotton Seed</td>
<td>9.29</td>
</tr>
<tr>
<td>Corn Hominy</td>
<td>7.64</td>
</tr>
<tr>
<td>Canola Meal</td>
<td>7.58</td>
</tr>
<tr>
<td>Almond Hulls</td>
<td>3.77</td>
</tr>
<tr>
<td>Distillers Dry Grains</td>
<td>2.27</td>
</tr>
<tr>
<td>Tallow</td>
<td>1.62</td>
</tr>
<tr>
<td>Blood Meal</td>
<td>2.96</td>
</tr>
<tr>
<td>Calcite</td>
<td>0.64</td>
</tr>
<tr>
<td>Vitamin and Mineral Premix</td>
<td>1.62</td>
</tr>
</tbody>
</table>

**NUTRIENT ANALYSIS**

- Crude Protein: 19.46
- Acid Detergent Fiber: 26.84
- Neutral Detergent Fiber: 41.49
- Copper (ppm): 9.08
- Manganese (ppm): 18.54
- Zinc (ppm): 16.25

1Consisted of 15.2% Ca; 3.5% P; 2.0% Mg; 2.85% S; 9.0 ppm Se; 1.9 ppm Co; 1900 ppm Fe; 3800 ppm Mn; 4200 ppm Zn; 750 ppm Cu; 18.0% NaCl; 180,531 IU Vitamin A/kg; 35,225 IU Vitamin D/kg; 330 IU Vitamin E/kg.

2Treatment and Control Zn, Mn, Cu Composition.

**Treatment:**
- 50% ZnSO₄ - 50% Zn Proteinate
- 50% MnSO₄ - 50% Mn Proteinate
- 50% CuSO₄ - 50% Cu Proteinate

**Control:**
- 100% ZnSO₄
- 100% MnSO₄
- 100% CuSO₄
intervals and analyzed for plasma Cu and Zn levels. Plasma samples were analyzed with the use of a Thermo-Jarrell Ash - ICAP 9000 analyzer following a nitro perchloric acid digestion. Feed samples were collected weekly. Fecal samples were collected twice daily for 2 consecutive days during the last week of the trial. Feed and fecal samples were dried at 60 degrees Celsius for 72 h, ground through a Wiley mill (Thomas Wiley Laboratories, Swedesboro, N.J.) equipped with a 1-mm screen, and composited by cow treatment. Feed and feces were analyzed for DM (4), CP (14), ADF, and NDF according to Van Soest (53). Acid insoluble ash (AIA) was determined according to Undersander et al. (47) and used as an internal marker to determine apparent nutrient digestibility. The feet of each heifer were critically evaluated and scored according to six separate criteria at the start and at the finish of the trial using the same scoring methods as Moore et al. (36). Reproductive parameters were measured throughout the 20-week postpartum period. Observations for estrus behavior were completed twice daily, once in the AM hours and once in the PM hours (0700 and 1700 h). Days to first estrus, days to first service, days open, and services per conception were evaluated between treatment groups.

The data were analyzed by ANOVA with the general linear models procedure of SAS (42). Five separate statistical models were employed in the analysis of the data collected in this study. Digestibility, reproduction, and cow body weight gain prepartum were analyzed under a completely randomized model that included treatments as the only main effect. Calf birth weight was analyzed under a randomized block model with treatment and sex as main effects. Hoof score was analyzed under a split plot model with treatments and foot identification as main effects and cows nested within treatments. Mean separation was done
by Duncan multiple range test, with cow within-treatment effect as the error term. Blood characteristics were analyzed under a split plot model with treatment and collection (replicate) as main effects and cows nested within treatments. Dry matter intakes, body weight, milk yield, and milk composition were analyzed under a split plot model that included treatment and week as main effects, treatment by week interaction, and cow effects nested within treatments. Results are presented as least square means with significance defined at $P < .05$ unless otherwise noted.
RESULTS AND DISCUSSION

Prepartum Period

Effects of treatment on dry matter intake (DMI) and calf birth weights are presented in Figure 1. Dry matter intakes were similar for control heifers and those supplemented with metal proteinates. These results agree with other studies (25, 43) that have not detected a significant DMI performance response when treatment groups have been fed the same basal ration supplemented with metal proteinates or chelates. Calf birth weights are also presented in Figure 1. The mean calf birth weights were not significantly different for calves on the control group compared to the treatment group receiving the organic mineral supplement. Although not significantly different, control heifers weighed more throughout the prepartum period than did treatment heifers (Figure 2). It may be possible that heavier body-weight heifers yielded heavier birth-weight calves.

Postpartum Period

Effects of treatment on dry matter intake (DMI) are presented in Figure 3. Dry matter intake increased during the postpartum period although no significant difference was found between treatment groups. Weekly body weights are displayed in Figure 4. No significant differences were found in weekly body weight change. These results agree with Kincaid (24), where the addition of metal proteinates did not affect body weight. No differences were found in daily milk yield (Figure 5) throughout the 20-week lactation period. Treatment effects on milk composition is presented in Table 4. There were no significant difference in milk fat, protein, solids not fat, and somatic cell count. Percent
Figure 1. Effect of prepartum treatment on calf birth weight and dry matter intake.
Figure 2. Effect of treatment on prepartum body weights over time.
Figure 3. Effect of treatment on postpartum dry matter intake over time.
Figure 4. Effect of treatment on postpartum body weights over time.
Figure 5. Effect of treatment on average daily milk yield over time.
TABLE 4. Effect of treatment on milk composition.

<table>
<thead>
<tr>
<th>Component</th>
<th>Treatment</th>
<th>Control</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Mean</td>
<td>SEM</td>
</tr>
<tr>
<td>Butterfat (%)</td>
<td>3.95</td>
<td>0.11</td>
</tr>
<tr>
<td>Protein (%)</td>
<td>3.01</td>
<td>0.03</td>
</tr>
<tr>
<td>Lactose (%)</td>
<td>5.20</td>
<td>0.02</td>
</tr>
<tr>
<td>Solids not fat (%)</td>
<td>8.68</td>
<td>0.03</td>
</tr>
<tr>
<td>SCC Ln/ml²</td>
<td>9.99</td>
<td>0.17</td>
</tr>
</tbody>
</table>

*Means differ significantly (P < .05)

1Standard error of the mean.

2Somatic Cell Count Natural Logarithm.

Milk lactose was significantly higher (P < .05) for the metal proteinate-treated cows. The reason for improvement in milk lactose percent in treatment cows is not clear. One possible theory explaining increase in lactose percent in the treatment group is that chelates and proteinates participate in some enzymatic cofactor in lactose production. Therefore it would be incorrect to dismiss this difference as not being a treatment effect.

Treatment effect on apparent total tract nutrient digestibility for crude protein, acid detergent fiber, and neutral detergent fiber is listed in Table 5. No significant differences in nutrient digestibility were detected. Blood plasma mineral composition results for Cu and Zn are summarized in Figure 6. Concentrations of Cu and Zn in blood plasma did not differ significantly. The theory that metal proteinate inclusion increases mineral blood plasma concentrations has been previously pursued. Kincaid (24) observed higher plasma Cu levels in calves fed Cu proteinate during a nutritional Cu deficient period, suggesting that certain factors may contribute to a treatment effect on bioavailability at the blood level.
TABLE 5. Effect of treatment on total tract apparent nutrient digestibility.

<table>
<thead>
<tr>
<th>Item</th>
<th>Treatment</th>
<th>Control</th>
<th>SEM(^1)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Crude Protein (%)</td>
<td>73.8</td>
<td>72.00</td>
<td>0.56</td>
</tr>
<tr>
<td>Acid Detergent Fiber (%)</td>
<td>41.11</td>
<td>42.62</td>
<td>0.81</td>
</tr>
<tr>
<td>Neutral Detergent Fiber (%)</td>
<td>58.39</td>
<td>56.88</td>
<td>0.72</td>
</tr>
</tbody>
</table>

No significance between treatments.

\(^1\) Standard error of the mean.

Figure 6. Effect of treatment on mineral blood plasma composition.
Effects of treatment on hoof evaluation scores are shown in Table 6. A significant difference (P < .05) was found in the texture category, favoring the treatment group supplemented with the metal proteinates. Although no significance was shown, there was a numerical trend showing lower, more favorable hoof scores in the heel crack, laminitis, and white line disease categories for the treatment group. These results agree with Miller (34), who reported a highly significant difference (P < .01) in the texture category, favoring cows supplemented with zinc methionine throughout an entire lactation.

Treatment effects on reproductive parameters are reported in Table 7. In our study, days to first estrus were significantly lower (P < .05) in the treatment group supplemented with the metal proteinates. These results agree with Manspeaker et al. (27), who reported more ovarian follicular activity and increased estrus behavior in animals supplemented with amino acid-chelated minerals versus control animals. Although not significantly different, days to first service were greater in the treatment group. This difference can be explained by the mandatory 60-day waiting period employed prior to breeding. Because treatment cows returned to estrus sooner after parturition than did control cows, most treatment cows were not initially bred until the second or third postpartum heat. This could account for the anomaly that the average days to first service was higher for the treatment cows than for the control cows, even though treatment cows showed signs of estrus behavior earlier.
TABLE 6. Effect of treatment on hoof evaluation scores.

<table>
<thead>
<tr>
<th>Category</th>
<th>Initial Score</th>
<th>Post Treatment Score</th>
<th>Minimum Critical Range</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>TRT</td>
<td>CONT</td>
<td>TRT</td>
</tr>
<tr>
<td>Texture</td>
<td>2.10</td>
<td>2.48</td>
<td>1.62(^a)</td>
</tr>
<tr>
<td>Heel Cracks</td>
<td>1.61</td>
<td>1.77</td>
<td>1.38</td>
</tr>
<tr>
<td>Laminitis</td>
<td>1.68</td>
<td>1.80</td>
<td>1.38</td>
</tr>
<tr>
<td>White Line Disease</td>
<td>1.82</td>
<td>1.98</td>
<td>1.46</td>
</tr>
<tr>
<td>Interdigital Dermatitis</td>
<td>1.56</td>
<td>1.55</td>
<td>1.22</td>
</tr>
</tbody>
</table>

\(^a\,^b\) = Means are significantly different (P < .05).

Scoring: 1-5 1 = perfect, 2 = good, 3 = fair, 4 = poor, 5 = severe.

TABLE 7. Effect of treatment on reproductive parameters.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Treatment</th>
<th>Control</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Mean</td>
<td>SEM(^1)</td>
</tr>
<tr>
<td>Days to First Heat</td>
<td>39.51</td>
<td>3.84</td>
</tr>
<tr>
<td>Days to First Service</td>
<td>81.41</td>
<td>4.44</td>
</tr>
<tr>
<td>Days Open</td>
<td>94.83</td>
<td>8.88</td>
</tr>
<tr>
<td>Services per Conception</td>
<td>1.58</td>
<td>0.34</td>
</tr>
</tbody>
</table>

\(^*\) Means differ significantly (P < .05).

\(^1\) Standard error of the mean.
CONCLUSIONS AND IMPLICATIONS

This study was conducted to measure the response of feeding a chelated metal proteinate supplement in a TMR diet fed to Holstein cows. It has been shown that under certain conditions ruminants respond to trace mineral proteinates or chelates through increased growth, milk production, reproduction, and immune responsiveness. In this study we found marked improvement in both reproductive performance and hoof quality. When compared to the control group, the treatment group had a significantly shortened interval to first detectable estrus. In a comparison of hoof integrity, the treatment group had significantly better hoof texture and overall improved hoof quality. Based on these findings, it can be concluded that the bioavailability of dietary minerals is enhanced by chelation, resulting in beneficial effects in primiparous dairy cattle. Conducting a similar trial using mature multiparous cows in a longer treatment period may show more significant differences with regard to treatment effects seen in this study.

Further research with metal proteinates is needed to: 1) define the optimal level of proteinates added to the diet, 2) better define conditions where performance responses may be seen, and 3) more closely determine the mode of action of metal proteinates to improve performance in ruminant animals.
REFERENCES


