Relationship of Copper, Zinc and Selenium Status with Udder Health and Mastitis Incidence in the Cal Poly Holstein Herd

by

Michelle Van Ryn

Dairy Science Department

College of Agriculture, Food and Environmental Sciences

California Polytechnic State University

San Luis Obispo

2009

ACKNOWLEDGMENT

I am very grateful for the proposal that Dr. Leanne Berning and Dr. Edwin Jaster created in order for this study to be funded and implemented. I am also very appreciative that they chose me of the many students to perform this study. I greatly thank Dr. Berning for her many mornings of kind assistance in collecting milk and blood samples. The financial support provided from the Virgil Leinardi Trust Fund is greatly appreciated along with the financial support received from my parents, Bill and Arlene Van Ryn, who paid for my tank of gas that brought me to the Cal Poly dairy every two weeks during the past two summers.

TABLE OF CONTENTS

Page

LIST OF TABLES

Table Page

LIST OF FIGURES

Figure Page

ABSTRACT

Mastitis is one of the main diseases that affects the profitability of dairy farmers. Mastitis results from immune system failure of the cow. There is a strong relationship between blood concentrations of micronutrients and immune function. There are many interrelationships of the nutrients and effects of over-supplementing certain nutrients on immune function that can create health problems. Nutrient uptake and micronutrient requirement vary due to stage of lactation and health status. In this study the baseline micronutrient status of a portion of the Cal Poly Holstein herd was determined with respect to copper, zinc, and selenium. Micronutrient status with various measures of udder health was analyzed. With this information, recommendations are formulated to the dairy regarding current micronutrient feeding practices relative to improvement of udder health. Cows were tested for blood micronutrient concentrations, somatic cell count, and teat end condition score at 3 points during a lactation: within 2 weeks after calving, in the fifth month of lactation, and within 2 weeks before dry off. Copper deficiencies were found at all 3 points of the lactation. Zinc deficiencies occurred in cows within 2 weeks after calving and within 2 weeks before dry off. There were no selenium deficiencies. Recommendations were made to increase copper levels in the diet and to review the use of copper sulfate. It is suggested that yeast culture concentration be increased in the diet to further aid in the absorption of zinc.

INTRODUCTION

Milk quality can most often be defined in terms of somatic cell count. Milk with a low somatic cell count is visibly normal and is considered high quality. Mastitis is a costly and common disease in the dairy industry. Nationally, mastitis costs producers approximately six percent of the value of production (27). Mastitis is caused from immune system failure of the cow. The effectiveness of mastitis treatment varies widely according to the pathogen involved and many other factors. The best approach for dealing with mastitis in a dairy herd is prevention. Strong attention needs to be placed toward the health of the cow's immune system. It is critical to the success of mastitis prevention.

There has been numerous work documented on the relationship between blood concentrations of micronutrients and various aspects of immune function, however significant questions remain unanswered. Among these questions are 1) The interrelationship of one nutrient on the requirement for another. 2) The effect(s) of over supplementation on immune function and associated health problems. The cow receives her micronutrient requirements from her diet. There are negative factors that affect the micronutrient levels that are available in different feedstuffs which include soil type, harvest, and storage conditions. The cow's uptake and requirement of micronutrients can also vary due to stage of lactation and health status. The main objective of this study is to determine the baseline micronutrient status of the Cal Poly Holstein herd with respect to copper, zinc, and selenium. Once determined, a

second objective is to relate micronutrient status to various measures of udder and teat health and clinical mastitis treatment data. The final objective of this study is to formulate recommendations to the dairy regarding current micronutrient feeding practices relative to improvement of udder health.

LITERATURE REVIEW

Mastitis

Mastitis is an inflammation of the mammary gland in response to injury for the purpose of destroying the infectious agents that have entered the udder. Inflammation can be caused by physical trauma or infectious agents and their toxins. Mastitis is most commonly caused by bacteria that invade the udder and multiply in the milk secretory tissue. The udder is composed of 4 quarters and each quarter consists of a teat cistern, gland cistern, milk ducts, and glandular tissue (29). The glandular tissue contains millions of sacs called alveoli that are lined with milk epithelial cells. There is muscle-like tissue surrounding the alveoli (myoepithelial cells) that contracts in order for milk to be released during the milking process. Nutrients are carried to the alveoli through blood vessels in order for epithelial cells to convert the nutrients to milk.

The teat end is the first barrier the cow has to protect against mastitis infection. A smooth sphincter surrounds the streak canal and helps to keep the streak canal closed in order to prevent milk from escaping and inhibit microorganisms from entering into the udder. The cells lining the streak canal produce keratin. The keratin forms a barrier against bacteria. Trauma to the teat end can damage the keratin causing the udder to be more susceptible to bacteria invasion. Contagious organisms are most common in the cow's milking environment (2). Milking equipment may be

contaminated or the cow may have an unclean udder or teat end that would allow bacteria to enter the udder when the teat end is opened for milk to be released.

Milk Quality

Mastitis reduces milk yield and alters milk composition. The severity of these effects depends on the duration and type of infection. The toxin produced by microorganisms can damage milk producing tissue and cause inflammation. The inflammation affects the quality of the milk produced. Mastitis is considered the most limiting factor to profitable dairy production (25). The National Mastitis Council estimates that mastitis costs dairy producers in the United States over 2 billion dollars annually (24). Milk quality is primarily measured through somatic cell count. Somatic cell count measures the amount of leukocytes, which are white blood cells, that include macrophages, lymphocytes, and polymorphonuclear neutrophilic leukocytes. Milk that is from uninfected quarters generally has a somatic cell count of 200,000/ml or less. A somatic cell count of 300,000 or more indicates an inflammation of the udder (12). Incidences of mastitis are not only costly to the dairy producer, but to the processors as well. With increased levels of somatic cell count, the types of proteins in the milk change dramatically. The cheese yield, quality of product, and flavor are affected. Many processors pay a premium to dairy producers who fall within a certain level of bulk tank somatic cell count.

Mastitis Prevention

The best approach to deal with mastitis is prevention. Strong attention needs to be paid toward the environment of the cow and the health of her immune system. There are contagious pathogens and environmental pathogens that most commonly cause mastitis. The National Mastitis Council recommends a 10 point control program to prevent mastitis that includes: 1) establishment of goals for udder health 2) maintenance of a clean, dry, comfortable environment 3) proper milking procedures 4) proper maintenance and use of milking equipment 5) good record keeping 6) appropriate management of clinical mastitis during lactation 7) effective dry cow management 8) maintenance of biosecurity for contagious pathogens and marketing of chronically infected cows 9) regular monitoring of udder health status 10) periodic review of mastitis control program (4). The health of the cow's immune system depends on quality and nutrient availability in the feed ration. Meeting the nutritional needs of the dairy cow is a crucial point to consider when preventing mastitis. The cow's uptake and nutrient requirements will vary due to stage of lactation and health status. That is why it is very important to have a well planned feeding program.

Contagious Mastitis Pathogens

Major contagious mastitis pathogens include *Streptococcus agalactiae* and *Staphylococcus aureus.* The primary source of these organisms is the udder of the infected cow. The spread most commonly occurs during the milking process from infected to uninfected cows through teat cups, the hands of the workers who milk, or

contaminated cloths used for wiping teats. Common indicators of the presence of contagious mastitis is a bulk tank somatic cell count over 300,000, more than 15 percent of cows with a DHIA somatic cell score of 5 or greater, frequent flare ups in the same cows, or a bacterial culture that indicates that these particular microorganisms are present (29).

Environmental Mastitis

Environmental mastitis caused by coliforms includes *Escherichia coli, Klebsiella pneumoniae, Klebsiella oxytoca,* and *Enterobacter aerogenes*. Environmental streptococci include *S. uberis, S. bovis, S. dysgalactiae, Enterococcus faecium*, and *Enterococcus faecalis*. The primary source of environmental pathogens causing mastitis is the environment of the cow. Infections usually occur between milkings, when the cow is exposed to her environment (24). It is important for the cow's bedding to be dry and clean. This would require manure removal on a daily basis and complete bedding replacement on a regular basis as well. Implementing these practices will improve the cleanliness of the udder and teat ends upon arrival into the milking parlor.

Control Strategies

Various strategies should be implemented to control mastitis. Pre-milking and post-milking teat disinfecting solutions are essential for cleaning the teat ends before milking and after milking to reduce pathogen contamination. Vaccines may also be

used before the onset of lactation to help reduce severity or duration of mastitis. Culling chronically infected cows is also very important to reduce the spread of infection from an infected cow to an uninfected cow. The transition cow diet is also very important to monitor closely and cows should receive adequate nutrients based upon the nutrient requirements that are presented from the National Research Council.

Nutrition and Udder Health

The risk that the cow will develop mastitis is primarily due to the quantity of pathogens present at the teat end and the cow's ability to prevent bacterial infection. Nutrition has a direct effect on the cow's immune system, therefore indicating that nutrition indirectly influences infection rate and severity of mastitis. High requirements of specific nutrients are necessary for optimal immune system function. At the 2009 National Mastitis Council (NMC) regional meeting, Dr. Bill Weiss from Ohio State University presented a review on nutritional influences on immune function and mastitis during the periparturient period. Three of his messages on how to improve mammary gland health were described in the August 2009 NMC newsletter (22) as follows:

> 1. Feed and manage late-lactation and dry cows to maintain proper body condition. Normal, healthy cows may lose .25 to .5 body condition score units in early lactation.
> 2. Prevent hypocalcemia through proper mineral nutrition for dry cows. Cows with milk fever are much more likely to get clinical mastitis than cows without milk fever.
> 3. Feed adequate, but not excessive, amounts of trace minerals and vitamins. Key nutrients include vitamin A, beta-carotene, copper, zinc, selenium, and

vitamin E. Increasing vitamin E supplementation should be considered during pre-fresh period.

Antioxidant Systems

When the mammary gland becomes infected, there are a substantial amount of free radicals or reactive oxygen species that are produced during an inflammation response. The major free radicals found in biological systems are superoxide, hydrogen peroxide, hydroxyl radical, and fatty acid radicals (32). When there are adequate levels of antioxidants available, the free radicals are kept under control and the lifespan of certain immune cells increases. If there are limited antioxidants available, the lifespan of certain immune cells is reduced and the infection can become established and increase in severity (33). Free radicals are toxic to cells and can cause cell damage or death when reacting with enzymes, cell membranes, and DNA. In order to control these free radicals, the cow has an antioxidant system that relies on antioxidant nutrients (Table 1). Trace minerals that are a part of enzymes and some vitamins are key components of the antioxidant system. According to W.P. Weiss from the Agricultural Research and Development Center, "Known antioxidant pathways suggest that the requirements of antioxidant nutrients are interrelated. A deficiency of one antioxidant may increase the requirement of another nutrient. However, a deficiency of a particular antioxidant nutrient cannot be alleviated fully by another nutrient" (32).

TABLE 1. Some of the antioxidant systems found in mammalian cells (32).

Component (location in cell)	Nutrients Involved	Function
Superoxide dismutase (cytosol)	Copper and zinc	An enzyme that converts superoxide to hydrogen peroxide
Superoxide dismutase (mitochondria)	Manganese and zinc	An enzyme that converts superoxide to hydrogen peroxide
Ceruloplasmin	Copper	An antioxidant protein, may prevent copper, from participating in oxidation reactions
Glutathione peroxidase (cytosol)	Selenium	An enzyme that converts hydrogen peroxide to water
Catalase (cytosol)	Iron	An enzyme (primarily in liver) that converts hydrogen peroxide to water
Alpha-tocopherol (membranes)	Vitamine E	Breaks fatty acid peroxidation chain reactions
Beta-carotene (membranes)	Beta-carotene	Prevents initiation of fatty acid peroxidation chain reactions

Supplementing the cow's diet with a nutrient that is involved with the antioxidant system will not necessarily improve the cow's health. Also, excessive supplementation of any of the nutrients listed in Table 1 may increase oxidative stress, decrease immune function, and increase health problems (32).

Mastitis and Antioxidants

The cow is working to maintain a natural balance between the formation of free radicals that occur during normal metabolism of the cells and the capacity of the antioxidant system that prevents free radicals from accumulating and harming cells. Oxidative stress occurs when the free radicals exceed the antioxidant capacity of the animal. High producing dairy cows are more prone to oxidative stress and can undergo severe stress in certain environmental, physiological, and dietary conditions (31). There have been numerous experiments that have examined the influence of nutrients on immune function.

Copper

Copper is involved in the antioxidant system through its presence in several significant proteins. Copper is present most commonly in the proteins ceruloplasmin and superoxide dismutase (SOD). Ceruloplasmin activity is diminished or absent without sufficient copper. It is released into the blood from the liver and constitutues about 60% of the circulating copper in the blood after meals (9). Ceruloplasmin functions include transportation of copper in the blood to various tissues, oxidizing minerals most notably iron and manganese, and scavenging oxygen radicals to protect cells. As a modulator of the inflammatory process, ceruloplasmin serves as an acute-phase protein. Acute phase proteins rise in the blood with infection and other inflammatory events (9). The enzyme SOD, which is found both in the cytosol of cells and extracellularly, is copper and zinc dependent. Without the presence of SOD,

superoxide radicals can form more destructive hydroxyl radicals that damage both unsaturated double bonds in cell membranes, fatty acids, and other molecules in cells. Therefore, SOD assumes a very important protective function (9). A study was conducted on first lactation Holstein heifers to assess a potential role for dietary copper in enhancing resistance to *E. coli* mastitis. Conclusions were made that copper supplementation reduced the severity of clinical signs during experimental E. coli mastitis but the duration of mastitis was unaffected (26).

Zinc

Functions of zinc include tissue or cell growth, cell replication, bone formation, skin integrity, cell-mediated immunity, and generalized host defense (8). The mammary gland is an organ that is derived from the skin, thus making zinc necessary to maintain the integrity of the keratin that lines the streak canal. Zinc has a significant effect on gene expression and cellular growth. An experiment was done that included 12 lactation trials addressing zinc supplementation. Supplementing zinc resulted in a 33% reduction in somatic cell count. However, not all forms of organic zinc showed a positive effect on mastitis (30). Cell mediated immunity has also been found to be altered by zinc deficiency. Zinc deficiency has been associated with reduced formation of both T and B lymphocytes and phagocytes (28). T and B cells are the major cellular components of the adaptive immune response. Once they have recognized an invader, the cells generate specific responses that work to eliminate pathogens or pathogen infected cells. As previously discussed, zinc is also involved

in the removal of free radicals by SOD. Extracellular and cytosolic SOD require both zinc and copper (6). Another important factor to note in regard to the cow's immune response is that immunoactive substances such as vitamin A have been found to react with zinc in several ways. Zinc is necessary for the hepatic synthesis of retinol-binding protein, which transports vitamin A in the blood (8).

Selenium

One of the most clearly established functions of selenium is that it is a cofactor for the enzyme glutathione peroxidase. Glutathione peroxidase (GPX) is mainly found within the cytosols (70%) of cells and the mitochondrial matrix (30%) (10). The enzyme, GPX, is responsible for catalyzing the removal of hydrogen peroxide and the reduction of other peroxides from tissues, which is an essential process of the immune system. The enzyme requires selenium as a cofactor or else its activity is impaired. A study on the relation of selenium to udder health was conducted by Malbe et al. in 1995 (16). The effects of feeding organic selenium in the form of selenized yeast and sodium selenite were compared in a feeding experiment on 100 dairy cows. Feed was supplemented at a rate of .2 ppm for 8 weeks. The results showed that the yeast selenium yielded the greater blood level of selenium. The GPX level went from .22 to 3.0 and to 2.3 microKat/g of hemoglobin from selenium yeast and selenite, respectively. A microKat is a unit of measurement used in chemistry to describe enzyme reactions. Enzymes are proteins that act as catalysts in the body. A catalyst measures 1 katal if it causes a chemical reaction at 1 mole per second (5). Blood

GPX continued to increase up to 10 weeks after the supplementation had stopped. The bioavailability of yeast selenium was found to be superior to selenite. The percentage of quarters harboring mastitis dropped along with the somatic cell count in the milk. It is important to note that the test cows began the trial with a significantly low blood selenium status.

National Research Council Requirements for Trace Minerals and Vitamins

To determine some of the trace nutrient levels that are required, immune function and clinical mammary gland health data was used. When formulating rations, it is important to begin with the National Research Council's (NRC) requirements on trace minerals and vitamins. Supplementing over the NRC levels will not improve mammary gland health except in certain limited situations (32). The NRC requirements assume the bioavailability of minerals and vitamins to be normal. It is important to note that, if excessive antagonists are being fed, it may reduce the availability of certain nutrients, making the NRC requirements inadequate. NRC trace mineral requirements are given as milligrams per day and not as dietary concentrations. In order to determine dietary concentrations, dry matter intake must be known as well as the absorption coefficient of the mineral. Average absorption coefficients are made available in the NRC (2001). For vitamins, NRC recommendations are expressed as IU needed per unit of body weight (32).

NRC Copper Requirements

The new NRC has increased the copper requirement of lactating and dry cows. An average Holstein cow producing 50 or 100 pounds of milk needs to consume 225 mg or 300 mg of copper per day, respectively. The recommendations assume normal concentration of sulfur and molybdenum. The copper recommendations will not be adequate when diets or drinking water contain excessive concentrations of sulfur or molybdenum. Copper is the most toxic mineral that is routinely supplemented. The recommended safe concentration of feeding is only four to five times the requirement (32).

NRC Zinc Requirements

The new NRC zinc requirement is about 900 mg/day for a cow producing 50 pounds of milk and 1400 mg/day for a cow producing 100 pounds of milk. Clinical data is lacking making it unclear whether these concentrations are adequate for optimal mammary gland health (32). Cows can tolerate relatively high dietary concentrations of zinc, which is about ten times the requirement. Excessive zinc supplementation, however, can cause a secondary copper deficiency because of their interaction. Zinc should not exceed five times the concentration of dietary copper.

NRC Selenium Requirements

The concentration of supplemental Se is regulated by the United States FDA at .3 ppm, and the new NRC requirement sets the requirement at .3 ppm for lactating and

dry cows. There is no clinical data that indicates feeding over these levels will improve mammary gland health.

Recommended Dietary Concentrations

The requirements set by the NRC take into account important sources of variation that include intake and bioavailability. The NRC requirements should be considered minimum requirements and they do not include any safety margins. Diets should be formulated to provide more than the recommended amounts of some minerals because of variation in intake, environment, and feed composition in order to ensure all cows consume adequate amounts of nutrients. Table 2 illustrates the values for a Holstein cow with an average body weight (1500 Lbs) for various stages of lactation and gestation. Pre-fresh is for cows in the last two weeks of gestation. Fresh is for cows in the first three weeks of lactation.

TABLE 2. Suggested dietary concentrations (dry matter basis) of trace nutrients (32).

	Nonlactating cows		Lactating cows (milk yield)		
	Dry	Pre-fresh	Fresh Cow	50 lb	100 lb
Est. intake, Lb/day	30	22	30	44	58
Vitamin A, IU/day	3300	4500	3300	1850	1500
Vitamin E, IU/day	35	50	25	12	10
Selenium, PPM	0.3	0.3	0.3	0.3	0.3
Copper, PPM	20	20	15-20	15-20	15-20
Manganese, PPM	30-50	40-50	40-50	30-40	30-40
Zinc, PPM	40-60	50-70	60-80	50-70	60-80

MATERIALS AND METHODS

Animals and Management

Twenty-four Holstein milking cows housed at the California Polytechnic State University dairy facility in San Luis Obispo were each monitored during one complete lactation cycle. The cows stayed in one group and received a common ration throughout their lactation. The feed ration was adjusted throughout their lactation based on changing feed prices and milk prices. As of September 21, 2009, the Holstein cows were receiving a diet that consisted of 52 pounds of dry matter that was 18.339 percent protein. Details of the feed ingredients and nutrients at three different stages of lactation are illustrated in table 3. Table 4 includes information for the mineral mix the cows received consistently throughout the lactation. The group consisted of 14 first lactation cows, 4 second lactation cows, 4 third lactation cows, and 2 fourth lactation cows. The cows were tested at 3 different stages of their lactation cycle: Within 2 weeks after freshening, in the fifth month of lactation, and within 2 weeks before drying off. Testing began on June 25, 2008 and calving proceeded until March 25, 2009.

TABLE 3. Diet summary for Holstein lactating cows reported within testing dates on April 21, 2009 and September 21, 2009.

Ingredient/Nutrient	4/21/09 DM, Lb/Conc.	9/21/09 DM, Lb/Conc.
Almond hulls	4.38	2.67
Alfalfa hay Nipomo	6.74	
Alfalfa hay Delta	6.57	13.95
Oat hay CP		1.70
Winter forage silage	8.78	8.00
Total forage	26.47	26.32
MEGALAC PLUS	.45	.34
MEGA LAC R	.48	.36
Cal Poly Lact TX	24.60	24.98
Total concentration	25.53	25.68
Total ration	52.00	52.00
Crude protein	18.381 %	18.339 %
Sol protein, % CP	39.80 ratio	37.72 %
RDP, % of CP	62.03 ratio	62.10 ratio
RUP, % of CP	37.93 ratio	37.86 ratio
ADF	16.505 %	16.758 %
NDF	28.734%	29.087 %
Digestible NDF	15.864 %	14.496 %
Roughage NDF	18.422%	19.559 %
Fat	4.591 %	4.498 %
Unsaturated fat	2.659 %	2.705 %
NF carbohydrates	39.980 %	40.438 %
NE lact 3X	78.981 mcal/cwt	77.644 mcal/cwt
Calcium	.950 %	.899 %
Phosphorus	.373 %	.438 %
Potassium	1.911 %	1.419 %
Magnesium	.320 %	.381 %
Sulfur	.248 %	.268 %
Added salt	.300 %	.296 %
Dietary cation/anion	36.973 meq/100g	20.930 meq/100g
Vitamin A	8.265 IU/g	8.248 IU/g
Vitamin D	1.413 IU/g	1.413 IU/g
Vitamin E	38.429 IU/kg	38.646 IU/g

TABLE 4. Cal Poly milk cow mix designed for lactating dairy cattle that was provided to the 24 cows throughout their lactation at a rate of 27.8 Lbs per head per day.

Guaranteed Analysis:				
Crude protein	Min.	18.00 %		
Crude Fat	Min.	3.50 %		
Crude Fiber	Max.	3.50 %		
Calcium	Min.	0.30 %		
Phosphorus	Min.	0.35 %		
Sodium	Max.	1.00 %		
Added Selenium	Min.	0.50 PPM	Max.	0.60 PPM

Ingredients:
Steam flaked corn, ground corn, wheat middlings, corn germ meal, maize distillers dried grains with solubles, canola meal, ground grains, pima cottonseed, animal blood dried, Sodium sesquicarbonate, dehulled soybean meal, urea, calcium carbonate, salt, magnesium oxide, yeast culture, DL- methionine hydroxyl, analogue, sodium selenite, zinc methionine, manganese methionine, copper lysine, cobalt glucoheptonate, vitamin E supplement, biotin, zinc oxide, manganous oxide, ferrous sulfate, vitamin A supplement, vitamin D3 supplement, ethylenediamine dihydriodide, mineral oil, cobalt carbonate, copper oxide, copper sulfate, cobalt sulfate

Blood Sampling

At each stage of sampling for each cow, coccygeal blood samples were collected in the morning following the 3:30 AM milking shift. Upon completion of milking, cows would return to their corral where a total mixed ration was available for consumption. Once cows arrived at the manger, stanchion lockups would keep them in place to allow for blood collection. Before sampling, the skin was cleaned with alcohol. Blood was collected using a 10 cc syringe with a 20 gauge needle. The blood collected was then injected into a vacuum tube designed for trace mineral analysis. A

10 milliliter royal blue top test tube (BD Vacutainer®, BD Diagnostics Franklin Lakes, NJ) was used to allow for serum testing of trace mineral and a 3 milliliter lavender top test tube was used to allow for blood testing of selenium. The blood samples were then shipped that morning for analysis to the California Animal Health and Food Safety Lab at the University of California-Davis.

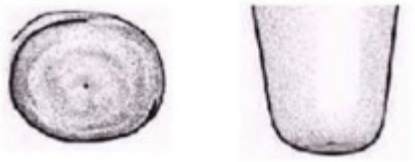
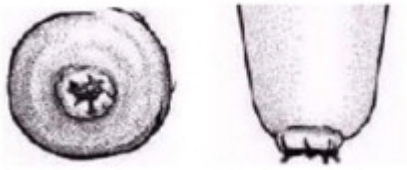
Somatic Cell Determinations

Somatic cell concentrations (SCC) were assessed on composite milk samples at each stage of the lactation. To perform this task, a DeLaval portable somatic cell analyzer was used. When the cows displayed clinical symptoms of mastitis or indicated a SCC of 400,000 or greater, quarter samples were evaluated using a CA mastitis test.

Teat End and Teat Skin Condition Analysis

All cows in the study were analyzed at the three different stages of lactation for teat end condition. The method of Mein and coworkers (20) was used for this evaluation. Table 5 illustrates the scoring guide. Evaluation of the results was used to interpret the incidence of teat condition problems. The numbers of cows with lower teat end scores were compared with the number of cows with higher scores to identify if their teat end condition resulted in a greater proportion of mastitis.

TABLE 5. Bovine teat-end condition scoring system (14, 20).

Teat score	Description	Illustration
1 (N)	No ring The teat-end is smooth with a small, even orifice. This is typical status for many teats soon after the start of lactation.	
2 (S)	Smooth or slightly rough ring A raised ring encircles the orifice. the surface of the ring is smooth or it may feel slightly rough but no fronds of old keratin are evident.	
3 (R)	Rough ring A raised, roughened ring with isolated fronds or mounds of old keratin extending 1-3 mm from the orifice.	
4 (VR)	Very Rough ring A raised ring with rough fronds or Mounds of old keratin extending 4 mm or more from the orifice. The rim of the ring is rough and Cracked, often giving the teat-end A “flowered” appearance.	

Statistical Analysis

Correlation of blood micronutrient status with clinical and subclinical mastitis data as well as teat condition was conducted. All data was entered into a Microsoft Excel spreadsheet where graphs and charts were generated using the data. Micronutrients in

the blood samples were analyzed and considered marginal/deficient (M/D), or adequate (A). The level considered A for copper is above .80 PPM and the level for M/D is below .50 PPM (3). The level considered A for zinc is above .80 PPM and the level for M/D is below .60 PPM (11). The level considered A for selenium is above .08 PPM and the level for M/D is below .06 PPM (11). For observing the correlation of stage of lactation, somatic cell count, and micronutrient levels, a linear model was used. Frequency probability was used to examine the number of times when a particular event occurred over a certain number of trials. Copper, zinc, and selenium were evaluated using this method. The marginal and deficient occurrences of the 3 micronutrients were correlated with a somatic cell count (SCC) that was over 200,000. In this case, the total number of trials will be the M/D occurrences (denominator) and the event that occurred will be when SCC is over 200,000 (numerator).

RESULTS AND DISCUSSION

Copper

Stage of lactation had a noticeable effect on the concentration of serum Cu, and the lowest concentration was found within 2 weeks before dry off. The highest concentration was within 2 weeks post-calving and the levels in the fifth month of lactation varied (Table 6). Within 2 weeks after calving, 20.88 % (5/24) of the cows had a Cu concentration of M/D. In the fifth month of lactation, 41.67 % (10/24) of the cows had a Cu concentration of M/D. Within 2 weeks before dry off, 62.5 % (15/24) of the cows had a Cu concentration of M/D.

TABLE 6. Mean concentration of serum copper, zinc, and selenium in blood samples taken at 3 points during lactation from each of the 24 dairy cows.

		Stage of lactation (days relative to calving)		
Variable	Units	14 days	155 days	Dry off
Cu	PPM	.970 (±.22)	.896 (±.27)	.739 (±.13)
Zn	PPM	.930 (±.14)	1.050 (±.13)	1.079 (±.19)
Se	PPM	.178 (±.059)	.253 (±.02)	.238 (±.01)
SCC		424,958	146,167	495,583
Teat end score		1.33 (±.58)	2 (±.75)	2 (±.55)

Figure 1 illustrates the Cu concentration for each cow at the 3 stages of lactation.

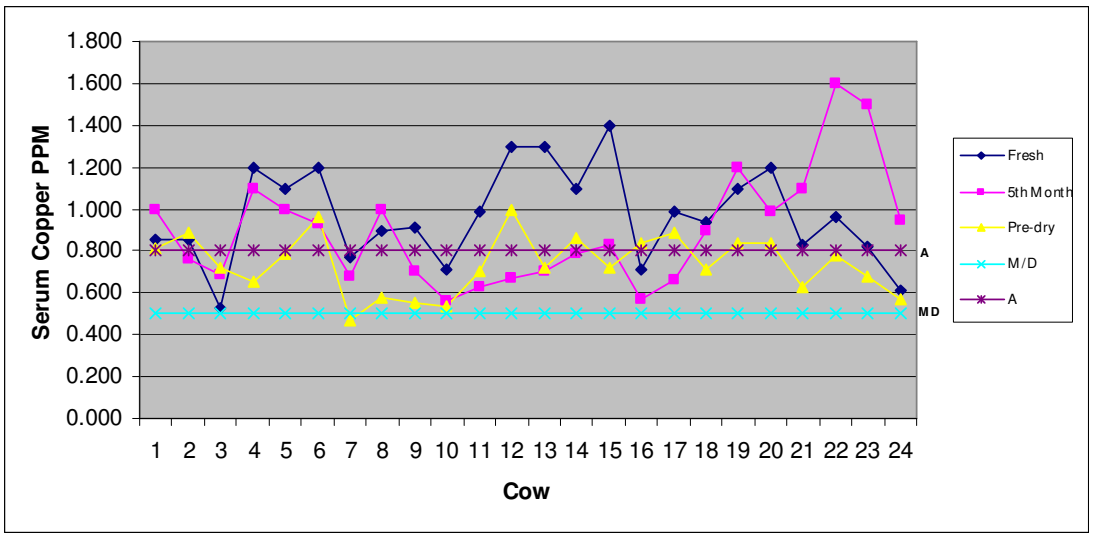


Figure 1. Serum copper concentration in blood samples taken from 24 cows at 3 different stages of lactation.

Zinc

Zinc concentrations were low at the onset of lactation, however, over the lactation the levels of zinc increased in 70.83 % (17/24) of the cows. Within 2 weeks after calving, 12.5 % (3/24) of the cows had a Zn concentration of M/D. In the fifth month of lactation, 0 % (0/24) of the cows had a Zn concentration of M/D. Within 2 weeks before dry off, 8.33 % (2/24) of the cows had a Zn concentration of M/D. There were increases in Zn concentration from within 2 weeks of lactation and in the fifth month of lactation for 79.17% (19/24) of the cows. However, the concentrations then decreased from the fifth month of lactation to within 2 weeks before dry off for 83.33% (20/24) of the cows. The average increase of Zn concentration from the first

stage to the second stage of lactation was .19 PPM. Figure 2 illustrates the Zn concentrations of each cow at the 3 stages of lactation.

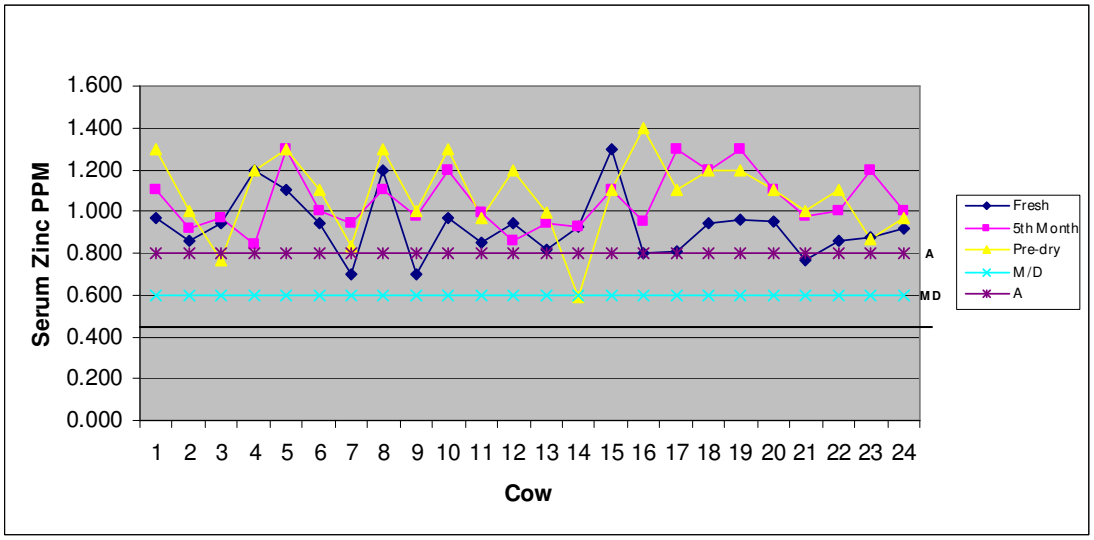


Figure 2. Serum zinc concentration in blood samples taken from 24 cows at 3 different stages of lactation.

Selenium

There were no deficiencies of Se in the blood samples at all 3 different stages of lactation. Figure 3 illustrates the Se concentrations of each cow at 3 stages of lactation. Selenium was found to be added in the Cal Poly milk cow mix provided from Cargill at a concentration of .50 to .60 PPM. Mean selenium concentrations were found to be lower within 2 weeks of lactation at .178 PPM, and then increased to .253 PPM in the fifth month of lactation. The levels decreased within 2 weeks of dry off to .238 PPM indicating a lower dietary se concentration in the dry cow diet.

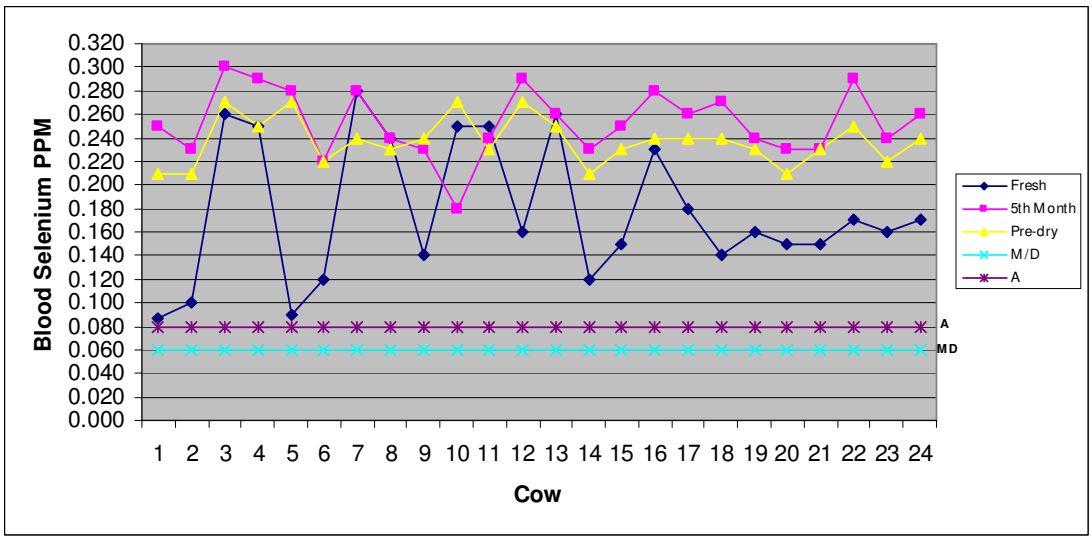


Figure 3. Blood selenium concentration in blood samples taken from 24 cows at 3 different stages of lactation.

Somatic Cell Count

Within 2 weeks of lactation, the SCC of 29.17 % (7/24) of the cows was over 200,000. In the fifth month of lactation, 25 % (6/24) of the cows had a SCC over 200,000. Within 2 weeks before dry off, 54.17 % of the cows had a SCC over 200,000. Figure 4 illustrates the somatic cell count of the cows at 3 different stages of lactation. Average somatic cell count for cows within 2 weeks of calving was 424,958. Average somatic cell count for cows in the fifth month of lactation was 146,167 and average somatic cell count for cows within 2 weeks before dry off was 495,583.

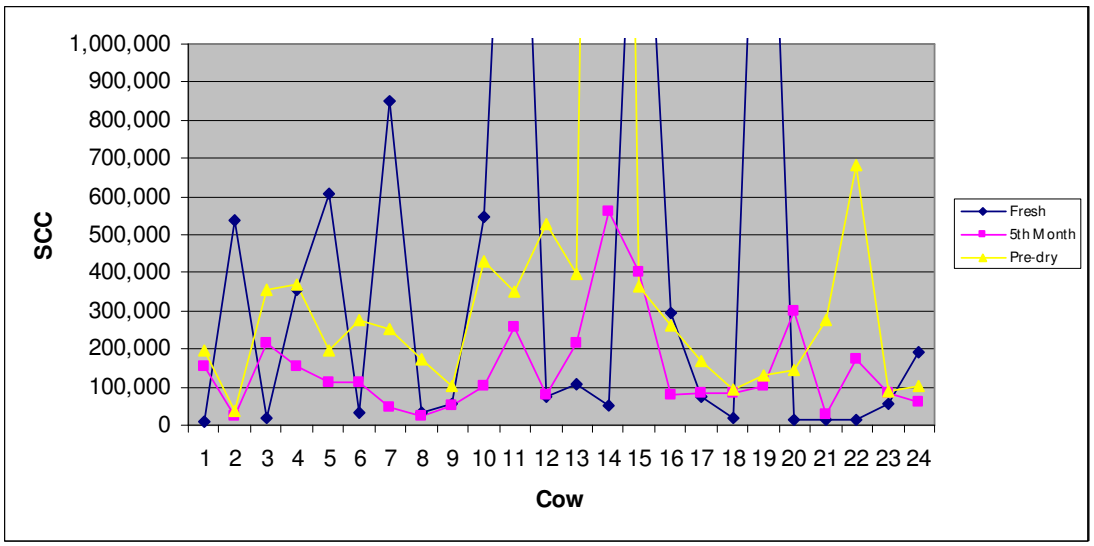


Figure 4. Somatic cell counts of 24 cows at 3 different stages of lactation.

Frequency probability was used to examine the number of times when a particular event occurred over a certain number of trials. Copper, zinc, and selenium were evaluated using this method. The marginal and deficient occurrences of the 3 micronutrients were correlated with a somatic cell count (SCC) that was over 200,000. In this case, the total number of trials will be the M/D occurrences (denominator) and the event that occurred will be when SCC is over 200,000 (numerator) within those animals that have a M/D level of nutrient concentration. A copper deficiency at the beginning of lactation was more closely associated to elevated SCC at the beginning of lactation than it was at 5 months into lactation. Low zinc concentrations at the end of lactation also had an effect on somatic cell count. Table 7 illustrates the frequency of a SCC over 200,000 for each micronutrient M/D level.

TABLE 7. Frequency probability of SCC over 200,000 for each micronutrient MD level.

Lactation Stage	Copper	Selenium	Zinc
1	60 % (3/5)	no deficiency	33.33 % (1/3)
2	30 % (3/10)	no deficiency	no deficiency
3	60 % (9/15)	no deficiency	100 % (2/2)

The lactation number of each cow did not have a significant effect on the somatic cell count. Table 8 illustrates the mean somatic cell count for each lactation number and each stage of lactation.

TABLE 8. Lactation number and mean somatic cell count at the 3 different stages of lactation of the 24 cows.

	Mean Somatic Cell Count		
Lactation Number	Stage 1	Stage 2	Stage 3
1 n=14	254,643	125,286	232,500
2 n=4	113,500	238,000	1,704,000
3 n=4	1,334,500	155,000	282,500
4 n=2	421,000	91,000	346,500

Daily milk production from Cal Poly's Dairy Herd Improvement computer program was evaluated for the 24 cows and was correlated with SCC at the 3 different stages of lactation. Figure 5 illustrates the relationship between milk production and SCC at

the 3 different stags of lactation. Variance in SCC over a lactation increased for the higher producing cows above 75 Lbs of production.

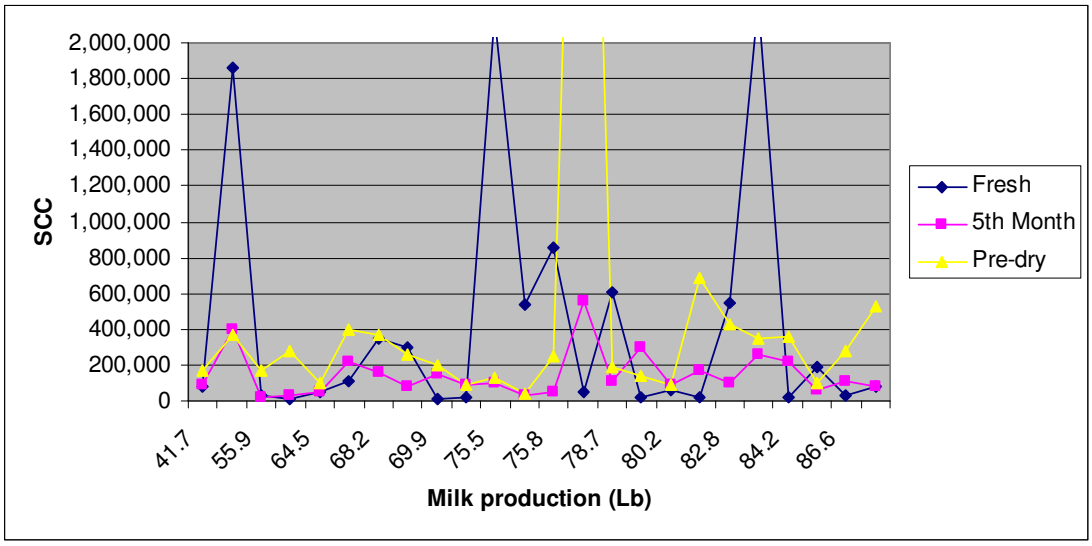


Figure 5. Daily milk production of the 24 cows and SCC at 3 different stages of lactation.

Teat End Score

A teat end score was given to each cow at 3 stages of lactation. Frequency was used to examine the relationship between micronutrient concentrations of M/D levels and teat end score. For each micronutrient M/D level that occurred, the probability of a teat end score of 2 or greater was measured. Table 9 illustrates these probabilities at the 3 different stages of lactation.

TABLE 9. Probability of a teat condition score of 2 or greater with a micronutrient deficiency.

Lactation Stage	Copper	Selenium	Zinc
Fresh	60 % (3/5)	no deficiency	33.33 % (1/3)
5th month	90 % (9/10)	no deficiency	no deficiency
Pre-dry	80 % (12/15)	no deficiency	100 % (2/2)

Parity had an effect on teat end score within 2 weeks of lactation. First lactation cows had 0 % (0/24) of the cows with a teat end score of 2 or greater. Second lactation cows had 75 % (3/4) of the cows with a teat end score of 2 or greater. Third lactation cows had 50 % (2/4) of the cows with a teat end score of 2 or greater and fourth lactation cows had a 50 % (1/2) probability as well.

CONCLUSION

There is a strong relationship between blood concentrations of micronutrients and immune function. There are many interrelationships of the nutrients and effects of over supplementing certain nutrients on immune function that can create health problems. The cow's uptake and requirement of micronutrients vary due to stage of lactation and health status. In this study the baseline micronutrient status of the Cal Poly Holstein herd with respect to copper, zinc, and selenium was determined. Micronutrient status with various measures of udder health was analyzed. With this information, recommendations are formulated to the dairy regarding current micronutrient feeding practices relative to improvement of udder health.

The results indicate that analyses of blood micronutrient concentrations at 3 different stages of lactation can be used as a tool to evaluate the need for extra supplementation of copper, zinc, and selenium. Copper was found to be deficient in the cows at all 3 stages of lactation and mean concentration levels continued to decline through lactation. Zinc was found to be deficient at calving and within 2 weeks before dry off. There were no selenium deficiencies in any of the cows at any stage of lactation.

Copper deficiency can be the result of a low Cu fed diet or a substance that inhibits cu absorption such as molybdenum, iron, or sulfate found in the feed or water. Diets should contain 225 mg or 300 mg of copper per day (32). A normal copper to molybdenum ratio is 5:1 to 10:1 (15). When it falls to 2:1 or less, severe interference

of copper utilization will occur. Molybdenum exerts its influence on copper through the association with sulfur in the formation of ruminal thiomolybdates (1). The relationship between Cu, Mo, and S has been widely investigated and Mo has been found to seldom affect tissue Cu storage when S levels are limiting. If there is adequate S provided, Mo combines with Cu to form an insoluble complex in the rumen, rendering Cu unavailable for absorption (1). The formation of thiomolybdates is directly dependent upon available dietary S, and S intake is a major factor influencing the sensitivity to Mo (18). Thiomolybdates can impact Cu nutrition by 2 means: 1) irreversibly bind Cu in the gut, thereby preventing absorption, and 2) post-absorption systemic depletion of Cu from tissue sites (17). Dietary S must first be reduced to sulfide before it can react to form thiomolybdates (19). Sulfur can also decrease Cu availability independent of Mo with the formation of insoluble copper sulfide complexes in the gut. An increase in S content in the diet may result in an increase in dietary Cu requirement. The Cal Poly diet in April 2009 had a sulfur content of .2489 % and this increased to .268% in a ration change that occurred in September 2009. There are many factors that can result in the increase of S, and the feed ration is one point to take notice in. Levels of copper were not analyzed in the diet; however, it can be suggested that increasing levels of sulfur had a negative impact on the absorption of Cu and may have contributed to the higher deficiency incidences that occurred toward the end of lactation. The forms of Cu found in the Cal Poly milk cow mix includes: Cu lysine, Cu oxide, and Cu sulfate. The levels of concentration in the mix are unknown, but it can be suggested that they are relatively

small amounts from the sequential order of the ingredients label. The use of Cu sulfate is suggested for review. It is recommended to increase copper levels in the diet. Dietary Cu supplements are usually given either as part of a concentrate or free choice salt mix. Chemical forms of available Cu that can be added to the diet include copper sulfate, oxide, chelates, and proteinate (13). When Cu deficiencies are due to dietary Mo, organically bound forms of Cu may have a higher biological availability. A trial was done to compare directly the efficacy of dietary Cu supplementation as Cu proteinate and Cu sulfate to cattle fed low Cu diets with Mo. It was found that Cu was not affected until the twelfth week into the trail at which time the cattle fed Cu proteinate supplement had higher plasma Cu than controls fed Cu sulfate supplement (13). Copper is the most toxic mineral that is routinely supplemented, so it is recommended that safe concentration of feeding should be a maximum of 4 to 5 times the requirement (32).

Mean concentrations of zinc in the 24 cows were not found to be deficient, however a small number of cows (12.5%) were deficient within 2 weeks after calving and 8.33% of cows were deficient within 2 weeks before dry off. Although Zn deficiency is not found to be a major concern in the Cal Poly Holstein herd, the causes of the few incidences should be reviewed. Phytate is a compound that can inhibit Zn absorption. Phytate, also called phytic acid, inositol hexaphosphate, or inositol polyphosphate, is found in plant foods particularly cereals such as maize, bran, and legumes. It binds to Zn (as well as other minerals) via oxygen. The zinc-phytate complex is large, insoluble, and poorly absorbed. Also, interactions between Zn and nutrients such as

the vitamin folic acid and a variety of divalent cations (Fe, Ca, Cu) may occur and inhibit Zn absorption (7). Although phytate affects the bioavailabilty of zinc, the enzyme, phytase, that hydrolyzes phytate aids in healing Zn deficiencies. Phytase is present in yeast, rye bran, wheat bran, barley, triticale, and many bacteria and fungi. Zinc nutrition and bioavailability can be enhanced by addition of phytase to animal feed (23). The forms of zinc found in the Cal Poly milk cow mix include zinc methionine and zinc oxide. Yeast culture is also in the milk cow mix which may help with the bioavailability of Zn with the presence of phytase. The amount of yeast in the ration is unknown. It is suggested that levels be increased for the Cal Poly Holstein herd to further aid in increased Zn absorption.

Selenium concentration levels were found to be adequate for the Cal Poly Holstein herd. No recommendations are needed for improvement of selenium concentrations. The forms of Se found in the Cal Poly milk cow mix include: added selenium and sodium selenite. The bioavailabilty of organic selenium, selenised yeast, has been found to be superior over sodium selenite and have a positive effect on udder health (26). It is recommended for use in the future should Se deficiencies in the herd ever become a problem; however, it is not necessary for current feeding.

REFERENCES

1 Arthington, J. Copper antagonist in cattle nutrition. http://dairy.ifas.ufl.edu /rns/2003/Arthington.pdf. Accessed Nov. 24, 2009.

2 Berning, Dr. Leanne. 2008. Mastitis prevention and control strategies. Lactation Physiology. 52.

3 Capello, Z. J., L.K. King, S.T. Marcum and E.A. Stanley. Chemical Analysis of selenium and copper levels in Virginia cattle herds. http://www.saanen doah.com/cu_virginia.pdf. Accedded Oct. 26, 2009.

4 Dunkey, W. L., A.A. Franke, L. Robb, and M. Ronning. 1968. Influence of dietary copper and ethylene diaminetetraacetate on copper concentration and oxidative stability on milk. J. Dairy Sci. 51(6):863.

5 Essig, Maria G. Microkats per liter. Healthwise, Incorporated. http://www. health.com/health/library/mdp/0,,tw12860,00.html. Accessed Nov. 30, 2009.

6 Gropper, S.S., J. Smith, and J. Groff. 2005. Advanced Nutrition and Human Metabolism: Hydrogen peroxide. 4th ed. Wadsworth. Belmont, CA. 370.

7 Gropper, S.S., J. Smith, and J. Groff. 2005. Advanced Nutrition and Human Metabolism: Inhibitors of zinc absorption. 4th ed. Wadsworth. Belmont, CA. 437-438.

8 Gropper, S.S., J. Smith, and J. Groff. 2005. Advanced Nutrition and Human Metabolism: Zinc-dependent enzymes. 4th ed. Wadsworth. Belmont, CA. 441-443.

9 Gropper, S.S., J. Smith, and J. Groff. 2005. Advanced Nutrition and Human Metabolism: Copper transport and uptake. 4th ed. Wadsworth. Belmont, CA. 449-451.

10 Gropper, S.S., J. Smith, and J. Groff. 2005. Advanced Nutrition and Human Metabolism: Glutathione peroxidase. 4th ed. Wadsworth. Belmont, CA. 458.

11 Hotenius, K., G.E. Meglia, P. Ohagen, L. Petersson and K. Persson Waller. 2004. Prediction of vitamin A, vitamin E, selenium and zinc status of periparturient dairy cows using blood sampling during the mid dry period. ACTA Vet. Scand. 45(2):119-128.

12 Jones, G.M. Understanding the basics of mastitis. Virginia Cooperative Extension. Pub 404-233. http://pubs.ext.vt.edu/404/404-233/404-233.pdf. Accessed Oct. 24, 2009.

13 Kincaid, R. L., R.M. Blauwiekel, and J.D. Cronrath. 1986. Supplementation of copper as copper sulfate or copper proteinate for growing calves fed forages containing molybdenum. J. Dairy Sci. 69:160-163.

14 Kirk, John H. Teat end conditions and scoring systems. http://www.vet med.ucdavis.edu/ vetext/ INF-DA/Abnormal_teats.pdf. Accessed Oct. 26, 2009.

15 Maas, J. 1997. Copper nutrition of beef cattle. http://www.vetmed. ucdavis.edu/vetext/INF-BE_cca/INF-BE_cca97/INF-BE_cca9705.html. Accessed Nov. 24, 2009.

16 Malbe, M., M. Klaassen, W. Fang, V. Myllys, M. Vikerpuur, K. Nyholm, S. Sankari, K. Suoranta and M. Sandholm. 1995. Comparisons of selenite and selenium yeast feed supplements on Se-incorporation, mastitis and leukocyte `function in Se-defecient dairy cows. J. Vet. Med. A. 42, 111-121.

17 Maon, J. The biochemical pathogenesis of molybedenum-induced copper deficiency syndromes in ruminants: towards the final chapter. Irish Vet. J. 43:18-22.

18 Mason, J. 1981. Molybedenum-copper antagonism in ruminants: a review of the biochemical basis. Irish Vet. J. 35:221-229.

19 Mason, J. 1986. Thiomolybdates: mediators of molybdenum toxity and enzyme inhibitors. Toxicol. 42:99-109.

20 Mein et al. 2001. A scoring system for teat-end condition.http://www.uwex.edu/milkquality/PDF/newteatdiagrams.pdf. Accessed Oct. 26, 2009.

21 National Mastitis Council. Dec. 2006. NMC Mastitis control program checklist updated. The NMC Newsletter: Udder Topic. 29:6.

22 National Mastitis Council. Aug. 2009. Nutrition's role in mammary gland health. The NMC Newsletter: Udder Topic. 32:3.

23 Oberleas, D., B. F. Harland. 2008. Treatment of zinc deficiency without zinc fortification. J. Zheljiang Univ. Sci. B. 9(3):192-196.

24 Oliver, Stephen P. 2009. Best management practices to reduce mastitis and improve milk quality. http://www.extension.org/pages/Best_Management_Practices_to_Reduce_Mastitis_and_Improve_Milk_Quality. Acessed Oct. 24, 2009.

25 Oliver, S.P., S.E. Murinda, and R.A. Almeida. Mastitis control, food safety, and quality milk production. Food Safety Center for Excellence and Dept. of Animal Sci. http://aged.ces.uga.edu/browseable_folders/TeacherResources/Mastitis_Control.doc. Accessed Nov. 30, 2009.

26 O'Rourke, D. 2009. Nutrition and udder health in dairy cows: a review. J. Irish Vet. 62:15-20.

27 Ott, Stephen L. 1999. Cost of herd-level production losses associated with subclinical mastitis in U.S. dairy cows. Page 152 in Natl. Mastitis Council Annual Mtg. http://www.nmconline.org/articles/costs.htm. Accessed Nov. 30, 2009.

28 Sherman, A.R. 1992. Zinc, copper, and iron nutriture and immunity. J. Nutr. 122:604.

29 Shroeder, J.W. 1997. Mastitis control programs: bovine mastitis and milking management. http://www.ag.ndsu.edu/pubs/ansci/dairy/as1129w.htm. Accessed Oct. 24, 2009.

30 Van Suan, Robert J. Nutrition, immunity and mastitis. http://vbs.psu.edu/ext/resources/pdf/small-ruminant/Goat-nutritionimmunitymastitis-VanSaun.pdf. Accessed Oct. 24, 2009.

31 Vazquez-Anon, M., J. Nocek, G. Bowen, T. Hampton, C. Atwell, P. Vazquez, and T. Jenkins. 2008. Effects of feeding a dietary antioxidant in diets with oxidized fat on lactation performance and antioxidant status of the cow. J. Dairy Sci. 91:3165-3172.

32 Weiss, W. P. 2009. Antioxidant nutrients and milk quality. http://www.extension.org/pages/Antioxidant_Nutrients_and_Milk_Quality. Accessed Oct. 24, 2009.

33 Weiss, W.P. Nutritional influences on the prevalence and severity of mastitis in dairy cows. http://nmconline.org/articles/nutrition.pdf. Accessed Oct 24, 2009.