Selenium and Mammary Resistance to Infectious Disease

R. J. Erskine, R.J. Eberhart,
P.J. Grasso and R. W. Scholz
Department of Veterinary Science
The Pennsylvania State University
University Pk, PA 16802

The element Selenium (Se) was discovered by Berzelius in 1818, who named it after the Greek word for the moon, selene. Although of interest to chemists, it aroused little biological interest until the 1930's, when Se became recognized as the causative agent of "alkali disease."[1] This is a toxicosis of grazing animals, in areas where accumulator plants generally have a Se concentration >5.0ppm. The ability to concentrate Se is inherent in some plant species, and is especially likely in areas that have a low annual rainfall and alkaline soil.[1] Geographical regions with these conditions occur widely in some parts of the western United States.

In 1957, Schwarz and Foltz reported the first evidence for the essential nature of dietary Se, as it was found to prevent hepatic necrosis in rats fed a diet deficient in Se and Vitamin E.[2] Ironically, that same year, Mills reported that the enzyme glutathione peroxidase (GSH-Px) protects erythrocytes from oxidative damage when incubated with hydrogen peroxide (H_2O_2).[3] Not until 1973 however, when Rotruck et al. discovered that Se is an essential component of GSH-Px, was a biochemcal role of Se determined.[4] To date, this is the only enzyme of higher animals known to require Se for catalytic activity.[1]

Glutathione-peroxidase catalyzes the reduction of H_2O_2 to H_2O, and organic peroxides to alcohols.[1]

$$\underset{(R\text{-}O\text{-}OH)}{H_2O_2} + 2GSH \xrightarrow[GSH\text{-}Px]{} \underset{(R\text{-}OH)}{H_2O} + GS\text{-}SG$$

In this reaction, the tripeptide glutathione (GSH) serves as the electron donor, which is oxidized to form GSSG. The oxidized GSSG molecules are then reduced through the activity of the enzyme glutathione reductase, and are again ready for use as a substrate by GSH-Px.[1]

$$GS\text{-}SG + NADPH + H^+ \xrightarrow[GSH\text{-}Red]{} NADP^+ + 2GSH$$

Selenium responsive diseases exist in several species. Many of the Se responsive diseases in animals are responsive to vitamin E as well.[1] This is not surprising, as both GSH-Px and vitamin E have a similar biochemical function of protecting cells from oxidative attack. Thus, any discussion of the effect of Se on disease must consider the synergistic effect of vitamin E.

Cattle and sheep can have Se and vitamin E responsive disorders that are nutritional myopathies, commonly termed "white muscle disease."[1] This disorder is typically seen when dietary Se levels are at 0.02-0.03ppm. Neonatal death and myocardial lesions are typical of the congenital form of this disease. If the onset of symptoms appears at 3 to 8 weeks of age, muscular weakness is the cardinal sign. These symptoms can be prevented if the dam is fed Se at the level of 0.1ppm.[1]

Reproductive disorders and mastitis are the Se and vitamin E responsive diseases of the dairy cow that have generated the most interest. We will focus our discussion on the effect of Se on mastitis. Smith et al. first reported that selenium and vitamin E supplementation reduced the incidence and duration of clinical mastitis in cows and heifers, as compared to unsupplemented controls.[5,6] In addition, Se and vitamin E supplementation decreased the prevalence of all intramammary infections in heifers during the first 8 months of lactation. In a field study of 16 high somatic cell count (SCC) and 16 low SCC dairy herds, low SCC herds had higher mean blood Se concentrations, and GSH-Px activity, than did high SCC herds.[7] In bovine blood there is a high correlation between Se concentration and GSH-Px activity,[7,8] and we believe blood GSH-Px activity is an excellent indicator of long-term dietary Se status. In addition, for all 32 herds, herd prevalence of infection was negatively correlated with mean herd blood GSH-Px activities.[7] Thus, previous research suggests that Se and vitamin E has a positive effect on mammary resistance to infectious disease.

We designed an experimental mastitis trial to study, under more controlled conditions, the effect of Se on mammary resistance to disease. We divided twenty Holstein heifers into two groups of ten. One group was maintained on a Se deficient (Se-) diet with 0.04ppm Se on a total ration basis. The other group was fed a selenium adequate (Se+) diet that contained 0.14ppm Se. We fed both groups identical rations, except the Se+ group received an additional 2 mg of Se per head per day. All other nutrients, including Vitamin E, were fed at or above NRC recommended levels. We challenged the heifers at about 14 weeks post-partum with 30 colony forming units (cfu) of *Escherichia coli* in one quarter. Quarters selected for challenge had SCC <250,000 cells/ml and no prior

infections.

Peak bacterial concentrations in milk were 100 times higher in Se- heifers than in Se+ heifers. The mean duration of infection was 162 hrs in Se- heifers,and 114 hours in Se+ heifers. As a result of greater bacterial numbers, Se- heifers likely had greater exposure to bacterial endotoxin. Endotoxin is thought to be the primary mediator of the inflammatory and systemic signs associated with acute coliform mastitis. Consequently, Se- heifers exhibited diarrhea, dehydration, quarter agalactia, and residual gland atrophy more frequently than Se+ heifers.

While quarter SCC increased rapidly in response to infection in both groups, the increase was more rapid in the Se+ heifers. Selenium adequate heifers also maintained a higher SCC:bacteria ratio in their milk than did Se- heifers. Thus, Se may improve mammary resistance to acute coliform infection by enhancing the influx of PMN into the gland, particularly during the early phase of infection.

Se also directly affects the bactericidal activity of bovine PMN. After ingesting foreign particles, phagocytes markedly increase their O_2 consumption, an event termed the respiratory burst.[9,11] The cell then converts the acquired O_2 to reactive oxygen species, such as H_2O_2, superoxide (O_2^-), the hydroxyl radical (·OH), and hypohalous acids.[9,11] Some of these compounds are extremely bactericidal, and thus enhance PMN killing of bacteria.[9,11] Normally, this process is well sequestered in the phagolysosome, an organelle that functions to protect the host cell from the reactive oxygen species produced. If these radicals leak into the cytosol, they can be extremely damaging to the functions of the host cell as well. Cytosolic enzymes such as GSH-Px and superoxide dismutase (which reduces O_2^- to H_2O_2), protect the phagocyte from potential harm by reducing the oxygen metabolites as they diffuse from the phagolysosome. If, due to a selenium deficiency, GSH-Px activity is reduced, the phagocyte can become susceptible to injury from H_2O_2. Bovine PMN have very low catalase activity,[12] an enzyme that also protects cells from H_2O_2. Consequently, bovine PMN are particularly dependent on GSH-Px activity for protection from H_2O_2 attack.

Serfass and Ganther first reported that GSH-Px activity is reduced in Se- as compared to Se+ PMN isolated from rats.[13] This reduction in activity decreased the killing ability of Se- PMN for *Candida albicans.*[14] Selenium deficiency reduces both GSH-Px activity, and killing ability of *C albicans* and *Staphylococcus aureus* in bovine blood PMN.[15,16] By use of a flourescent acridine orange assay, we found that Se- PMN of mammary origin did not kill *S aureus* or *E. coli* as effectively as Se+ PMN.[17] Therefore, it can be concluded from *in vitro* studies that selenium deficiency decreases phagocytic killing efficiency.

In addition, Aziz and Klesius demonstrated decreased chemotactic ability in Se- caprine PMN.[18] Selenium deficient PMN also produce leukotriene B_4 at lower levels than Se+ PMN.[19] Leukotriene B_4 is an arachidonic acid metabolite, and a potent chemotactic agent.[19]

To summarize the effect of Se on acute coliform mastitis; Se+ heifers maintain lower bacterial numbers in milk than Se- heifers. Selenium supplementation reduces bacterial numbers by enhancing PMN influx into the gland, and improving phagocyte killing efficiency. As a result of lower bacterial concentrations, Se+ heifers are exposed to less endotoxin. Consequently, the infections in Se+ heifers are less severe, and the clinical signs, including milk loss, are less pronounced.

Is Se a mastitis panacea? Not likely. We performed a study similar to the *E coli* trial, but instead we challenged heifers with *S aureus*. The preliminary results suggest that Se does not affect the long term course of infection with this organism. However, *S aureus* infections generally follow a very different course than do those of *E coli.* Much of the benefit gained by Se supplementation in the *E coli* challenge was due to the rapid influx of PMN into the gland. This rapid PMN response is typical of acute coliform

Herd Vitamin E and Selenium Program

Nutrient	Dietary Level (Dry Matter Basis)	Amount/ Head/Day†	Comments	Metabolic Profile Average	Range	Critical	Units
Selenium	0.3 ppm	6 mg	FDA approved, 1987	.26	.22-.40	>1.0	mcg/ml*
			GSH-Px activity	65	45-85	>100	mU/mgHb*
	0.1 ppm	2 mg	Minimum adequate dietary level	.12	.10-.15	<.08	mcg/ml*
			GSH-Px activity	36	22-50	<15	mU/mgHb*
Vitamin E	7 IU/lb	300 IU§	NRC recommendation IIU=1mg α-tocophereol	484	227-741	<150	mcg/dl‡

†For a mature Holstein cow, total ration.

*Whole blood in EDTA.

§PSU recommendation 800-1,000 IU/head/day.

‡Serum.

mastitis, but not *S aureus* mastitis. *S aureus* infections are generally chronic, and subclinical,[20,21] and they rarely induce an early acute inflammatory response on the part of the host. Consequently, Se will most likely affect the severity of intramammary infection when the infection is acute.

Practical feeding of Se must include knowledge of the element's availability in the soil. Plants grown in areas that have low soil Se availability will be a deficient dietary source of Se.[1] Thus, farms that are in Se deficient areas will need to supplement their forages and grain rations in order to maintain adequate dietary Se levels. Dietary Vitamin E must also be monitored as it will vary seasonally with forage quality and availability. A table of recommended dietary levels for Se and Vitamin E is below. In addition, values used by our diagnostic laboratory for evaluating herd status of these nutrients is included. Herd profiles are usually done by collecting whole blood (in EDTA) and serum from 6-7 cows in each of three groups: early lactation, mid-lactation, and late lactation/or dry cows. We prefer GSH-Px analysis to indicate long term Se feeding status.

References

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