

Effect of Vitamin E and Selenium Supplementation on Incidence of Clinical Mastitis and Duration of Clinical Symptoms^{1,2}

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ABSTRACT

Incidence of clinical mastitis and duration of clinical symptoms for complete lactations were evaluated for 80 cows randomly assigned to one of four groups: vitamin E supplemented- and selenium injected, selenium injected, vitamin E supplemented, and controls. Vitamin E supplementation and selenium injection were during the dry period. Log-linear analysis of incidence data revealed a significant 37% reduction of clinical mastitis by vitamin E. Incidence was not affected by selenium alone, nor was there any evidence for interaction of vitamin E with selenium on incidence. However, duration of clinical symptoms (calendar months clinical/quarter lactating) was reduced by 46% for the selenium group, 44% for the vitamin E group, and 62% for the vitamin E-selenium group as compared to controls. We conclude that dairy cow diets deficient of vitamin E may elevate incidence of clinical mastitis. Selenium deficiency may result in greater duration of clinical symptoms, and selenium may interact with vitamin E. Coliform bacteria and species of streptococcus other than *Streptococcus agalac-*

tiae were isolated from 70% of the clinical cases.

INTRODUCTION

Mastitis continues as a problem in many dairy herds despite proper application of proven control methods (7, 22) of teat dipping (TD) and total dry cow therapy (TDCT). A frequent finding in such dairy herds is that the mastitis is caused by coliform bacteria and species of streptococci other than *Streptococcus agalactiae*. These bacterial species collectively are referred to as environmental pathogens. Origin of these pathogens for infection of uninfected quarters is the environment of the cow in contrast to the infected quarter (7, 8, 22, 34).

Herds that experience unacceptable frequency of environmental mastitis frequently have a history of long application of TD and TDCT, and as a result, percent of quarters infected with *Staphylococcus aureus* is low and *S. agalactiae* is eliminated frequently from the herd (8, 23, 34). Other frequent observations of affected herds are confinement housing, high cow densities per unit area, use of free stalls, and bedding materials that are finely chopped organic matter (9, 34). Under conditions of confinement housing, these bedding materials can support substantial growth of environmental pathogens and result in marked increase of exposure of teat ends (9, 32, 34). Thus, risk of infection is greater (7).

Diets of herds in confinement housing are often heavily dependent upon ensiled forages as a roughage source. Ensiled forages contain only one-fifth to one-sixth the amount of vitamin E (E) in freshly cut forages in the vegetative stage (18, 21). Thus, many dairy cow rations contain less than adequate E. Additionally, diets of dairy cows in Ohio and other important dairy

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states are deficient of selenium (Se). Reports (13, 17, 40) show that dairy cow diets deficient of E and Se result in increased incidence of disease associated with reproduction, i.e., retained placenta, metritis, and cystic ovaries. This relationship was studied in the dairy research herd of the Ohio Agricultural Research and Development Center (13). We report here the influence of supplemental E and Se on incidence of clinical mastitis caused by environmental pathogens.

EXPERIMENTAL PROCEDURE

Cows and Rations

Seventy-eight multiparous cows were in a 2×2 factorial experiment. Cows were assigned randomly to one of two dietary groups at drying off and subdivided for Se treatment at 21 days prior to anticipated calving. Treatments were: 1) Se + E; 2) E; 3) Se; and 4) control. Numbers of cows per group were: Se + E = 21, E = 20, Se = 19, and control = 20.

All quarters of all cows were dry treated with a commercially available product⁵ at drying off. Cows were in confinement housing during the dry period. Housing was freestalls bedded with untreated recycled manure,⁶ and new bedding was added twice per week.

All cows received a legume-grass haylage provided for ad libitum consumption and supplemented with .5 kg of concentrate per cow per day as a total mixed ration. Details of ingredients and nutrient content of the ration are in (13). Groups supplemented with vitamin E received supplemental [d,l]-alpha-tocopherol acetate to provide an average of .74 g vitamin E ([d]- α -tocopherol equivalents/cow per day). Ad libitum consumption of haylage provided an estimated .32 g vitamin E per cow per day. Selenium was injected i.m. (.1 mg/kg of body weight) at 21 days prior to anticipated calving. Selenium injections were prepared to contain

5.0 mg Se (sodium selenite, 5-hydrate)⁷ and 250 mg polysorbate (polyoxyethylene sorbitan mono-oleate)⁸/mo. Postpartum rations were not supplemented with Se or E, and details of these rations are in (13).

Housing and Milking Postpartum

Three to five days prepartum cows were moved to a maternity unit. Housing was in box stalls bedded with pelleted corn cobs.⁹ Box stalls were cleaned, and fresh bedding was added once per day. Cows were milked twice per day postpartum with a Surge¹⁰ bucket milker, and calves were permitted to nurse for 3 days. Machine milking was followed by post-milking teat end disinfection with 5% sodium hypochlorite.¹¹

Four days postpartum cows were returned to the milking herd. Housing was total confinement and was either freestalls or comfort stalls bedded with recycled manure.⁶ Fresh bedding was added twice per week to freestalls and as required to comfort stalls. Milking was in a double three, side-opening parlor with prep stalls, and milking was with the Surge QTO¹⁰ System. Udders were dried with individual paper towels, and postmilking teat end disinfection was as described.

Detection and Diagnosis of Clinical Mastitis

Milkers were requested to examine every quarter of every cow for swelling and foremilk for abnormal secretion at all milkings. Quarters abnormal were coded and entered into a computer terminal located in the milking parlor. Reports were available at the conclusion of each milking and were reviewed by mastitis laboratory technicians every 24 h. All cows with symptoms of clinical mastitis were examined by mastitis laboratory technicians. Strict foremilk was examined by a strip cup for clots, flakes, or otherwise obviously abnormal secretion, and quarters were palpated for swelling. All quarters then were sampled for microbiological culture. Only quarters found to be clinical by technicians were recorded permanently as clinical. Once a quarter was designated as having a clinical case of mastitis, a new clinical case could be diagnosed only when the pathogen changed or when culture data and Direct Microscopic Somatic Cell Count (DMSCC) data indicated that the pathogen had been eliminated

⁵ Dryguard, The Upjohn Co., Kalamazoo, MI.

⁶ TRU Solids Separator, Babson Bros., Co., Oak Brook, IL.

⁷ J. T. Baker Chemical Co., Phillipsburg, NJ.

⁸ Sigma Chemical Co., St. Louis, MO.

⁹ The Andersen Co., Maumee, OH.

¹⁰ Babson Bros., Co., Oak Brook, IL.

¹¹ The Clorox Co., Oakland, CA.

and that reinfection had occurred by a similar pathogen. Number of antibiotic treatments had no bearing on diagnosis of a new clinical case.

Microbiological Culture of Milk

Samples of foremilk were obtained by aseptic techniques (4). The majority of samples were obtained 3 to 6 h post a.m. milking. Samples were stored on ice and transported to the laboratory within 2 h. Culture of milk was basically by described procedures (4). Sample (.01 ml) was streaked on one-fourth plate of trypticase soy agar¹² containing 5% whole bovine blood and aesculin¹³ (1 g/liter). An additional aliquot of each sample (.1 ml) was streaked on one-half plate of MacConkey agar¹³ to aid detection of coliform bacteria (35). All plates were observed and all growth recorded after 24 and 48 h incubation at 37°C. Colonies tentatively were identified as staphylococci, streptococci, coliform, *Corynebacterium bovis*, or other on colony morphology and appearance, growth characteristics, hemolytic patterns, and aesculin hydrolysis. All staphylococcal isolates were tested for production of coagulase and DNase and for mannitol fermentation. Streptococcal isolates were tested for Christie, Atkins, Munch-Petersen (CAMP) reaction (6), aesculin hydrolysis, inulin and raffinose fermentation, and growth in the presence of 6.5% NaCl. Coliform isolates were tested for lactose fermentation, utilization of citrate as a sole carbon source, and motility. Direct microscopic somatic cell counts were determined for all milk samples (20).

Therapy of Clinical Quarters

Clinical cases of mastitis were treated routinely by intramammary infusion of a commercially available intramammary infusion product.¹⁴ All infusions were by mastitis laboratory technicians. Clinical quarters were sampled for microbiological culture on day 0, and therapy was initiated on day 1. Therapy

regimen was dictated by pathogen isolated following 24-h incubation. Isolation of a coliform dictated that all quarters of that cow requiring therapy were infused thrice at 12-h intervals. Therapy was twice at a 24-h interval where coliforms were not involved. All quarters of all cows were resampled for microbiological culture and DMSCC at 7 and 14 days postinitiation of therapy. An additional course of therapy could be initiated no sooner than 14 days after a course.

Summarization and Analysis of Data

All data regarding clinical mastitis were obtained and summarized for each cow lactation without prior knowledge of the identity of cows within vitamin E and selenium supplementation groups. Only new clinical cases of mastitis were summarized, and duration of clinical symptoms was expressed on the basis of calendar months clinical. Thus, a quarter clinical on any day in June, July, and August was clinical for 3 calendar mo.

Effects of E and Se on clinical mastitis and all possible interactions were tested for statistical significance in log-linear analysis (1, 3). Analysis was on a quarter basis, and quarters either had a clinical case of mastitis or they did not. Additional analyses were by least squares analysis of variance and Duncan's multiple range test.

RESULTS

Results of sorting the 320 quarters on clinical mastitis, E, and Se for log-linear analysis are in Table 1. Multiple clinical cases within a quarter was not a factor in this analysis. Results of testing all possible models by log-linear analysis to determine which main effects and

TABLE 1. Results of sorting 320 quarters on clinical mastitis, vitamin E, and selenium (Se).

Quarter status	Se	Vitamin E	
		Yes	No
Clinical	Se	20	26
	No Se	17	29
Not clinical	Se	64	50
	No Se	63	51

¹² BBL Microbiology Systems, Becton Dickinson and Co., Cockeysville, MD.

¹³ Difco Laboratories, Detroit, MI.

¹⁴ 17900 Forte, The Upjohn Co., Kalamazoo, MI.

interactions were required to explain the cross-sectional data in Table 1 are in Table 2. A large likelihood ratio for Chi square indicated lack of fit between the model and the data. The model EC (Table 2) explains the data significantly better than any model without interaction. Thus, a significant ($P<.05$) influence of vitamin E on clinical mastitis was less clinical mastitis when supplemental vitamin E was fed. Log-linear analysis provided no evidence of Se effect on clinical mastitis because the model ES, EC did not fit the data significantly better than the EC model alone.

The incidence of clinical mastitis within the four groups of cows, expressed as clinical cases per quarter per lactation, is in Table 3. Diet supplementation with E reduced clinical cases per quarter per lactation by 37%. Incidence in the Se supplemented group was reduced slightly (12%) compared to controls. Again there was no evidence for interaction between E and Se when the data were expressed in this manner as incidence did not differ between the group receiving E and that receiving E + Se.

Data also were expressed as calendar months clinical per quarter lactating (Table 3) to take

TABLE 2. Results of testing all possible models by log-linear analysis to determine which main effects and interactions were required to explain cross-sectional data in Table 3.

Model ¹	df	Likelihood ratio Chi-square	P
E	6	66.42	0
S	6	66.42	0
C	6	6.94	.33
E, S	5	66.42	0
E, C	5	6.74	.24
S, C	5	6.94	.23
E, S, C	4	6.74	.15
ES	4	66.22	0
EC*	4	.43	.98
SC	4	6.9+	.14
ES, C	3	6.54	.09
EC, S	3	.43	.94
SC, E	3	6.74	.08
ES, EC	2	.22	.89
SE, SC	2	6.54	.04
CE, CS	2	.43	.81
ES, EC, SC	1	.22	.64

¹ E = Vitamin E; S = selenium; C = clinical mastitis.

* $P<.05$.

TABLE 3. Effect of vitamin E and selenium supplementation on incidence of clinical mastitis and duration of clinical symptoms.

Experimental group	(n)	Clinical cases per quarter lactating			Months ¹ clinical per quarter lactating			Months ¹ clinical per clinical case		
		Clinical cases per quarter lactating			Months ¹ clinical per quarter lactating			Months ¹ clinical per clinical case		
		̄X	SE	(Reduction) ²	̄X	SE	(Reduction) ²	̄X	SE	(Reduction) ²
E + Se ³	22	.26	.05	37	.36	.10 ^b	62	1.36	.17 ^c	41
E	21	.26	.06	37	.53	.14 ^b	44	2.00	.37 ^c	13
Se	27	.36	.06	12	.51	.12 ^b	46	1.44	.23 ^c	37
Control	33	.41	.07	0	.95	.19 ^a	0	2.30	.32 ^c	0

^{a,b} Means followed by different superscripts differ ($P<.05$).

^c Probability of a significant treatment effect was .071.

¹ All months are calendar months.

² % Reduction = $100 - [(Experimental \div Control) \times 100]$.

³ E = Vitamin E, Se = selenium.

into account not only incidence but also duration of clinical manifestations of the infection. These data together with incidence (clinical cases per quarter lactating) suggest that Se effect on mastitis should not be ruled out by log-linear analysis. Calendar months clinical per quarter lactating was reduced by 46% when cows with supplemented Se were compared to controls. These data also suggest a possible interaction of E with Se as percent reduction for cows supplemented with E + Se (62%) was greater than for either the E (44%) or Se (46%) cows. There was a significant ($P < .05$) treatment effect, and all three treatments differed from controls (Table 3).

Durations of clinical symptoms (months clinical per clinical case) are also in Table 3 and show that duration of clinical symptoms was greatest for control cows whereas cows supplemented with E + Se had shortest clinical cases. These data again suggest a possible effect of Se and indicate that Se supplementation of cow diets known to be deficient in Se may reduce duration of clinical symptoms and duration of infection.

The probable cause of clinical mastitis in experimental cows is in Table 4. These data reflect a herd mastitis problem associated with environmental pathogens as 70% of the isolates from clinical quarters were coliform bacteria and species of streptococci other than *S. agalactiae*. Less than 1% of quarters in this herd of approximately 130 lactating cows were infected at any one time with coagulase positive species of staphylococci, and *S. agalactiae* had been eradicated completely. Thus, effects of E and possible effects of Se shown here may not be applicable to herds in which clinical mastitis is caused primarily by *S. agalactiae* or *S. aureus*.

The third leading cause of clinical mastitis was by unknown pathogens as culture of milk samples resulted in no bacterial growth. Data from experimental cows were consistent with data from the entire herd. All such clinicals were judged to be clinical at cow side and those judgments supported by DMSCC's of greater than 10^6 /ml. Such clinical cases were consistently mild, short, and tended not to repeat within quarters of a cow, indicating that there is little reason to suspect pathogens not culturable by techniques of this study. We are of the opinion that a more logical explanation would be that these clinical cases were caused by

TABLE 4. Probable cause of clinical mastitis based on microbiological culture of aseptically obtained foremilk samples from clinical quarters.

Pathogens isolated from clinical cases of mastitis	E + Se		E		Se		Control		All groups	
	(n)	(% Clinicals)	(n)	(% Clinicals)	(n)	(% Clinicals)	(n)	(% Clinicals)	(n)	(%)
Streptococci ¹	11	50	7	33	8	30	16	49	42	41
Coliform	4	18	8	38	9	33	9	27	30	29
No isolation	6	27	2	10	6	22	4	12	18	17
Staphylococci ²	2	9	2	10	3	11	5	15	12	12
<i>C. bovis</i>	...	...	2	10	3	11	2	6	7	7
Other pathogens	...	...	1	5	2	7	...	...	3	3
Mixed infections ³	1	5	1	5	5	19	3	9	10	10

¹ 86% of isolates were aesculin positive and 0% were CAMP positive and aesculin negative.

² 2 of 12 isolates were coagulase positive.

³ Four were streptococci and coliform, 4 coliform and *C. bovis*, 1 streptococci and *C. bovis*, and 1 streptococci and staphylococci.

TABLE 5. Duration of clinical symptoms by infection type.¹

	(n)	Clinical ²	
		$\bar{X}$	SE
Streptococci	36	2.03	.27 ^{ab}
Coliform	22	2.45	.44 ^a
No isolation ³	18	1.11	.11 ^b
Staphylococci ³	11	1.09	.09 ^b

^{a,b} Means followed by different superscripts differ ($P < .05$).

¹ Mixed infections not included in summary.

² Months are based on calendar months.

³ Only one infection was clinical in more than 1 calendar mo.

streptococci or coliform bacteria but that the pathogen was eliminated effectively by the cow prior to our obtaining milk samples for microbiological culture.

As would be expected with relatively small numbers of cows per group, pathogen types responsible for clinical mastitis were not distributed evenly among experimental groups and could have influenced duration of clinical

TABLE 6. Duration of clinical symptoms in quarters infected with streptococci or coliform bacteria.¹

Experimental group ^a	(n)	Months ² clinical		(% Reduction)
		$\bar{X}$	SE	
E + Se	14	1.57	.25 ^b	42
E	14	2.36	.52	14
Se	12	2.00	.48	27
Controls	22	2.73	.45 ^b	0

^a Analysis of data by least squares analysis of variance indicated that the probability of a significant treatment effect was $P = .281$.

^b Analysis of the E + Se and control data only by least squares analysis of variance indicated that the probability of a significant difference was $P = .057$.

¹ Data include streptococci and coliform mixed infections but not other mixed infections.

² Months are calendar months.

³ % Reduction = $100 - [(\text{Experimental} \div \text{Control}) \times 100]$.

symptoms. Clinical cases caused by streptococci and coliform bacteria were longer as compared to those caused by staphylococci and no bacterial isolation (Table 5). Duration of clinical symptoms for quarters infected by coliform tended to be greater than for quarters infected by streptococci and probably reflects lower drug efficacy against coliform bacteria for antibiotics used to treat clinical mastitis in this herd (data not shown).

Duration of clinical symptoms for the two major pathogen groups were combined and summarized for treatment groups (Table 6). Duration was reduced by 42% in the E + Se group compared to controls, and E (14%) and Se (27%) groups were intermediate. Thus, data indicate that an unequal distribution of pathogen types among experimental groups was not a major factor in the reduced duration of clinical symptoms for E, Se, and E + Se groups.

DISCUSSION

There is evidence that the nutritional status of dairy cows can influence resistance and susceptibility to disease (2, 13, 17, 40), and the relationship of nutrition to incidence of mastitis is not a new concept (19, 30, 36, 39). Pounden and coworkers published numerous papers on this subject from 1952 to 1968 (10, 11, 26, 27, 28, 29, 30, 31). Despite these early efforts, nutritional factors directly relating to incidence of mastitis were not described. Frank et al. (11) suggested that forage estrogens may influence incidence of mastitis, but this contention never was substantiated.

Reports have begun to show the relationship of diet, and in particular, vitamins and trace minerals, to disease processes (2, 12, 13, 14, 15, 17, 33, 40). That dietary deficiencies of vitamin E and selenium could increase susceptibility of the mammary gland to new intramammary infection can be hypothesized on known interactions of Se and E with resistance mechanisms and their role in protecting cellular membranes against oxidative degradation (12, 14, 15, 33). Boyne and Arthur (2) reported that the polymorphonuclear neutrophils (PMN) of selenium deficient cattle were deficient of selenium-dependent glutathione peroxidase and that the PMN had reduced ability to kill ingested *Candida albicans*. The PMN participates in defense of the mammary gland (24), and

decreased ability to kill invading pathogens likely would lead to increased incidence of mastitis and possibly longer infections. Vitamin E deficiency can decrease immune response and may be mediated in part through an antioxidant effect on one or more cell types of the immunopoietic system (37, 38). Supplementation of weanling pig diets with E and Se increased humoral antibody response to antigen (25). The data indicated that E and Se independently enhanced immune response and that their combined effects were additive.

Other than our data, we are aware of only one other attempt to relate Se and E deficiency to incidence of mastitis. Ishak et al. (16) reported no significant effect of Se and vitamins A, D, and E supplementation on incidence of clinical mastitis. The major thrust was reproduction, not mastitis, and the mastitis data dealt with clinical cases only. Methods of detection and diagnosis of causative agents were not defined. In spite of no significant effect, incidence of clinical mastitis for the first 3 mo of lactation was lower in Se, vitamins, and Se plus vitamins groups as compared to controls. Recently, Chew et al. (5) reported that less vitamin A in plasma and milk was associated with increased California Mastitis Test (CMT) reactions.

Thus, there is evidence that deficiencies of E and Se can lead to impaired resistance to disease. Dairy cow rations in Ohio and other major dairy producing states are deficient of Se when feedstuffs are grown locally, and many diets for dairy cows are deficient of E as a result of increasing dependence on ensiled forages (13, 18, 21). Data in our report were based on incidence of clinical mastitis and duration of clinical symptoms. Data were from relatively small numbers of cows per group, and the experimental design did not originally include mastitis. However, from the data, dietary deficiencies of E and possibly Se elevate incidence of mastitis from environmental pathogens, and additional research on the relationship of E and Se to mastitis would benefit dairy producers.

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