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Zinc-Methionine Supplementation for Dairy Cows—A Study of Effects on Plasma Zinc, Wound Healing, Mammary Health, and Immune Responses

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Zinc-Methionine Supplementation for Dairy Cows—A Study of Effects on Plasma Zinc, Wound Healing, Mammary Health, and Immune Responses[1]

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SUMMARY

Twenty lactating dairy cows were used in a series of experiments to examine the effects of adding a zinc supplement (0.056% of the grain mixture) to a ration containing 60% alfalfa haylage and 40% grain for a period of 60 days.

Plasma zinc levels due to zinc supplementation were not found to be different. Nor was there any effect on wound healing, incidence of mastitis, and mammary health, or immune responses as measured by white blood cell counts and T cell function.

Thus, feeding this level of supplemental zinc appeared to have no beneficial effects on the cows.

INTRODUCTION

Zinc, an essential mineral for all animals, was first reported to be required by rats by Todd *et al.* in 1934 (20). Zinc is widely distributed throughout the body and plays an essential role in many body processes. It is present in enzyme systems and plays a vital role in the fundamental processes of protein synthesis and metabolism.

The estimated zinc requirement for dairy cattle is 40 ppm in the diet (12); however, there may be certain conditions or interrelationships with other nutrients which may increase the level of zinc required. Zinc deficiency has been reported in dairy cattle on many occasions (6, 11, 17). Regardless of the level of zinc previously fed, cattle fed diets insufficient in zinc develop a deficiency over a period of a few weeks, as there are no appreciable long-term stores of zinc in the body (13).

One of the first signs of a zinc deficiency in dairy cattle is a decrease in feed intake (10). It has been found that these animals grow less rapidly because of both decreased feed intake and feed utilization.

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Additional symptoms of zinc deficiency are skin parakeratosis of the legs, head, and neck; hair loss; general unthriftiness; failure of wounds to heal normally; stiffness of joints; teeth gnashing; retarded testicular growth; and excessive salivation (2).

Zinc absorption occurs predominantly in the small intestine, primarily in the duodenum. The overall absorption from conventional ruminant diets is only 8 to 10% (4). Absorption is affected by several factors, including the age of the animal, the level of intake of zinc, the amount and proportion of several other minerals and dietary components, and the chemical form in which the zinc is ingested (7). Neither phytic acid nor calcium have been shown to reduce zinc absorption in ruminants; however, the presence of anionic complexes of zinc in forages may have a significant effect on the availability of zinc (1) which might be protected by certain ligands.

The zinc content of whole blood and plasma has been shown to be responsive to changes in dietary zinc levels (9). Large increases in supplemental zinc have been shown to increase blood zinc concentrations dramatically in several species of animals, including cattle (14, 15, 16). Cattle given various increments of supplemental zinc, which provided total intakes of 18 to 189 ppm, revealed increases in serum zinc from 1.5 up to 2.7 μ/ml (18).

The organic zinc-methionine used in this study is used in the feed industry on the basis of improving healing rate of lesions from infections such as "foot rot" at the level of 80 ppm of zinc in cattle diets. The objective of this study was to investigate zinc-methionine's effect on plasma zinc concentrations, healing of hock injuries, and immune status of dairy cows fed recommended levels. Zinc-methionine added at the manufacturer's recommended amount was used because Miller *et al.* (10, 12) found it would take up to 400 ppm of zinc as zinc sulfate to increase plasma zinc concentrations and improve wound healing.

EXPERIMENTAL DESIGN

Twenty lactating Holstein dairy cows were used in this experiment to determine the effects of supplementary zinc on various parameters. Cows were paired at the start of the trial according to age, milk production, and stage of lactation. All cows were fed a control diet for the first 2 weeks of the experiment; after this 2-week preliminary period, paired animals were randomly assigned to either the control grain or zinc-supplemented grain mix. The two grain mixes were:

Ingredient	Control	Zinc-supplemented
	percent	percent
Ground shelled corn	80.0	80.0
Soybean meal	18.0	18.0
Selenium	0.1	0.1
Biofos	0.9	0.9
Salt, TM	1.0	0.944
ZinPro-40*	—	0.056

*ZinPro Corp., Chaska, MN.

The total ration consisted of 60% alfalfa haylage and 40% grain. All rations were fed *ad libitum* and adjusted weekly for a 5% refusal.

The analysis of the haylage showed that it contained 32 ppm of zinc, while the grains contained 57 and 93 ppm for the control and zinc-supplemented grains, respectively. The cows received between 0.8 and 1.0 gram of zinc per day.

The average analysis of the haylage used which contained 300 to 450 mg of zinc was:

	Percent
Dry Matter	51.59
Crude Protein	18.55
VFA's	μmol/g DM
acetic	251.0
propionic	1.73
butyric	1.06
iso-valeric	0.45
valeric	1.31
pH	5.1

Blood samples were obtained at 0, 30, and 60 days after the start of the zinc supplementation. Plasma samples were frozen (4° C) until analyzed for plasma zinc (5) using atomic absorption spectroscopy.

RESULTS

Plasma Zinc

Plasma zinc levels obtained at the stated times after initial zinc supplementation are shown in Table 1.

No differences occurred in plasma zinc levels between the treated and control groups. Therefore there was no evidence to support the concept that elevated plasma zinc levels can be achieved from feeding

TABLE 1.—Plasma Zinc Levels.

Time Point	Plasma Zinc μg/Zn/ml	
	Control	Zinc-supplemented
Day 0	1.41 ± 0.10	1.41 ± 0.11
Day 30	1.63 ± 0.08	1.52 ± 0.07
Day 60	1.47 ± 0.11	1.65 ± 0.07

this dietary zinc compound at the supplemental level of 0.056% of the grain mix for 60 days.

The results of this trial indicate that initial plasma zinc levels were in the normal physiological range for dairy cows. Possibly due to limited absorption of dietary zinc in presence of normal amounts in blood (4), the extra zinc supplemented through this grain mix was insufficient to enhance plasma zinc levels of cows already on a zinc adequate diet.

Wound Healing

Zinc has long been associated as a factor involved with wound healing. It has been shown with calves that wound healing is impaired when zinc-deficient diets are fed. It has been suggested that there is an increased metabolic demand for zinc by the tissues during healing (8, 11).

The animals used in these studies were housed in an indoor comfort stall area. Many of these animals started the trial with a condition of bruised and/or swollen hocks. These wounds were self-incurred from bumping metal gutter grates.

In an attempt to monitor any increases in wound healing during this trial, photographs of rear hocks of all cows were taken prior to and at the end of the trial. The photographs were evaluated on a scale of 1 to 3, with 1 the normal condition of the hocks, 2 showing evidence of unthriftiness and hair loss, and 3 showing evidence of swelling, open sores, and loss of hair on an area of skin.

The results of scoring by three persons independently showed that the control group averaged 2.84 before and 2.50 after, while the zinc group averaged 2.63 before and 2.31 after. Both groups showed a 12% decrease in the amount of wounds present by the end of the trial. Therefore there appeared to be no effect of supplemental zinc on wound healing at the level of zinc fed and under these housing conditions.

Mammary Health and Mastitis

The total cell count or somatic cell count of bovine mammary gland secretions is influenced by numerous factors. While there is a wide range

of maximal permissible cells in milk from a normal gland, 500,000 cells/ml or less is the usual acceptable cell count from a normal gland. Cell counts in excess of 500,000/ml are considered abnormal. The presence of an infection or inflammation in a quarter will cause an increase in the total cell count of the milk. In the case of infection in the mammary gland, the polymorphonuclear leukocyte is the major cell type responsible for the increase in the cell count. Other cell types present in mammary gland secretions include: lymphocytes, macrophages, monocytes, eosinophils, and epithelial cells. Numbers and types of these cells present depend on infection status and stage of lactation.

Total somatic cell counts are also known to vary according to stage of lactation and lactation age. During involution of the gland and at parturition, cell counts are high. With advancing lactation age, somatic cell counts increase. In an individual cow, cell counts have been shown to have diurnal fluctuations, and different fractions of milk from a gland will have varying total cell counts.

An aseptic foremilk sample was obtained from all quarters of all 20 cows at 2 weeks prior to the initiation of the experiment, on the first day of the experiment, and 30 and 60 days later. All quarter samples were streaked for bacteriological examination onto one quadrant of an esculin blood agar plate and one-half of a MacConkey agar plate. Total somatic cells of each quarter milk sample were determined by direct microscopic somatic cell counts (DMSCC). All cell counts were done in duplicate.

Clinical quarters were treated with an approved lactation antibiotic preparation. A clinical quarter was defined as abnormal appearance of the milk by observation with a strip cup.

TABLE 2.—Somatic Cell Counts of Control and Experimental Mammary Quarter Milk Samples.*

Time Point	Control	Zinc-supplemented
Pretrial sample (9-18-81)	5.7524 ± 0.2887† (40)	5.6784 ± 0.3104 (39)
Day 1 (10-1-81)	5.2265 ± 0.2904 (40)	5.6850 ± 0.2615 (39)
Day 30 (10-28-81)	5.0346 ± 0.2406 (40)	5.5672 ± 0.2339 (39)
Day 60 (12-2-81)	4.9970 ± 0.1151 (36)	5.4220 ± 0.1347 (39)

*Values are expressed as total cell/ml, $\log_{10}$.
†Values represents x ± SEM.

TABLE 3.—MSCC of Normal and Clinical Quarters in Control and Experimental Animals.†.

Time Point	Control Quarters		Zinc-supplemented Quarters	
	Normal	Clinical	Normal	Clinical
Pretrial	5.1343 ± 0.4221	7.2978 ± 0.1256 (5)‡	5.0240 ± 0.1459	7.3143 ± 0.2998 (5)
Day 1	4.7377 ± 0.1186	6.8561 ± 0.5051 (4)	5.1543 ± 0.1522	7.0121 ± 0.2054 (5)
Day 30	4.9185 ± 0.2330	6.1960 ± 0.0000* (1)	5.1449 ± 0.1836	6.6230 ± 0.2386* (4)
Day 60	4.9327 ± 0.0982	7.2497 ± 0.0000 (1)	5.3341 ± 0.1264	7.0478 ± 0.1521 (2)

†Values expressed as x ± SEM.
‡Numbers in parentheses indicate number of clinical quarters.
*P $<$0.05.

TABLE 4.—Bacterial Isolations from Quarter Milk Samples of Control and Experimental Cows.*

	Pretrial		Day 1		Day 30		Day 60	
	C†	Z	C	Z	C	Z	C	Z
Staphylococci (Coagulase —)	7.5	17.9	5.0	21.0	7.5	25.6	5.6	7.7
Streptococci (Esculin + and —)	10.0	7.7	10.0	7.7	7.5	2.6	5.6	2.6
Coliforms	0.0	2.6	2.5	2.6	2.5	7.6	0.0	0.0
C. bovis	15.0	12.8	25.0	23.1	17.5	23.1	8.3	20.5
Other	0.0	0.0	0.0	2.7	0.0	0.0	0.0	2.6
No Isolation	67.5	58.9	60.0	46.2	67.5	46.2	80.5	66.6

*Values expressed as percent of total quarters.
†Control (C) (n = 40, n = 36 for day 60); zinc-supplemented (Z).

Table 2 shows the mean quarter somatic cell counts for the first three sampling points of the control and experimental animals. There were no significant ($P > 0.05$) differences between the two groups of cows for the first 30 days of the dietary regimen.

The mean cell counts for normal quarters and clinical quarters are shown in Table 3. There were no significant differences ($P > 0.05$) with time in the normal quarters in either group. Clinical quarter cell counts in both groups were significantly lower ($P < 0.05$) when comparing day 30 with the pretrial sample.

Bacterial isolations from all quarter milk samples are shown in Table 4. *C. bovis* was the major bacterial isolation. Of the ten clinical quarters present at the beginning of the study, esculine + streptococci were isolated from five of these quarters, coliforms from one, and a *Cornybacterium* spp. from one. Three clinical quarters had no isolation.

In conclusion, it was found that after 60 days of zinc supplementation, there was no effect on mammary somatic cell counts in normal quarters. The decrease in DMSCC of clinical quarters in both groups of cows was likely due to the antibiotic treatment administered.

Previous research has suggested a correlation between plasma zinc levels and the incidence of udder infections (4). In this study, statistical analysis of the data did not show a correlation between the two parameters.

Immune Responses

T cell blastogenesis induced by the mitogens phytohemagglutinin (PHA) and concanavalin A (Con A), and erythrocyte and leukocyte counts were measured 2 days before starting treatments, at 4 weeks, and at 8 weeks in all animals. Sterile blood samples were taken via tail vein into vacutainer tubes and in the laboratory were subsampled for cell counting. The remaining sample was centrifuged at 200 x g, 18° C, for 30 min to produce a "buffy coat" of leukocytes. The buffy coats were removed and individually layered onto a preparation of Lymphoprep (ficoll 400 - sodium metrizoate) to isolate mononuclear cells (lymphocytes). The suspension was centrifuged at 400 x g, 18° C, for 30 min, after which the lymphocytes were removed and washed three times with tissue culture medium RPMI-1640.

The cells from each cow were then suspended at 5×10^6/ml in enriched RPMI (RPMI-1640 with 5% steer serum, 2 ml of 200 mM glutamine/100 ml, 100 IU penicillin/ml, and 100 μg streptomycin/ml). One hundred μl of each lymphocyte suspension was used in a culture. All cultures were performed in triplicate.

Control cultures (for spontaneous blastogenesis), cultures stimulated with 1 μg PHA, and cultures stimulated with 1 μg Con A were

TABLE 5.—Lymphocyte Blastogenesis in Zinc-treated Dairy Cows.*

Time Point	Con A†		PHA‡	
	Control (n = 10)	Zinc-supplemented (n = 9)	Control (n = 10)	Zinc-supplemented (n = 9)
Day 0 (9/29/81)	32.14[d]**	35.0[d]	31.40[d]	32.27[d]
Day 30 (10/29/81)	9.72[e]	12.58[e]	8.98[e]	9.84[e]
Day 60 (12/1/81)	24.63[d]	27.49[d]	28.37[d]	29.23[d]

*Data are least squares means, net pmol thymidine incorporated/10^5 cells.
†Error mean square = 94.08.
‡Error mean square = 110.00.
**Means with different superscripts within mitogen experiments are different ($P < .01$), Duncan's new multiple range test (19).

run for each cow. Cells were harvested after 3 days of culture by the procedures of Murray and Chenault (12), and computations were done by the method of Burford-Mason and Gyte (3). Cell counts were made with a hemocytometer.

Blastogenesis was not affected by zinc treatment, but blastogenesis at 4 weeks was significantly lower than at other periods.

Cell counts generally followed the same pattern as blastogenesis rate (although no significant correlation was detected), indicating that the immunological capacity of the cows in both groups was not constant throughout the experiment.

The fact that the immunological functions were affected by period suggests that either there is an unknown environmentally related factor capable of influencing immune functions, or that the natural fluctuation

TABLE 6.—Cell Numbers in Blood of Cows Treated with Zn.

Time Point	RBC*		WBC†	
	Control (n = 10)	Zinc-supplemented (n = 9)	Control (n = 10)	Zinc-supplemented (n = 9)
Day 0 (9/29/81)	6743	6031	9355	7611
Day 30 (10/29/81)	6454	5743	7876	6132
Day 60 (12/1/81)	6984	6273	9184	7440

*Data are least squares means X 10^{-6} cells/ml. Error M.S. = 1325035.
†Data are least squares means X 10^{-3} cells/ml. Error M.S. = 1183399.

in immune response function was somehow synchronized in these animals. It is interesting to note that the significant reduction in rate of blastogenesis occurred in the same time period in which a significant reduction in somatic cell count occurred in clinical quarters. There was no significant correlation between these values, however. These points are of potential importance for future work. It is clear that the zinc treatment did not affect immunological function in these animals.

REFERENCES

1. Bremmer, I. and A. H. Knight. 1970. The complexes of zinc, copper and manganese present in rye grass. Brit. J. Nutr., 24:279.
2. Brink, M. F., D. E. Becker, S. W. Terrilll, and A. H. Jensen. 1959. Zinc toxicity in the weanling pig. J. Anim. Sci., 18:836.
3. Buford-Mason, A. P. and G. M. L. Gyte. 1979. An alternative method of expressing results of lymphocyte transformation experiments. J. Immunol. Methods, 28:391.
4. Feaster, J. P., S. L. Hansard, J. T. McCall, F. H. Skipper, and G. K. Davis. 1954. Absorption and tissue distribution of radiozinc in steers fed high-zinc rations. J. Anim. Sci., 13:781.
5. Henry, R. J., D. C. Cannon, and J. W. Winkelman, eds. 1974. Clinical Chemistry, Principles and Techniques. Harper and Row Inc., New York, pp. 704-705.
6. Legg, S. P. and L. Sears. 1960. Zinc sulphate treatment of parakeratosis in cattle. Nature (London), 186:1061.
7. Miller, W. J., D. M. Blackmon, R. P. Gentry, W. J. Pitts, and G. W. Powell. 1967. Absorption, excretion, and retention of orally administered zinc-65 in various tissues of zinc-deficient and normal goats and calves. J. Nutr., 92:71.
8. Miller, W. J., D. M. Blackmon, J. M. Hiers, Jr., P. R. Fowler, C. M. Clifton, and R. P. Gentry. 1967. Effects of adding two forms of supplemental zinc to a practical diet on skin regeneration in Holstein heifers and evaluation of a procedure for determining rate of wound healing. J. Dairy Sci., 50:715.
9. Miller, W. J., C. M. Clifton, P. R. Fowler, and H. F. Perkins. 1965. Influence of high levels of dietary zinc on zinc in milk performance and biochemistry of lactating cows. J. Dairy Sci., 48:450.
10. Miller, J. K. and W. J. Miller. 1962. Experimental zinc deficiency and recovery in calves. J. Nutr., 76:467.

11. Miller, W. J., J. D. Morton, W. J. Pitts, and C. M. Clifton. 1965. Effect of zinc deficiency and restricted feeding on wound healing in the bovine. Proc. Soc. Exp. Biol. Med. 118:427.
12. Murray, F. A. and J. R. Chenault. 1982. Effects of steroids on bovine T-lymphocyte blastogenesis *in vitro*. J. Anim. Sci. (in press).
13. National Research Council. 1978. Nutrient requirements of domestic animals. 3. Nutient requirements of dairy cattle. 5th rev. ed. Nat. Acad. Sci., Washington, D. C.
14. Ott, E. A., W. H. Smith, R. B. Harrington, and W. M. Beeson 1966. Zinc toxicity in ruminants. II. Effect of high levels of dietary zinc on gains, feed consumption and feed efficiency of beef cattle. J. Anim. Sci., 25:419.
15. Ott, E. A., W. H. Smith, R. B. Harrington, H. E. Parker, and W. M. Beeson. 1966. Zinc toxicity in ruminants. IV. Physiological changes in tissues of beef cattle. J. Anim. Sci., 25:432.
16. Ott, E. A., W. H. Smith, R. B. Harrrington, M. Stob, H. E. Parker, and W. H. Beeson. 1966. Zinc toxicity in ruminants. III. Physiological changes in tissues and alterations in rumen metabolism in lambs. J. Anim. Sci., 25:424.
17. Ott, E. A., W. H. Smith, M. Stob, H. E. Parker, and W. M. Beeson. 1965. Zinc deficiency syndrome in the young calf. J. Anim. Sci., 24:735.
18. Perry, T. W., W. M. Beeson, W. H. Smith, and M. T. Mohler. 1968. Value of zinc supplementation of natural rations for fattening beef cattle. J. Anim. Sci., 27:1674.
19. Steel, R. G. D. and J. H. Torrie. 1980. Principles and Procedures of Statistics, 2nd ed. McGraw-Hill, Inc., New York.
20. Todd, W. R., C. A. Elvehjem, and E. B. Hart. 1934. Zinc in the nutrition of the rat. Am. J. Physiol., 107:146.

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