

The effect of injectable trace mineral (selenium, copper, zinc, and manganese) on health and production of lactating Holstein cows

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ABSTRACT

The objective of this study was to evaluate the effect of subcutaneous supplementation of 300 mg of zinc, 50 mg of manganese, 25 mg of selenium, and 75 mg of copper (Multimin North America, Inc., Fort Collins, Co) at 230 days of gestation, 260 days of gestation, and 35 days postpartum, on health, milk production and reproduction of lactating Holstein cows. A randomized field trial was conducted on 3 large commercial dairy farms located near Ithaca, New York and 1416 cows were enrolled. Subcutaneous TMS significantly decreased linear Somatic Cell Count (SCC) scores as compared to control cows. The interaction of parity (1, 2, > 2) and treatment group (Control vs. TMS) was significant; the effect of treatment on linear score increased as parity group increased and the greatest reduction in linear scores was observed in parity > 2 cows treated with TMS. Control cows had 1.31 times higher odds of subclinical mastitis than TMS cows. The main effect of treatment on clinical mastitis was not significant, however; the interaction of treatment and parity was significant with a reduction of 39% in the cumulative incidence of clinical mastitis observed for multiparous cows treated with TMS. Additionally, control cows had 1.69 and 1.30 increased odds of having stillbirth parturitions and endometritis, respectively.

INTRODUCTION

Trace minerals play an important role in dairy cows' immune function (Spears, 2000), fertility (Wilde, 2006) and growth (Wikse et al., 1992). Zn forms a structural component of over 300 enzymes (Spears, 2000; Andrieu, 2008) and Zn deficiency impairs immunity and reduces disease resistance (Shankar and Prasad, 1998). Manganese (Mn) is an important part of enzymes that are involved in immune system, reproduction, lipid and carbohydrate metabolism, bone growth, nervous function, and anti-oxidant defense (Andrieu, 2008). A recent study has shown that lactating cows need a Mn intake of 1.6 times higher and dry cows need a Mn intake of 2.7 times higher than the intake suggested by the NRC, to meet estimated fecal losses of Mn (Weiss and Socha, 2005). Copper (Cu) is also a component of several enzymes important for anti-oxidant resistance, cellular respiration, bone formation, immune function, carbohydrate and lipid metabolism and cardiac function (Andrieu, 2008) and it seems to play an important role on udder health (Scaletti et al., 2003). Selenium (Se) is also an essential component of enzymes of the antioxidant system (Spears and Weiss, 2008). Se deficiency impairs the anti-oxidant and immune system, and consequently disease resistance (Grasso et al., 1990; Hogan et al., 1990; Spears, 2000). Additionally, serum concentration of Se seems to be associated with udder health (Erskine, 1987; Weiss, 1990); and its synergistic action with vitamin E has for long been established (Smith, et al., 1984).

The transition period (usually defined as the period from three weeks before to three weeks after calving) is extremely challenging for the dairy cow that goes through physiological stress, preparing for and recovering from parturition (Drackley, 1999) dramatically altering her metabolism to supply the mammary gland with nutrients necessary for milk synthesis (Bauman and Currie, 1980; Goff et al., 2002) and dealing with reduced dry matter intake, negative energy balance (Roche et al., 2009) and oxidative stress (Sordillo and Aitken, 2009).

Nockels et al. (1993) reported that calves under stress reduced their trace mineral retention ability. Given the fact that the transition period is a stressful period for the cow, a similar reduction in trace mineral retention ability could also happen in transition cows. Moreover, either the act of parturition or the beginning of lactation have been found to be related with a decrease in plasma minerals levels, such as calcium (Ca) and zinc (Zn) (Goff et al., 2002), suggesting that other mineral levels could also be affected during the same period. Hence, it is possible that transition cows may have increased trace minerals needs that a diet formulated to meet the current NRC recommendations for Holstein cows may not satisfy, especially since dietary mineral supplements may not be absorbed properly due to interactions with other nutrients at the ruminal level (Suttle, 1986, Goonerate et al., 1989) or due to modifications in the rumen, while antagonists located in drinking water may also have a negative effect on the efficiency of trace minerals absorption from the digestive tract (Spears, 2003). Dietary sulfur seems to reduce the bioavailability of Se and Cu; moreover, high dietary iron reduces Cu bioavailability (Spears, 2003). Additionally, high dietary Ca and phosphorus may reduce Mn absorption in ruminants (Spears, 2003). An injectable trace mineral solution could potentially provide an alternative way of extra trace mineral supplementation during the transition period. Some positive effects of such supplementation on cows' reproductive traits have already been shown (Harrison et al. 1984, Sales et al. 2011). However, studies regarding the effect of injectable trace minerals supplementation during the transition period, on health and milk production traits remain, to the best of our knowledge, scarce.

Thus, the objective of this study was to evaluate the effect of subcutaneous supplementation of a trace mineral supplement containing Zn, Mn, Se, and Cu (Multimin North

America, Inc., Fort Collins, Co) at 230 days of gestation, 260 days of gestation and 35 days postpartum, on health traits, milk production and reproductive performance of lactating Holstein cows.

MATERIALS AND METHODS

Farms and Management

In this study 1416 cows kept in three dairy farms located near Ithaca, New York, were enrolled from August 30th of 2010 until June 30th of 2011. These farms were selected because of their long history of working relationship with the Ambulatory and Production Medicine Clinic at Cornell University. Farm A milked 3,700 cows, farm B milked 1,600 cows and farm C milked 3,500 cows. Cows were fed a diet that exceeded the NRC recommendations for trace minerals

Study Design and Treatments

A randomized field trial was conducted on 3 large commercial dairy farms. Dry cows and pregnant heifers were blocked by parity group (groups 1, 2, and >2 for first, second and third or greater lactation cows respectively) and randomly allocated into one of two treatments; trace mineral supplemented (TMS) or control. Randomization was completed in Excel (Microsoft, Redmond, WA) using the random number function and imported into the farms' Dairy Comp 305[®] program. Cows that were randomly assigned to the treatment group received 3 injections of trace minerals (Multimin North America, Inc., Fort Collins, Co) at approximately 230 days of gestation, 260 days of gestation, and 35 days

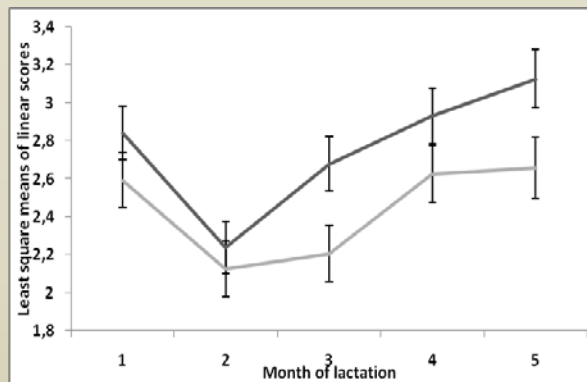
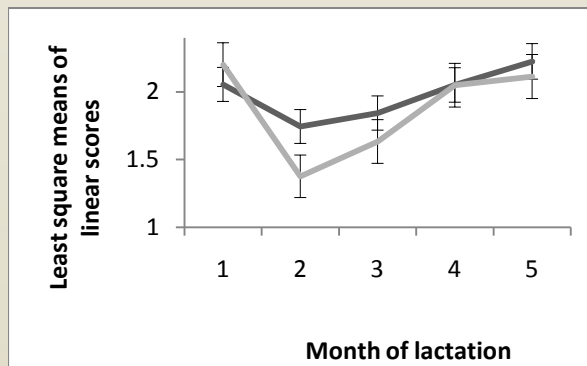
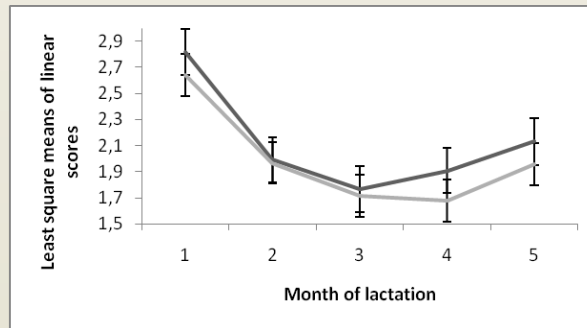


Figure 1: Least square means of linear scores by month of lactation for primiparous cows. The dark gray line represents the controls and the light gray lines represent the trace mineral supplemented cows. The first, second and third figures represent parities 1, 2, and 3 respectively

postpartum; each injection contained 300 mg of zinc oxide, 50 mg of manganese carbonate, 25 mg of sodium selenite, and 75 mg of copper carbonate. Control cows were not injected with a negative placebo. Body condition scores (BCS) were assessed for all study cows at 230 days of gestation and at 35 ± 3 DIM. A five-point scale (1 = emaciated, 5 = obese, scored in 0.25-point intervals) as described by Edmonson et al. (1989) was used.

RESULTS

Effect of subcutaneous trace minerals supplementation on udder health

Effects of trace mineral supplementation on linear scores during the first five months of lactation are presented in Figure 1. Briefly, the Least Square Means (LSM) of linear scores of control and TMS cows were 2.3 and 2.1, respectively ($P = 0.021$).

Additionally, LSM of linear scores for cows in parity 1, 2 and > 2 were 2.0, 1.9 and 2.6, respectively ($P < 0.001$), while, the LSM of linear scores for cows in the first, second, third, fourth and fifth month of lactation were 2.5, 1.9, 2.0, 2.2 and 2.4, respectively ($P < 0.001$).

Figures 1, illustrates the three way interaction between the independent variables treatment, parity and month of lactation. The effect of TMS treatment on linear scores increased as parity increased; the decreased in linear scores observed in TMS treated primiparous cows was small compared to linear score drops observed for parity 2 and parity 3 or greater (Figures 1).

The effect of treatment, parity and month of lactation on subclinical mastitis was also evaluated. All three independent variables were found to have a statistically significant effect on subclinical mastitis. Control cows were at 1.31 times higher odds of having subclinical mastitis ($P = 0.005$). Moreover, second lactation cows and third lactation or older cows were at 1.2 and 2.6 times higher odds of having subclinical mastitis, respectively ($P < 0.001$). Additionally, cows in the second, third, fourth and fifth month of lactation had 7.0, 9.6, 11.2 and 10.3 increased odds of developing subclinical mastitis,

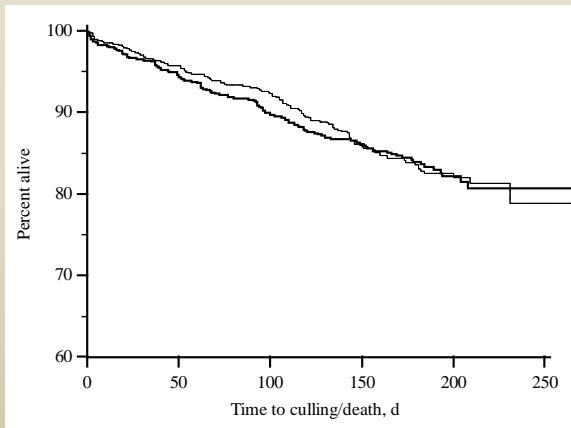
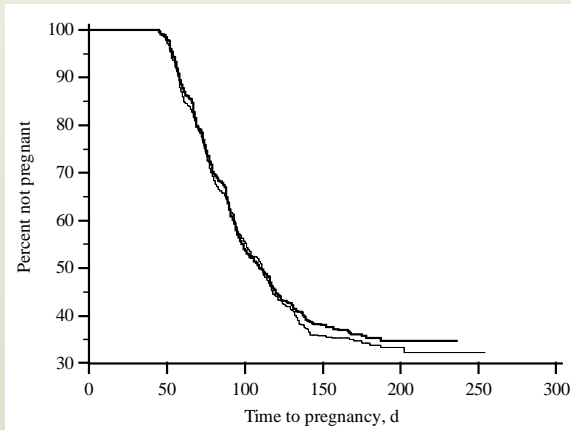


Figure 2: Reproduction and Survivability

respectively ($P < 0.001$). Additionally, primiparous cows were at 1.82 times increased odds of having clinical mastitis compared to multiparous cows ($P < 0.01$).

Effect of subcutaneous trace minerals supplementation on other health traits

The effect of treatment on the odds of stillbirth parturition, endometritis, metritis, retained placenta and displaced abomasum is presented in Table 7. Metritis, retained placenta and displaced abomasum were not affected by treatment. However, control cows had 1.69 and 1.30 increased odds of having stillbirth parturition and endometritis, respectively ($P = 0.039$ and 0.028 , respectively).

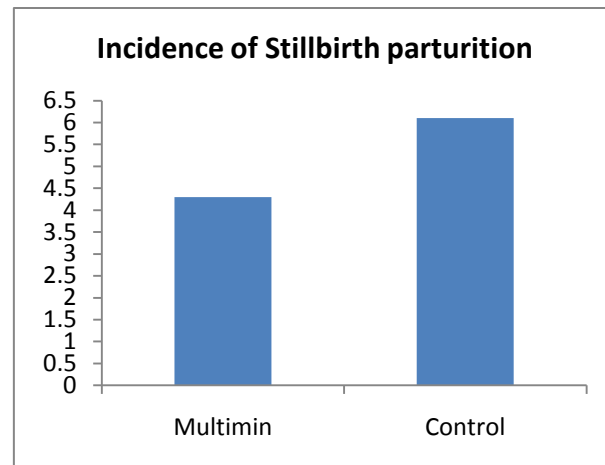


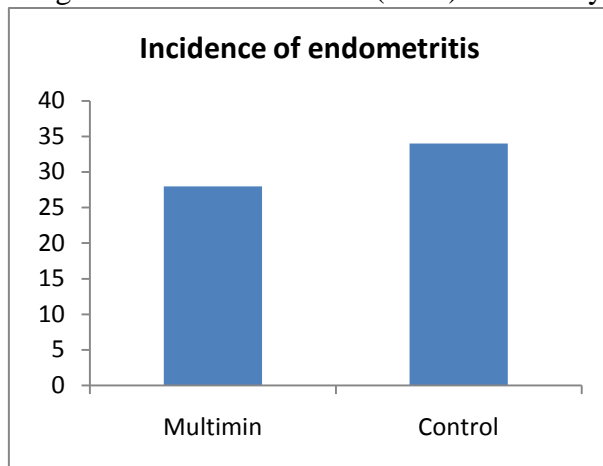
Table 7: Mixed logistic regression model that evaluated the effect of treatment on the odds of stillbirth, endometritis, metritis, retained placenta, and displaced abomasum. The models were fitted in SAS using the GLIMMIX procedure and included the variable “farm” as a random effect.

	Control	TMS ¹	Adjusted odds ratio 95% C. I.	<i>P</i> -value
Stillbirth	6.1 %	4.3 %	1.69 (1.03 – 2.80)	0.039
Endometritis	34.2 %	28.6%	1.30 (1.03 – 1.64)	0.028
Metritis	11.5 %	11.8 %	1.04 (0.74 – 1.46)	0.827
Retained Placenta	6.7 %	6.8 %	1.00 (0.65 – 1.53)	0.999
Displaced abomasum	2.6 %	1.3 %	1.73 (0.76 – 3.93)	0.194

¹TMS = Trace mineral supplemented. See table 3 for remainder of key.

DISCUSSION

The most remarkable effect of trace minerals supplementation in this study was on udder health. Linear scores were significantly lower for TMS treated cows and the effect was particularly important for multiparous cows. Additionally, TMS treated cows were less likely to have subclinical mastitis. There was also a significant reduction in clinical mastitis cases in TMS treated multiparous cows; which was not observed for primiparous cows. This effect on udder health may be due to the known positive association of cows' Se status with their immune response (Salman, et al., 2009) and consequently with udder health (Erskine, et al., 1987; Weiss, et al., 1990). It has also been proven that Cu plays an important role in enhancing resistance to *Escherichia coli* mastitis. Kruze et al. (2007) found that cows receiving a single subcutaneous shot of Se at drying-off had lower SCC after an intramammary challenge with *Staphylococcus aureus*, which is also in accordance with results presented here. On the other hand, Ceballos et al. (2010), reported that one injection of a long-acting form of Se at drying-off did not affect udder health in the subsequent lactation for pasture-based multiparous cows. It should be noted though that Ceballos et al. (2010) used only 49 cows in their study that were reared under



different conditions than the cows in our study. Added to that, the combination of trace minerals used in the present study could be more beneficial than administering Se alone. Nevertheless, the same study found that supplemented cows had higher serum concentrations of glutathione peroxidase, an antioxidant regulated by Se, which contributes in the defense of the mammary gland (Sordillo, et al., 2007). Our findings regarding the effect of treatment on heifers' udder health are in partial agreement with Ceballos-Marquez et al., (2010), who found that either dietary or

injectable supplementation of Se did not show any effect on SCC and clinical mastitis in pasture-based heifers. The effect of trace mineral supplementation on udder health may be increased by adding vitamin E to the supplement (Smith, et al., 1984) and the investigation of the effects of a combination of the product used in this study with vitamin E on udder health seems justified.

Trace mineral supplementation in this study had a significant positive effect only on multiparous cows' udder health while it did not seem to be beneficial for primiparous cows. Negative energy balance and some of its consequences (BCS loss, high blood concentration of non-esterified fatty acids and β -hydroxybutyric acid) are usually more severe in multiparous cows (Wathes, et al., 2007) and have already been correlated with immunosuppression (Lacetera, et al., 2004; Hammon, et al., 2006; Grinberg, et al., 2008). Mehrzad, et al. (2009) reported a more pronounced decrease in the phagocytic and bactericidal activities of both blood and milk polymorphonuclear neutrophils against *Staphylococcus aureus* in multiparous cows, as compared to primiparous cows, during early lactation. Additionally, multiparous cows usually have higher peak milk yields and earlier in the lactation period and hence their trace minerals needs during early lactation might also be higher than the needs of primiparous cows. In other words, multiparous transition cows may be in greater need of trace mineral supplementation in order to

maintain their udder health during early lactation and this assumption may partially explain our results.

Although trace minerals are related to fertility (Wilde, 2006; Andrieu, 2008), this study did not reveal any effect of additional trace mineral supplementation on dairy cows' reproductive performance. In this study, cows were fed a diet that exceeded the NRC recommendations for trace minerals and apparently this diet was alone enough for maintenance of reproductive functions. The effect of additional trace mineral supplementation on reproductive performance is controversial, studies have reported that additional supplementation of trace minerals could either have a negative (Olson, et al., 1999; Vanegas, et al., 2004), positive (Sales, et al.,), or neutral effect on reproductive performance (Vanegas, et al., 2004; Hackbart, et al., 2010). Vanegas et al. (2004) used the same source of trace minerals as the present study and they reported that one shot postpartum did not affect cows' reproductive performance. However, the same study showed a negative effect on reproductive performance when cows received two shots of trace minerals, one before and one after parturition. Additionally, Olson et al. (1999) reported that the combination of Cu, Mn, cobalt (Co) and Zn fed at higher levels than the required had a negative effect on reproduction. Moreover, embryo quality and follicular growth were not altered by oral supplementation of Zn, Mn, Cu and Co (Hackbart, et al., 2010). On the other hand, a study performed with crossbred heifers showed an increment in the conception rate (embryo survival) of heifers that received subcutaneously the trace mineral source that was used in the present study 17 days prior embryo transfer (Sales, et al., 2011). Moreover, additional Co, Cu, Mn and Zn effectively reduced days to first observed estrus in Holsteins and Jersey cows (Campbell, et al., 1999).

Although trace mineral supplementation in this study did not have any effect on reproductive performance, it was found to decrease incidence of stillbirths and endometritis; conditions that impair reproductive performance (Bicalho et al., 2007; Gilbert et al., 2005) and are both associated with immunosuppression (Chassagne et al., 1999; Galvao et al., 2010). (Chassagne et al., 1999) found that cows with prepartum circulating neutrophil counts higher than 1950 per mm³ had lower risk of stillbirths, while (Galvao et al., 2010) reported that cows that developed endometritis experienced a decrease in neutrophil glycogen concentration during the first 3 weeks postpartum, which could result in reduction of neutrophil activity. Immunosuppression is associated with trace mineral deficiencies. Research with laboratory animals and humans revealed that Zn deficiency impairs immune response and disease resistance (Shankar and Prasad, 1998). Additionally, it has been shown that Cu affects neutrophils and specific immune functions (Spears, 2000). Moreover, Se deficiency in dairy cows reduced the ability of blood neutrophils to kill bacteria (Hogan et al., 1990). Therefore, supplementation of trace minerals during the dry period in this study could have increased the immune response of the cows and hence decreased stillbirths and endometritis incidence. However, this immune response increment observed in supplemented cows was probably not sufficient to reduce incidence of retained placenta and metritis, which are disorders associated with impaired immune function too (LeBlanc, 2008).

In conclusion, the administration of three subcutaneous trace mineral injections (at 230 days of gestation, 260 days of gestation, and 35 days postpartum) had a positive impact on udder health, decreasing linear scores and the incidence of mastitis. Additionally, TMS treatment also decreased the incidence of stillbirth parturition and endometritis.