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Effect of an injectable trace mineral supplement containing selenium, copper, zinc, and manganese on the health and production of lactating Holstein cows

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ABSTRACT

The objective of this study was to evaluate the effect of a subcutaneous injection of a multimineral preparation containing 300 mg of zinc, 50 mg of manganese, 25 mg of selenium, and 75 mg of copper at 230 and 260 days of gestation and 35 days postpartum, on the health, milk production and reproductive performance of lactating Holstein cows. A randomized field trial was conducted on three large commercial dairy farms located near Ithaca, New York, USA, with 1416 cows enrolled. All cows were housed and offered a total mixed ration consisting of approximately 55% forage and 45% concentrate on a dry matter basis of the diet, which supplied 2–6 times the NRC requirements for the supplemented elements. Dry cows and pregnant heifers were blocked by parity and randomly allocated to one of two treatments: Trace mineral supplemented (TMS) or control.

For multiparous cows, subcutaneous TMS significantly decreased linear somatic cell count scores (normalized data) as compared to control cows. The incidence of subclinical mastitis for TMS and control cows was 10.4% and 8.0%, respectively ($P = 0.005$). The main effect of treatment on clinical mastitis was not significant but the interaction of treatment and parity was significant. For primiparous cows, the incidence of clinical mastitis was 11.8% and 15.6% for control and TMS cows, respectively ($P = 0.33$); for multiparous cows, the incidence of clinical mastitis for control and TMS cows was 25.4% and 19.7%, respectively ($P = 0.03$). Additionally, control cows had increased odds of stillbirth and endometritis (odds ratios 1.69 and 1.30, respectively). The incidence of endometritis was 34.2% and 28.6% for control and TMS cows, respectively ($P = 0.039$) but treatment had no effect on reproductive performance, milk production or other health traits. Further research is required to confirm these findings and to establish whether the response seen in this study was related to the supplementation of a particular mineral.

Introduction

During the transition period, dairy cows undergo physiological stress preparing for and recovering from parturition, dramatically altering their metabolism to supply the mammary gland with nutrients necessary for milk synthesis (Goff et al., 2002), and also dealing with reduced dry matter intake (DMI), negative energy balance (Roche et al., 2009) and oxidative stress (Sordillo and Aitken, 2009).

Trace minerals play an important role in dairy cow immune function (Shankar and Prasad, 1998), fertility (Rabiee et al., 2010), and growth (Enjalbert et al., 2006). Nockels et al. (1993) reported that stressed calves reduced their trace mineral retention ability. Given that the transition period is a stressful time for the cow, a similar reduction in trace mineral retention ability could also happen in transition cows (Xin et al., 1993). The act of parturition or the beginning of lactation has been found to be associated with a reduction in plasma concentrations of calcium (Ca) and zinc (Zn) (Goff and Stabel, 1990; Goff et al., 2002), suggesting that other mineral concentrations could also be affected during the same period.

Inclusion of minerals in the diet does not ensure intake or absorption. DMI is already decreased during the transition period (Roche et al., 2009); additionally, there are dietary and animal factors contributing to DMI variation between animals (Hayirli et al., 2002) and consequently to variation in the intake of trace minerals. Dietary mineral supplements may not be absorbed properly due to interactions with other nutrients at the rumen level (Suttle, 1986). Antagonists in drinking water (e. g. iron) can also have a negative effect on trace mineral absorption from the digestive tract (Spears, 2003).

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An injectable trace mineral solution could potentially provide an alternative way of delivering extra trace minerals during the transition period. Pogge et al. (2012) reported that the use of an injectable trace mineral solution increased liver concentrations of Cu and Se for at least a 15-day period, and increased plasma Zn and Mn for several hours. Positive effects of such supplementation on cow reproductive traits have already been shown (Harrison et al., 1984; Sales et al., 2011). Additionally, Cu and Se seem to play an important role in udder health (Scaletti et al., 2003; Weiss et al., 1990). However, to the best of our knowledge, studies regarding the effect of injectable trace mineral supplementation during the transition period on health and milk production traits remain scarce. It is possible that supplementing cows with extra trace minerals twice in the dry period may help them accumulate trace minerals in stores which can be used during early lactation, while an injection during early lactation could have a positive effect on reproductive efficiency.

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) 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.

Material and methods

Farms and management

One thousand, four hundred and sixteen cows kept on three dairy farms located near Ithaca, New York, USA, were enrolled from 30th August until 4th November 2010, and data were collected until 30th June 2011. Farm A milked 3700 cows, farm B 1600 cows and farm C 3500 cows. The cows on farms A and C were housed in free-stall (cubicle) barns with concrete stalls covered with mattresses and bedded with digested manure solids and waste paper–pulp, respectively. Farm B housed the cows in free-stalls with sand bedding.

All cows were offered a total mixed ration consisting of approximately 55% forage (corn silage, haylage, and wheat straw) and 45% concentrate (corn meal, soybean meal, canola, cotton seed, and citrus pulp) on a DM basis of the diet. The diets were formulated to meet or exceed the National Research Council nutrients requirements for lactating Holstein cows weighing 650 kg and producing 45 kg of 3.5% fat corrected milk. The chemical composition (mineral and vitamins) of pre-fresh and lactating cow diets for study farms A, B, and C is shown in Table 1.

Study design and treatments

Dry cows and pregnant heifers were blocked by farm and parity group (groups 1, 2, and >2, for first, second and third or greater lactation cows, respectively) and randomly allocated to one of two treatments, namely, (1) trace mineral supplemented (TMS) or (2) control. All dry cows and pregnant heifers that were available during the enrollment period were included in the study. Randomization was completed in Excel (Microsoft) using the random number function and imported into the farms' Dairy Comp 305 program (Valley Agricultural Software).

Cows that were randomly assigned to the treatment group were given three injections of trace minerals (Multimin) at approximately 230 days of gestation, 260 days of gestation, and 35 days postpartum; each injection (5 mL) contained 300 mg of zinc, 50 mg of manganese, 25 mg of selenium, and 75 mg of copper. Ethylenediamine tetra-acetate (Na_2EDTA) was used to dissolve insoluble Cu, Mn and Zn at a concentration of 450 mg/mL; 0.1% chlorocresol was added to the solution as a preservative. Control cows were not injected with a negative placebo. Body condition scores (BCS) were assessed at 230 days of gestation and at 35 ± 3 days in milk (DIM). A 5-point scale was used (Edmonson et al., 1989). Assessors were blinded to treatment status.

The project proposal was approved by the Cornell University Animal Care and Use Committee (2009-0001) and owner consent was obtained before the study was started.

Case definitions

'Stillbirth' was defined as the death of a calf occurring just prior to, during, or within 48 h of parturition. 'Retained fetal membranes' was defined as failure to release fetal membranes within 24 h of calving. Metritis and clinical mastitis were diagnosed and treated by trained farm personnel who followed a specific diagnostic protocol designed by the staff of the Ambulatory and Production Medicine Clinic, Cornell University. 'Metritis' was defined as the presence of fetid, watery, red–brown uterine discharge. 'Clinical mastitis' was defined by the diagnosis of abnormal changes in the udder and/or milk. Composite milk somatic cell count (SCC) was determined monthly by Dairy Herd Improvement Association (DHIA). 'Subclinical mastitis' was defined as a cow having a somatic cell count >200,000 and not diagnosed with clinical mastitis (Oliveira et al., 2011).

Data regarding survivability, reproduction (calving to conception interval), health traits, milk yield and SCC during the subsequent lactation were extracted from the farms' DairyComp 305 database. Displaced abomasum diagnosis made by the farm personnel was confirmed by veterinarians. Signs of uterine inflammation were evaluated at 35 ± 3 DIM by visual inspection of a uterine lavage sample as previously described (Machado et al., 2012a). Cows that had pus in the lavage sample were considered to have clinical endometritis.

Statistical analysis

Descriptive statistics analysis was undertaken in SAS using the FREQ procedure (SAS Institute). Five mixed general linear models were fitted to the data using the MIXED procedure of SAS (SAS Institute). The dependent variables evaluated in this study were: average daily milk production (kg/day), average daily fat-corrected milk (FCM) production (kg/day), milk protein (%), milk fat (%), and linear somatic cell count (SCC) score. The latter was calculated using the formula:

$$\text{Linear score} = [\ln(SCC/100,000)/0.693147] + 3$$

This formula is automatically calculated and inputted into the dairy farm software of participating dairy farms by Dairy Herd Improvement Association laboratories (Radostits, 2000). Visual evaluation of the distribution plot of the studentized residuals was used to confirm that the residuals were normally distributed.

Table 1
Chemical composition (minerals and vitamins) of pre-fresh and lactating cow diets for study farms A, B, and C.

	NRC 2001 recommendations		Farm A		Farm B		Farm C	
	Pre-fresh	Lactation	Pre-fresh	Lactation	Pre-fresh	Lactation	Pre-fresh	Lactation
Calcium (%)	0.45	0.67	1.37	0.83	1.35	0.80	1.52	0.88
Phosphorus (%)	0.23	0.36	0.30	0.36	0.31	0.42	0.34	0.38
Magnesium (%)	0.12	0.2	0.42	0.32	0.37	0.35	0.39	0.33
Potassium (%)	0.52	1.06	1.11	1.24	1.07	1.28	1.55	1.54
Sodium (%)	0.10	0.22	0.12	0.46	0.11	0.47	0.14	0.53
Chloride (%)	0.15	0.28	0.52	0.50	0.36	0.50	0.45	0.59
Sulfur (%)	0.20	0.20	0.41	0.25	0.36	0.24	0.45	0.25
Cobalt (ppm)	0.11	0.11	0.96	1.49	0.87	1.13	0.75	1.90
Copper (ppm)	13.0	11.0	19.2	20.0	16.3	21.6	16.5	25.7
Iodine (ppm)	0.40	0.44	0.96	0.99	0.18	0.69	0.92	1.08
Iron (ppm)	13.0	17.0	225	205	220	190	316	213
Manganese (ppm)	18.0	13.0	109	72.8	81.0	81.5	95.8	90.2
Selenium (ppm)	0.30	0.30	0.38	0.47	0.36	0.53	0.55	0.57
Zinc (ppm)	22.0	52.0	63.8	80.8	53.7	84.4	70.90	91.76
Vitamin A (kIU/day)	82.6	75.0	178	170	138	172	178	187
Vitamin D (kIU/day)	21.5	21.0	35	39	27	43	34	42
Vitamin E (IU/day)	1202	545	1772	726	1560	611	1760	874

Pre-fresh diets were fed from 3 weeks prepartum through parturition and lactation diets were fed from parturition through week 35 postpartum.

The data comprised a series of repeated measures of each dependent variable throughout the 5 months of lactation. To account appropriately for within-cow correlation, the error term was modeled by imposing a first-order autoregressive covariance structure for all statistical models (which assumed that the within-cow correlation of the repeated measures of milk weights decreased as the time between the test dates increased; Sawalha et al., 2005). The model described below was fitted to all five mixed general linear models:

$$Y = X\beta + Z\gamma + e$$

where Y is the linear SCC score or average daily milk production (kg/day) or average daily FCM production (kg/day) or milk protein (%) or milk fat (%), X is the matrix of all independent variables, β the vector of all fixed effect parameters, $Z\gamma$ the random effect of farm (the covariance structure assumed for this term was a compound symmetry) and e is the random residual.

The independent variables offered to the model were: treatment, prepartum and postpartum BCS, parity, and month of lactation. All two-way interactions and the three-way interaction term between treatment, parity, and month of lactation were offered to the model. In all models variables and their respective interaction terms were retained in the model only when they had a significant effect ($P < 0.05$).

To assess the effect of injectable trace mineral treatment on the odds of subclinical mastitis, clinical mastitis, endometritis, metritis, stillbirth, retained placenta, and displaced abomasum, mixed logistic regressions were fitted to the data using the Glimmix procedure of SAS. The models included the fixed effects of treatment (TMS and control), parity (1, 2, >2) and month of lactation (1, 2, 3, 4, and 5). Additionally, the variable 'farm' was included in all models as a random effect and the variable 'cow id' was also included in the subclinical mastitis model to control for the within-cow correlation of data across the 5 months of observation of subclinical mastitis. All two-way interactions and the three-way interaction term between treatment, parity, and month of lactation were offered to the model. The only significant two-way interaction encountered was the interaction between treatment group and parity on the mixed logistic regression model that evaluated the effect of treatment on clinical mastitis. To obtain strata-specific odds ratio parameters for the different concentrations of the interaction term treatment * parity, the lsmeans option of the GLIMMIX procedure (binary distribution) was used.

Finally, the effect of treatment on reproduction and survival was analyzed by Cox's Proportional Hazard using the proportional hazard regression procedure in SAS. For analysis of reproduction, cows were right-censored if not diagnosed pregnant before culling, death, or the end of the data-collection period. For analysis of survival, cows were censored if they were alive at the end of the data-collection period. Variables offered to the models included treatment, parity, and farm. Two-way interactions between treatment and parity were tested. To evaluate the effect of treatment on reproductive performance and survivability, Kaplan–Meier survival analysis was carried out using MedCalc version 11.5.1.0 software (MedCalc Software).

Results

Descriptive statistics

Descriptive statistics regarding average age at enrollment (days), average BCS at enrollment, average gestation length at enrollment, and number of animals enrolled in each farm are presented in Table 2.

Table 2
Descriptive statistics of treatment groups.

	Control	Trace mineral supplemented
Average age (days) at enrollment (±SE)	1335 (±420)	1320 (±411)
Average body condition score at enrollment (±SE)	3.64 (±0.56)	3.65 (±0.54)
Average days of gestation at enrollment (±SE)	228.2 (±19.2)	230.2 (±17.5)
Enrolled animals on farm A (%)	198 (56)	153 (44)
Enrolled animals on farm B (%)	140 (48)	151 (52)
Enrolled animals on farm C (%)	350 (48)	371 (52)
Enrolled animals in parity 1 (%)	168 (45)	197 (55)
Enrolled animals in parity 2 (%)	295 (51)	288 (49)
Enrolled animals in parity 3 (%)	247 (53)	221 (47)
Total enrolled animals (%)	710 (50.1)	706 (49.9)

Effect of subcutaneous trace mineral supplementation on udder health

Effects of trace mineral supplementation on linear scores during the first 5 months of lactation are presented in Table 3. 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, the 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 ($P < 0.001$).

Linear SCC for the TMS and control cows, by parity and month of lactation, are presented in Fig. 1. The effect of TMS treatment on linear scores increased as parity increased; the decrease 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.

Effects of treatment, parity and month of lactation on subclinical mastitis, and the incidence of subclinical mastitis by treatment, parity and month of lactation are presented in Table 4. Control cows were at 1.3 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, respectively ($P < 0.001$).

Effects of treatment and parity on clinical mastitis, and the incidence of clinical mastitis by treatment, parity and the interaction of treatment and parity can be seen in Table 5. Primiparous cows were at 1.82 times increased odds of having clinical mastitis compared to multiparous cows ($P < 0.01$). Treatment had no significant effect on the odds ratio of clinical mastitis ($P = 0.14$). However, the interaction between treatment and parity significantly affected the odds of clinical mastitis; multiparous TMS treated cows had 39% less chances of developing clinical mastitis than multiparous control cows ($P = 0.03$) but there was no significant effect of treatment for primiparous cows ($P = 0.33$).

Table 3
Least squares means of linear scores. A mixed effect general linear model was used in this analysis and the variable 'farm' was included in the model as a random effect. To account appropriately for within-cow correlation of the repeated linear scores, the error term was modeled by imposing a first-order autoregressive covariance structure for all statistical models.

	LSM of linear scores (95% C.I.)	*P*-value
Treatment		
Control	2.3 (2.2–2.4)	0.021
TMS	2.1 (2.0–2.2)	
Parity		
1	2.0 (1.9–2.2)	<0.001
2	1.9 (1.8–2.0)	
>2	2.6 (2.5–2.7)	
Month of lactation		
1	2.5 (2.4–2.6)	<0.001
2	1.9 (1.8–2.0)	
3	2.0 (1.8–2.1)	
4	2.2 (2.1–2.3)	
5	2.4 (2.2–2.5)	
Treatment * parity * month	Refer to Fig. 1	<0.001

Treatment * parity * month, three-way interaction term between the independent variables treatment, parity, and month of lactation; LSM, least squares means; TMS; trace mineral supplemented. Treatment group received three injections of trace minerals (at 230 and 260 days of gestation, and 35 days postpartum). Each injection contained 300 mg of zinc, 50 mg of manganese, 25 mg of selenium, and 75 mg of copper.

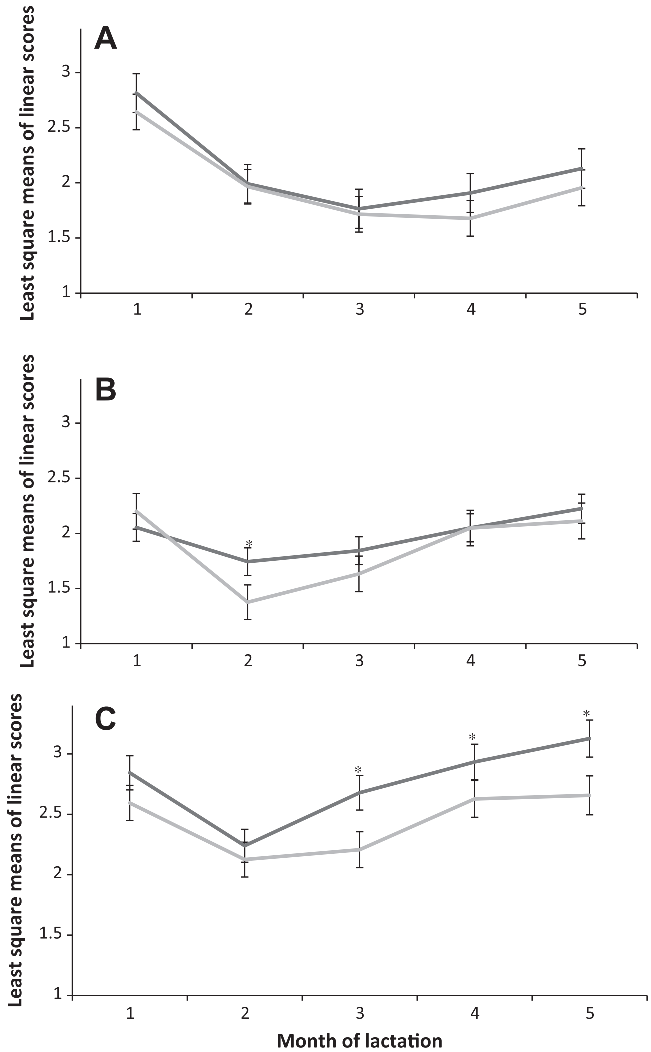

Fig. 1. Least squares means of linear scores by month of lactation for primiparous cows (A), for second lactation cows (B) and for third or higher lactation cows, and (C). The dark gray line represents the controls and the light gray lines represent the trace mineral supplemented cows. The error bars stand for the 95% confidence interval for the mean and were calculated for the mixed effect general linear model that included 'farm' as a random effect. An asterisk (*) indicates monthly means differ ($P < 0.05$).

Table 4
Effect of treatment, parity and month of lactation on the odds of subclinical mastitis. Subclinical mastitis was defined as somatic cell count >200,000 and negative for clinical mastitis.

	Incidence of subclinical mastitis	Adjusted odds ratio	*P*-value
Treatment			
Control	10.4	1.3	0.005
TMS	8.0	1.0	
Parity			
>2	14.2	2.6	<0.001
2	7.4	1.2	
1	6.0	1.0	
Month of lactation			
5	10.3	1.3	<0.001
4	11.2	1.4	
3	9.6	1.2	
2	7.0	0.8	
1	8.2	1.0	

TMS, trace mineral supplemented. See Table 3 for details of treatment.

Table 5
Mixed logistic regression model that evaluated the effect of treatment on the odds of clinical mastitis.

	Incidence of clinical mastitis (%)	Adjusted odds ratio	*P*-value
Treatment			
Control	22.4	1.25	0.14
TMS[a]	18.7	1.00	
Parity			
Multiparous	22.6	1.82	<0.01
Primiparous	13.8	1.00	
Treatment ∗ parity			
Control and primiparous	11.8	0.72	0.33
TMS and primiparous	15.6	1.00	
Control and multiparous	25.4	1.39	0.03
TMS and multiparous	19.7	1.00	

Strata-specific statistical comparisons were made comparing the effect of treatment for primiparous and multiparous cows separately using the lsmeans option of the GLIMMIX procedure of SAS and the binary distribution option; the *P* values for these comparisons are presented in this table.

[a] TMS, trace mineral supplemented. See Table 3 for details of treatment; *treatment ∗ parity*, interaction term between treatment and parity on the odds of clinical mastitis.

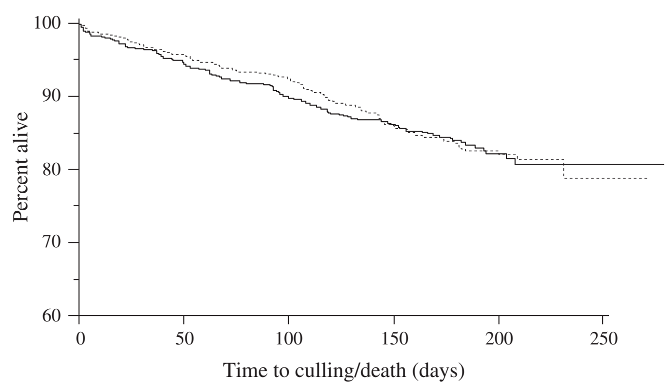

Fig. 2. Kaplan–Meier survival analysis of probability of death/culling by treatment group. Median time until culling/death was not estimated because more than 50% of the cows in both treatment groups were still alive by the end of the follow up period. There was no significant difference on survivability between trace mineral supplemented and controls ($P = 0.99$).

Effect of subcutaneous trace mineral supplementation on reproduction, survivability, milk production traits and on other health traits

There was no significant difference in reproduction between TMS and control cows. The median calving-to-conception intervals for the control and TMS groups were 110 and 111 days, respectively ($P = 0.61$). There was no significant difference in survivability between TMS and control cows (Fig. 2, $P = 0.99$). Treatment had no effect on reproduction and survivability when controlled for parity and farm in the models: The hazard ratio for pregnancy by treatment group was 1.008 (0.83–1.22) and the hazard ratio for death/culling by treatment group was 0.998 (0.77–1.29). Treatment also had no effect on average daily milk production, average daily FCM production, and percentage of fat and protein in the milk (Table 6).

The incidence of and effect of treatment on the odds of stillbirth parturition, endometritis, metritis, retained fetal membranes and displaced abomasum are 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

Table 6
Least squares means of milk (kg/day), fat corrected milk (kg/day), fat (%), and protein.

	Treatment		P-value
	TMS (95% C.I.)	Control (95% C.I.)	
Milk (kg/day)	40.3 (39.6–41.0)	40.5 (39.7–41.3)	0.66
Fat corrected milk (kg/day)	43.0 (42.4–43.5)	43.0 (42.4–43.6)	0.95
Fat (%)	3.7 (3.6–3.7)	3.7 (3.6–3.7)	0.75
Protein (%)	3.1 (3.07–3.11)	3.1 (3.07–3.11)	0.84

TMS, trace mineral supplemented. See Table 3 for details of treatment.

Table 7
Mixed logistic regression model that evaluated the effect of treatment on the odds of stillbirth, endometritis, metritis, retained placenta, and displaced abomasum.

	Control (%)	TMS (%)	Adjusted odds ratio (95% C.I.)	P-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

TMS, trace mineral supplemented. See Table 3 for details of treatment.

stillbirth and endometritis, respectively ($P = 0.039$ and 0.028, respectively).

Discussion

This study evaluated a multimineral supplementation so it was not possible to relate any response to a particular mineral. However, in a previous, recently published study using the same product that was also used here, it was reported that supplementation increased Se and Cu over 15 days, while plasma Zn and Mn concentrations were increased over 24 h (Pogge et al., 2012). These findings suggest that only Se and Cu were likely to be stored effectively, while the Mn and Zn supplementation was not likely to have a long term effect. However, further research is needed to validate what responses are due to which supplemented mineral, and to identify whether these minerals are stored in other tissues than the liver or if they are excreted 24 h after supplementation.

Our objective was to evaluate the effect of a subcutaneous supplementation of a trace mineral supplement on health traits performance of lactating Holstein cows. It is important to highlight that the trace mineral status of the study cows were not established. However, the study was performed in well-managed farms that did not have any history of mineral deficiencies and where the diets were also well managed. The study showed a positive effect of trace mineral supplementation on udder health. Linear SCC scores were significantly lower for TMS cows, particularly multiparous cows in the third to fifth month of lactation (Fig. 1). This was reflected in TMS having a lower incidence of subclinical mastitis than control cows (8% vs. 10.4%, respectively). The incidence of clinical mastitis cases was reduced by trace mineral supplementation, but only in multiparous cow (from 25.4% to 19.7%). In primiparous cows the incidence of mastitis was higher in treated than in control cows (15.6% vs. 11.8%, respectively). Although the difference was not significant ($P = 0.33$) this may have been due to lack of power so a larger scale replication of the study using more primiparous cows is required to establish whether the biologically important difference seen in this study is consistently found.

This effect on udder health, especially for multiparous cows, is consistent with the literature which suggests that there is a positive association between a cow's Se status and its immune response (Salman et al., 2009) and, consequently, with udder health (Weiss et al., 1990). The effect seen in this study is also consistent with the report by Kruze et al. (2007) who showed that cows receiving a single injection of Se at drying-off had lower SCC after an intramammary challenge with *Staphylococcus aureus*. In contrast, 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. However, that study lacked power (it used only 49 cows) and the animals were based at pasture rather than housed as in this study. Additionally, it could be that using a combination of trace minerals is more beneficial than administrating Se alone but this needs further research. The lack of an impact on udder health in heifers is consistent with the findings of Ceballos-Marquez et al. (2010), who reported that neither dietary nor injectable supplementation of Se reduced SCC or clinical mastitis in pasture-based heifers.

Although trace mineral supplementation can improve fertility (Rabiee et al., 2010), the present study did not reveal any effect of additional trace mineral supplementation on dairy cow calving-to-conception interval. Our study cows were fed a diet that exceeded NRC recommendations for trace minerals and apparently was enough for maintenance of reproductive functions but not for optimizing udder health. This difference is consistent with the finding by Jukola et al. (1996) that the whole blood Se concentration needed to optimize udder health was two times higher than the Se concentration needed to maintain reproductive parameters.

The effect of additional trace mineral supplementation on reproductive performance is controversial; researchers have reported that additional supplementation of trace minerals can have a negative (Vanegas et al., 2004), positive (Sales et al., 2011), or neutral effect on reproductive performance (Vanegas et al., 2004). Vanegas et al. (2004) used a similar trace mineral supplement to that used in the present study but with lower concentrations of Cu, Mn and Zn. They reported that one shot postpartum did not affect cow reproductive performance but two shots of trace minerals (one before and one after parturition) reduced reproductive performance. On the other hand, in a study performed with crossbred heifers, there was an increase in the conception rate (embryo survival) of heifers that received a subcutaneous injection, 17 days prior to embryo transfer, of the same trace mineral source used in the present study (Sales et al., 2011).

Trace mineral supplementation in our study was found to decrease the incidence of stillbirths and endometritis, conditions which are known to impair reproductive performance (Bicalho et al., 2007). In a recently published report, it was found that systemic trace mineral supplementation significantly decreased the proportion of cows with intrauterine contamination by *Fusobacterium* spp. and *Trueperella* spp. (Machado et al., 2012b), which are bacteria associated with uterine diseases, especially metritis and clinical endometritis (Williams et al., 2005; Bicalho et al., 2011).

The incidence of uterine diseases and mortality in the present study was similar to that seen in other work in North America. Leblanc et al. (2011) reported that the incidence of metritis typically ranged from 10% to 20%, which is similar to the incidence recorded here. Endometritis incidence was slightly higher in the current study than reported in previous studies performed in North America (Cheong et al., 2012; Dubuc et al., 2010) but it is important to highlight that the method we used to diagnose endometritis differs from other studies; we evaluated the presence of pus in a uterine lavage sample, while others used cytology and the presence of purulent vaginal discharge (Cheong et al., 2012; Dubuc et al., 2010). Culling rate (<25%) was also comparable to other studies performed in North America (Bonneville-Hebert et al., 2011; Brown et al., 2012).

In our study, we observed an effect of subcutaneous trace mineral supplementation on udder health, endometritis and stillbirth incidences. We believe that this was due to some of the trace minerals, particularly Se and Cu, being stored in the liver after supplementation (Pogge et al., 2012), and thus being available during periods of deficiency or high demands (Xin et al., 1993). However, it is important to highlight that many other factors than trace mineral status can influence the incidence of stillbirth, endometritis and mastitis. Our results on the effect of trace mineral supplementation might be different for different herds and the generalization of these results should be made cautiously. In particular, the response to treatment could be region-, farm- system or country-specific. Further research is needed to determine if the results presented here are repeatable in different geographical locations and different farm systems and also to determine the mechanism through which some health parameters are improved with additional trace mineral supplementation subcutaneously.

Conclusions

Administration of three subcutaneous injections of trace minerals had a positive impact on udder health, decreasing linear SCC scores, the incidence of subclinical mastitis, and (in multiparous cows) the incidence of clinical mastitis. Additionally, trace mineral supplementation decreased the incidence of stillbirth parturition and endometritis. However, treatment did not have effect on reproduction performance, milk production and other health traits.

Conflict of interest statement

This study was funded by Multimin North America, Inc. (Fort Collins, Co). The funders had no role in study design, data collection and analysis, decision to publish, or preparation of the manuscript.

References

Bicalho, R.C., Galvao, K.N., Cheong, S.H., Gilbert, R.O., Warnick, L.D., Guard, C.L., 2007. Effect of stillbirths on dam survival and reproduction performance in Holstein dairy cows. Journal of Dairy Science 90, 2797–2803.

Bicalho, M.L., Machado, V.S., Oikonomou, G., Gilbert, R.O., Bicalho, R.C., 2011. Association between virulence factors of *Escherichia coli*, *Fusobacterium necrophorum*, and *Arcanobacterium pyogenes* and uterine diseases of dairy cows. Veterinary Microbiology 157, 125–131.

Bonneville-Hebert, A., Bouchard, E., Tremblay, D.D., Lefebvre, R., 2011. Effect of reproductive disorders and parity on repeat breeder status and culling of dairy cows in Quebec. Canadian Journal of Veterinary Research (Revue Canadienne de Recherche Vétérinaire) 75, 147–151.

Brown, D.E., Dechow, C.D., Liu, W.S., Harvatine, K.J., Ott, T.L., 2012. Hot topic: Association of telomere length with age, herd, and culling in lactating Holsteins. Journal of Dairy Science 95, 6384–6387.

Ceballos, A., Kruze, J., Barkema, H.W., Dohoo, I.R., Sanchez, J., Uribe, D., Wichtel, J.J., Wittwer, F., 2010. Barium selenate supplementation and its effect on intramammary infection in pasture-based dairy cows. Journal of Dairy Science 93, 1468–1477.

Ceballos-Marquez, A., Barkema, H.W., Stryhn, H., Wichtel, J.J., Neumann, J., Mella, A., Kruze, J., Espindola, M.S., Wittwer, F., 2010. The effect of selenium supplementation before calving on early-lactation udder health in pastured dairy heifers. Journal of Dairy Science 93, 4602–4612.

Cheong, S.H., Nydam, D.V., Galvao, K.N., Crosier, B.M., Ricci, A., Caixeta, L.S., Sper, R.B., Fraga, M., Gilbert, R.O., 2012. Use of reagent test strips for diagnosis of endometritis in dairy cows. Theriogenology 77, 858–864.

Dubuc, J., Duffield, T.F., Leslie, K.E., Walton, J.S., LeBlanc, S.J., 2010. Definitions and diagnosis of postpartum endometritis in dairy cows. Journal of Dairy Science 93, 5225–5233.

Edmonson, A.J., Lean, I.J., Weaver, L.D., Farver, T., Webster, G., 1989. A body condition scoring chart for Holstein dairy cows. Journal of Dairy Science 72, 68–78.

Enjalbert, F., Lebreton, P., Salat, O., 2006. Effects of copper, zinc and selenium status on performance and health in commercial dairy and beef herds: Retrospective study. Journal of Animal Physiology and Animal Nutrition 90, 459–466.

Goff, J.P., Kimura, K., Horst, R.L., 2002. Effect of mastectomy on milk fever, energy, and vitamins A, E, and beta-carotene status at parturition. Journal of Dairy Science 85, 1427–1436.

Goff, J.P., Stabel, J.R., 1990. Decreased plasma retinol, alpha-tocopherol, and zinc concentration during the periparturient period: Effect of milk fever. Journal of Dairy Science 73, 3195–3199.

Harrison, J.H., Hancock, D.D., Conrad, H.R., 1984. Vitamin E and selenium for reproduction of the dairy cow. Journal of Dairy Science 67, 123–132.

Hayirli, A., Grummer, R.R., Nordheim, E.V., Crump, P.M., 2002. Animal and dietary factors affecting feed intake during the prefresh transition period in Holsteins. Journal of Dairy Science 85, 3430–3443.

Jukola, E., Hakkarainen, J., Saloniemi, H., Sankari, S., 1996. Blood selenium, vitamin E, vitamin A, and beta-carotene concentrations and udder health, fertility treatments, and fertility. Journal of Dairy Science 79, 838–845.

Kruze, J., Ceballos, A., Stryhn, H., Mella, A., Matamoros, R., Contreras, P.A., Leyan, V., Wittwer, F., 2007. Somatic cell count in milk of selenium-supplemented dairy cows after an intramammary challenge with *Staphylococcus aureus*. Journal of Veterinary Medicine A, Physiology, Pathology, Clinical Medicine 54, 478–483.

LeBlanc, S.J., Osawa, T., Dubuc, J., 2011. Reproductive tract defense and disease in postpartum dairy cows. Theriogenology 76, 1610–1618.

Machado, V.S., Knauer, W.A., Bicalho, M.L., Oikonomou, G., Gilbert, R.O., Bicalho, R.C., 2012a. A novel diagnostic technique to determine uterine health of Holstein cows at 35 days postpartum. Journal of Dairy Science 95, 1349–1357.

Machado, V.S., Oikonomou, G., Bicalho, M.L., Knauer, W.A., Gilbert, R., Bicalho, R.C., 2012b. Investigation of postpartum dairy cows' uterine microbial diversity using metagenomic pyrosequencing of the 16S rRNA gene. Veterinary Microbiology 159, 460–469.

Nockels, C.F., DeBonis, J., Torrent, J., 1993. Stress induction affects copper and zinc balance in calves fed organic and inorganic copper and zinc sources. Journal of Animal Science 71, 2539–2545.

Oliveira, L., Rodrigues, A.C., Hulland, C., Ruegg, P.L., 2011. Enterotoxin production, enterotoxin gene distribution, and genetic diversity of *Staphylococcus aureus* recovered from milk of cows with subclinical mastitis. American Journal of Veterinary Research 72, 1361–1368.

Pogge, D.J., Richter, E.L., Drewnoski, M.E., Hansen, S.L., 2012. Mineral concentrations of plasma and liver following injection with a trace mineral complex differ among Angus and Simmental cattle. Journal of Animal Science 90, 2692–2698.

Rabiee, A.R., Lean, I.J., Stevenson, M.A., Socha, M.T., 2010. Effects of feeding organic trace minerals on milk production and reproductive performance in lactating dairy cows: A meta-analysis. Journal of Dairy Science 93, 4239–4251.

Radostits, O.M., 2000. Veterinary Medicine: A Textbook of the Diseases of Cattle, Sheep, Pigs, Goats and Horses. Saunders, London.

Roche, J.R., Friggens, N.C., Kay, J.K., Fisher, M.W., Stafford, K.J., Berry, D.P., 2009. Invited review: Body condition score and its association with dairy cow productivity, health, and welfare. Journal of Dairy Science 92, 5769–5801.

Sales, J.N.S., Pereira, R.V.V., Bicalho, R.C., Baruselli, P.S., 2011. Effect of injectable copper, selenium, zinc and manganese on the pregnancy rate of crossbred heifers (*Bos indicus* × *Bos taurus*) synchronized for timed embryo transfer. Livestock Science 142, 59–62.

Salman, S., Khol-Parisini, A., Schafft, H., Lahrssen-Wiederholt, M., Hulan, H.W., Dinse, D., Zentek, J., 2009. The role of dietary selenium in bovine mammary gland health and immune function. Animal Health Research Reviews/Conference of Research Workers in Animal Diseases 10, 21–34.

Sawalha, R.M., Keown, J.F., Kachman, S.D., Van Vleck, L.D., 2005. Evaluation of autoregressive covariance structures for test-day records of Holstein cows: Estimates of parameters. Journal of Dairy Science 88, 2632–2642.

Scaletti, R.W., Trammell, D.S., Smith, B.A., Harmon, R.J., 2003. Role of dietary copper in enhancing resistance to *Escherichia coli* mastitis. Journal of Dairy Science 86, 1240–1249.

Shankar, A.H., Prasad, A.S., 1998. Zinc and immune function: The biological basis of altered resistance to infection. The American Journal of Clinical Nutrition 68, 447S–463S.

Sordillo, L.M., Aitken, S.L., 2009. Impact of oxidative stress on the health and immune function of dairy cattle. Veterinary Immunology and Immunopathology 128, 104–109.

Spears, J.W., 2003. Trace mineral bioavailability in ruminants. The Journal of Nutrition 133, 1506S–1509S.

Suttle, N.F., 1986. Problems in the diagnosis and anticipation of trace element deficiencies in grazing livestock. Veterinary Record 119, 148–152.

Vanegas, J.A., Reynolds, J., Atwill, E.R., 2004. Effects of an injectable trace mineral supplement on first-service conception rate of dairy cows. Journal of Dairy Science 87, 3665–3671.

Weiss, W.P., Hogan, J.S., Smith, K.L., Hoblet, K.H., 1990. Relationships among selenium, vitamin E, and mammary gland health in commercial dairy herds. Journal of Dairy Science 73, 381–390.

Williams, E.J., Fischer, D.P., Pfeiffer, D.U., England, G.C., Noakes, D.E., Dobson, H., Sheldon, I.M., 2005. Clinical evaluation of postpartum vaginal mucus reflects uterine bacterial infection and the immune response in cattle. Theriogenology 63, 102–117.

Xin, Z., Waterman, D.F., Hemken, R.W., Harmon, R.J., 1993. Copper status and requirement during the dry period and early lactation in multiparous Holstein cows. Journal of Dairy Science 76, 2711–2716.