RESEARCH UPDATE: EFFECTS OF TRACE MINERAL SOURCE ON OXIDATIVE METABOLISM, ENDOMETRITIS, AND PERFORMANCE OF TRANSITION COWS

T. Yasui,[1] C. M. Ryan,[1] R. O. Gilbert,[2] K. E. Perryman,[3] and T. R. Overton[1]
[1] Cornell University Department of Animal Science; [2] Cornell University Department of Clinical Sciences; [3] Micronutrients, Inc., Indianapolis, IN

INTRODUCTION

Trace minerals are critical for immune function, oxidative metabolism, nutrition and energy metabolism, and reproductive function in dairy cows (NRC, 2001; Spears and Weiss, 2008). The specific roles of zinc (**Zn**), copper (**Cu**), and manganese (**Mn**) have received substantial attention in dairy cows, and studies have been conducted to evaluate effects of Zn, Cu, and Mn on productive and reproductive performance (Siciliano-Jones et al., 2008; Formigoni et al., 2011; Nemec et al., 2012).

Oxidative stress is increased during the transition period due to high demands of metabolism (Sordillo and Aitken, 2009) and likely contributes to periparturient disorders including metabolic diseases (Sharma et al., 2011). Bernabucci et al. (2005) reported that cows with higher plasma BHBA and NEFA had higher concentrations of reactive oxygen metabolites and thiobarbituric acid-reactive substances (**TBARS**) and lower levels of antioxidants in plasma during the transition period. Furthermore, Sordillo and Aitken (2009) suggested that oxidative stress during the transition period can be a major underlying cause of inflammatory and immune dysfunction in dairy cattle. Because of their antioxidant function, trace minerals can contribute to counterbalancing oxidative stress, which could reduce metabolic and immune problems during the transition period.

Cytological endometritis (**CE**) is characterized by inflammation of the endometrium that results in a significant reduction in reproductive performance in the absence of signs of clinical endometritis (Sheldon et al., 2009). The incidence of CE was reported to be 37–74% of dairy cows (Gilbert et al., 2005; Sheldon et al., 2009). Work conducted in five commercial dairy farms demonstrated that the incidence of CE was high (~53% of cows between 40 and 60 DIM) across those farms and that this had profoundly negative effects on first service conception rate and median days to pregnancy (Gilbert et al., 2005). Given the role of trace minerals in immune function, it is possible that their interaction with immunity and hence CE may underpin some of the responses of reproduction to trace mineral supplementation outlined above.

Substantial interest has focused on the potential to enhance function of dairy cows by feeding trace mineral sources with greater bioavailability (NRC, 2001; Andrieu, 2008) or to maintain high function by feeding lower overall levels of trace minerals using these more bioavailable sources (Nocek et al., 2006). Indeed, recent research conducted by our research group suggested that trace mineral amount and source can modulate aspects of oxidative metabolism (Yasui et al., 2009). Hydroxy trace mineral sources (Micronutrients, Inc. Indianapolis, IN) have shown higher bioavailability than sulfates in steers (Spears et al., 2004) and weanling pigs (Fry et al., 2012) and recently have

become available for potential use in the dairy industry; however, research has not been conducted to determine how these sources compare with either sulfates or currently available chelated sources of trace minerals in dairy cows. Therefore, the objectives of this experiment were to evaluate a new source of trace minerals with potentially higher bioavailability and determine whether trace mineral source affects aspects of oxidative metabolism, CE, and performance of cows during the transition period and early lactation.

EXPERIMENTAL APPROACH

Sixty Holstein cows entering second lactation or greater from the Cornell University Teaching and Research Center Dairy were enrolled in this experiment at 28 d before expected calving, housed in tiestalls, and assigned to one of three topdress treatments with randomization restricted by previous lactation 305-d mature-equivalent milk production. Treatments were initiated 21 d before expected calving and continued through 84 d post calving: 1) Inorganic sources based upon sulfates of Zn, Cu, and Mn (**ITM**); 2) a blend (75:25) of sulfates and chelated sources of Zn, Cu, and Mn (**ITM/OTM**); and 3) hydroxy trace minerals (**HTM**) of Zn, Cu, and Mn (IntelliBond; Micronutrients, Inc., Indianapolis, IN). The target concentrations of supplemental Zn, Cu, and Mn across treatments were the same for both prepartum and postpartum periods: 60, 15, and 40 ppm, respectively. Based upon actual DMI of cows during the prepartum and postpartum periods, actual concentrations of supplemental Zn, Cu, and Mn for the prepartum period were essentially the same among treatments: 40, 10, and 27 ppm, respectively. Actual concentrations of supplemental Zn, Cu, and Mn for the postpartum period were similar among treatments: 59, 15, and 39 ppm for ITM treatment; 61, 15, and 41 ppm for ITM/OTM treatment; and 58, 14, and 39 ppm for HTM treatment.

Ingredient and chemical composition of the diets fed during the experiment are described in Table 1. Formulated dietary ingredients and composition were typical of those fed in the Northeastern U.S., except that the mineral supplements fed omitted Zn, Cu, and Mn as minerals targeted for treatment and also omitted Se, Co, and I. All three experimental topdress premixes also contained Se, Co, and I at targeted concentrations in the final TMR of 0.3 ppm (added), 0.4 ppm (added) and 0.8 ppm (added), respectively. Fresh feed was provided each morning at 0800 h, orts were weighed and recorded daily, and water was made available at all times. Samples of the forages and concentrate mixtures were obtained weekly throughout the experiment, used to adjust DM content of the component feeds, and composited at 4-wk intervals for subsequent analysis (Cumberland Valley Analytical Services, Hagerstown, MD) for analysis.

Cows were milked twice daily (0900 and 2100 h) and milk yields were recorded at all milkings for the 84-d postpartum treatment period. Milk samples were collected on the same day each week from both milkings. Samples were composited and analyzed (Dairy One Cooperative Inc., Ithaca, NY) within 24 h for fat, true protein, lactose, total solids, SCC, and MUN. Body condition scores (**BCS**, 1 to 5 scale; Wildman et al., 1982) and body weights (**BW**) were measured weekly from the preliminary period through the

Table 1. Ingredient and chemical composition (DM basis, % of DM unless otherwise noted) of experimental diets

Item	Prepartum diet	Postpartum diet
Corn silage, processed	42.40	39.16
Wheat straw	25.12	2.04
Legume silage	-	16.31
Ground shelled corn	-	7.51
Soybean meal	-	5.10
Wheat middlings	-	4.52
Corn germ meal	8.18	3.60
Distillers grain (with solubles)	6.99	5.12
Citrus pulp	-	2.99
Cereal fines	-	2.94
Soy Chlor[1]	3.26	-
Amino Plus[2]	3.22	-
Soybean hulls	3.15	1.79
Canola meal	3.21	2.59
Blood meal	-	1.31
Molasses	1.04	1.19
Bypass fat	-	1.10
Alimet[3]	-	0.10
Urea	-	0.33
Minerals and vitamins	3.45	2.35
Chemical composition		
CP	12.4	16.8
ADF	29.1	21.4
NDF	47.6	33.9
Starch	18.1	24.7
Ca	1.25	0.84
P	0.39	0.42
K	1.12	1.47
Mg	0.35	0.32
Na	0.14	0.45
Cl	0.52	0.48
S	0.29	0.24
Fe (ppm)	232.2	205.1
Zn (ppm)	39.4	43.1
Cu (ppm)	6.0	7.7
Mn (ppm)	35.0	35.1
DCAD, mEq/100g DM[4]	2.0	29.0

[1]Anionic feed supplement; West Central, Ralston, IA.
[2]Rumen undegradable protein supplement; AGP Inc., Omaha, NE.
[3]D,L-2-hydroxy-4-methylthiobutanoic acid; Novus International Inc., St. Louis, MO.
[4]Calculated as mEq [(Na + K) - (Cl + S)] / 100 g DM (NRC, 2001).

whole treatment period. Gait scores were measured once during the week before assignment to treatment and at 4, 8, and 12 wk postpartum using a five-point scale (1 = normal to 5 = severely lame; Sprecher et al. 1997).

Samples of blood were obtained from each cow via coccygeal blood vessel puncture on one day before assignment to treatment and once weekly from 21 d prepartum through 84 d postpartum. Plasma samples collected from -3 wk prepartum through 12 wk postpartum were analyzed for total antioxidant capacity (**TAC**) and TBARS. Plasma samples collected from 1 wk postpartum through 8 wk postpartum were analyzed for concentrations of haptoglobin (**Hp**).

Evaluation of endometrial cytology by low volume lavage (Gilbert et al., 2005) was determined on all cows at 7 d postcalving (1st lavage) and on one day between 40 and 60 d (2nd lavage) post calving as previously described (Cheong et al., 2011). Two hundred cells were counted from each slide, and results were expressed as the percentage of polymorphonuclear neutrophils (**PMN**) in total cells (excluding erythrocytes). All the slides were read masked to treatment by the same investigator (TY). The percentage of PMN was compared among treatments as a continuous variable for both 1st and 2nd lavage. Incidence of CE diagnosed with cut-off point of 10% PMN (Cheong et al., 2011) was dichotomously analyzed in 2nd lavage.

Data for DMI and milk yield were reduced to weekly means prior to analysis. Baseline values collected during the pretreatment week prior to assignment to treatment were used as covariates for DMI, BW, BCS, gait score, plasma TAC, and plasma TBARS. Previous 305-d mature equivalent milk yields were used as covariates for milk yield and milk composition. Weekly variables were analyzed using the MIXED procedure of SAS version 9.1 (SAS Institute, Cary, NC) for a completely randomized design with repeated measures. Model terms were the fixed effects of covariate, treatment, week, and the interaction of treatment and week.

The percentage of PMN in the total cells in low volume lavage was analyzed as a continuous variable for data collected during the 1st and 2nd lavage. Incidence of CE was analyzed as a dichotomous variable using the cutpoint described above for 2nd lavage. Statistical significance was declared at $P < 0.05$ and trends were discussed at $0.05 < P < 0.10$. Least squares means and standard error of the mean were reported throughout.

RESULTS

Production results are presented in Table 2. Overall effects of treatment on DMI, milk yield, and milk components were not significant; however, an interaction of treatment and week existed ($P = 0.02$) for milk yield such that cows fed HTM increased milk yield faster than cows fed the other two treatments (Figure 1). This interaction was also present and similar for yields of 3.5% FCM ($P = 0.03$) and lactose ($P = 0.06$; data not shown). Content and yield of milk fat, true protein, and total solids were not affected by treatment, and neither somatic cell count nor concentrations of milk urea N were

affected by treatment (Table 2). Cows fed HTM had higher BW than those fed ITM during the prepartum period (P = 0.02) and than those fed both other treatments during the postpartum period (P = 0.04; Table 2). BCS were not affected by treatment during either the prepartum or postpartum periods. Gait scores were not affected by treatment.

Analyses conducted in plasma suggested that trace mineral source modulated aspects of oxidative metabolism. Plasma TAC was lower in cows fed HTM than ITM treatment during whole, prepartum, and postpartum study period (P = 0.03, 0.03, and 0.04, respectively; Table 3). Although effects of treatment on plasma TBARS were not significant when assessed separately by prepartum and postpartum periods, cows fed HTM tended (P = 0.07) to have lower plasma TBARS than those fed ITM when analyzed across the entire study period (Table 3). Although effects of treatments on plasma Hp were not significant when assessed using all weekly data from wk 1 through 8 postpartum, there was a tendency (P = 0.10) for lower plasma Hp in cows fed HTM than those in ITM/OTM treatment during wk 1 postpartum (Table 3).

The percentages of PMN in both 1st and 2nd lavage were not affected by treatments (Table 4). One 2nd lavage from one cow fed the ITM/OTM treatment was not obtained because of cervix deformity for unknown reason, which resulted in 15 cows total for the ITM/OTM treatment. There were no treatment effects on incidence of cytological endometritis assessed at 2nd lavage (Table 4).

CONCLUSIONS

Supplementation of HTM, a newly available source of trace minerals, resulted in improvements in milk production compared to both ITM and ITM/OTM treatments. Feeding HTM modulated markers related to oxidative metabolism during the periparturient period; cows fed HTM had lower plasma TAC than those fed ITM along with a tendency for decreased plasma TBARS in cows fed HTM compared to ITM. Plasma Hp was lower in cows fed HTM at 1 wk postpartum than those fed ITM/OTM. Although the effects of trace mineral sources on endometrial cytology were not significant in this experiment, the results indicate that aspects of production and oxidative metabolism can be modulated by trace mineral source during the periparturient period.

ACKNOWLEDGEMENTS

The authors sincerely appreciate the assistance of the following students and staff at Cornell University in completing the study: S. H. Cheong, S. Pelton, K. Donnan, S. Honda, Z. Hernandez, V. Kim, E. Diehl, A. Jones, Y. Bing, E. Martens, B. Pomeroy, J. Huzzey, K. Schoenberg, R. Higgs, and the staff at the Cornell University Dairy Teaching and Research Center. Partial funding for this experiment was provided by Micronutrients, Inc.

Table 2. Least squares means for productive performance and gait score in cows fed varying sources of Zn, Cu, and Mn during the periparturient period and early lactation.

	Treatment[1]				*P*–value		
Item	ITM	ITM/OTM	HTM	SEM	Trt	Wk	Trt x Wk
DMI, kg/d							
Prepartum	15.0	14.8	14.8	0.3	0.87	<0.001	0.68
Postpartum	25.4	24.5	25.8	0.5	0.18	<0.001	0.93
Milk,[2] kg/d	45.3	45.7	46.6	1.1	0.65	<0.001	0.02
Fat. %	3.35	3.29	3.29	0.06	0.67	<0.001	0.92
Fat, kg/d	1.48	1.45	1.49	0.05	0.80	<0.001	0.11
Protein, %	3.01	2.96	3.01	0.05	0.75	<0.001	0.71
Protein, kg/d	1.34	1.33	1.37	0.04	0.66	0.002	0.19
Lactose, %	4.71	4.70	4.66	0.04	0.53	<0.001	0.94
Lactose, kg/d	2.14	2.16	2.18	0.05	0.83	<0.001	0.05
SCC (x 1000)	347	285	382	67	0.56	0.71	0.74
Urea N, mg/d	11.0	11.8	11.2	0.3	0.14	<0.001	0.84
Total solids, %	12.0	11.9	11.9	0.1	0.65	<0.001	0.67
Total solids, kg/d	5.38	5.35	5.46	0.11	0.74	<0.001	0.16
3.5% FCM,[3] kg/d	43.6	43.3	44.3	1.2	0.80	<0.001	0.03
ECM,[4] kg/d	43.2	42.9	44.0	0.9	0.62	<0.001	0.18
BW, kg							
Prepartum	719[b]	740[ab]	765[a]	14	0.07	0.69	0.48
Postpartum	638	644	682	14	0.04	<0.001	0.19
BCS							
Prepartum	3.34	3.44	3.37	0.07	0.56	0.02	0.99
Postpartum	2.86	2.79	2.86	0.05	0.59	<0.001	0.84
Gait score	1.8	1.8	1.6	0.1	0.31	0.51	0.93

[a-b]Means within a row with different lower-case superscripts differ (P < 0.05).
[1]ITM = inorganic sources based upon sulfates; ITM/OTM = a blend (75:25) of sulfates and chelated sources; HTM = hydroxy trace minerals.
[2]Represents milk yields collected daily from parturition through 84 d postpartum and then reduced to weekly means prior to analysis.
[3]FCM = (0.4324 × kg of milk) + (16.216 × kg of milk fat).
[4]ECM = [(0.323 × kg of milk) + (12.82 × kg of fat) + (7.13 × kg of protein)].

Table 3. Plasma total antioxidant capacity (TAC), thiobarbituric acid reactive substances (TBARS), and haptoglobin (Hp) in cows fed varying sources of Zn, Cu, and Mn during the periparturient period and early lactation.

Item	Treatment			SEM[4]	*P*-value		
	ITM[1]	ITM/OTM[2]	HTM[3]		Trt[5]	Wk[6]	Trt x Wk
TAC, mM							
Whole study	2.14[a]	2.07[ab]	1.93[b]	0.07	0.09	<0.001	0.94
Prepartum period[8]	2.09[a]	1.93[ab]	1.84[b]	0.08	0.09	0.01	0.85
Postpartum period[9]	2.16[a]	2.09[ab]	1.95[b]	0.08	0.12	<0.001	0.87
TBARS, uM							
Whole study	2.11[A]	1.98[AB]	1.95[B]	0.07	0.16	<0.001	0.40
Prepartum period[8]	1.47	1.49	1.38	0.06	0.37	0.16	0.74
Postpartum period[9]	2.26	2.11	2.10	0.09	0.29	<0.001	0.23
Hp, mg/mL							
Postpartum	0.83	0.91	0.78	0.09	0.55	<0.01	0.66
1 wk postpartum[11]	1.05[AB]	1.26[A]	0.90[B]	0.16	0.25	-	-

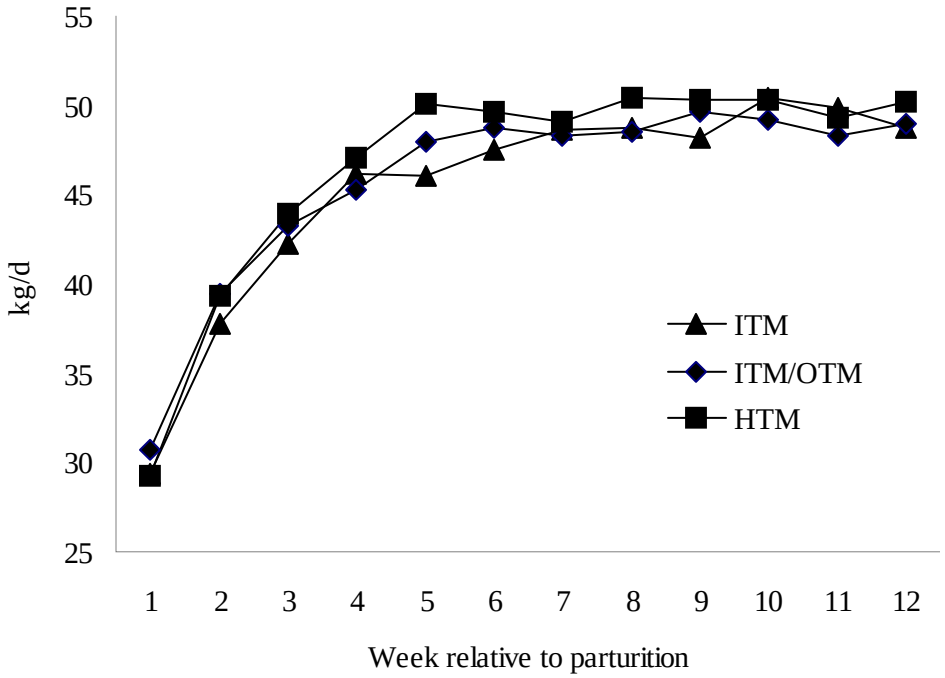
[a-b]Means within a row with different lower-case superscripts differ ($P < 0.05$).
[A-B]Means within a row with different upper-case superscripts tend to differ ($P < 0.1$).
[1]ITM = inorganic sources based upon sulfates.
[2]ITM/OTM = a blend (75:25) of sulfates and chelated sources.
[3]HTM = hydroxy trace minerals.
[4]SEM = standard error of the mean.
[5]Trt = treatment.
[6]Wk = week.
[7]Represents plasma samples collected weekly from 3 wk prepartum through 12 wk postpartum.
[8]Represents plasma samples collected weekly from 3 wk prepartum through 1 wk prepartum.
[9]Represents plasma samples collected weekly from 1 wk postpartum through 12 wk postpartum.
[10]Represents plasma samples collected weekly from 1 wk postpartum through 8 wk postpartum.
[11]Represents plasma samples collected from 1 wk postpartum only.

Table 4. Percentages of neutrophils in 1st and 2nd uterine lavage and incidence of cytological endometritis (CE) in 2nd uterine lavage from cows fed varying sources of Zn, Cu, and Mn during the periparturient period and early lactation.

	Treatment			SEM[4]	*P*-value
Item	ITM[1]	ITM/OTM[2]	HTM[3]		Trt[5]
% of PMN[6]					
1st lavage[7]	40.4	40.2	41.2	6.2	0.99
2nd lavage[8]	12.5	9.9	8.9	2.4	0.48
Cows with CE, n[9]	7	5	7		
Cows without CE, n	12	10	12	-	0.97

[1]ITM = inorganic sources based upon sulfates.
[2]ITM/OTM = a blend (75:25) of sulfates and chelated sources.
[3]HTM = hydroxy trace minerals.
[4]SEM = standard error of the mean.
[5]Trt = treatment.
[6]Percentage of polymorphonuclear neutrophils (PMN) in total 200 cells, excluding erythrocytes.
[7]Lavage at 7 d postpartum.
[8]Lavage at one day between 40 and 60 d postpartum.
[9]Number of cows that developed CE (cytological endometritis diagnosed positive if more than 10% PMN were counted in 2nd lavage).

Figure 1. Milk yield for cows fed three sources of trace minerals during the transition period and early lactation. Values are least square means, n = 19 for ITM (inorganic sources based upon sulfates), n = 16 for ITM/OTM (75:25 blend of sulfates and chelated sources), and n = 19 for HTM (hydroxy trace minerals); SEM averaged 1.7 kg/d; the P-value for the interaction of treatment × week was 0.02.



REFERENCES

Andrieu, S. 2008. Is there a role for organic trace element supplements in transition cow health? Vet. J. 176:77-83.

Bernabucci, U., B. Ronchi, N. Lacetera and A. Nardone. 2005. Influence of body condition score on relationships between metabolic status and oxidative stress in periparturient dairy cows. J. Dairy Sci. 88:2017-2026.

Cheong, S. H., D. V. Nydam, K. N. Galvão, B. M. Crosier and R. O. Gilbert. 2011. Cow-level and herd-level risk factors for subclinical endometritis in lactating Holstein cows. J. Dairy Sci. 94:762-770.

Formigoni, A., M. Fustini, L. Archetti, S. Emanuele, C. Sniffen and G. Biagi. 2011. Effects of an organic source of copper, manganese and zinc on dairy cattle productive performance, health status and fertility. Anim. Feed Sci. Technol. 164:191-198.

Fry, R. S., M. S. Ashwell, K. E. Lloyd, A. T. O'Nan, W. L. Flowers, K. R. Stewart and J. W. Spears. 2012. Amount and source of dietary copper affects small intestine morphology, duodenal lipid peroxidation, hepatic oxidative stress,and mRNA

expression of hepatic copper regulatory proteins in weanling pigs. J. Anim. Sci. 90:3112-3119.
Gilbert, R. O., S. T. Shin, C. L. Guard, H. N. Erb and M. Frajblat. 2005. Prevalence of endometritis and its effects on reproductive performance of dairy cows. Theriogenology. 64:1879-1888.
National Research Council. 2001. Nutrient Requirements of Dairy Cattle. 7th rev. ed. National Academy Press, Washington, D.C.
Nemec, L. M., J. D. Richards, C. A. Atwell, D. E. Diaz, G. I. Zanton and T. F. Gressley. 2012. Immune responses in lactating Holstein cows supplemented with Cu, Mn, and Zn as sulfates or methionine hydroxy analogue chelates. J. Dairy Sci. 95:4568-4577.
Nocek, J. E., M. T. Socha and D. J. Tomlinson. 2006. The effect of trace mineral fortification level and source on performance of dairy cattle. J. Dairy Sci. 89:2679-2693.
Sheldon, I. M., J. Cronin, L. Goetze, G. Donofrio and H. Schuberth. 2009. Defining postpartum uterine disease and the mechanisms of infection and immunity in the female reproductive tract in cattle. Biol. Reprod. 81:1025-1032.
Siciliano-Jones, J. L., M. T. Socha, D. J. Tomlinson and J. M. DeFrain. 2008. Effect of trace mineral source on lactation performance, claw integrity, and fertility of dairy cattle. J. Dairy Sci. 91:1985-1995.
Sordillo, L. M. and S. L. Aitken. 2009. Impact of oxidative stress on the health and immune function of dairy cattle. Vet. Immunol. Immunopathol. 128:104-109.
Spears, J. W., E. B. Kegley and L. A. Mullis. 2004. Bioavailability of copper from tribasic copper chloride and copper sulfate in growing cattle. Anim. Feed Sci. Technol. 116:1-13.
Spears, J. W. and W. P. Weiss. 2008. Role of antioxidants and trace elements in health and immunity of transition dairy cows. Vet. J. 176:70-76.
Sprecher, D. J., D. E. Hostetler and J. B. Kaneene. 1997. A lameness scoring system that uses posture and gait to predict dairy cattle reproductive performance. Theriogenology. 47:1179-1187.
Wildman, E. E., G. M. Jones, P. E. Wagner, R. L. Boman, H. F. Troutt Jr and T. N. Lesch. 1982. A dairy cow body condition scoring system and its relationship to selected production characteristics. J. Dairy Sci. 65:495-501.
Yasui, T., R. M. Ehrhardt, G. R. Bowman, M. Vazquez-Anon, J. D. Richards, C. A. Atwell, T. D. Wineman and T. R. Overton. 2009. Effects of trace mineral amount and source on aspects of oxidative status and immune function in dairy cows. J. Dairy Sci. 92 (E. Suppl. 1):725 (Abstr.).