

# Effect of feeding a combination of zinc, manganese and copper amino acid complexes, and cobalt glucoheptonate on performance of early lactation high producing dairy cows

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## Abstract

This study determined the effect of feeding a combination of zinc amino acid (AA), manganese AA and copper AA complexes, and cobalt glucoheptonate on productive and reproductive performance of early lactation high producing dairy cows. The product used as a source of the organic trace mineral complexes was Availa4<sup>®</sup> (Zinpro Corp., Eden Prairie, MN). Forty pregnant Holstein cows were blocked and randomly assigned to one of two dietary treatments at calving. After 48 ± 30 days, cows were moved from the fresh groups to the high production groups within their treatment assignments. Cows were housed in a free-stall barn and group-fed total mixed rations (TMR) containing inorganic minerals (control) or inorganic minerals and Availa4<sup>®</sup> (Availa4) for 80 days, or until first breeding service. The TMR were formulated to exceed NRC (1988) trace mineral requirements for lactating dairy cows. The Availa4 TMR was formulated to supply each cow with 7 g per day of Availa4<sup>®</sup> by partially substituting the inorganic minerals in the concentrate mix of the control TMR. Each 7 g of Availa4<sup>®</sup> supplied 360 mg of zinc, 200 mg of manganese, 125 mg of copper, and 12 mg of cobalt. Cows fed the Availa4 TMR tended to have fewer days to first service ( $P = 0.071$ ), fewer services per conception ( $P = 0.069$ ), and fewer days to conception ( $P = 0.012$ ) as compared to cows fed with the control TMR. Feeding a combination of zinc AA, manganese AA and copper AA complexes, and cobalt glucoheptonate to early lactation Holstein cows improved conception rate with fewer days in milk to pregnancy but had no effect on milk production, milk fat and protein content, linear somatic cell count, animal body condition score, or lameness score. © 2001 Elsevier Science B.V. All rights reserved.

**Keywords:** Dairy cow; Organic trace minerals; Milk production; Reproductive performance

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## 1. Introduction

Trace minerals have traditionally been added to ruminant diets in the form of inorganic salts. However, in recent years, there has been considerable interest in feeding ruminants organic trace minerals that increase the bioavailability of the mineral above that of soluble inorganic forms (Kratzer and Vohra, 1986; Henry et al., 1992; Formigoni et al., 1993; Henry, 1995; Rojas et al., 1995; Luo et al., 1996; Spears, 1996; Socha and Johnson, 1998; Miles and Henry, 1999). Definitions of organically-bound mineral compounds sold in the USA are provided by the Association of American Feed Control Officials (AAFCO, 1997). A metal amino acid (AA) complex results from complexing a soluble metal salt with an AA in a ratio of 1 mol of metal to 1 mol of AA. A metal AA chelate is a product resulting from the reaction of a soluble metal salt with AA in the ratio of 1 mol of metal to 1–3 mol of AA to form coordinate covalent bonds. The molecular weight of the chelate is below 800.

The metal complex, or chelate, is stable in the digestive tract and is thus protected from forming complexes with other dietary components that would otherwise inhibit its absorption (Heinrichs and Conrad, 1983; Ward et al., 1992; Formigoni et al., 1993; Spears, 1996). In the animal, trace minerals occur and function as organic complexes, or chelates, and not as free inorganic ions (Spears, 1996). Hence, utilization of inorganic trace minerals is dependent on the animal's ability to convert them to biologically active organic forms. The trace minerals that occur naturally in feeds also exist primarily as organic chelates or complexes (Spears, 1996). However, Beutler et al. (1998), using monkey kidney cells and human epithelial cells incubated in media containing zinc (Zn) methionine complex or Zn from inorganic sources, showed that there could be dissociation of trace mineral AA complexes prior to uptake by the cells. Rojas et al. (1995) also showed, using sheep fed Zn sulfate and Zn oxide, that the organic forms of Zn could be metabolized differently from the inorganic forms.

Studies have shown improved growth, milk yield, reproductive performance, and/or immune response in ruminants fed diets containing organic trace minerals (Kropp, 1993; Manspeaker and Robl, 1993; Spears, 1996; Socha and Johnson, 1998; Gunter et al., 1999). The objective of this study was to determine the effect of feeding a combination of Zn AA, manganese (Mn) AA, copper (Cu) AA complexes, and cobalt (Co) glucoheptonate to high producing dairy cows on milk yield, milk composition, and reproductive performance.

## 2. Materials and methods

### 2.1. Animals and treatments

Pregnant Holstein cows ( $n = 40$ ) were blocked by parity and milk production from their previous lactation, and randomly assigned to one of two treatments at calving. Cows in each block were in the same parity and had similar milk production in the previous lactation. One of the treatment diets did not contain (control) and the other contained (Avalia4), a combination of Zn AA, Mn AA, Cu AA complexes, and Co glucoheptonate (Avalia4<sup>®</sup>; Zinpro Corp., Eden Prairie, MN). Following calving, all multiparous cows received 500 ml of 23% calcium solution, 500 ml of 50% dextrose solution and 5 ml

oxytocin intravenously, as well as 10 ml of Vitamin E-selenium, 10 ml of Vitamin B complex and 2 ml of *Escherichia coli* vaccine intramuscularly. All cows received 500 mg-injections of bST every 2 weeks (Posilac<sup>®</sup>; Monsanto, St. Louis, MO, USA) beginning when cows were 100 days in milk or were confirmed pregnant and greater than 70 days in milk, whichever came first.

Cows in each treatment were housed in a free-stall barn in separate fresh cow group pens at calving and then moved at  $48 \pm 30$  days to separate high production group pens. The animal pens were of the same size with similar bunk space, water accessibility and animal density. The free stalls were bedded with sawdust, which were changed weekly for all pens. Non-treatment cows were used to maintain same animal density across pens. Cows were group-fed total mixed rations (TMR) containing inorganic minerals (control) or inorganic minerals and Availa4<sup>®</sup> (Availa4) for 80 days, or until first breeding service. Animals were fed once a day starting at 7.30 h, and the stalls and alleys were cleaned three times a day at 3.00, 11.00, and 19.00 h.

The TMR (Table 1) were formulated using the CPM Dairy<sup>®</sup> nutrition model (version 1.0; Cornell-Penn-Miner, Cornell University, Ithaca, NY, USA) to exceed NRC (1988) trace mineral requirements for lactating dairy cows at these levels of milk production (Table 2). The trace mineral concentrations in the TMR were well below toxicity levels established by NRC (1988) (Table 2). The Availa4 TMR was formulated, assuming a total dry matter intake (DMI) of 25 kg per cow per day, to supply each cow with 7 g per day of Availa4<sup>®</sup> by partially substituting the inorganic minerals supplied by trace mineral mix-2 in the concentrate mix of the control TMR. Each 7 g of Availa4<sup>®</sup> supplied 360 mg of Zn, 200 mg of Mn, 125 mg of Cu, and 12 mg of Co. The control TMR was formulated to supply each cow with similar amounts of Zn, Mn, Cu, and Co from inorganic sources.

The TMR were mixed in a Reel-Auggie<sup>®</sup> mixer wagon (model 3300; Knight, Brodhead, WI, USA) in the order: concentrate mixes, whole cottonseed, ground corn, beet pulp, grass hay, alfalfa silage, and corn silage, and allowed to mix for about 5 min prior to feeding. Corn silage was ensiled in a bunk silo, while the alfalfa silage was ensiled in silage bags made of 9-mm thick plastic (Ag-Bag Int., Warrenton, OR, USA). Because of possible mycotoxin contamination in the alfalfa silage, cows were fed a sodium calcium aluminosilicate compound during the study.

Corn and alfalfa silages were analyzed prior to the beginning of the study and monthly thereafter. Mixed cool season grass hay, whole cottonseed, beet pulp, ground corn, and concentrate mixes were analyzed at the beginning of the study and in new deliveries. The TMR were sampled weekly from the mixer wagon and approximately 500 g of sample dried in a conventional forced-air oven at 100°C for 48 h to determine DM concentration for estimation of group intake by cows in each pen. Cows were milked three times per day in a double-six herringbone milking parlor at 4.30, 12.30, and 20.30 h and milk yield recorded for each milking for each cow. Milk samples were collected once a week during the afternoon milking from week 1 to 8 and sent to Dairy One (Ithaca, NY, USA) for analyses of fat and CP by infrared procedures (Foss 4000; Foss Technology, Eden Prairie, MN, USA), and analysis of somatic cell count by infrared procedures (Foss 5000; Foss Technology, Eden Prairie, MN, USA). Milk samples collected from other commercial dairy farms were used as internal standards in analysis of milk components. These standards were stored under refrigeration at 0–4°C for a maximum of 14 days. Standards

Table 1  
Composition (% DM) of TMR containing inorganic minerals (control) or inorganic minerals and Availa4<sup>®</sup> (Availa4)

	Fresh group cows		High group cows	
	Control	Availa4	Control	Availa4
<b>Ingredient composition</b>				
Corn silage	31.33	31.33	27.52	27.52
Alfalfa silage	11.77	11.77	14.13	14.13
Mixed cool season grass hay <sup>a</sup>	4.72	4.72	3.40	3.40
Whole cottonseed	3.62	3.62	5.70	5.70
Beet pulp	4.69	4.69	4.22	4.22
Energy II <sup>b</sup>	0.69	0.69	0.71	0.71
Sodium bicarbonate	0.52	0.52	0.56	0.56
Ground corn	19.86	19.86	21.13	21.13
Sucrose	1.39	1.39	1.67	1.67
Concentrate mix <sup>c</sup>	21.40	21.40	20.96	20.94
<b>Chemical composition<sup>d</sup></b>				
Crude protein	17.5	17.5	18.0	18.0
Soluble protein	6.18	6.18	6.37	6.37
Neutral detergent fiber	32.8	32.8	32.2	32.2
Fat	4.87	4.87	5.21	5.21
Calcium	0.91	0.91	0.92	0.91
Phosphorus	0.38	0.38	0.39	0.39
Magnesium	0.31	0.31	0.31	0.31
Potassium	1.23	1.23	1.25	1.25
Sulfur	0.25	0.25	0.25	0.25
Sodium	0.59	0.59	0.60	0.60
Chloride	0.55	0.55	0.55	0.56
<b>(DM basis)</b>				
Iron, mg/kg	284.1	282.3	281.6	279.9
Selenium, mg/kg	1.47	1.30	1.44	1.27
Iodine, mg/kg	0.99	0.57	0.97	0.55

<sup>a</sup> Reed canary grass, timothy grass and orchard grass in ratio of 40:40:20, respectively, harvested at seed heading stage of maturity.

<sup>b</sup> Energy II (Bioproducts, Inc., Fairlawn, OH, USA) contains 90% fat and 10% calcium on DM basis.

<sup>c</sup> The Control and Availa4 TMR concentrate mixes contained on DM basis 9.20%, ground corn, 13.97% soybean hulls, 2.61% urea, 2.87% blood meal, 12.25% microwave roasted soybeans, 18.94% canola meal, 18.73% soybean meal (49% CP), 0.09% corn gluten meal (67% CP), 9.53% fishmeal (Sea Lac), 2.12% salt, 3.77% sodium bicarbonate, 2.21% calcium supplement, 1.36% trace mineral mix-1, and 2.19% selenium supplement. In addition, the Control TMR concentrate mix contained on DM basis 0.16% trace mineral mix-2, and the Availa4 TMR concentrate mix contained 0.07% trace mineral mix-2 and 0.15% Availa4<sup>®</sup>.

<sup>d</sup> Calculated using chemical composition (DM basis) of dietary ingredients shown in Table 3. The concentration of zinc, manganese, copper, and cobalt are in Table 2.

were analyzed for fat (Method 905.02; AOAC, 1995), N (Method 991.22; AOAC, 1995), and somatic cell count (Method 975.16; AOAC, 1995) to provide the wet chemistry values that were used to calculate standard curves used in the respective infrared procedures.

A sample of drinking water was collected from the tap that supplied the water troughs. The water was analyzed for *E. coli* using presence–absence broth (Clesceri et al., 1998), for

Table 2

Zinc, manganese, copper, and cobalt balances (in mg/kg (DM basis)) in the TMR containing inorganic minerals (control) or inorganic minerals and Availa4<sup>®</sup> (Availa4)

Cow group	Nutrient	Requirement by dairy cows <sup>a</sup>	Toxicity level <sup>a</sup>	Control TMR	Availa4 TMR <sup>b</sup>	
					Total	Contribution from Availa4 <sup>®c</sup>
Fresh	Zinc	40.0	1000	148.6	121.2	17.1
	Manganese	40.0	1000	81.4	75.7	9.1
	Copper	4.0–10.0	100	27.6	24.9	5.9
	Cobalt	0.10	10	1.21	1.25	0.58
High	Zinc	40.0	1000	146.5	119.7	16.8
	Manganese	40.0	1000	78.6	73.0	8.9
	Copper	4.0–10.0	100	27.1	24.5	5.8
	Cobalt	0.10	10	1.19	1.23	0.57

<sup>a</sup> NRC (1988) guidelines.

<sup>b</sup> Availa4<sup>®</sup> comprised on DM basis 0.0321% (or 321 mg/kg) and 0.03141% (or 314.1 mg/kg), respectively of the diet for fresh and high groups of cows.

<sup>c</sup> Calculated by multiplying the concentration of mineral in Availa4<sup>®</sup> determined by wet chemistry (Table 4) by the total amount of Availa4<sup>®</sup> in the Availa4 TMR, e.g. for the fresh group: zinc = 5.34% × 321 mg/kg = 17.14 mg/kg.

sulfur by colorimetry, and for Fe, Zn, Cu, Mn, and Mo by atomic absorption spectrophotometry (Method 968.08; AOAC, 1995).

## 2.2. Animal body condition, lameness, and reproduction

Cows were scored for body condition (Wildman et al., 1982) and lameness (Sprecher et al., 1997) every two weeks from week 1 to 11. Body condition scores were based on a five-point scale with 0.25-unit intervals, where 1, emaciated and 5, obese. Lameness scores were also based on a five-point scale, where 1, normal gait; 2, mild lameness; 3, moderate lameness; 4, lame and 5, severely lame. Reproductive performance was assessed as days to first service, services per conception, and days to conception. Animals were monitored for health problems throughout the study. Cows were checked for general health 20–30 days post-calving by a veterinarian and if no health problems were found, they were bred on the subsequent heat by artificial insemination without observing a voluntary waiting period. In order to avoid potential bias the investigators who scored the cows for body condition and lameness, and recorded health problems were not told the treatment assignments of the cows. Reproductive management practices, such as the use of prostaglandins, were similar for both treatment groups.

## 2.3. Chemical analyses

Samples of forages were dried to a constant weight at 60°C in a forced air oven and ground to pass a 1-mm screen in a Wiley mill (Arthur H. Thomas Co., Philadelphia, PA, USA) and submitted to Woodson-Tenent Laboratories Inc. (Goldston, NC, USA) for

analyses of Co, Se, and Cr by atomic absorption spectrophotometry (Method 968.08; AOAC, 1995). Freshly sampled forage samples, and samples of feed by-products and concentrate mixes used in the experiment were sent to Dairy One (Ithaca, NY, USA) where, after being dried (60°C) and ground to pass a 1-mm screen in a Wiley mill, they were analyzed for DM by drying a 1 g sample in duplicate at 100°C in a conventional oven for 24 h, ash by burning a 2 g sample in duplicate at 600°C for 2 h in a muffle furnace (Method 942.05; AOAC, 1995), fat (Method 920.39; AOAC, 1995), N (Method 984.13; AOAC, 1995), neutral detergent fiber with residual ash (NDF, using  $\alpha$ -amylase and sodium sulfite), acid detergent fiber (ADF), and acid detergent lignin (ADL) (Van Soest et al., 1991). Soluble N was determined as the difference in N concentration of sample before and after soaking in borate phosphate buffer at pH 6.8 (Krishnamoorthy et al., 1982). The non-structural carbohydrates were calculated as the difference between 100 and the sum of CP, NDF, fat, and ash. Analyses of Ca, P, Mg, K, Na, Fe, Zn, Cu, Mn, and Mo were conducted using a Thermo Jarrell Ash IRIS Advantage Inductively Coupled Plasma Radial Spectrometer (model ICAP 61; Thermo Jarrell Ash, Ithaca, NY, USA). Sulfur was analyzed using a Leco model SC-432 (Leco, St. Joseph, MI, USA). Chloride ion was analyzed using a Brinkman Metrohm 716 Titrimetric titration unit with a silver electrode (model 716; Brinkman Instruments Inc., Westbury, NY, USA).

#### 2.4. Statistical analysis

Data were analyzed as a randomized block using the general linear models procedure of SAS (1993) and results presented as least square means. Data were analyzed as a split plot design in which the factors in the main plot were treatment and block, and the factor in the subplot was week. The error term used to test treatment effects was treatment  $\times$  block interaction. The following model was used:

$$Y_{ijk} = \mu + \tau_i + \gamma_j + \tau\gamma_{ij} + \beta_k + \tau\beta_{ik} + \gamma\beta_{jk} + e_{ijk}$$

where  $\mu$  is the overall mean,  $\tau_i$  the effect of the  $i$ th treatment,  $\gamma_j$  the effect of the  $j$ th block,  $\tau\gamma_{ij}$  the treatment  $\times$  block interaction,  $\beta_k$  the  $k$ th week,  $\tau\beta_{ik}$  the treatment  $\times$  week interaction,  $\gamma\beta_{jk}$  the block  $\times$  week interaction and  $e_{ijk}$  the residual error.

Since animals were group-fed with no replications of pens within treatment, the investigators assumed (1) no pen effect, and (2) that errors within pens were independent (St-Pierre and Jones, 1999), allowing for cow to serve as the experimental unit. No statistical tests were performed on DMI because pens were not replicated.

### 3. Results and discussion

#### 3.1. Diets

The nutrient composition of corn silage, alfalfa silage, mixed cool season grass hay, whole cottonseed, sugar beet pulp, and ground corn (Table 3) was consistent with values reported by NRC (1988) for the respective feedstuffs. The concentrations of CP, NDF, fat, and macro-minerals were similar for the concentrate mixes used in the control and Availa4

Table 3  
Chemical composition of major feedstuffs used in diets fed to lactating Holstein cows in fresh and high groups

	Corn silage	Alfalfa silage <sup>a</sup>	Alfalfa silage <sup>b</sup>	Mixed grass hay <sup>c</sup>	Whole cottonseed	Beet pulp	Ground corn <sup>d</sup>	Concentrate mix		Avalia4 <sup>®</sup>	Trace mineral mix	
								Control	Avalia4		1	2
Dry matter (%)	34.6	31.9	25.3	91.5	87.6	90.8	87.4					
Composition (% of DM)												
Crude protein	7.7	22.8	18.1	10.3	25.7	9.5	7.9	40.9	40.8	23.6		0.3
Soluble protein	3.85	13.89	10.15	2.58	7.45	0.67	1.11					
Neutral detergent fiber	44.2	44.1	52.8	63.2	52.9	44.3	10.3	20.4	20.0	22.7		2.8
Acid detergent fiber	26.4	34.8	40.0	46.5	39.1	32.9	3.8			11.5		2.0
Acid detergent lignin	3.4	11.1	8.3	8.9	15.7	5.7	2.6					
Nonstructural carbohydrates	40.4	16.7	14.1	15.5	1.1	36.7	75.4			12.8		
Fat	3.8	6.1	5.6	3.2	18.4	0.7	4.8	5.70	6.10	1.0		
Ash	3.9	10.3	9.4	7.8	4.1	8.8	1.6			39.9	97.0	
Calcium	0.28	1.44	1.52	0.57	0.15	1.04	0.06	2.51	2.42	4.47	0.70	7.12
Phosphorus	0.24	0.36	0.25	0.19	0.57	0.14	0.33	0.82	0.84	0.05	0.10	0.04
Magnesium	0.17	0.31	0.23	0.22	0.37	0.36	0.13	0.79	0.79	0.19	23.00	0.48
Potassium	1.21	2.88	2.91	1.43	1.19	0.52	0.45	1.58	1.63	0.28	11.00	0.25
Sodium	0.02	0.05	0.05	0.01	0.02	0.11	0.02	2.32	2.32	1.95	0.20	0.22
Sulfur	0.11	0.34	0.26	0.19	0.25	0.14	0.08	0.55	0.56	1.62	14.00	8.64
Chloride	0.21	0.67	0.48	0.52	0.08	0.21	0.03	1.42	1.49	11.34	0.20	0.57
Composition (mg/kg (DM basis))												
Iron	163	242	180	724	62	972	73	448	453	2010	6666	23600
Zinc	24	31	39	29	36	31	22	583	599	53400	10000	182300
Manganese	20	36	34	190	14	62	9	213	235	28400	5050	85000
Copper	6	8	13	11	6	17	0.1	125	130	18400	1333	40300
Cobalt								7	9	1769	169	1676
Molybdenum	0.6	1.4	1.3	2.0	0.5	2.1	0.5	2.0	4.1	0.5		39.7
Selenium								5.13	4.54		40	754
Iodine								1.64	0.86			
Chromium								5.13	4.54	14.00		14.00

<sup>a</sup> Third-cut mainly alfalfa silage was fed to cows during the first two-thirds of the study. Values are averages of five separate analyses.

<sup>b</sup> First-cut mainly alfalfa silage was fed to cows during the last one-third of the study. Values are averages of two separate analyses.

<sup>c</sup> Reed canary grass, timothy grass and orchard grass in ratio of 40:40:20, respectively, harvested at seed heading stage of maturity.

<sup>d</sup> Ground to pass through a 1.18 cm screen in a feed mill hammer mill.

TMR (Table 3). Chemical composition of Availa4<sup>®</sup> and mineral supplements are in Table 3. No coliforms were detected in the drinking water, and concentrations of S, Fe, Cu, and Mn, were 42.7, 0.2, 0.006, and 0.136 mg/l, respectively. Zinc, Co, and Mo were below detectable limits. Therefore, the contribution of trace minerals from the drinking water was low.

### 3.2. Dry matter intake, milk yield, milk composition, body condition, and lameness score

Since cows were group-fed, DMI data were not subjected to statistical analysis (Table 4). DMI by cows fed the control TMR, respectively were 21.2 and 18.5 kg for the fresh groups, and 25.6 and 25.4 kg for the high production groups. The average daily DMI by each cow for all treatments was approximately 22.7 kg per cow. Cows reached a peak of about 49 kg of milk in week 5, which they maintained through week 11. Feeding Availa4<sup>®</sup> had no effect on milk yield, milk fat, milk protein, linear somatic cell count, body condition score, and lameness score (Table 4).

Others have reported increased milk production by lactating dairy cows fed 4-Plex<sup>®</sup> (Zinpro Corp., Eden Prairie, MN, USA), a product similar to Availa4<sup>®</sup> (Socha and Johnson, 1998), or Zn methionine (Kellogg et al., 1989; Spears, 1996). In contrast, Formigoni et al. (1993) and Campbell et al. (1999) reported no increase in milk yield from feeding organic trace mineral complexes. Lack of a performance response to feeding metal-AA complexes in our study may partially reflect the high mineral content of the basal diet and naturally occurring organic trace mineral complexes in soybean protein (Baker and Ammerman, 1995; Spears, 1996). A decline in somatic cell count has also been observed in milk produced by lactating dairy cows fed Zn methionine (Kellogg, 1990; Spears, 1996).

### 3.3. Reproductive performance

Cows fed the Availa4 TMR tended ( $P = 0.071$ ) to have fewer days to first service (Table 5). The number of cows that were fed the control TMR and Availa4 TMR,

Table 4

Milk production, body condition score and lameness score of Holstein cows fed TMR containing inorganic minerals (control) or inorganic minerals and Availa4<sup>®</sup> (Availa4)

	Treatment		S.E.	P
	Control	Availa4		
Dry matter intake (kg per day)	23.4	22.0	–	–
Milk (kg per day)	44.9	44.4	0.44	0.820
Fat				
In percentage	4.05	4.23	0.06	0.382
In kg per day	1.77	1.84	0.03	0.761
Crude protein				
In percentage	3.18	3.25	0.02	0.448
In kg per day	1.42	1.42	0.02	0.934
Linear somatic cell count	4.10	3.74	0.08	0.198
Body condition score	3.09	3.13	0.02	0.365
Lameness score	1.39	1.46	0.06	0.738



Table 5

Reproductive performance of lactating Holstein cows fed TMR containing inorganic minerals (control) or inorganic minerals and Availa4<sup>®</sup> (Availa4)

	Treatment		S.E.	P
	Control	Availa4		
Days to first service				
<i>n</i>	20	20		
Median	66	54		
Mean	68	57	4.05	0.071
Services per conception				
<i>n</i>	20	20		
Median	2	1		
Mean	2.60	1.80	0.29	0.069
Days to conception				
<i>n</i>	20	20		
Median	120	77		
Mean	148	86	15.80	0.012

respectively, that were serviced within 60 days was 8 and 15 ( $P = 0.990$ ). Services per conception were lower ( $P = 0.069$ ) for the cows that were fed the Availa4 TMR compared with cows fed the control TMR (Table 5). Days to conception were lower ( $P = 0.012$ ) for cows fed the Availa4 TMR compared with cows fed the control TMR (Table 5). The number of cows that were fed the control TMR and Availa4 TMR, respectively, that had less than 80 days to conception was 5 and 11 ( $P = 0.995$ ). A possible lack of a normal distribution suggested by the large difference between the median and mean values for days to conception among cows fed with the control TMR, is not supported by the Shapiro–Wilk  $W$ -test for normality (SAS, 1993), which indicate a normal distribution ( $P = 0.067$ ).

In a study in which a 60-day voluntary waiting period postpartum was observed, Campbell et al. (1999) reported no differences in days to first service, days open, days from first service to conception, and services per conception between cows fed with inorganic minerals and cows fed with organic minerals. In this study, lack of a voluntary waiting period postpartum before an animal was bred may have therefore made it possible to detect differences in the reproductive performance by the cows. However, our results are consistent with Socha and Johnson (1998), who found that dairy cows fed organic trace minerals had fewer days to first service and days open than cows fed inorganic trace minerals. Zinc methionine has also been shown to improve pregnancy rate within the first 21 days of the breeding season in beef cows (Spears, 1996). Others have also reported that feeding organic trace minerals improved reproductive performance in beef cattle (Kropp, 1993), dairy heifers (Manspeaker and Robl, 1993), and dairy cows (Formigoni et al., 1993) even when the basal diet contained adequate concentrations of trace minerals. Feeding supplemental organic trace minerals to lactating Holstein cows improved reproductive performance, while maintaining their high levels of milk production.

#### 4. Conclusions

Feeding a combination of Zn AA, Mn AA and Cu AA complexes, and Co glucoheptonate to lactating Holstein cows did not increase milk production, milk fat or protein content, linear somatic cell count, animal body condition or lameness scores. However, it reduced the number of days cows were in milk until they were confirmed pregnant.

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