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Supplementing Zn, Mn, and Cu from amino acid complexes and Co from cobalt glucoheptonate during the peripartal period benefits postpartal cow performance and blood neutrophil function

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ABSTRACT

The physiologic and metabolic stresses that dairy cows experience during the transition into early lactation can promote oxidative stress, inflammation, and immune dysfunction. Optimal supply of micronutrients such as trace minerals (e.g., Zn, Mn, Cu, and Co) via more bioavailable forms (e.g., AA complexes) might minimize these negative effects. Multiparous Holstein cows were enrolled at 60 d before dry-off (~110 d before calving) and remained on experiment until 30 d in milk (DIM). Cows were offered a common diet supplemented entirely with inorganic trace minerals (INO) from −110 to −30 d before calving. From −30 to calving cows received a common prepartal [1.5 Mcal/kg of dry matter (DM), 15% crude protein] diet, and from calving to 30 DIM a common postpartal (1.76 Mcal/kg of DM, 18% crude protein) diet. Both diets were partially supplemented with an INO mix of Zn, Mn, and Cu to supply 35, 45, and 6 mg/kg, respectively, of the total diet DM. Cows were assigned to treatments in a randomized complete block design to receive an oral bolus with a mix of INO (n = 21) or organic AA complexes (AAC; n = 16) of Zn, Mn, Cu, and Co to achieve supplemental levels of 75, 65, 11, and 1 mg/kg, respectively, in the total diet DM. Inorganic trace minerals were provided in sulfate form and AAC were supplied via Availa Zn, Availa Mn, Availa Cu, and COPRO (Zinpro Corp., Eden Prairie, MN). Liver tissue was harvested on −30, −15, 10, and 30 d, and blood samples for biomarker analyses were obtained more frequently from −30 to 30 DIM. Short-term changes in blood ketones were measured via Precision Xtra (Abbott Diabetes Care, Alameda, CA) every other day from 1 to 15 d postpartum. Prepartal DM intake was lower in AAC cows. In contrast, a tendency for a diet by time (D × T) interaction resulted in greater postpartal DM intake of approximately 2 kg/d in cows fed AAC. Milk and milk protein yield had a D × T interaction because AAC cows produced approximately 3.3 kg/d more milk and 0.14 kg/d more protein during the first 30 DIM. Although blood glucose, fatty acids, and liver triacylglycerol were not affected by diet, the Precision Xtra ketones (1.44 vs. 2.18 mmol/L) and γ-glutamyltransferase (liver function biomarker) were lower in AAC than INO. Furthermore, feeding AAC increased (D × T) polymorphonuclear neutrophilic lymphocyte phagocytosis, antioxidant capacity postpartum, and overall concentration of liver tissue Co and Cu. Overall, the positive response in milk yield and milk protein in AAC cows might be partly explained by the beneficial effects of AAC on postpartal DM intake driven at least in part by better liver and immune function as a result of improved antioxidant status.
Key words: nutrition, lactation, dairy cattle, transition period

INTRODUCTION

An adequate supply of macro and micronutrients, such as trace minerals (e.g., Zn, Mn, Cu, Co), is important for ensuring an optimal transition from pregnancy to lactation (Andrieu, 2008). For instance, trace minerals have critical roles in a variety of physiological process, particularly antioxidant defense, and a deficiency may depress immunity especially in peripartal or transition cows (Spears and Weiss, 2008). The challenge in supplying adequate amounts of trace minerals lies in the negative interactions among them and certain dietary factors that ultimately affect their bioavailability (NRC, 2001). Both sharing of common uptake mechanisms by Fe, Mn, and Co and the fact

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that certain sugars and fiber might bind metals (McDonald et al., 1996) are important aspects affecting the absorption of trace minerals through the small intestine. Other factors also could influence the absorption of minerals including age, the intake of the mineral relative to requirements, and even sunlight (Underwood and Suttle, 1999). Therefore, the use of organic forms [e.g., complexed, covalently-bonded to AA] of these trace minerals has been widely adopted by the dairy industry.

The most commonly reported positive responses to supplementing greater amounts of trace minerals or organic forms of trace minerals to dairy cows include greater milk yield (Rabiee et al., 2010), improved reproductive performance (Rabiee et al., 2010), decreased SCC (Kellogg et al., 2004), decreased lameness, and improved foot health (Nocek et al., 2000; Siciliano-Jones et al., 2008; Overton and Yasui, 2014). However, limited data have been published on systemic and tissue indicators of biological effects as a result of supplementation of organic trace minerals, such as Zn, Mn, Cu, and Co, particularly in transition dairy cows. Although some have observed that organic trace mineral supplementation produces moderate alterations in glucose and fatty acids (Nayeri et al., 2014), it is conceivable that organic trace minerals have beneficial effects on oxidative stress and inflammation (e.g., can help increase overall health and epithelial tissue integrity while reducing the energy required to maintain these systems and consequently improving available energy; Nayeri et al., 2014). As such, they would have an added benefit in terms of liver function. Such alterations could be evaluated via the use of plasma or serum and tissue concentrations of selected biomarkers. Additionally, the onset of inflammation and related disorders (e.g., ketosis and mastitis), which commonly occur after calving, are likely to induce a redistribution of trace minerals such as Zn, Fe, and Cu from peripheral blood to tissues (Erskine and Bartlett, 1993; Zhang et al., 2010). However, the extent of the effect of inflammation on redistribution or availability of organic trace minerals remains unknown.

Based on the previous research with transition dairy cows (Ballantine et al., 2002; Siciliano-Jones et al., 2008), we hypothesized that AA-complexed (**AAC**) trace minerals would improve DMI and milk yield as well as decrease blood BHB and fatty acids while increasing glucose concentrations. Furthermore, we hypothesized that AAC could enhance immune function. Therefore, the objective of this experiment was to evaluate the effects of supplementing AAC trace minerals during the peripartal period (−30 through 30 DIM) on performance, blood and tissue biomarkers, and systemic PMNL function.

MATERIALS AND METHODS

Experimental Design and Dietary Treatments

The Institutional Animal Care and Use Committee (**IACUC**) of the University of Illinois approved all protocols for this study (protocol no. 12097). The experiment was conducted as a randomized complete block design where 44 multiparous Holstein cows were blocked according to parity, previous lactation milk yield, and expected day of calving. Per IACUC guidelines, cows with a clinical disorder could not continue on experiment; thus, a total of 7 cows had to be removed from the experiment due to clinical ketosis, clinical mastitis, retained placenta, displaced abomasum, or leg fracture (Table 1). Cows were enrolled at 60 d before dry-off (~110 d before calving) and a total of 37 remained on experiment until 30 DIM. Cows were offered a common diet supplemented entirely with inorganic trace minerals (**INO**) from −110 to −30 d prior calving. From d −30 to calving, cows received a common prepartal (1.5 Mcal/kg of DM, 15% CP) diet, and from calving to 30 DIM a common postpartal (1.76 Mcal/kg of DM, 18% CP) diet. Both diets were partially supplemented with an INO mix of Zn, Mn, and Cu to supply 35, 45, and 6 mg/kg, respectively, of the total diet DM. Cows were assigned to treatments in a randomized completed block design, receiving a daily oral bolus using a balling gun (Torpac, Fairfield, NJ) with a mix of INO (n = 21) or AAC (n = 16) containing supplemental Zn, Mn, Cu, and Co to achieve 75, 65, 11, and 1 mg/kg, respectively, in the total diet DM. Inorganic trace minerals were provided in sulfate form and AAC were supplied via Availa Zn, Availa Mn, Availa Cu, and COPRO (Zinpro Corp., Eden Prairie, MN). Per IACUC guidelines, a total of 12 cows per treatment were randomly selected for liver biopsies. However, only 11 in INO and 9 in AAC had the complete set of biopsies and corresponding blood samples, hence, they were used for the liver and blood biomarkers analyses.

Animal Management

Prior to calving, cows were fed individually once daily at 0630 h using an individual gate system (American Calan, Northwood, NH). Cows were housed in a ventilated enclosed barn during the dry period and had access to sand-bedded freestalls until 3 d before expected parturition, when they were moved to individual maternity pens bedded with straw until parturition. After parturition, cows were housed in a tiestall barn, were fed a common lactation diet once daily (Table 2), and were milked 3 times daily. At 30 DIM cows returned

to the farm herd. Feed offered was adjusted daily to achieve 5 to 10% refusal.

Individual BW was measured weekly before the midday milking at the same time after the morning feeding. Cows were body condition scored (scale 1 = thin to 5 = obese, with quarter-point increments) weekly by 2 individuals and the average score was used for statistical analysis. Intake of DM was recorded daily. Milk yield was recorded at each milking during the first 30 DIM. Weekly milk composition was analyzed whereas ECM and energy balance (**EB**) were calculated from calving to 30 DIM.

Feed and Milk Samples

Dry matter of individual feed ingredients was determined weekly and rations were adjusted accordingly to maintain formulated DM ratios of ingredients in the TMR. Weekly samples of ingredients and TMR were frozen at −20°C and composited monthly for analysis of DM, CP, NDF, ADF, Ca, P, K, Mg, Zn, Mn, Cu, and Co by standard wet chemistry techniques at a commercial laboratory (Dairy One, Ithaca, NY; http://dairyone.com/wp-content/uploads/2014/02/Forage-Lab-Analytical-Procedures-Listing-Alphabetical-July-2015.pdf). Consecutive morning, midday, and evening milk samples were taken weekly until 30 DIM. Composite milk samples were prepared in proportion to milk yield at each milking, preserved (800 Broad Spectrum Microtabs II; D & F Control Systems Inc., San Ramon, CA), and analyzed for contents of fat, protein, lactose, SNF, MUN, and SCC (Dairy Lab Services, Dubuque, IA). Based on milk sample analysis, the ECM (3.5% fat) was calculated daily as ECM = (12.82 × fat yield, kg) + (7.13 × protein yield, kg) + (0.23 × milk yield, kg) (Tyrrell and Reid, 1965).

Energy balance was calculated for each cow using equations from the NRC (2001). Net energy intake ($\mathbf{NE_I}$) was determined using daily DMI multiplied by the laboratory-calculated NE_L density of the diet. Net energy for maintenance was calculated as $BW^{0.75} \times 0.080$. Requirements of NE_L for milk production were

Table 1. Frequency of occurrence of health problems in cows supplemented with inorganic (INO) or amino acid complexes (AAC) of organic trace minerals during the peripartal period

	Diet	
Event	INO	AAC
Total	22	22
Ketosis[1]	0	2
Staphylococcus aureus[2]	0	1
Leg fracture	1	0
Retained placenta[3]	0	2
Left-displaced abomasum	0	1
Total excluded cows[4]	1	6

[1]Defined as cows having moderate (~40 mg/dL) or large ketone concentrations (>80 mg/dL) in urine after calving, as detected using a reagent strip and treated by veterinarians with oral propylene glycol or intravenous dextrose.

[2]Positive mammary gland infection with *Staph. aureus*.

[3]Defined as fetal membranes retained >24 h postpartum.

[4]Actual number of cows excluded from the experiment per Institutional Animal Care and Use Committee guidelines of University of Illinois due to clinical disease.

Table 2. Ingredient and analyzed chemical composition of diets fed during far-off (−50 to −31 d relative to expected calving), close-up (−30 d to calving), and early lactation[1]

Component[1]	Far-off	Close-up	Early lactation
Ingredient, % of DM			
Alfalfa silage	12.23	7.58	4.90
Alfalfa hay	—	3.50	3.90
Corn silage	33.63	38.85	33.13
Wheat straw	34.81	8.39	2.64
Cottonseed	—	—	3.86
Wet brewers grains	—	6.11	9.42
Ground shelled corn	4.87	18.77	22.60
Soy hulls	2.03	4.07	3.90
Soybean meal, 48% CP	8.92	3.03	5.59
Expeller soybean meal[2]	—	0.67	3.15
SoyChlor[3]	0.16	2.25	—
Blood meal 85% CP	1.04	0.63	0.29
Molasses	—	0.42	—
Urea	0.28	—	0.74
Rumen-inert fat[4]	—	—	1.97
Limestone	0.81	2.23	1.56
Salt (plain)	0.33	—	0.26
Ammonium chloride	—	1.14	—
Dicalcium phosphate	0.13	0.31	0.43
Magnesium oxide	—	0.11	0.13
Magnesium sulfate	0.16	1.36	0.26
Sodium bicarbonate	—	—	0.71
Calcium sulfate	—	—	0.10
Mineral-vitamin mix[5]	0.21	0.17	0.20
Vitamin A[6]	0.02	0.03	0.04
Vitamin D[7]	0.01	0.02	0.02
Vitamin E[8]	0.36	0.36	0.20
Chemical analysis			
NE_L, Mcal/ kg DM	1.25	1.59	1.67
CP, % DM	14.4	14.3	18.7
NDF, % DM	53.0	39.1	35.9
ADF, % DM	34.5	23.9	22.2
Zn, mg/kg of DM	103	83	69
Mn, mg/kg of DM	84	76	70
Cu, mg/kg of DM	15.5	14.4	12.3
Co, mg/kg of DM	0.83	0.72	0.19

[1]Basal close up and lactation diets were considered as basal diet plus inorganic trace minerals, or basal diet plus organic trace minerals.

[2]SoyPLUS (West Central Soy, Ralston, IA).

[3]SoyChlor (West Central Soy).

[4]Energy Booster 100 (MSC, Carpentersville, IL).

[5]Contained a minimum of 4.3% Mg, 8% S, 6.1% K, 2.0% Fe, 3.0% Zn, 3.0% Mn, 5,000 mg/kg of Cu, 250 mg/kg of I, 40 mg/kg of Co, 150 mg/kg of Se, 2,200 kIU/kg of vitamin A, 660 kIU/kg of vitamin D_3, and 7,700 IU/kg of vitamin E.

[6]Contained 30,000 kIU/kg.

[7]Contained 5,009 kIU/kg.

[8]Contained 44,000 IU/kg.

calculated as NE_{MILK} = (0.0929 × fat, % + 0.0547 × protein, % + 0.0395 × lactose, %) × milk yield. Net energy requirement for pregnancy (NE_P; Mcal/d) was calculated as [(0.00318 × day of gestation − 0.0352) × (calf birth weight/45)]/0.218. The equation used to calculate prepartal EB (Mcal/d) was $EB_{PRE} = NE_I - (NE_M + NE_P)$ and that used to calculate EB (% requirements) was $EB_{PRE} = [NE_I/(NE_M + NE_P)] \times 100$. The equation used to calculate postpartal EB was EB_{POST} (Mcal/d) = NE_I – (NE_M + NE_{MILK}) and that for EB (% requirements) was = $[NE_I/(NE_M + NE_{MILK})] \times 100$.

Blood and Liver Samples and Biomarker Analyses

Blood was sampled from the coccygeal vein every Monday and Thursday before the morning feeding from −30 to 30 d relative to calving. Samples were collected into evacuated serum tubes (BD Vacutainer, Becton Dickinson and Co., Franklin Lakes, NJ) containing either clot activator or lithium heparin for serum and plasma, respectively. After blood collection, tubes with lithium heparin were placed on ice and tubes with clot activator were kept at 21°C until centrifugation (~30 min). Serum and plasma were obtained by centrifugation at 1,900 × *g* for 15 min at 4°C. Aliquots of serum and plasma were frozen (−20°C) until further analysis. Liver was sampled via puncture biopsy (Dann et al., 2006) from cows under local anesthesia at approximate 0800 h on d −30, −15, 10, and 30 relative to parturition. Liver was frozen immediately in liquid nitrogen and stored until further analysis for concentration of triacylglycerol (**TAG**).

Blood samples were analyzed for fatty acids, BHB, glucose, creatinine, urea, and γ-glutamyltransferase (**GGT**) using kits purchased from Instrumentation Laboratory (Lexington, MA) following the procedures described previously (Bionaz et al., 2007; Trevisi et al., 2012) using a clinical auto-analyzer (ILAB 600, Instrumentation Laboratory). Whole blood concentration of BHB was measured via Precision Xtra (Abbott Diabetes Care Inc., Alameda, CA) every other day from 1 to 15 d postpartum. The suitability of this assay for accurately measuring blood ketones has been verified (Iwersen et al., 2009).

The analyses of total reactive oxygen metabolites (**ROMt**) and oxygen radical absorbance capacity (**ORAC**) have been previously described (Bionaz et al., 2007). Briefly, blood samples were analyzed for ROMt by measuring its oxidant ability toward a modified aromatic amine (i.e., chromogen) substance used as an indicator; in turn, ORAC was analyzed by measuring the ability of a given sample to object to a massive oxidative insult induced in vitro by a solution of hypochlorous acid, which is one the most powerful oxidizing agents produced by the body (Diacron, Grosseto, Italy).

For liver tissue TAG analysis, a total of 50 mg of tissue was first homogenized in 1.5 mL of PBS/10 m*M* EDTA using a hand-held homogenizer (Tissue-Tearor, Biospec Products, Bartlesville, OK). Subsequently, 200 μL of PBS/10 m*M* EDTA containing 2 drops of 0.1% green food coloring (cat. #S05376, Fisher Scientific, Pittsburgh, PA) along with 3 mL of isopropanol-hexane-water (80:20:2 vol/vol) were added to each sample, the tube was covered with aluminum foil, and the mixture was incubated for 30 min at room temperature. One milliliter of hexane-diethyl ether (1:1) was then added to each sample followed by vortexing and incubating for 10 min at room temperature (protected from light). One milliliter of water was added to each sample to separate the lipid phase and the mixture was vortexed. Samples were incubated covered with aluminum foil for ~20 min at room temperature. The organic phase was then aspirated and placed into glass vials, before evaporation under a stream of N gas. An 8-point TAG standard was prepared with Infinity TG reagent (cat. #10010509, Cayman Chemicals, Ann Arbor, MI). Each 150-μL sample was mixed with 540 μL of Infinity TG reagent before vortexing. A total of 160 μL of this sample mixture was pipetted into a flat-bottom 96-well plastic microplate. The plate was incubated for 15 min at 37°C before determining absorbance at 540 nm using a microplate reader. Concentration of TAG was calculated from the standard curve.

Determination of Co, Cu, Mn, Se, and Zn concentration in liver samples was by flame atomic absorption spectroscopy (Siciliano-Jones et al., 2008). Frozen tissue was shipped to the Diagnostic Center for Population and Animal Health at Michigan State University (Lansing, MI).

Whole-Blood Phagocytosis

The method to assess the phagocytic capacity of PMNL has been previously described (Osorio et al., 2013). Phagocytic capacity of PMNL from heparinized whole blood was determined at −30, −15, 10, and 30 d relative to parturition using the Phagotest kit (Orpegen Pharma GmbH, Heidelberg, Germany) according to the manufacturer's instructions. In brief, 20 μL of *Escherichia coli* was added to 2 of 3 whole-blood samples (100 μL) in test tubes (Falcon, Becton Dickinson and Co.) and incubated for 10 min at 37°C. The cells were resuspended in 200 μL of DNA-staining solution, and were light-protected in an ice bath until analyzed by flow cytometry (LSR II, Becton Dickinson, San Jose, CA).

Statistical Analysis

Data were analyzed using the MIXED procedure of SAS according to the following model:

$$Y_{ijklm} = \mu + D_i + P_j + DP_{ij} + B_k + C_{ijkl} + T_m + DT_{im} + DPT_{ijm} + e_{ijklm},$$

where Y_{ijklm} is the dependent, continuous variable; μ is the overall mean; D_i is the fixed effect of the ith diet (i = 1 and 2); P_j is the fixed effect of the jth parity (j = 1, 2, 3); B_k is the random effect of the kth block (k = 1, ...15); C_{ijkl} is the random effect of lth cow within the ith treatment, the jth parity, and the kth block (l = 1,...., n_{ijk}); T_m is the fixed effect of the mth time (d or wk) of the experiment (m = 1,...n); DT_{im} is the fixed effect of the ith treatment by the mth time of the experiment interaction; DPT_{ijm} is the fixed effect of the ith treatment by the jth parity by the mth time of the experiment interaction; and e_{ijklm} is the residual error. Parity was removed from the model any time it was nonsignificant ($P > 0.05$). Blood and liver biomarkers were analyzed at various time points that were not equally spaced; therefore, an exponential correlation covariance structure SP (POW) was used for repeated measures. Blood and liver biomarker results were log-scale transformed if needed to comply with normal distribution of residuals. For blood biomarkers and liver TAG, the data on d −30 relative to parturition were used as covariate. The covariate of previous 305-d milk yield was maintained in the model for all variables for which it was significant ($P < 0.05$). Statistical differences were declared significant at $P \leq 0.05$ and tendencies were discussed when $P \leq 0.15$.

RESULTS

Ingredient and Nutrient Composition of Diets

Ingredient and chemical composition of the basal diets are presented in Table 2. The chemical composition was determined by analyzing each individual feed ingredient for its chemical composition and then entering the feed analysis results into the Spartan Dairy Ration Evaluator 3.0 (Michigan State University, East Lansing; http://spartandairy.msu.edu/spartandairy/home). The software was developed to evaluate and balance rations for replacements, dry cows, and lactating cows based on NRC (2001) requirements. The estimated supply and balance of trace minerals (i.e., Zn, Mn, Cu, and Co) in TMR as well as the balance of trace minerals after bolus administration in prepartal and postpartal diets are presented in Table 3.

Prepartal DMI, BW, and BCS

Main effects and interactions for postpartal BW, BCS, DMI, DMI as percent BW, and prepartal EB are presented in Table 4. A trend for a diet by time interaction ($\mathbf{D} \times \mathbf{T}$, $P = 0.10$) was detected for prepartal energy balance (Table 4). This response was associated with greater energy balance (Mcal/d, $P = 0.05$, Figure

Table 3. Estimated trace minerals supplied and balance in the TMR as well as balance after bolus administration in prepartal and postpartal diets fed to cows supplemented with inorganic (INO) or amino acid complexes of organic (AAC) trace minerals during the peripartal period[1]

	Prepartum				
		Close-up		Postpartum	
Item	Far-off	INO	AAC	INO	AAC
Trace mineral supplied					
Zn, mg/d	1,425	1,131	1,007	1,109	1,238
Mn, mg/d	1,158	1,031	917	1,017	1,135
Cu, mg/d	214	196.3	174.6	192.4	214.8
Co, mg/d	11.4	9.8	8.7	7.4	8.2
Trace mineral balance					
Zn, mg/d	1,149	547	418	−133	−93
Mn, mg/d	942	637	521	678	783
Cu, mg/d	48.2	−22.8	−45.3	−67.4	−56.9
Co, mg/d	9.9	8.0	7.0	4.5	5.3
Trace mineral balance plus bolus					
Zn, mg/d	—	990	858	519	524
Mn, mg/d	—	856	739	1,086	1,089
Cu, mg/d	—	30.6	16.1	16.9	29.2
Co, mg/d	—	21.3	18.4	23.5	21.3

[1]Evaluation of diets was based on mean DMI, BW, and production data and feed analysis using the Spartan Ration Evaluator, version 3.0 (East Lansing, MI).

Table 4. Effects of supplementing cows with inorganic (INO) or amino acid complexes of organic (AAC) trace minerals during the peripartal period on DMI, BW, BCS, and energy balance

	Diet			*P*-value			
Item	INO	AAC	SEM[1]	Diet	Parity[2]	Time	D × T[3]
Total, no.	21	16					
Prepartum							
BW, kg	761.9	759.3	7.9	0.81	—	0.03	0.88
BCS	3.32	3.34	0.05	0.76	—	0.74	0.18
DMI, kg/d	13.6	12.1	0.65	0.12	—	0.03	0.93
DMI, % BW	1.85	1.58	0.10	0.06	—	<0.01	0.84
Energy balance, Mcal/d	6.33	4.00	1.51	0.28	—	0.06	0.10
Energy balance,[4] %	142.3	125.6	9.8	0.24	—	0.05	0.09
Postpartum							
BW, kg	661.7	655.3	13.9	0.73	0.10	<0.01	0.01
BCS	3.15	3.10	0.06	0.55	—	<0.01	0.03
DMI, kg/d	14.6	16.3	1.1	0.24	—	<0.01	0.11
DMI, % BW	2.25	2.44	0.18	0.42	0.10	<0.01	0.19
Energy balance, Mcal/d	−14.61	−14.49	2.66	0.97	—	0.04	0.34
Energy balance,[5] %	64.8	67.1	5.3	0.75	—	0.01	0.27

[1]Largest SEM is shown.

[2]Parity effect was used in the model depending on significance ($P \leq 0.15$).

[3]Interaction of diet × time.

[4][Net energy intake/(NE_M + net energy for pregnancy)] × 100.

[5][Net energy intake/(NE_M + net energy for milk production)] × 100.

1A; % requirements, $P = 0.06$, Figure 1C) for INO compared with AAC cows at −2 wk relative to parturition. The numerically greater ($P = 0.12$) prepartal DMI in INO compared with AAC cows was associated with a trend for greater ($P = 0.06$) DMI as percent BW in the same group of cows (Table 4). Prepartal BW and BCS were not affected ($P > 0.05$) by dietary treatments. Parity was not significant ($P > 0.15$) in the analyses of any of the prepartal variables measured.

Postpartal DMI, BW, and BCS

Main effects and interactions for postpartal BW, BCS, DMI, DMI as percent BW, and postpartal EB are presented in Table 4 and Figure 1. Diet × time interactions for postpartal BW ($P = 0.01$) and BCS ($P = 0.03$) were detected. In the case of BCS, this response is partly explained by a greater ($P = 0.03$) BCS at 2 wk postpartum for INO compared with AAC (Figure 2D). The trend ($P = 0.11$) for differences in postpartal DMI was attributed to cows in the AAC group, at several time points, tending ($P < 0.15$) to consume more DMI than INO cows. Responses were particularly evident after 15 d postpartum (Figure 2F).

Milk Production and Composition

Main effects and interactions for postpartal production variables and ECM-to-DMI ratio are presented in Table 5. A D × T interaction ($P \leq 0.02$) was observed for milk yield, milk protein yield, and ECM due to greater responses in cows fed AAC than INO. Milk and milk protein yield responses to AAC were greatest between 15 and 20 d postpartum (Figure 3D and E). The D × T interaction ($P = 0.02$) for ECM yield (Figure 3F) was attributed to increased ECM yield of AAC cows at several time points between 15 and 30 DIM. A strong trend ($P = 0.07$) for a D × T interaction was detected for ECM-to-DMI ratio, where AAC had greater ECM-to-DMI at several time points from 5 to 30 DIM (Figure 3G). Similarly, the strong ($P = 0.07$) trend for D × T interaction for milk protein percentage was in part due to greater milk protein percentage at 2 wk postpartum (Figure 3C) in AAC compared with INO cows. Yield of ECM was the only production variable with a strong overall trend ($P = 0.06$) for a parity effect (Table 5).

Blood and Liver Biomarkers

Main effects and interactions for blood and liver biomarkers are presented in Table 6 and Figures 4 to 7. The D × T interactions for GGT ($P = 0.04$), ORAC ($P = 0.01$), and Co ($P = 0.04$) had a strong trend ($P = 0.08$) for PMNL phagocytosis and a mild trend for Mn ($P = 0.13$) and Se ($P = 0.12$). The D × T interaction for GGT seemed to be associated with a trend for greater ($P < 0.10$) GGT in INO cows compared with AAC at 15 and 30 d postpartum (Figure 4). In the case of ORAC, the D × T interaction was associated with a trend ($P \leq 0.15$) for greater values on d −14 for INO cows but with a significant increase in ORAC for

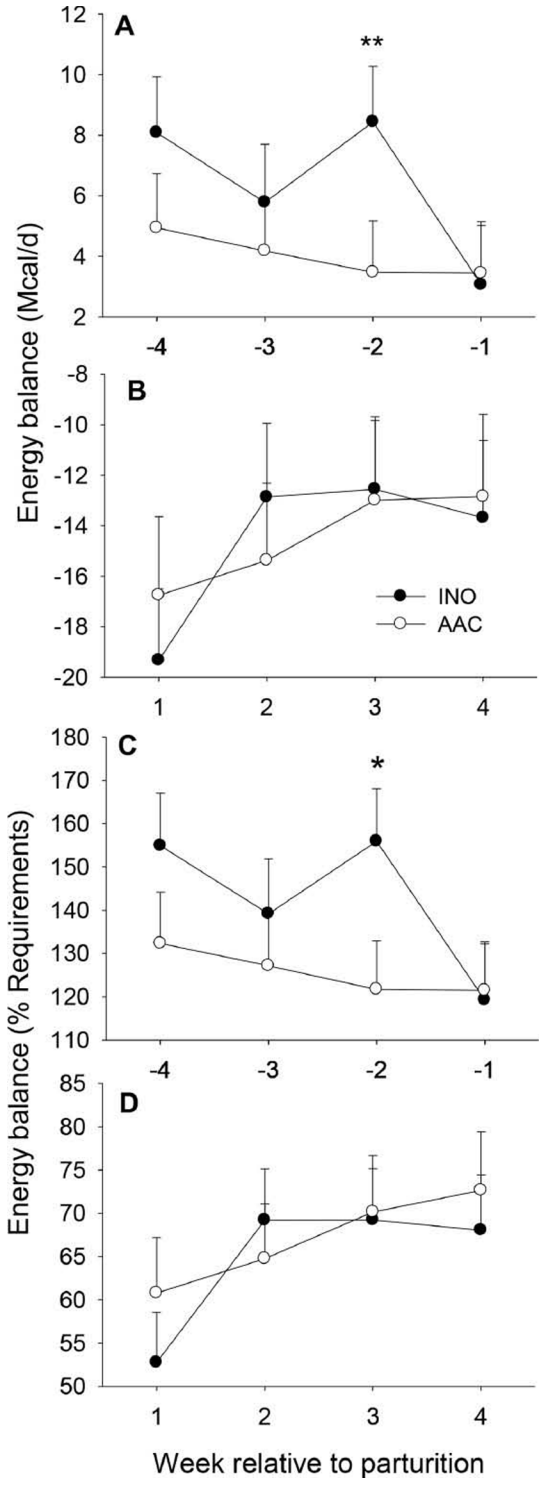

Figure 1. Prepartal energy balance (EB) as megacalories per day (A), postpartal EB as megacalories per day (B), prepartal EB as percent requirements (C), and postpartal EB as percent requirements (D), in cows supplemented with inorganic (INO; n = 21) or amino acid complexes of organic (AAC; n = 16) trace minerals during the peripartal period. Mean separations between diets at a given time point were evaluated at a diet × time interaction ($P \leq 0.10$) and differences (*) were declared at $P \leq 0.05$ and trends (**) at $P \leq 0.15$. Values are means ± SD.

ORG cows, such that by 30 d they achieved the highest values ($P = 0.01$) compared with −14 d. No D × T interaction ($P = 0.41$) was noted for ORAC in INO cows between −14 and 30 d. The D × T interaction for liver Co was associated with greater liver concentration in AAC than INO cows at 10 ($P = 0.002$) and 30 d ($P = 0.07$; Figure 6). In the case of liver Mn, the D × T interaction was due to greater ($P = 0.02$) concentration in AAC than INO cows at 10 d. Similar to Mn, the D × T interaction for Se was due to greater ($P < 0.01$) concentration in AAC than INO cows at 10 d.

For PMNL phagocytosis, the D × T interaction was associated with an overall strong trend for greater ($P = 0.06$) phagocytosis in AAC cows compared with INO cows at 30 d postpartum (Figure 7). Furthermore, the better PMNL response in AAC cows compared with INO cows also was associated with overall lower ($P = 0.02$) blood BHB measured using the Precision Xtra system (Table 6). The overall liver concentrations of Cu ($P = 0.01$) were greater in AAC than INO cows and a trend ($P = 0.08$) for overall greater concentration of Se also was detected in ACC than INO cows (Table 6). No D × T ($P > 0.05$) or main effect of diet ($P > 0.05$) was detected for concentration of ROMt (Figure 5). With the exception of Cu ($P = 0.13$) and Se ($P = 0.15$), all the blood and liver biomarkers were affected ($P < 0.04$) by time. Glucose, creatinine, and Zn concentrations decreased and fatty acids, BHB, liver TAG, GGT, urea, ROMt, ORAC, and ketone concentrations increased over time regardless of dietary treatment.

DISCUSSION

Ingredient and Nutrient Composition of Diets

Mean chemical composition of feed ingredients throughout the experiment were used to evaluate, in particular, the content of trace minerals of interests in prepartal and postpartal diets through the Spartan Dairy Ration Evaluator 3.0. This software package is partly based on recommendations from NRC (2001), with some additional improvements developed at Michigan State University (East Lansing, MI). Although the original diets were formulated to supplement 35, 45, 6, and 0 mg/kg for Zn, Mn, Cu, and Co, respectively, the estimated concentrations of these trace minerals in the diets, based on chemical composition of samples, surpassed the targeted concentrations (Table 2). This is likely due to endogenous trace minerals in feedstuffs. However, it is likely that negative interactions that are known to occur among ingested trace minerals (NRC, 2001) and certain dietary factors (McDonald et al., 1996), to some extent, reduced the actual absorption of these trace minerals.

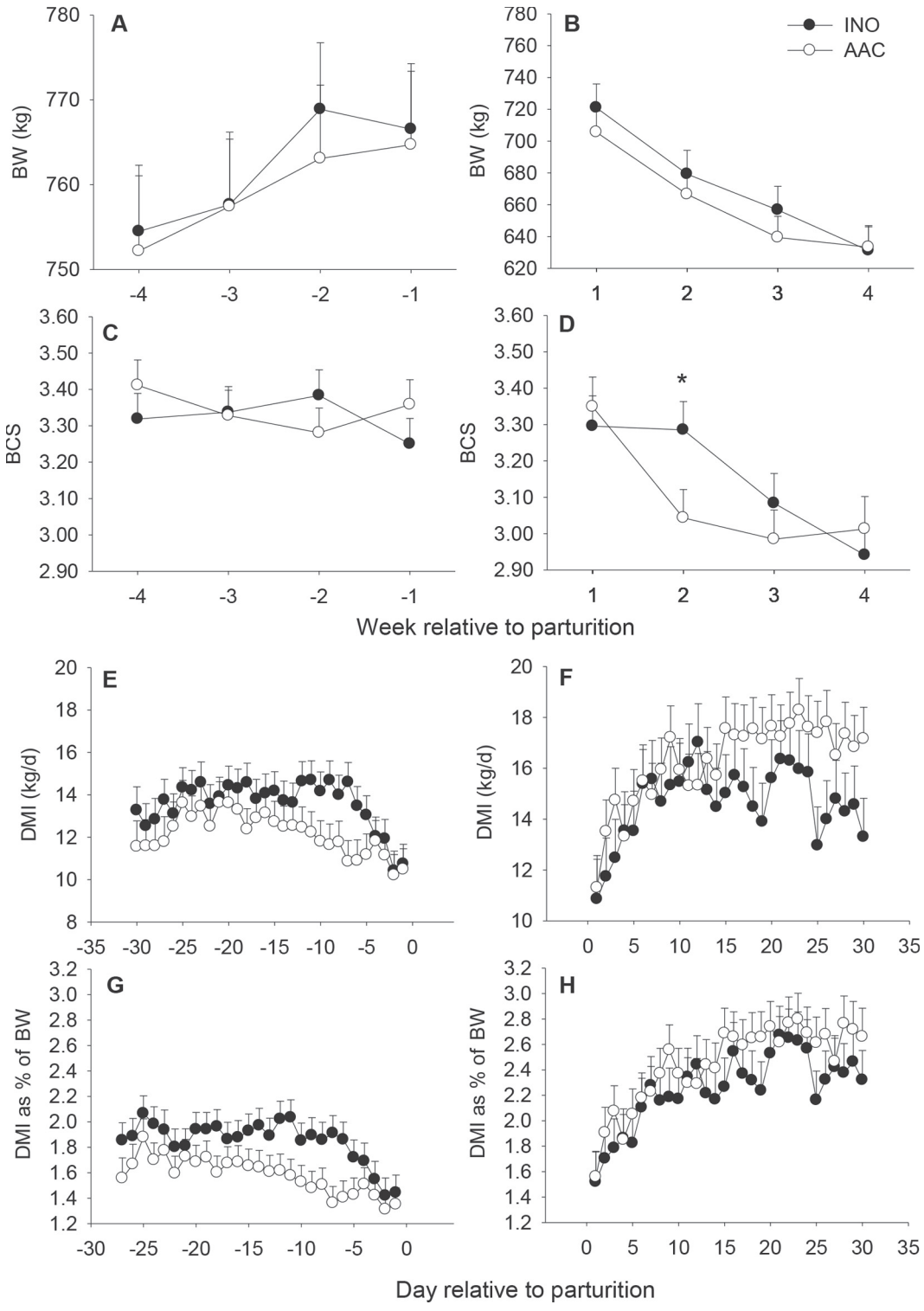

Figure 2. Prepartal (A) and postpartal (B) BW, BCS (C, D), DMI (E, F), and DMI as percentage of BW (G, H) in cows fed a basal diet supplemented with inorganic (INO; n = 21) or amino acid complexes of organic (AAC; n = 16) trace minerals during the peripartal period. Mean separations between diets at a given time point were evaluated at a diet × time interaction ($P \leq 0.10$) and differences (*) were declared at $P \leq 0.05$. Values are means ± SD.

Table 5. Effects of supplementing cows with inorganic (INO) or amino acid complexes of organic (AAC) trace minerals during the peripartal period on production variables

	Diet			*P*-value			
Item	INO	AAC	SEM[1]	Diet	Parity[2]	Time	D × T[3]
Total, no.	21	16					
Milk yield, kg/d	38.30	41.60	2.00	0.27	—	<0.01	0.02
Milk fat, %	4.70	4.50	0.23	0.42	—	<0.01	0.30
Milk protein, %	3.07	3.08	0.05	0.93	—	<0.01	0.07
Milk fat yield, kg/d	1.63	1.75	0.10	0.46	—	<0.01	0.15
Milk protein yield, kg/d	1.12	1.26	0.07	0.19	—	<0.01	<0.01
ECM, kg/d	44.20	46.10	2.50	0.60	0.06	<0.01	0.02
ECM:DMI	2.57	2.84	0.15	0.19	—	<0.01	0.07
Log-transformed SCC[4]	3.24	3.45	0.20	0.41	—	0.06	0.17

[1]Largest SEM is shown.

[2]Parity effect was used in the model depending on significance ($P \leq 0.15$).

[3]Interaction of diet × time.

[4]Original SCC in weekly milk samples were transformed to Log10.

From a more mechanistic standpoint, a limitation of the current study is the lack of data on concentrations of these trace minerals (i.e., Zn, Mn, Cu, and Co) in feces and urine, which could provide a better assessment of mineral retention or availability. However, although an important route of excretion, fecal excretion exogenous or endogenous trace minerals is quiet variable (Underwood and Suttle, 1999). In addition, the increased requirements at the end of pregnancy and the onset of lactation coupled with a low DMI might have further exacerbated the amount of trace minerals such as Co and Zn needed in the diet (Underwood and Suttle, 1999). The latter was reflected in the estimated negative balance of Cu during the transition period as well as the postpartal negative balance of Zn in the TMR diets before bolus administration (Table 3).

Effects on DMI, BW, and BCS

Data reporting an alteration in DMI before the end of pregnancy by source of trace minerals are scarce in both ruminants and monogastrics. In fact, a recent study where either AA complexes or hydroxy forms of trace minerals (Zn, Mn, and Cu) were supplemented during the transition period did not detect changes in prepartal DMI (Yasui et al., 2014). Similarly, in monogastrics, growing gilts supplemented with either INO or organic trace minerals until farrowing did not exhibit differences in prepartal intake (Peters and Mahan, 2008). In contrast, when the ratio of AA complexed to Zn sulfate was increased during the transition period, it was associated with a decrease in prepartal DMI (Nayeri et al., 2014). Overall, the discrepancies among experiments could arise from differences in breed, stage of lactation, duration of treatments, and the combination of trace minerals. Therefore, the relationship between Zn status or other trace minerals and prepartal appetite regulation remains to be determined.

Despite the fact that neither postpartal EB nor liver TAG were affected by trace mineral source, the D × T interaction for postpartal BCS (Figure 2D) suggests that AAC cows might have been subjected to transient periods of greater mobilizing body reserves. However, the lack of dietary treatment effect on EB indicates that the amounts of lipid and AA mobilized were negligible in AAC INO cows and did not elicit major metabolic effects (Table 4). It also is possible that the liver of AAC cows was able to sustain such stress better than that in INO cows without compromising its functionality. Support for this idea comes from the gradual increase postpartum in antioxidant capacity (i.e., ORAC; Figure 5), as well as the well-known effect of trace minerals as activators of antioxidant systems [e.g., Zn and Cu are needed for the functionality of the enzyme superoxide dismutase (**SOD**); Spears and Weiss, 2008]. Therefore, it is conceivable that greater bioavailability of AAC trace minerals promoted better liver function and antioxidant status during the transition period. In fact, Zn deficiency has been long known to be associated with liver disease (Vallee et al., 1957).

The rate at which postpartal DMI increases could be considered as one the most important signs of dairy cow health, particularly during stressful periods such as the transition into lactation. The importance of Co as precursor in the ruminal biosynthesis of vitamin B_{12} in ruminants has long been known, and specifically its positive effects on DMI (Smith and Loosli, 1957). Therefore, the trend for D × T interaction in postpartal DMI resulting in an overall greater DMI (approximately 2 kg/d) in AAC specifically toward the end of the experimental period could be partly associated with greater ruminal-bioavailable Co in the AAC supplement.

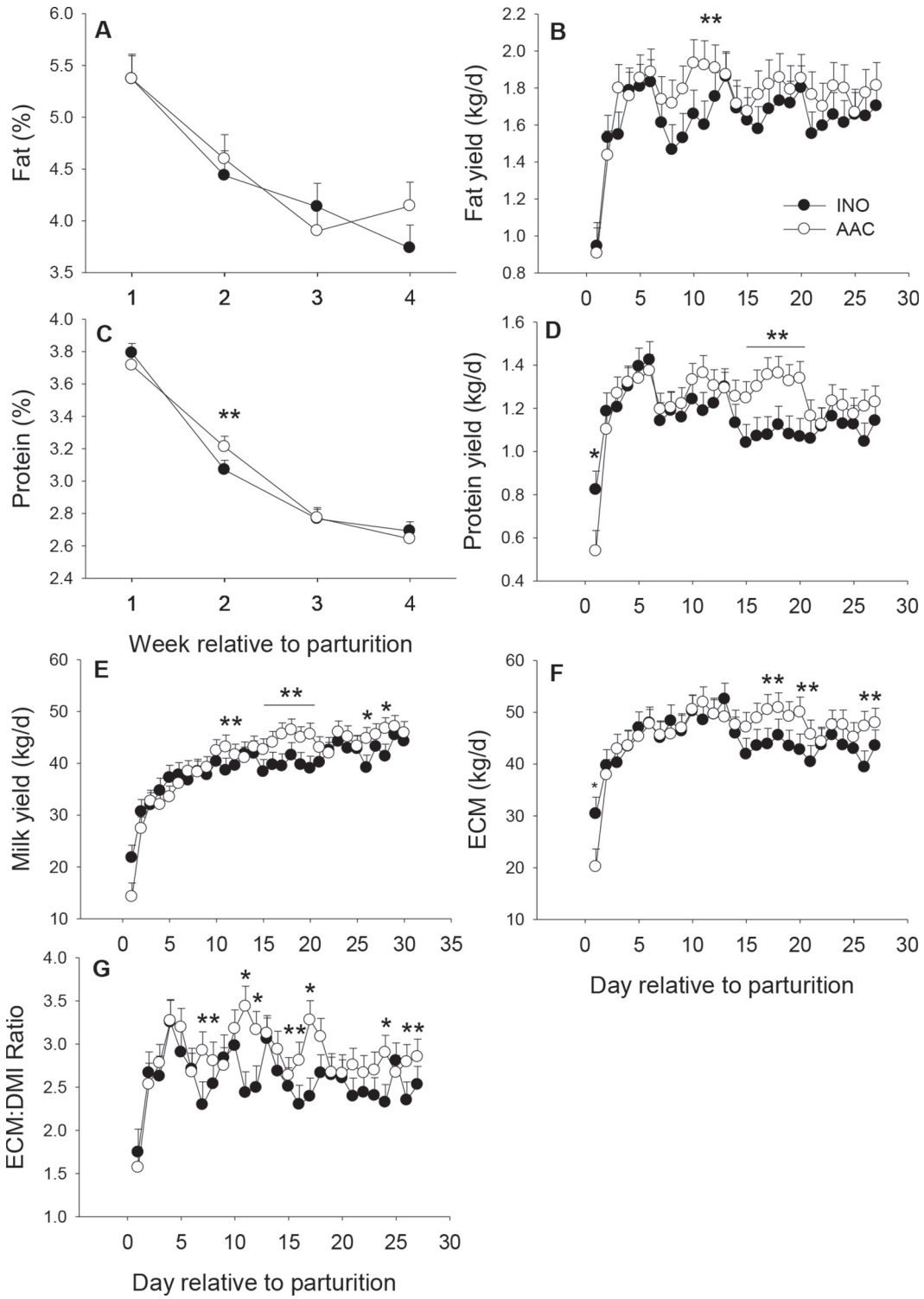

Figure 3. Milk fat percentage (A), milk fat yield (B), milk protein percentage (C), milk protein yield (D), milk yield until 30 DIM (E), ECM (F), and ECM-to-DMI ratio (G) in cows fed a basal diet supplemented with inorganic (INO; n = 21) or amino acid complexes of organic (AAC; n = 16) trace minerals during the peripartal period. Mean separations between diets at a given time point were evaluated at a diet × time interaction ($P \leq 0.10$) and differences (*) were declared at $P \leq 0.05$ and trends (**) at $P \leq 0.15$. Values are means ± SD.

Milk Production and Composition

The greater milk yield in AAC cows at several time points (D × T interaction, $P = 0.02$) resulted in an overall milk yield increase of 3.3 kg/d. Through a meta-analysis that included 20 research papers, Rabiee et al. (2010) estimated that supplementing AAC trace minerals increases milk yield approximately 1 kg/d, which our results support. Through an evaluation of 12 studies, Kellogg et al. (2004) estimated similar increases in milk yield (approximately 1.3 kg/d) with a concomitant decrease in SCC in Zn methionine complex-supplemented cows. They attributed this effect to an improvement of udder health.

Previous meta-analyses estimated an increased in milk protein yield of 0.03 kg/d in cows supplemented with AAC trace mineral complexes (Kellogg et al., 2004; Rabiee et al., 2010). The D × T interaction for milk protein percentage and milk protein yield observed in the present study was driven mainly by the greater response in AAC-supplemented than INO cows during wk 2 and 3 (Figure 3C and D). The protein yield response translated into an increase of 0.14 kg/d, which agrees with meta-analyses data.

Blood and Liver Tissue Biomarkers

Greater bioavailability of AAC likely produces a positive effect on several biological reactions known to require trace minerals such as Zn and Cu (NRC, 2001). An important organ in the metabolism of trace minerals is the liver, which also is a major storage site for some minerals (Table 6). Furthermore, in the liver, some of the trace minerals can be used for synthesis of functional molecules (e.g., ceruloplasmin and transferrin) that could, when needed, distribute the trace minerals to other tissues (Erskine and Bartlett, 1993; NRC, 2001). Therefore, maintaining adequate liver function during the transition period is of high priority for a successful transition.

The enzyme GGT is commonly used as a biomarker of liver damage across species (Bertoni et al., 2008; Oh et al., 2011). Although GGT typically increases after calving in dairy cows (Bertoni et al., 2008), the over-

Table 6. Effects of supplementing cows with inorganic (INO) or amino acid complexes of organic (AAC) trace minerals during the peripartal period on blood and liver biomarkers, and whole blood PMNL phagocytosis; data on d −30 for blood biomarkers and liver triacylglycerol (TAG) were used as covariate

	Diet			*P*-value			
Item	INO	AAC	SEM[1]	Diet	Parity[2]	Time	D × T[3]
Total, no.	11	9					
Blood							
Metabolism							
Fatty acids,[4] mmol/L	−0.89	−0.87	0.09	0.82	—	<0.01	0.82
BHB							
mmol/L[4]	−0.04	−0.08	0.08	0.72	0.02	<0.01	0.70
mmol/L[5]	2.18	1.44	0.22	0.02	—	<0.01	0.70
Glucose, mmol/L	3.67	3.62	0.07	0.67	—	<0.01	0.47
Creatinine, μmol/L	87.2	88.6	1.65	0.57	—	<0.01	0.39
Urea, μmol/L	5.33	5.76	0.33	0.34	—	<0.01	0.46
Liver function							
γ-Glutamyltransferase,[4] U/L	3.39	3.31	0.07	0.45	—	<0.01	0.04
Oxidative stress							
Reactive oxygen metabolites, mg of H_2O_2/100 mL	13.1	13.7	0.53	0.41	—	<0.01	0.92
Total antioxidant capacity, μmol/L	14.9	14.7	0.40	0.72	—	<0.01	0.01
Liver (% wet wt)							
Triacylglycerol, mg/g of tissue	12.5	13.3	1.0	0.56	—	<0.01	0.77
Liver trace minerals							
Co, μg/g dry	0.28	0.33	0.01	0.03	—	0.02	0.04
Cu, μg/g dry	321.1	482.0	42.4	0.01	—	0.13	0.24
Mn, μg/g dry	11.2	11.6	0.45	0.46	—	0.01	0.13
Se, μg/g dry	1.62	1.88	0.10	0.08	—	0.15	0.12
Zn, μg/g dry	99.9	98.5	10.8	0.92	—	<0.01	0.89
PMNL phagocytosis, (%)	41.8	47.1	4.6	0.40	—	0.04	0.08

[1]Largest SEM is shown.

[2]Parity effect was used in the model depending on significance ($P \leq 0.15$). When significant, the *P*-value is reported.

[3]Interaction of diet × time.

[4]Data were log-transformed before statistics.

[5]Measured using the Precision Xtra (Abbott Diabetes Care Inc., Alameda, CA).

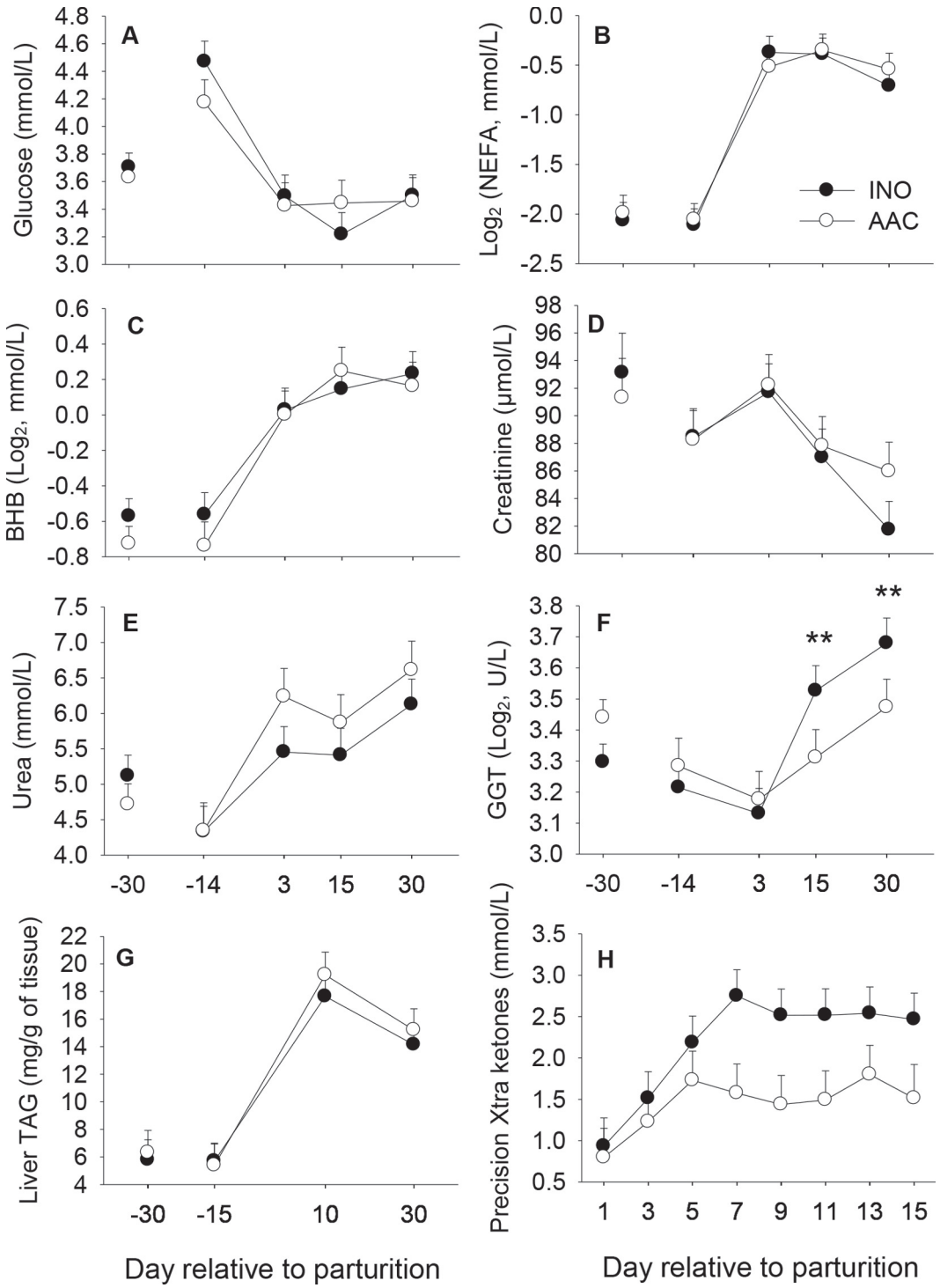

Figure 4. Effects of supplemental inorganic (INO; n = 11) or amino acid complexes of organic (AAC; n = 9) trace minerals on blood glucose (A), blood fatty acids (B), blood BHB (C), blood creatinine (D), blood urea (E), blood γ-glutamyltransferase (GGT; F), liver triacylglycerol (TAG) (G), and Precision Xtra (Abbott Diabetes Care Inc., Alameda, CA) BHB (H) in dairy cows during the transition period. Data on d −30 were used as covariate in the statistical analysis. Mean separations between diets at a given time point were evaluated at a diet × time interaction ($P \leq 0.10$) and trends (**) declared at $P \leq 0.15$. Values are means ± SD.

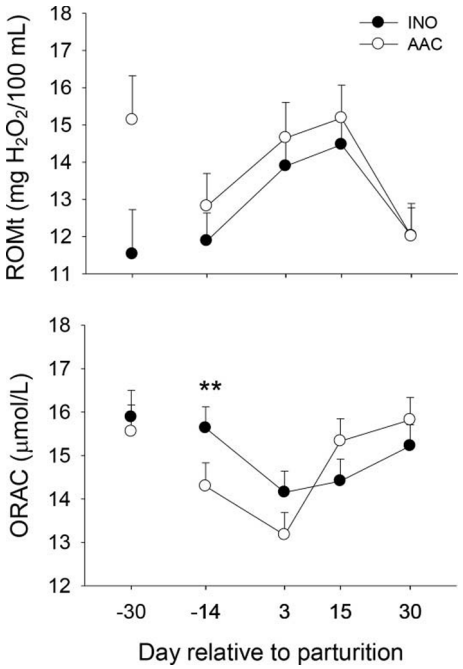

Figure 5. Effects of supplemental inorganic (INO; n = 11) or amino acid complexes of organic (AAC; n = 9) trace minerals on reactive oxygen metabolites (ROMt) and total antioxidant capacity (ORAC) in dairy cows during the transition period. Data on d −30 were used as covariate in the statistical analysis. Mean separations between diets at a given time point were evaluated at a diet × time interaction ($P \leq 0.10$) and trends (**) declared at $P \leq 0.15$. Values are means ± SD.

all lower blood GGT postpartum in response to AAC indicates a lesser degree of liver dysfunction. In fact, Bertoni et al. (2008) observed a decrease of 2 to 3 U/L in GGT between cows with a high compared with low liver function during the early postpartum. Similarly, toward the end of the study, the AAC compared with INO cows had a 1.6 U/L (back-transformed on Log10 scale from Table 6) increase in GGT. Thus, as reflected by the postpartal increase in total antioxidant capacity (Figure 5), better liver functionality with AAC could be partly related to a lower degree of oxidative stress and inflammation (Andrieu, 2008; Overton and Yasui, 2014), which could enhance metabolic processes such as gluconeogenesis. The latter was suggested by Nayeri et al. (2014), because replacing 16 mg/kg of Zn from Zn sulfate with 16 mg/kg of Zn from Zn AA complex in postpartal diets resulted in greater plasma glucose. Together, the data provide evidence that AAC might have a positive effect on liver metabolism.

Oxidative stress is driven by the imbalance between the production of ROMt and the neutralizing capacity of antioxidant mechanisms in tissues and in blood. Therefore, under scenarios such as the transition period of dairy cows, when DMI is insufficient to meet the demand for nutrients primarily for milk production,

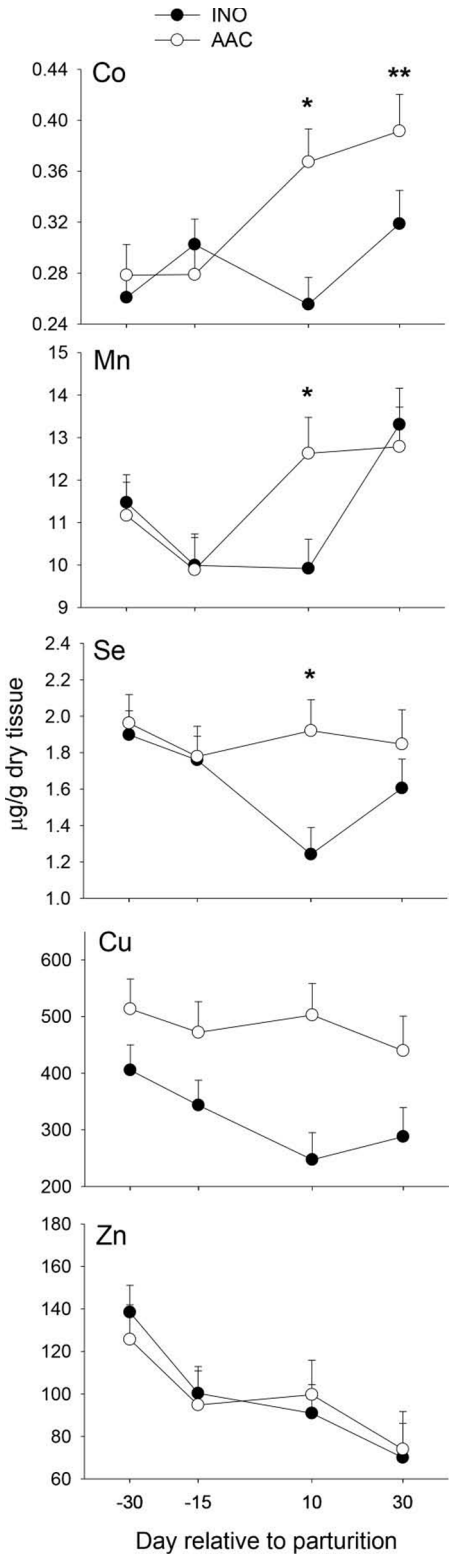

Figure 6. Effects of supplementing inorganic (INO; n = 11) or amino acid complexes of organic (AAC; n = 9) trace minerals on liver concentrations of Co, Cu, Mn, Se, and Zn. Mean separations between diets at a given time point were evaluated at a diet × time interaction ($P \leq 0.10$) and differences (*) were declared at $P \leq 0.05$ and trends (**) at $P \leq 0.15$. Values are means ± SD.

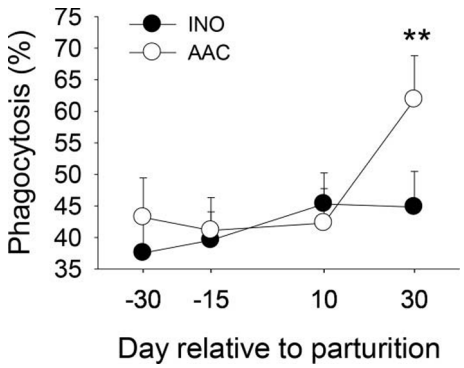

Figure 7. Effects of supplemental inorganic (INO; n = 11) or amino acid complexes of organic (AAC; n = 9) trace minerals on whole blood PMNL phagocytosis percent in dairy cows during the transition period. Mean separations between diets at a given time point were evaluated at a diet × time interaction ($P \leq 0.10$) and trends (**) at $P \leq 0.15$. Values are means ± SD.

antioxidant mechanisms such as SOD, glutathione, and vitamins A and E (Bernabucci et al., 2005) can be severely impaired, rendering cows prone to develop oxidative stress. Such a response is consistent with the overall increase detected in ROMt concentration soon after calving (i.e., 3 and 15 d postpartum; Figure 5). It is also noteworthy that the greater ORAC over time in AAC cows indicates that at least one of these antioxidant mechanisms was directly affected by supplementation of organic trace minerals during the transition period. For instance, it has been observed in rodents that Zn deficiency can affect the antioxidant defense system by affecting glutathione metabolism (Ozturk et al., 2003; Omata et al., 2013). Similarly, Cu deficiency has been associated with decreased activity of SOD and glutathione metabolism. One explanation for such an effect is that Cu deficiency decreases liver activity and mRNA expression of selenoglutathione peroxidase (Olin et al., 1994). This is an essential enzyme in the glutathione metabolism pathway, which is in charge of neutralizing hydrogen peroxide by converting it to water. The fact that hepatic Se availability is unresponsive to Cu deficiency (Prohaska et al., 1992) indicates that no competition or interaction occurs between these trace minerals, but rather Cu availability might indirectly modulate the liver activity and mRNA expression of selenoglutathione peroxidase.

The observed greater liver Co content as early as 10 d postpartum in AAC compared with INO (Figure 6) contrasts several studies (Siciliano-Jones et al., 2008; Akins et al., 2013) where Co supplementation had no response on liver Co content. Although previous studies have reported that Cu supplementation does not increase liver Cu, Xin et al. (1993) observed an increase in liver Cu content by supplementing basal diets with 20 mg/kg, which is similar to our results at lower dose of supplementation (i.e., <15.5 mg/kg of DM; Figure 6).

Creatinine is an important indicator of body muscle mass and its concentration typically decreases around parturition (Pires et al., 2013; Osorio et al., 2014). In contrast, urea has been reported to increase after calving (Douglas et al., 2006), and this effect has been primarily related to total protein intake (Roseler et al., 1993). The lack of dietary effects on creatinine and urea suggests that these metabolites are not sensitive to organic trace mineral supplementation, or that during the transition period these metabolites (i.e., creatinine and urea) might respond primarily to availability of macronutrients such as AA, thus masking any potential effect of organic trace mineral supplementation.

A strong negative correlation between blood concentrations of Zn and retained placenta incidence was noted in early postcalving cows (Trevisi et al., 2008; Moretti et al., 2015), such that concentrations of Zn were markedly lower whereas inflammatory status was markedly greater in afflicted cows. However, the connection between trace mineral supplementation and ketosis in dairy cows has not received much attention and is yet to be fully understood. Previous investigations have dealt with the relationships among serum Cu and Zn concentrations and those of BHB, NEFA, and glucose in healthy and subclinically ketotic cows (Zhang et al., 2010). Compared with healthy cows, only serum Zn decreased significantly in subclinically ketotic cows that had greater BHB, NEFA, and lower glucose. A decrease in serum Zn also is observed during mastitis induction, suggesting this is a natural response during inflammation or that the onset of a disease increases transfer of Zn from peripheral blood into the liver for synthesis of metallothionein or sequestration of Zn by binding proteins such as lactoferrin with the aim to decrease Zn availability for bacterial growth (Erskine and Bartlett, 1993; Underwood and Suttle, 1999). It is noteworthy that the decrease in liver concentration of Zn suggested that occurrence of inflammation was less likely as cows progressed through lactation (Figure 6).

The greater bioavailability of Zn in AAC trace minerals might partly explain the greater ketone bodies in INO compared with AAC cows, thus suggesting that they were more prone to or were undergoing a state of subclinical ketosis. The discrepancy between BHB and Precision Xtra ketones (i.e., no dietary effects were observed in BHB) could have been due to the lower frequency of BHB analysis. It must be kept in mind that the intent was not to correlate these data, but to use the Precision Xtra approach as a quick and effec-

tive way of measuring the short-term response in blood ketones (Iwersen et al., 2009). Only 2 time points (3 and 15 d postpartum) had a high correlation ($r = 0.75$; $P < 0.01$) between both assays; similar results have been reported previously (Iwersen et al., 2009; Pineda and Cardoso, 2015).

The greater demand for nutrients during the transition period is commonly accompanied by a reduction in immunological capacity (Goff and Horst, 1997; Overton and Waldron, 2004). Such an effect has been associated with a decrease in PMNL function that, in turn, can render cows more prone to disorders, including retained placenta, metritis, and mastitis (Cai et al., 1994). An increase in oxidative stress through production of ROMt as a result of greater metabolic activity during early lactation and negative energy balance have been regarded as important causes for decreased PMNL function (Sordillo and Aitken, 2009; Overton and Yasui, 2014). Furthermore, substantial evidence exists that trace minerals such as Se, Zn, Cu, and Mn can minimize the negative effects of oxidative stress by increasing the activity of antioxidant enzymes such as glutathione peroxidase and SOD (Spears and Weiss, 2008; Sordillo and Aitken, 2009). However, a lack of data exists on the response of AAC to physiological cues, such as early postpartal inflammation, when availability of peripheral blood inorganic trace minerals such as Zn are minimized to reduce its use for bacterial growth (Underwood and Suttle, 1999). Therefore, it is plausible that PMNL in AAC cows could have benefited from greater availability of trace minerals. Taken together, the greater PMNL phagocytosis detected in AAC cows at 30 d postpartum is likely due to a lower degree of oxidative stress induced by a greater bioavailability of AAC, such as Zn, Mn, and Cu. The postpartal increase in total antioxidant capacity with AAC supplementation offers support for this idea.

CONCLUSIONS

The better milk production, DMI, lower blood ketones, and better PMNL function from feeding AAC underscored the beneficial effects of supplementing trace minerals as AAC. Unlike previous studies, use of AAC can increase the concentration of some trace minerals in liver; thus, this organ could act as a buffer during periods of low availability of trace minerals by storing them or their intermediate functional compounds. Additional research to determine trace mineral excretion in feces and accumulation in key tissues seems warranted and will help better understand their metabolism during the periparturient period.

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