



Original Research Article

Colostrum and milk performance, and blood immunity indices and minerals of Holstein cows receiving organic Mn, Zn and Cu sources

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ABSTRACT

Effects of supplementing the basal diets with Mn, Zn and Cu, as sulphate, glycine or methionine salts, on colostrum and milk performance, some blood immunity indices and blood minerals of pre- and postpartum Holstein cows were assessed. Forty cows in different groups received: 1) a diet without supplementary Mn, Zn and Cu (control), 2) a diet containing Mn, Zn and Cu sulphates, 3) a diet containing Mn, Zn and Cu glycine, or 4) a diet containing Mn, Zn and Cu methionine with 10 cows per group from d 60 before calving (dry period) to d 100 of lactation. Dry matter intake (DMI), dry matter digestibility (DMD), colostrum and milk performance, milk somatic cell count (SCC), blood and milk total antioxidant capacity (TAC), immunoglobulin M (IgM) and immunoglobulin A (IgA), and blood Mn, Zn and Cu were determined. Dietary supplementation with Mn, Zn and Cu as methionine, glycine or sulphate salts had positive effects on DMD, DMI, colostrum and milk performance, milk SCC, and blood Mn and Zn. Addition of Mn, Zn and Cu in diets could increase ($P < 0.05$) blood and milk TAC and blood IgA and IgM in the cows and their new-born calves. There were no differences in DMI, DMD, colostrum and milk yields, milk SCC, blood Mn (except d 50 postpartum), Zn and Cu and TAC (except d 50 postpartum) among the organic and inorganic minerals-supplemented groups ($P > 0.05$), however, the blood concentrations of IgA (except d 1 postpartum) and IgM in the cows supplemented with organic Mn, Zn and Cu were higher ($P < 0.05$) than those in the cows receiving the sulphate sources of minerals. Overall, dietary supplementation of Mn, Zn and Cu as methionine, glycine or sulphate salts can improve colostrum and milk performance, blood Zn and Mn and immunity indices in Holstein cows and their new-born calves. Moreover, the organic sources of Mn, Zn and Cu have advantage over the sulphate forms in terms of the blood immunoglobulins.

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1. Introduction

Trace minerals, e.g., Mn, Zn and Cu, are essential for antioxidant protection, health maintenance and maximization of digestibility and productivity in dairy cows (Griffiths et al., 2007; Faulkner and

Weiss, 2017; Wu, 2018). Although Mn, Zn and Cu are usually supplemented as inorganic salts in livestock diets, some studies have shown that feeding dairy cows using organic trace mineral sources instead of inorganic sources improved health, production (Cope et al., 2009; Bach et al., 2015; Batistel et al., 2016; Osorio et al., 2016) and fertility (Chester-Jones et al., 2013), and may have environmental benefits due to their relative higher bioavailability (McDonald et al., 2011). Despite these findings, Formigoni et al. (2011) reported that productive performance, reproduction and pathologic events were not changed by supplementing dairy cows with organic trace minerals instead of the minerals in a sulphate form. Moreover, no nutritional effect of organic trace mineral sources on the milk performance of dairy cows was observed by Faulkner and Weiss (2017) and Zhao et al. (2015). The conflicting results regarding the nutritional effects of substituting organic

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micro-minerals (e.g., Zn, Mn and Cu) for the inorganic sources on the ruminant health and productivity could be owing to some factors such as the degree of purity of mineral supplements, presence or absence of stressors, previous mineral storages of the body, environment, performance and physiological stage of animals (Suttle, 2010; Alimohamady et al., 2019). On the other hand, some review papers have indicated that increasing dietary trace minerals above the recommended requirements can improve animal performance and health, especially in critical conditions such as the presence of stressors or during the transition period, probably due to the roles of trace minerals in the antioxidant systems (Overton and Yasui, 2014; Abuelo et al., 2015).

In this work, it was hypothesised that supplementation of the diet with organic sources of Mn, Zn and Cu would improve productivity, immunoglobulins and antioxidant status of dairy cows during the pre- and post-partum periods. Thus, the present study assessed the influence of feeding dry and lactating Holstein cows with organic Mn, Zn and Cu (with glycine- or methionine-as ligands) instead of their inorganic sources (as sulphate) on dry matter intake (DMI), dry matter digestibility (DMD), colostrum and milk performance, milk somatic cell count (SCC), blood and milk total antioxidant capacity (TAC), blood immunoglobulins A and M, and some trace minerals.

2. Materials and methods

The study was conducted at Golpayegan Agricultural Joint-Stock Company (Golpayegan, Isfahan province, Iran). The Guide for the Care and Use of Agricultural Animals in Research and Teaching (FASS, 2010) was followed in this study and all protocols were approved by the Animal Science Group of Tarbiat Modares University.

2.1. Animals, treatments and chemical analysis of diets

Forty dry Holstein cows with 772 ± 48.2 kg of body weight were assessed for a period of 60 d before predicted calving date to 100 d after calving. At the beginning of the dry period, the body weight of the animals was determined by a calibrated digital large animal scale. The animals were blocked based on their parity (second and third) and were randomly allocated to one of 4 experimental treatments (10 cows per treatment). The experimental cows were housed individually in tie stalls.

The pre- and post-partum diets (Table 1) were formulated according to the nutrient requirements of dairy cattle (NRC, 2001), except for those of Mn, Zn and Cu so that the Mn, Zn and Cu supplements were included in the trace mineral supplemented diets, not in the control diet. Accordingly, the experimental diets were 1) the control diet free of supplemental Zn, Cu and Mn, 2) a diet containing Zn, Mn and Cu sulphates, 3) a diet containing Zn, Mn and Cu as glycine salts, and 4) a diet containing Zn, Mn and Cu as methionine salts. The mineral premixes were supplied by KaniDam Co. (Tehran, Iran) (Table 2). The Cu, Mn and Zn concentrations of the control diet were lower than those of the diets containing supplemental minerals. The trace mineral-supplemented diets were balanced to provide the same levels of Cu, Mn and Zn, and the chemical forms of these minerals were the only differences among these diets. The chemical composition of the diets are shown in Table 3.

The diets, as total mixed rations, were offered freely (*ad libitum* feeding) to the cows at 07:00 and 19:00. During the pre- and post-partum periods, the representative samples of each total mixed ration (200 g) were obtained daily and dried. At the end of the experiment, the daily samples were pooled to obtain a composite per experimental diet and ground by a Wiley mill (Swedesboro, USA) equipped with a 1-mm screen. Subsequently, the organic

Table 1

Ingredients (g/kg DM) of the basal diets (free of supplementary Mn, Zn and Cu) fed to the cows before or after calving.

Item	Feeding phase ¹			
	Far-off	Close-up	Fresh	Lactation
Alfalfa	164.1	275.2	191.2	180.5
Wheat straw	305.3	–	–	6.70
Corn silage	297.7	283.3	200.7	193.5
Barley grain	–	155.7	107.2	83.1
Corn grain	126.7	122.0	173.6	163.7
Wheat bran	22.7	–	–	42.9
Sugar beet pulp	–	–	66.7	81.3
Soybean meal	71.5	49.5	124.9	94.3
Canola meal	–	59.1	21.8	–
Cottonseed meal	–	–	–	54.6
Full-fat soybean	–	8.90	44.3	31.4
Fish meal	–	9.70	28.1	17.2
Fat	–	–	13.5	11.4
NaHCO ₃	–	–	10.7	12.0
NaCl	–	1.0	1.70	5.10
CaHPO ₄	1.80	0.50	–	2.0
CaCO ₃	–	2.90	1.70	6.30
MgO	0.20	2.50	0.90	1.0
CaSO ₄	–	5.90	–	–
MgCl ₂	–	4.90	–	–
CaCl ₂	–	5.90	–	–
Vitamin premix ²	5.0	10.0	10.0	10.0
Mineral premix ³	5.0	3.0	3.0	3.0

¹ Far-off: d 60 to 22 prepartum; close-up: d 21 to 1 prepartum; fresh: d 1 to 21 postpartum; lactation: d 22 to 100 postpartum.

² Per kilogram of vitamin premix contained 3,000,000 IU of vitamin A, 700,000 IU of vitamin D₃ and 10,000 IU of vitamin E.

³ Mineral premix compositions were shown in Table 2.

Table 2

Compositions (mg/kg DM) of the mineral premixes included in diets used during different feeding phases.

Item	Feeding phase ¹			
	Far-off	Close-up	Fresh	Lactation
Co ²	15	10	15	10
I ²	120	150	200	110
Se ²	70	90	80	70
Mn ³	3,500	5,000	3,650	1,700
Zn ³	5,000	7,000	28,500	22,000
Cu ³	4,600	7,000	6,200	4,600

¹ Far-off: d 60 to 22 prepartum; close-up: d 21 to 1 prepartum; fresh: d 1 to 21 postpartum; lactation: d 22 to 100 postpartum.

² All diets contained supplementary Co, I and Se, as CoSO₄·4H₂O, KIO₃·H₂O and Na₂O₃Se, respectively.

³ The trace mineral-supplemented diets contained Mn as MnSO₄·H₂O, Mn-methionine or Mn-glycine, Zn as ZnSO₄·2H₂O, Zn-methionine or Zn-glycine, and Cu as CuSO₄·7H₂O, Cu-methionine or Cu-glycine.

matter, nitrogen, ether extract and neutral detergent fibre (NDF) were measured according to the AOAC (2002) methods (No. 924.05, 988.05, 920.3 and 2002.04, respectively). Determinations of Zn, Cu and Mn were carried out using an atomic absorption spectrophotometer (AA-6200, Shimadzu, Japan).

2.2. Feed intake and dry matter digestibility

In the pre- and post-partum periods, the feed distributed to each experimental cow and the resultant ort were weighed daily for determining the voluntary feed intake of each animal. The representative samples of the feed and orts were taken for determinations of dry matter (DM), Zn, Cu and Mn. Samples were oven dried (60 °C) to reach a constant weight, and ground to pass through a 1-mm sieve. Later, the samples were analysed for Zn, Cu

Table 3

Chemical composition (mean \pm SD) and trace minerals of the control (free of supplemental Mn, Zn and Cu) and mineral-supplemented diets fed to the cows before or after calving.

Item	Feeding phase ¹			
	Far-off	Close-up	Fresh	Lactation
Chemical composition, g/kg DM ²				
Crude protein	110 \pm 3.13	137 \pm 4.99	178 \pm 6.30	169 \pm 5.86
Organic matter	926 \pm 6.31	906 \pm 6.45	920 \pm 7.57	909 \pm 7.29
Neutral detergent fibre	475 \pm 5.00	329 \pm 5.60	285 \pm 4.28	319 \pm 6.91
Ether extract	25.6 \pm 0.30	29.0 \pm 0.83	36.5 \pm 0.61	44.8 \pm 0.98
NE _L , Mcal/kg DM	1.27 \pm 0.03	1.57 \pm 0.02	1.85 \pm 0.04	1.64 \pm 0.03
Mn, mg/kg DM				
Control diet	20.0	22.9	23.6	21.9
Mineral-sulphate diet ³	35.1	38.0	35.6	28.0
Mineral-glycine diet ³	35.6	37.7	35.5	27.5
Mineral-methionine diet ³	35.3	37.7	35.9	27.5
Zn, mg/kg DM				
Control diet	20.1	24.4	28.7	27.2
Mineral-sulphate diet ³	44.3	45.2	126	101
Mineral-glycine diet ³	44.8	45.0	122	102
Mineral-methionine diet ³	44.6	45.5	128	104
Cu, mg/kg DM				
Control diet	5.48	5.98	7.30	8.72
Mineral-sulphate diet ³	26.8	26.7	26.9	22.3
Mineral-glycine diet ³	26.7	27.0	26.4	22.7
Mineral-methionine diet ³	26.5	26.5	26.3	22.2

NE_L = net energy for lactation.

¹ Far-off: d 60 to 22 prepartum; close-up: d 21 to 1 prepartum; fresh: d 1 to 21 postpartum; lactation: d 22 to 100 postpartum.

² The chemical composition and trace minerals of the diets were determined using the standard chemical procedures, as described in the Materials and methods section.

³ The trace mineral-supplemented diets contained Mn as MnSO₄·H₂O, Mn-methionine or Mn-glycine, Zn as ZnSO₄·2H₂O, Zn-methionine or Zn-glycine, and Cu as CuSO₄·7H₂O, Cu-methionine or Cu-glycine.

and Mn as mentioned above. Finally, the daily intakes of DM and trace minerals were calculated as daily DM, Zn, Cu and Mn distributed to the cows subtracted from the corresponding orts.

Acid-insoluble ash, as an internal digestibility marker, was measured to calculate the in vivo DMD of the diets (Mc Geough et al., 2010). Spot samples of faeces (100 g) were obtained, for 5 d, from each animal in the final week of the pre- and post-partum periods. Spot sampling was done 3 h pre-feeding and 3 h post-feeding (i.e., 4 times during a 24 h period). The samples of faeces, diet distributed and orts from each cow on each experimental group were dried in an oven, set at 60 °C, and ground (1 mm). The same DM weights from faecal samples were pooled to obtain a composite for every animal and DMD was estimated after DM determination.

2.3. Colostrum and milk performance

Colostrum yield per individual cow was determined as the sum of the amounts obtained from the first and second milkings (d 1 of lactation) and colostrum samples were taken. Moreover, individual daily milk yields of the animals were recorded at all milkings (06:00, 14:00 and 22:00) and milk samples of each cow were taken, once a week, up to d 100 after calving.

A portion of the composite milk sample, per cow for every sampling day, and colostrum samples were analysed for solids non-fat, protein, lactose and fat (Milko Scan 133B; Foss Electric, Denmark). Milk SCC was determined using a Fossomatic apparatus (Foss Electric, Denmark). Another portion of milk was frozen (–20 °C) until analysis for TAC (as below). Feed efficiency was calculated by dividing milk yield by DMI.

2.4. Blood immunoglobulins and trace minerals

The blood samples (10 mL) of all the cows were collected via the jugular vein into the evacuated tubes (MediPlus, Sunphoria Co., Ltd,

China), without any anticoagulant, on d 23 and 6 before the calving date, and d 1, 21 and 50 after calving, 3 h after morning feeding. Moreover, blood samples of all calves were collected on d 3 after the birth. After centrifugation (1,500 \times g; 15 min) of the blood samples, the obtained serum was stored at –20 °C.

The immunoglobulin A (IgA) and immunoglobulin M (IgM) ELISA Kits (supplied by PT Co., Tehran, Iran) were used for spectrophotometrically determinations of the IgA and IgM concentrations at a wavelength of 450 nm. The spectrophotometric assays were used to measure the blood serum Zn and Cu using analytical kits of Biorexfars Co. (Shiraz, Iran). The Mn concentration was determined by an atomic absorption spectrometer (AA-6200, Shimadzu, Japan).

2.5. Total antioxidant capacity of blood and milk

The TAC of the milk and blood was evaluated by assay of the ferric reducing antioxidant power (FRAP) using ferrous sulphate solution as the standard. The technique is based on reduction of Fe³⁺-tripridyltriazine complex to Fe²⁺ form in the presence of antioxidants, and development of an intense blue colour detecting at 593 nm. The obtained results were expressed as μ mol Fe²⁺ formed per litre of the sample (μ mol Fe²⁺/L) (Benzie and Strain, 1996).

2.6. Statistical analyses of obtained data

Data were analysed by the PROC MIXED procedure of SAS 9.1 (Cary, NC, USA). Data on colostrum yield, blood parameters, DMD and antioxidant capacity were analysed in a randomized complete block (parity) design according to the following model:

$$Y_{ijkl} = \mu + T_i + R_j + e_{ij} + e_{ijkl},$$

where Y_{ijkl} = observation, μ = general mean, T_i = treatment effect, R_j = block effect, e_{ij} = experimental error (block \times treatment) and

e_{ijkl} = sampling error (cow \times block \times treatment \times sample). The effect of treatment was considered fixed. The effect of calving date on the investigated traits was not significant between groups; hence, the covariance effect of calving date was excluded from the statistical model. Obtained data on DMI and milk performance were analysed using repeated measurements, considering the fixed effects of treatment, either sampling day or sampling week, and their interactions. Least squares means were separated using the probability of difference (PDIF) option, which tests significance between all pairs of means (SAS User's Guide). The statistical significance of the means was defined by $P \leq 0.05$ and a trend was declared if $0.05 < P \leq 0.10$.

3. Results

3.1. Dry matter digestibility and feed intake

Dietary supplementation of both organic and inorganic Mn, Cu, and Zn increased ($P < 0.05$) the DMD of diets in the close-up transition and post-partum periods as compared to the control group (Table 4). Moreover, the pre- and post-partum DMI and the trace mineral intakes were higher ($P < 0.05$) for the cows receiving the supplemental Mn, Cu and Zn sources than those for the control animals except DMI in the close-up transition period. However, there were no significant differences ($P > 0.05$) among the minerals-supplemented cows in terms of DMD, DMI and trace mineral intakes.

3.2. Colostrum and milk performance

Use of the supplementary Mn, Zn and Cu had no effect ($P > 0.05$) on the colostrum and milk components (Table 5). Colostrum yield of the cows increased ($P < 0.05$) by the inclusion of Mn, Zn and Cu glycine in the diet. Milk yield of the fresh cows (d 1 to 21 postpartum) receiving Mn, Zn and Cu as sulphate or glycine salts was higher ($P < 0.05$) than that of the control animals. Feeding the fresh cows

with Mn, Zn and Cu methionine led to a lower ($P < 0.05$) milk SCC compared to the control group. During d 22 to 100 of lactation, milk yield of the cows increased ($P < 0.05$) and milk SCC decreased ($P < 0.05$) by the inclusion of Mn, Zn and Cu supplements in the diet.

3.3. Antioxidant capacity, IgM and IgA

According to Table 6, on d 1 postpartum, the blood TAC in the cows supplemented with Mn, Zn and Cu as methionine salts was higher than that in the control group ($P < 0.05$) and the cows receiving the supplementary minerals with sulphate and glycine as ligands were intermediate ($P > 0.05$). On d 21 and 50 postpartum, the minerals-sulphate and minerals-glycine treatments had higher ($P < 0.05$) blood TAC compared to the control group. Milk TAC in the cows receiving the inorganic trace minerals was higher ($P < 0.05$) than that in the control animals. Total antioxidant capacity in the milk of the organic trace mineral-supplemented cows was slightly higher ($P > 0.05$) than that in the control group. Blood TAC in new-born calves was improved ($P < 0.05$) by the dietary inclusion of Mn, Zn and Cu supplements compared to the control diet.

The blood IgA concentration in the cows receiving Cu, Mn and Zn as glycine and methionine salts was higher ($P < 0.05$) compared to the control group. The cows treated with the inorganic and organic sources of trace minerals had higher ($P < 0.05$) blood concentration of IgM than those in the control animals, the Mn, Zn and Cu sulphate supplemented animals were intermediate, and the control had the lowest concentrations of IgM and IgA. Feeding the mother cows with the organic sources of the trace minerals increased ($P < 0.05$) the concentrations of IgA and IgM in the blood of their new-born calves.

3.4. Blood Mn, Zn and Cu

Dietary treatments had no effect ($P > 0.05$) on the blood concentration of Mn in the pre-partum period (Table 7). On d 1

Table 4
Influence of different supplemental sources of Mn, Zn and Cu on dry matter digestibility (DMD) and dry matter intake (DMI) of the cows.

Item ¹	Diets				SEM	P-value
	Control	Mineral-sulphate	Mineral-glycine	Mineral-methionine		
DMD, g/kg						
Far-off	650	677	662	670	10.1	0.31
Close-up	654 ^b	678 ^a	680 ^a	689 ^a	7.32	0.042
Fresh	661 ^b	688 ^a	686 ^a	694 ^a	6.14	0.032
Lactation	683 ^b	722 ^a	705 ^a	727 ^a	9.21	0.041
DMI, kg/d						
Far-off	12.5 ^b	14.4 ^a	15.1 ^a	14.8 ^a	0.27	0.022
Close-up	9.71 ^b	10.6 ^a	10.3 ^{ab}	10.9 ^a	0.31	0.049
Fresh	22.7 ^b	25.0 ^a	24.7 ^a	25.2 ^a	0.46	0.037
Lactation	24.3 ^b	26.1 ^a	26.0 ^a	26.1 ^a	0.34	0.038
Mn intake, mg/d						
Far-off	231 ^b	451 ^a	462 ^a	450 ^a	37.1	0.019
Close-up	196 ^b	356 ^a	364 ^a	362 ^a	40.5	0.023
Fresh	471 ^b	802 ^a	791 ^a	816 ^a	51.9	0.014
Lactation	476 ^b	640 ^a	627 ^a	646 ^a	42.8	0.030
Zn intake, mg/d						
Far-off	230 ^b	566 ^a	589 ^a	581 ^a	61.4	0.017
Close-up	207 ^b	419 ^a	412 ^a	433 ^a	58.4	0.026
Fresh	576 ^b	2,845 ^a	2,658 ^a	2,701 ^a	92.3	0.004
Lactation	582 ^b	2,336 ^a	2,288 ^a	2,391 ^a	90.0	0.005
Cu intake, mg/d						
Far-off	69.5 ^b	340 ^a	347 ^a	340 ^a	63.0	0.025
Close-up	54.2 ^b	253 ^a	242 ^a	264 ^a	44.5	0.022
Fresh	248 ^b	575 ^a	570 ^a	561 ^a	48.8	0.017
Lactation	184 ^b	502 ^a	521 ^a	505 ^a	50.2	0.016

SEM = standard error of the means.

^{a, b} Within a row, means without a common uppercase superscript differ ($P < 0.05$).

¹ Far-off: d 60 to 22 prepartum; close-up: d 21 to 1 prepartum; fresh: d 1 to 21 postpartum; lactation: d 22 to 100 postpartum.

Table 5
Influence of different supplemental sources of Mn, Zn and Cu on colostrum and milk performance of the cows.

Item	Diets				SEM	P-value
	Control	Mineral-sulphate	Mineral-glycine	Mineral-methionine		
Colostrum						
Yield, kg/first 2 milkings	9.15 ^b	9.8 ^{ab}	10.9 ^a	10.0 ^{ab}	0.55	0.041
Protein, %	12.4	12.8	13.1	12.4	0.23	0.22
Fat, %	4.52	4.55	4.78	4.24	0.19	0.27
Lactose, %	3.23	3.11	3.28	3.08	0.07	0.26
Solids non-fat, %	21.0	20.7	21.4	20.4	0.37	0.32
Milk (d 1 to 21)						
Yield, kg/d	41.6 ^b	44.1 ^a	44.0 ^a	43.7 ^{ab}	0.75	0.039
Protein, %	3.45	3.44	3.44	3.47	0.12	0.94
Fat, %	3.68	3.82	3.89	3.78	0.11	0.51
Lactose, %	4.69	4.70	4.68	4.65	0.23	0.95
Solids non-fat, %	8.80	8.85	8.86	8.75	0.17	0.84
Somatic cells, × 10 ³ /mL	85.7 ^a	83.5 ^{ab}	83.7 ^{ab}	82.0 ^b	1.23	0.043
Milk/DMI	1.83	1.76	1.78	1.73	0.31	0.93
Milk (d 22 to 100)						
Yield, kg/d	43.8 ^b	46.3 ^a	46.1 ^a	46.5 ^a	0.61	0.025
Protein, %	3.22	3.14	3.19	2.99	0.09	0.36
Fat, %	3.66	3.60	3.45	3.61	0.18	0.70
Lactose, %	4.75	4.80	4.80	4.85	0.11	0.75
Solids non-fat, %	8.39	8.46	8.50	8.42	0.26	0.89
Somatic cells, × 10 ³ /mL	64.5 ^a	60.0 ^b	60.1 ^b	59.8 ^b	1.14	0.037
Milk/DMI	1.80	1.77	1.77	1.78	0.27	0.95

SEM = standard error of the means; DMI = dry matter intake.

^{a, b}Within a row, means without a common uppercase superscript differ ($P < 0.05$).

Table 6
Influence of different supplemental sources of Mn, Zn and Cu on total antioxidant capacity (TAC), immunoglobulin A (IgA) and immunoglobulin M (IgM) in the postpartum cows and their new-born calves.

Item	Diets				SEM	P-value
	Control	Mineral-sulphate	Mineral-glycine	Mineral-methionine		
TAC, μmol Fe²⁺/L						
Cow blood, d 1 postpartum	1,523 ^b	1,603 ^{ab}	1,788 ^{ab}	1,912 ^a	88.5	0.045
Cow blood, d 21 postpartum	1,919 ^b	2,258 ^a	2,270 ^a	2,099 ^{ab}	83.1	0.046
Cow blood, d 50 postpartum	1,606 ^c	2,102 ^a	1,984 ^a	1,794 ^{bc}	86.6	0.032
Milk	1,362 ^b	1,722 ^a	1,504 ^{ab}	1,531 ^{ab}	72.0	0.037
Calf, d 3 of age	1,574 ^b	1,820 ^a	1,986 ^a	1,825 ^a	80.9	0.040
IgA, g/L						
Cow blood, d 1 postpartum	0.745 ^b	0.857 ^{ab}	0.997 ^a	1.13 ^a	0.071	0.036
Cow blood, d 21 postpartum	0.807 ^b	0.841 ^b	1.23 ^a	1.17 ^a	0.073	0.040
Calf, d 3 of age	0.640 ^b	0.763 ^{ab}	0.862 ^a	0.891 ^a	0.061	0.043
IgM, g/L						
Cow blood, d 1 postpartum	1.95 ^c	2.17 ^b	2.71 ^a	2.49 ^a	0.093	0.035
Cow blood, d 21 postpartum	2.18 ^c	2.36 ^b	2.94 ^a	3.12 ^a	0.108	0.028
Calf, d 3 of age	1.87 ^c	2.12 ^{bc}	2.52 ^a	2.31 ^{ab}	0.089	0.031

SEM = standard error of the means.

^{a, b, c}Within a row, means without a common uppercase superscript differ ($P < 0.05$).

postpartum, the blood Mn concentration of the cows increased ($P < 0.05$) by the dietary inclusion of Mn, Zn and Cu as methionine salts. On d 21 of the lactation, the minerals-sulphate and minerals-glycine treatments had higher ($P < 0.05$) blood Mn compared to the control group. The blood Mn concentration of the 50 d postpartum cows receiving Mn, Zn and Cu sulphates was higher ($P < 0.05$) compared to the animals receiving the control diet. The blood Zn concentration on d 23 before calving ($P = 0.078$) and d 1 of lactation ($P = 0.064$) tended to increase. On d 50 of lactation, the cows treated with the sulphate and methionine sources of the trace minerals had the higher ($P < 0.05$) blood Zn concentration compared to the control group. The blood Cu concentration of the cows was not affected ($P > 0.05$) by the dietary treatments. Feeding the mother cows with the inorganic or organic sources of the trace minerals failed to change ($P > 0.05$) the Mn, Cu and Zn concentrations in the blood of their new-born calves.

4. Discussion

4.1. Dry matter digestibility and feed intake

The increased DMD, in the close-up transition and postpartum periods, by the dietary supplementation of Mn, Zn and Cu as glycine, methionine or sulphate salts could be due to the positive effects of such trace minerals (divalent cations) on the growth and function of the rumen microorganisms and enzymatic digestion of nutrients (Lopez-Guisa and Satter, 1992; NRC, 2001; Wu, 2018) and rumen fermentation (Zhang et al., 2007; Gaafar et al., 2011) compared to the mineral-deficient diets. However, no differences in the diet DMD among the minerals-supplemented cows (as sulphate, glycine or methionine salts) indicated that the Mn, Zn and Cu requirements of the animals and rumen microbes for enzymatic and microbial digestion of feedstuffs were sufficiently supplied by sulphate salts of the minerals. In the other study, the organic Mn, Zn

Table 7
Influence of different supplemental sources of Mn, Zn and Cu on the blood trace minerals in the cows and their new-born calves.

Item	Diets				SEM	P-value
	Control	Mineral-sulphate	Mineral-glycine	Mineral-methionine		
Mn, ng/dL						
Cow blood, d 23 prepartum	22.7	23.1	25.5	24.7	1.32	0.35
Cow blood, d 6 prepartum	20.4	19.8	21.8	25.3	1.57	0.11
Cow blood, d 1 postpartum	19.8 ^b	22.5 ^{ab}	22.9 ^{ab}	28.0 ^a	0.99	0.024
Cow blood, d 21 postpartum	13.6 ^b	16.3 ^a	16.4 ^a	14.7 ^{ab}	0.54	0.026
Cow blood, d 50 postpartum	13.0 ^c	17.9 ^a	16.9 ^{ab}	14.8 ^{bc}	0.68	0.023
Calf blood, d 3 of age	32.1	30.9	35.5	35.7	1.65	0.18
Zn, µg/dL						
Cow blood, d 23 prepartum	86.5	96.6	105	101	7.12	0.078
Cow blood, d 6 prepartum	93.9	102	98.0	100	7.72	0.73
Cow blood, d 1 postpartum	83.1	100	107	97.4	7.48	0.064
Cow blood, d 21 postpartum	89.8	108	108	105	5.62	0.17
Cow blood, d 50 postpartum	91.1 ^b	112 ^a	105 ^{ab}	110 ^a	5.21	0.047
Calf blood, d 3 of age	92.3	106	106	103	5.70	0.38
Cu, µg/dL						
Cow blood, d 23 prepartum	83.5	89.1	92.8	90.3	5.89	0.59
Cow blood, d 6 prepartum	84.4	94.5	96.9	92.5	6.50	0.48
Cow blood, d 1 postpartum	86.7	99.9	98.1	96.6	5.48	0.37
Cow blood, d 21 postpartum	91.4	103	108	106	6.63	0.35
Cow blood, d 50 postpartum	92.1	102	104	101	6.51	0.53
Calf blood, d 3 of age	72.0	78.3	75.2	79.9	5.96	0.72

SEM = standard error of the means.

^a, ^b, ^cWithin a row, means without a common uppercase superscript differ ($P < 0.05$).

and Cu supplementation instead of inorganic salt had no effects on the diet DMD in dairy heifers (Pino and Heinrichs, 2016). Moreover, Faulkner and Weiss (2017) observed that DMD in dairy cows was not influenced by feeding the organic source of Mn, Zn and Cu instead of the minerals in a sulphate form. However, they found that the cows receiving the organic minerals had greater NDF digestibility compared to those fed diets with sulphate sources.

The improving effect of the organic Mn, Zn and Cu sources, similar to ZnSO₄, on DMI of the pre- and post-partum cows was partly related to the higher diet DMD in the mineral supplemented animals, because of the positive relationship between diet digestion and feed consumption (NRC, 2001; Wu, 2018). Another reason could be attributed to the encouraging influence of the trace minerals on the appetite in animals (Wu, 2018). The greater Mn, Zn and Cu intakes were in line with the higher DMI in the cows receiving the supplemental trace minerals. On the other hand, the similar DMI of the mineral supplemented animals was in parallel with the same DMD among them. This result was comparable with the study conducted by Cope et al. (2009), who proposed that the chemical form (organic vs. inorganic salts) of dietary trace minerals has little effect on DMI. Other researchers (Hackbart et al., 2010; Bach et al., 2015) also reported no difference in DMI of cows when an inorganic source of Mn, Zn and Cu was replaced by the organic sources. In the other study, Nemeč et al. (2012) found that the replacement of Mn, Zn and Cu sulphates by the minerals in an organic form decreased DMI in cows.

4.2. Colostrum and milk performance

In the current study, the positive effect of the Cu, Zn and Mn-glycine on colostrum yield and the positive effect of the inorganic or organic sources of the trace minerals on milk yields of the fresh and lactating cows, compared to the control group, could be due to the higher DMI and DMD (NRC, 2001; Wu, 2018) and the lower SCC as an index of udder health (Cope et al., 2009) in the cows receiving the supplemental trace minerals. The higher colostrum and milk yields could also be attributed to the role of the trace minerals in structural proteins, enzymes and hormones necessary for biochemical and physiological functions (Suttle, 2010). However, no

positive influence of replacing Mn, Zn and Cu sulphates by the organic forms on the performance of the present animals may be due to providing sufficient levels of Mn, Zn and Cu for all mineral-supplemented cows. As suggested by Rojas et al. (1996), at a sufficient level of a dietary mineral, the chemical form and bioavailability of supplementary mineral sources are less important compared with under circumstances of limited dietary mineral or if great concentrations of supplementary mineral are fed.

The decreasing effect of Mn, Zn and Cu methionine on the milk SCC of the fresh cows as well as the decreasing effect of the inorganic or organic sources of the trace minerals on the milk SCC in the lactating cows were in accordance with the higher TAC of the blood and milk in the minerals-supplemented groups, as a valuable guide of the udder susceptibility to bacterial infections and oxidative damage (Andrei et al., 2016). Moreover, it has been noted that a higher available Zn could improve teat and mammary epithelial health, or both (Nayeri et al., 2014).

Unchanged milk protein and fat percentages with feeding cows with the organic trace minerals instead of the minerals in inorganic form were agree with the results reported by Nemeč et al. (2012), Bach et al. (2015) and Faulkner and Weiss (2017).

In another studies on dairy cows (Formigoni et al., 2011; Nemeč et al., 2012), no influence of substituting organic sources of trace minerals for inorganic salts on milk yield and SCC was reported. Bach et al. (2015), Zhao et al. (2015) and Faulkner and Weiss (2017), also, reported no significant alteration in the milk produced by dairy cows when supplementary Mn, Zn and Cu were provided as inorganic or organic salts (with methionine hydroxy-analogue as a ligand). In another study, replacing Mn, Cu and Zn sulphates with the organic salts, containing methionine as a ligand, improved the milk produced by high-performance dairy cows (El Ashry et al., 2012). Cortinhas et al. (2010) detected a decreased milk SCC of dairy cows by feeding organic Mn, Zn and Se instead of inorganic sources. Also, Kellogg et al. (2004) summarized the data from 12 trials, and suggested that the feeding Zn-methionine to dairy animals could be a suitable strategy to increase milk yield and to decrease milk SCC (i.e., subclinical mastitis).

Conflicted findings on the impact of different sources of micro-minerals on the animal performance in different experiments could

have been attributed to the previous mineral storages of the body, animal performance, physiological stage, nutritional conditions, the type of supplementation, stressors and environment, affecting the trace minerals absorption and metabolism (Suttle, 2010; Aditia et al., 2014; Alimohamady et al., 2019).

4.3. Antioxidant capacity, immunoglobulin M and immunoglobulin A

The higher TAC in the blood and milk of the cows receiving the different supplemental trace minerals was due to inhibitory effects of Mn, Zn and Cu on oxidative stress and declining the levels of free radicals in the body (NRC, 2001; Suttle, 2010). It is informed that Cu, Zn and Mn affect the livestock immunity via maintaining the integrity of membranes against infections and antioxidant pathways (Shankar and Prasad, 1998; McClure, 2008; Spears and Weiss, 2008). In the present work, the improved milk TAC indicates that the supplementation of diets with Mn, Zn and Cu may be a useful way to increase the antioxidant capacity and health quality of the milk in cows. Compared to the control group, the higher blood TAC of the new-born calves in the inorganic and organic trace minerals-supplemented treatments was in line with the greater TAC of their mothers.

However, the similar TAC in the blood and milk of the Mn, Zn and Cu-supplemented cows indicated that providing the minerals as sulphates had the equal potential as the organic sources in terms of the antioxidant defence. In the other study, Osorio et al. (2016) reported that dietary replacing of inorganic Cu, Zn and Mn with the trace minerals from amino acid complexes improved blood TAC in post-partum cows, but not during the pre-partum period. The improved antioxidant status was also detected in cows supplemented with Cu, Zn and Mn as metal methionine hydroxyl analogue instead of sulphate forms (Zhao et al., 2015).

Immunoglobulin A and IgM have important roles in protective functions and they can reflect the immune function of the body (Boes, 2000; Singh et al., 2014; Zhao et al., 2015). Compared to the control group, the greater blood IgM concentration in the inorganic and organic Mn, Zn and Cu-supplemented cows as well as the higher blood IgA concentration in the organic trace minerals-fed animals and their calves suggested that these minerals probably have important roles in the production of immunoglobulins and supplementing cows with these minerals may improve passive transfer of immunoglobulins in new-born calves, as it has been suggested by Nayeri et al. (2014) for Zn. Moreover, in confirmation of the result reported by Zhao et al. (2015), the higher immunoglobulins in the organic minerals-supplemented cows than that in the control group indicated that organic trace minerals may strengthen immunity system in the animals. Similarly, the positive effect of using the supplemental organic Zn, Mn and Cu instead of the sulphate form on total immunoglobulins in the blood of dairy cows and new-born calves was reported by Kinal et al. (2005). Moreover, Nagalakshmi et al. (2016) reported a higher humoral immune response in buffalo heifers by replacement of ZnSO₄ with Zn-proteinate. Also, Mandal et al. (2007) observed a positive influence of Zn-propionate on cell mediated and humoral immune response in growing bulls, due to its different function versus Zn from ZnSO₄ after absorption.

4.4. Blood Mn, Zn and Cu

The concentrations of blood biochemical parameters are indicators of the nutritional status and the sufficiency of nutrients supply for the body (Ndlovu et al., 2007). In the present study, the overall positive effects of the inorganic and organic mineral supplementations on the blood Mn and Zn, compared with the control group, could be attributed to the more absorption of these minerals

from the gastrointestinal tract into the blood compared with the diet free of the supplemental minerals (Suttle, 2010). However, no effect of the replacing Mn, Zn and Cu sulphates with the organic forms on the blood minerals could be due to the homeostatic regulatory mechanisms (Aliarabi et al., 2015) stimulating a decline in the mineral absorption efficiency (Brugger and Windisch, 2017). In other word, supplying Mn, Zn and Cu as sulphate salts was enough to maintain the normal concentrations of these minerals in the cows' blood.

In another work, replacing Mn, Zn and Cu sulphates with the organic forms as Bioplexes (produced by Rolimpex, Wroclaw, Poland) increased the blood Cu concentration of cows in the first month of lactation, but there was no effect of the organic minerals on the blood Zn in the 1st, 2nd and 3rd months of lactation and blood Cu in the 2nd and 3rd months of lactation (Kinal et al., 2007). In the other studies, Nemeč et al. (2012) and Faulkner et al. (2017) found that the replacement of Mn, Zn and Cu sulphates by the organic form (metal methionine hydroxy analogue) in the diet of dairy cows had no effect on the plasma Mn, Cu and Zn. Moreover, similar plasma mineral status of beef cattle was observed by feeding different sources (organic vs. inorganic) of Mn, Cu and Zn (Ahola et al., 2004).

5. Conclusion

Supplementation of the basal diets of pre- and post-partum Holstein cows with Mn, Zn and Cu as methionine, glycine or sulphate salts can improve digestion, DMI, colostrum and milk performance, milk SCC, blood and milk TAC, and blood Zn and Mn. Moreover, practically it may be possible to increase the blood TAC, IgA and IgM of the new-born calves by supplementing their mothers with different sources of Mn, Zn and Cu. On the other hand, the organic sources of Mn, Zn and Cu have advantage over the sulphate forms in terms of the blood immunoglobulins.

Conflict of interest

We declare that we have no financial and personal relationships with other people or organizations that can inappropriately influence our work, there is no professional or other personal interest of any nature or kind in any product, service and/or company that could be construed as influencing the content of this paper.

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