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DIETARY ORGANIC TRACE MINERALS LEVEL INFLUENCES EGGSHELL QUALITY AND MINERALS RETENTION IN HENS*

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Abstract

The objective of this study was to investigate the effect of reduced levels of Cu, Zn, Mn in combination from organic mineral source on eggshell quality and mineral retention in hens. After feeding the basal diet (8.82 mg/kg Cu, 24.94 mg/kg Zn, and 16.38 mg/kg Mn) without Cu, Zn, and Mn addition for 4 weeks, hens (39-week-old) were assigned to 5 treatments according to the equal body weight and egg production for 12-week experimental trial. The 5 treatments included the basal diet without Cu, Zn, and Mn (NCON), and NCON added with 16-80-60 mg/kg Cu-Zn-Mn from sulfates (ITM100%), or 4-20-15, 8-40-30 or 16-80-60 mg/kg Cu-Zn-Mn from 2-hydroxy-4-(methylthio)butanoic acid (HMTBA) mineral chelates (OTM25%, OTM50% or OTM100%). Supplementation of Cu, Zn, and Mn had no significant influences on the performance of hens. After 12 weeks feeding, eggshell breaking strength (EBS) decreased in the following order: OTM25% and ITM100% > OTM50% > NCON and OTM100%. The eggshell weight and thickness in OTM25% were greater than that in NCON, while not differing from that in ITM100%. The EBS and eggshell weight linearly decreased with increasing level of OTM. After 12 weeks feeding, supplementation of Cu, Zn, and Mn increased the concentrations of liver Zn, tibia Zn and Mn, and Zn and Mn retention in eggs as compared with NCON. No significant difference was observed in the concentrations of Cu, Zn, and Mn in liver and plasma, and Zn and Mn in eggs between any OTM treatment and ITM100% groups. Addition of OTM at increasing level had quadratic effect on tibia Cu, Zn, Mn concentrations, with the greater retention of Cu, Zn, and Mn in OTM50%. In conclusion, the OTM25% from HMTBA mineral chelates can substitute for ITM100% evaluated by the eggshell quality in the diet of laying hens.

Key words: copper, manganese, zinc, 2-hydroxy-4-(methylthio)butanoic acid mineral chelates, eggs

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Approximately eight percentages of all losses in egg production are direct result of poor eggshell quality (Klecher et al., 2002). A great deal of effort has been applied to improving eggshell quality in the fields of genetics, management, and nutrition, especially mineral nutrition (Nys, 2001). Trace minerals can influence eggshell quality either by their catalytic properties as key enzymes involved in the process of membrane and eggshell formation or by interacting directly with the calcite crystals in the forming eggshell (Rucker et al., 1998; Akagawa et al., 1999; Nys et al., 1999). The enzyme lysyl oxidase, a cuproenzyme, is involved in conversion of lysine to cross-linked desmosine and isodesmosine, two components of eggshell membrane (Baumgartner et al., 1978; Rucker et al., 1998; Akagawa et al., 1999). Zinc (Zn), as a component of carbonic anhydrase, is involved in the supply of carbonate ions during eggshell formation (Nys et al., 1999). Manganese (Mn) can activate the glycosyl transferases involved in the formation of mucopolysaccharides, which are components of proteoglycans (Leach, 1976). The above trace mineral deficient diet can lead to a decrease in egg production and eggshell quality (Kienholz et al., 1961; Xie et al., 2014; Xiao et al., 2015). However, no literature was found about the minimum amount of Cu, Zn, and Mn in combination without negative effect on the eggshell quality in the diet for hens.

In practice, 2 and 10 times more trace minerals than NRC recommended levels (NRC, 1994) were used from inorganic trace minerals (ITM), in order to avoid trace mineral deficiencies by the presence of antagonists in the poultry diet, or maximize performance of animals (Esenbuga et al., 2008). However, increased ITM levels can interfere with the bioavailability of other nutrients (Suttle, 2010), and their low retention rates resulted in very high levels of minerals in excreta. Many studies indicated that organic minerals (OTM) have higher bioavailability than traditionally inorganic forms (oxide and sulfate) for broiler (Henry et al., 1989; Wedekind et al., 1992; Li et al., 2005) and laying hens (Świątkiewicz and Koreleski, 2008; Sun et al., 2012). Several studies also showed that organic sources of Cu, Zn and Mn improved eggshell quality as compared with ITM (Klecher et al., 2002; Stefanello et al., 2014; Yenice et al., 2015). However, several reports showed the inconsistent results (Lim and Paik, 2003; Mabe et al., 2003). The discrepancy in the relative bioavailability of OTM was partly due to differences in the chelation strength or ligands of organic minerals used in these studies (House et al., 1997; Li et al., 2004).

The 2-hydroxy-4-(methylthio)butanoic acid (HMTBA) mineral chelates are atoms of Cu, Zn, or Mn, each chelated by two molecules of DL-HMTBA (Richards et al., 2007). Previous studies in broilers showed that HMTBA-Zn (Yi et al., 2007), HMTBA-Mn (Yan and Waldroup, 2006) and HMTBA-Cu (Wang et al., 2007) had greater bioavailability than sulfate minerals evaluated by minerals retention in tissues. Sun et al. (2012) found that the HMTBA mineral chelate had higher bioavailability than sulfate forms evaluated by the performance of laying hens. However, no literature was found about the bioavailability of HMTBA mineral chelate evaluated by eggshell quality in hens. In this study, we hypothesized that the low level of Cu-Zn-Mn from HMTBA mineral chelates replacing relatively higher level of Cu-Zn-Mn from sulfate minerals had no negative effect on eggshell quality and minerals retention in tissues, because of their potential higher bioavailability than sulfate minerals in hens.

Material and methods

Birds husbandry and experimental diets

The animal experiment was conducted in accordance with the guidelines approved by Animal Health and Care Committee of Sichuan Agricultural University. All the Lohmann pink-shell laying hens were raised in the 4-tier ladder type cages at Animal Nutrition Research Center in a commercial farm (Mianyang, Sichuan, China). Prior to the initiation of the experiment, the hens were selected according to equal body weight, and then the hen-day egg production was determined for each cage (5 birds per cage; 45 × 60 × 43 cm). After that, a total of 750 39-week-old hens were allocated to 5 treatments (Table 1) according to the equal egg productions, with 10 replicates of 15 hens each (3 adjacent cages) in a randomized complete block design according the location of cages. The ambient temperature was kept at 25°C with 16L:8D light program during experimental period. Feed and water were provided *ad libitum*.

Table 1. Dietary level of added trace minerals from organic or inorganic sources

Items	NCON[1]	ITM[2]	OTM[3]25%	OTM50%	OTM100%
Cu (mg/kg)	0	16	4	8	16
Zn (mg/kg)	0	80	20	40	80
Mn (mg/kg)	0	60	15	30	60

[1]NCON = no trace minerals addition; the basal diet contained 8.82 mg/kg Cu, 24.94 mg/kg Zn, and 16.38 mg/kg Mn.

[2]ITM = NCON added with 16 mg/kg Cu ($CuSO_4{\cdot}5H_2O$), 80 mg/kg Zn ($ZnSO_4{\cdot}7H_2O$), and 60 mg/kg Mn ($MnSO_4{\cdot}H_2O$).

[3]OTM = NCON added with Cu, Zn, and Mn from Zn-bis-(2-hydroxy-4-methylthiobutyrate) (Zn-MHA), Cu-MHA, and Mn-MHA respectively.

Prior to the experiment, all hens were fed the basal diet (containing 8.82, 24.94, and 16.38 mg Cu, Zn, and Mn per kg) without Cu-Zn-Mn addition for 4 weeks (from 35 to 38 week-old) in order to decrease body minerals storage. And then, the hens were fed the different experimental diet for 12 weeks. The 5 treatment diets included the basal diet (Table 2) without Cu, Zn, and Mn addition (NCON), and NCON added with 16-80-60 mg/kg Cu-Zn-Mn from sulfates (ITM100%), or 4-20-15, 8-40-30 or 16-80-60 mg/kg Cu-Zn-Mn from HMTBA mineral chelates (OTM25%, OTM50% or OTM100%). The HMTBA mineral chelates are atoms of Cu, Zn, or Mn, each chelated by two molecules of DL-HMTBA (Richards et al., 2007). The HMTBA-Cu, HMTBA-Zn, and HMTBA-Mn (Novus, USA) contained 15.05% Cu and 78.00% HMTBA, 16.00% Zn and 80.00% HMTBA, and 13.08% Mn and 75.00% HMTBA, respectively. In order to keep all experimental diets containing equal levels of HMTBA (Novus, USA), variable amounts of HMTBA and cornstarch were added to respective experimental diet according to the amount of HMTBA from supplemental HMTBA mineral chelates.

Table 2. Composition and nutrients level of the basal diet

Items	Composition (%)
Ingredients	
ground yellow corn	61.28
soybean meal	14.24
rapeseed meal	3.20
cottonseed meal	4.00
corn gluten meal	4.20
corn germ meal	1.00
soybean oil	1.70
ground limestone	8.10
dicalcium phosphate	1.30
L-Lysine sulfate (65%)	0.20
sodium chloride	0.40
choline chloride (50%)	0.10
vitamin premix[1]	0.03
minerals premix[2]	0.25
Calculated nutrient concentrations	
AME (MJ/kg)	11.54
crude protein	16.50
calcium	35.60
nonphytate phosphorus	3.40
lysine	7.60
methionine	3.70
methionine and cystine	6.70
threonine	6.20
tryptophan	1.80
Analysed mineral concentrations	
zinc (mg/kg)	24.94
copper (mg/kg)	8.82
manganese (mg/kg)	16.38

[1]Provided per kg of diet: trans-retinyl acetate, 2.4 mg; cholecalciferol, 0.04 mg; DL-α-tocopheryl acetate, 10 mg; menadione 1.25 mg; thiamine 0.5 mg; riboflavin, 4.0 mg; D-pantothenic acid, 6.25 mg; niacin, 8.75 mg; pyridoxine, 1.5 mg; biotin, 0.0125 mg; folic acid, 0.125 mg; cyanocobalamin, 0.008 mg.

[2]Provided per kg of diet: iron ($FeSO_4 \cdot H_2O$), 60.00 mg; iodine ($Ca(IO_3)_2$), 0.35 mg; selenium (Na_2SeO_3), 0.30 mg. For the minerals supplementation diet, the test mineral supplement contained variable amounts of cornstarch, 2-hydroxy-4-(methylthio)butanoic acid and mineral sources.

Data collection

Total eggs, cracked and broken eggs, and eggs weight were recorded daily per replicate. Feed consumption was recorded weekly during the experimental period. Hen-day egg production, feed conversion ratio (feed:egg), and egg weight were calculated every 4 weeks. At week 4, 8 and 12 of the trial, 2 eggs per replicate were collected randomly to measure eggshell quality, including eggshell breaking strength (EBS), eggshell weight, eggshell thickness, and the percentage of eggshell. Another 2 eggs of each replicate were mixed to 1 sample for analyzing Cu, Zn, and

Mn concentrations. The EBS (kg/cm^2) was measured using eggshell intensity meter (Mitutoyo digital caliper, Tokyo, Japan). The eggshell weight was determined by washing the interior egg membrane and after drying overnight at 80°C. The eggshell thickness (mm) was determined measuring the thickness mean values taken at three locations on egg (air cell, equator, and sharp end) by using micrometer screw. The eggshell percentage was calculated as following: (eggshell weight/egg weight) ×100.

Sample collections

The 10 ml blood was collected using a heparin sodium coated tube from one bird per replicate with the body weight close to the plot mean after a 12-h fast after 12 weeks of the trial. And then, the hen was killed by cervical dislocation, and the right tibia and the right lobe of liver were collected for Cu, Zn, and Mn concentrations measurement. The collected blood was centrifuged (3000 g for 10 min) to obtain plasma sample for mineral concentrations analysis.

Mineral analysis

The Zn, Cu, and Mn concentrations in tibia were determined on a moisture-free, fat-free basis. After removing the adhering tissues, the tibia was ashed in a muffle furnace at 600ºC for 24 h. The concentrations of Zn, Cu, and Mn in egg and liver were determined on a moisture-free basis. The entire contents of egg or the right lobe of liver was homogenized and then freeze-dried to constant weight. After pretreatment, approximately 0.5 g of tibia, egg, or liver sample was digested according to the method as described by Li et al. (2005). Plasma was digested in concentrated nitric acid as described by Mohanna and Nys (1999). Concentrations of Zn, Cu, Mn in the diet, tibia, liver, and egg, and plasma Zn concentration were determined by flame atomic absorption spectrometry, while concentrations of Cu and Mn in plasma were measured by graphite furnace atomic absorption spectrometry in atomic absorption spectrophotometer (Model PE-800, PerkinElmer, USA). Validation of the mineral analysis was conducted using green tea or bovine liver powder as a standard reference material (National Institute of Standards and Technology, Beijing, China).

Statistical analysis

Data were analyzed by one-way ANOVA using GLM procedure of SAS 9.03 (SAS Inst. Inc., USA.) (SAS, 2003). One replicate plot, bird, or egg was served as the statistical unit. When significant effects were detected by ANOVA, treatment means were compared using Tukey's test. Statistical significance was detected at $P<0.05$. Linear and quadratic tread analysis was performed in a contrast statement to evaluate the effect of different levels of OTM on the various parameters determined.

Results

Performance and eggshell quality

In all experimental periods, supplementation of Cu, Zn, and Mn in combination did not significantly influence the hen-day egg production, egg weight, and feed conversion ratio of hens (Table 3). However, all Cu, Zn, and Mn supplementation treatments increased (P<0.01) ESB at week 4, and OTM25%, OTM50%, and ITM100% significantly increased EBS at week 8 and 12 after treatment when compared with NCON (Table 4). No significant difference was observed in EBS between OTM100% and NCON groups at week 8 and 12 after treatment. The EBS in OTM25% was similar to that in ITM 100% at any sampling time. Minerals supplementation did not significantly influence eggshell weight and thickness at week 4 and 8. However, OTM25% increased (P<0.05) eggshell weight and thickness as compared with NCON, and no significant difference was observed in above parameters among NCON, ITM100%, OTM50%, and OTM100% groups at week 12 after treatment. Minerals supplementation did not significantly influence eggshell percentage at any sampling time. Eggshell weight and EBS linearly decreased (P<0.03) with increasing level of OTM in the diet for hens at week 8 and 12. Eggshell percentage and eggshell thickness linearly decreased (P<0.03) at week 8, and also had the trend of decrease at week 12 after treatment with increasing level of OTM in the diet for hens.

Table 3. Influences of dietary level of organic trace minerals on the performance of laying hens (39 to 50 weeks) in different periods after treatment[1]

Items	NCON[2]	ITM100%[2]	OTM25%[2]	OTM50%[2]	OTM100%[2]	SEM[3]	P-value	Linear[4]	Quadratic[4]
Hen-day egg production[5] (%)									
week 1 to 4	94.6	94.7	93.6	94.7	95.1	0.85	0.768	0.231	0.782
week 5 to 8	92.7	94.2	92.5	92.8	93.8	0.91	0.632	0.349	0.791
week 9 to 12	90.2	92.2	92.2	91.1	92.5	0.85	0.293	0.854	0.285
Egg weight[5] (g)									
week 1 to 4	63.4	63.3	63.3	63.2	62.8	0.37	0.762	0.317	0.737
week 5 to 8	63.6	63.3	63.7	63.5	63.0	0.35	0.665	0.225	0.74
week 9 to 12	63.7	63.1	63.8	63.4	62.9	0.30	0.181	0.064	0.836
Feed conversion ratio[5] (g feed/g egg)									
week 1 to 4	1.81	1.83	1.87	1.83	1.82	0.02	0.381	0.110	0.719
week 5 to 8	1.85	1.84	1.86	1.85	1.83	0.02	0.916	0.346	0.771
week 9 to 12	1.91	1.9	1.89	1.91	1.9	0.02	0.834	0.773	0.399

[1]Hens (39-wk-old) had been fed with the basal diet without Zn-Cu-Mn supplementation for 4 weeks before the experimental diet introduction.

[2]NCON = no trace minerals addition (containing 8.82 mg/kg Cu, 24.94 mg/kg Zn, and 16.38 mg/kg Mn); ITM100% = NCON added with 16 mg/kg Cu ($CuSO_4 \cdot 5H_2O$), 80 mg/kg Zn ($ZnSO_4 \cdot 7H_2O$), and 60 mg/kg Mn ($MnSO_4 \cdot H_2O$); OTM25%, OTM50%, and OTM100% represented NCON added with 4-20-15, 8-40-30, and 16-80-60 mg/kg Cu-Zn-Mn from Zn-bis-(2-hydroxy-4-methylthiobutyrate) (Zn-MHA), Cu-MHA, and Mn-MHA respectively.

[3]SEM = standard error means.

[4]Linear and quadratic tread analysis was performed in a contrast statement to evaluate the effect of different levels of OTM on the various parameters determined.

[5]Means calculated for n = 10 replicates of 15 hens per replicate.

Table 4. Influences of dietary level of organic trace minerals on the eggshell quality in laying hens (39 to 50 weeks) at different times after treatment[1]

Items	NCON[2]	ITM100%[2]	OTM25%[2]	OTM50%[2]	OTM100%[2]	SEM[3]	P-value	Linear[4]	Quadratic[4]
Eggshell percentage[5] (%)									
week 4	10.72	10.54	10.71	10.58	10.55	0.183	0.254	0.548	0.829
week 8	10.61	10.68	10.88	10.48	10.36	0.141	0.288	0.024	0.358
week 12	10.41	10.69	10.81	10.74	10.54	0.133	0.413	0.080	0.742
Eggshell weight[5] (g)									
week 4	6.89	6.61	6.75	6.64	6.60	0.097	0.536	0.376	0.821
week 8	6.66	6.59	6.74	6.70	6.46	0.094	0.278	0.020	0.345
week 12	6.63 b	6.92 ab	7.12 a	6.89 ab	6.73 b	0.136	0.01	0.010	0.780
Eggshell breaking strength[5] (kg/cm^2)									
week 4	3.863 b	4.498 a	4.223 a	4.481 a	4.331 a	0.116	0.002	0.574	0.225
week 8	4.041 b	4.330 a	4.467 a	4.329 a	4.008 b	0.075	<0.001	<0.001	0.369
week 12	3.654 c	4.717 a	4.669 a	4.149 b	3.851 bc	0.129	<0.001	<0.001	0.521
Eggshell thickness[5] (mm)									
week 4	0.375	0.391	0.380	0.396	0.384	0.006	0.146	0.673	0.096
week 8	0.366	0.370	0.378	0.364	0.362	0.004	0.189	0.017	0.281
week 12	0.356 b	0.377 ab	0.386 a	0.382 ab	0.363 b	0.007	0.028	0.076	0.493

a, b – within a row, means with no common letter were significantly different at $P<0.05$.

[1]Hens (39-wk-old) had been fed with the basal diet without Zn-Cu-Mn supplementation for 4 weeks before the experimental diet introduction.

[2]NCON = no trace minerals addition (containing 8.82 mg/kg Cu, 24.94 mg/kg Zn, and 16.38 mg/kg Mn); ITM100% = NCON added with 16 mg/kg Cu (CuSO4·5H2O), 80 mg/kg Zn (ZnSO4·7H2O), and 60 mg/kg Mn (MnSO4·H2O); OTM25%, OTM50%, and OTM100% represented NCON added with 4-20-15, 8-40-30, and 16-80-60 mg/kg Cu-Zn-Mn from Zn-bis-(2-hydroxy-4-methylthiobutyrate) (Zn-MHA), Cu-MHA, and Mn-MHA respectively.

[3]SEM = standard error means.

[4]Linear and quadratic tread analysis was performed in a contrast statement to evaluate the effect of different levels of OTM on the various parameters determined.

[5]Means calculated for n = 20 eggs.

Minerals retention in tissues of hens

Supplementation of Cu, Zn, and Mn in combination increased (P<0.01) the concentrations of Zn in liver, Zn and Mn in tibia, and Cu in plasma, but did not significantly influence the concentrations of liver Mn, tibia Cu, and plasma Zn when compared with NCON (Table 5). No significant difference was observed in the concentrations of liver Zn and plasma Cu among all minerals supplementation groups. Tibia Zn concentration in OTM25% did not significantly differ from that in ITM100% and OTM100%, while was lower than that in OTM50%. Tibia Mn concentration in OTM25% was lower (P<0.02) than that in ITM100% and OTM50%, while was similar (P>0.1) to that in OTM100%. Plasma Mn concentration was higher (P<0.05) in OTM25% than that in NCON or OTM100%, and no significant difference was observed among OTM25%, OTM50%, and ITM100%. Addition of OTM at increasing level had quadratic effect on the concentration of Cu in liver, and Cu, Zn, and Mn in tibia, with the lower liver Cu concentration and higher tibia Cu, Zn, and Mn concentrations in OTM50%.

Table 5. Influences of dietary level of organic trace minerals on trace minerals retention in the tissues of laying hens (50 weeks) at week 12 after treatment[1]

Items	NCON[2]	ITM100%[2]	OTM25%[2]	OTM50%[2]	OTM100%[2]	SEM[3]	P-value	Linear[4]	Quadratic[4]
Liver[5] (mg/kg)									
Cu	3.07 a	2.94 ab	3.05 a	2.55 b	2.94 ab	0.12	0.031	0.541	0.008
Zn	25.76 b	37.25 a	34.45 a	35.22 a	36.67 a	1.94	<0.001	0.427	0.888
Mn	2.18	2.67	2.64	2.47	2.66	0.14	0.081	0.929	0.349
Tibia[5] (mg/kg)									
Cu	7.48 ab	7.89 ab	6.84 b	8.21 a	6.89 b	0.34	0.026	0.904	<0.001
Zn	218.1 c	270.0 b	262.7 b	299.7 a	250.0 b	7.41	<0.001	0.170	<0.001
Mn	7.28 c	10.73 a	9.50 b	10.83 a	10.33 ab	0.35	<0.001	0.076	0.026
Plasma[5] (mg/L)									
Cu	0.233 b	0.324 a	0.333 a	0.314 a	0.353 a	0.025	0.018	0.590	0.352
Zn	5.28	6.38	5.98	5.36	6.15	0.35	0.142	0.120	0.177
Mn	2.65 b	4.09 ab	5.80 a	4.83 a	2.53 b	0.51	0.001	0.742	0.109

a, b, c – within a row, means with no common letter were significantly different at P<0.05.

[1]Hens (39-wk-old) had been fed with the basal diet without Zn-Cu-Mn supplementation for 4 weeks before the experimental diet introduction.

[2]NCON = no trace minerals addition (containing 8.82 mg/kg Cu, 24.94 mg/kg Zn, and 16.38 mg/kg Mn); ITM100% = NCON added with 16 mg/kg Cu ($CuSO_4 \cdot 5H_2O$), 80 mg/kg Zn ($ZnSO_4 \cdot 7H_2O$), and 60 mg/kg Mn ($MnSO_4 \cdot H_2O$); OTM25%, OTM50%, and OTM100% represented NCON added with 4-20-15, 8-40-30, and 16-80-60 mg/kg Cu-Zn-Mn from Zn-bis-(2-hydroxy-4-methylthiobutyrate) (Zn-MHA), Cu-MHA, and Mn-MHA respectively.

[3]SEM = standard error means.

[4]Linear and quadratic tread analysis was performed in a contrast statement to evaluate the effect of different levels of OTM on the various parameters determined.

[5]Means calculated for n = 10 hens.

Mineral concentrations in eggs

Supplementation of Cu, Zn, and Mn in combination increased (P<0.05) Zn concentration at week 4, 8 and 12, and Mn concentration at week 8 and 12 after treatment in eggs when compared to NCON (Table 6). No significant difference was observed in Zn concentration after 8 weeks treatment in eggs among minerals supplementation groups, although Zn concentration in eggs were lower (P<0.05) in OTM25% and ITM100% than that in OTM50% and OTM100% at week 4 after treatment. The Mn concentration in eggs significantly increased in the following order: OTM25% < OTM50% < OTM100% < ITM100% at week 4, and was also lower in OTM25% than that in ITM100% at week 8 after treatment. However, no significant difference was observed in Mn concentration in eggs among trace minerals supplementation treatments. The OTM25% and ITM100% decreased Cu concentration in eggs when compared with NCON and OTM100%, while no significant difference was observed for Cu concentration in eggs between OTM25% and ITM100% at week 12 after treatment. The concentration of Cu in egg linearly increased at week 4 and 8, and also had a trend of increase at week 12 after treatment with increasing level of OTM. The dietary level of OTM did not significantly influence the concentrations of Zn and Mn in egg at week 12, although the concentrations of Zn and Mn in egg linearly increased with increasing level of OTM at week 4 after treatment.

Table 6. Influences of dietary level of organic trace minerals on trace minerals deposition in the eggs laid by laying hens (39 to 50 weeks) at different times after treatment[1]

Items	NCON[2]	ITM100%[2]	OTM25%[2]	OTM50%[2]	OTM100%[2]	SEM[3]	P-value	Linear[4]	Quadratic[4]
Copper[5] (mg/kg)									
week 4	5.22 ab	4.96 ab	4.05 c	4.89 b	5.46 a	0.17	<0.001	<0.001	0.563
week 8	4.92 b	6.15 a	4.51 b	4.50 b	6.40 a	0.25	<0.001	<0.001	0.001
week 12	6.83 a	5.56 b	5.70 b	6.39 ab	6.80 a	0.34	0.021	0.061	0.774
Zinc[5] (mg/kg)									
week 4	33.09 c	39.63 b	38.06 b	42.64 a	43.57 a	0.91	<0.001	0.001	0.174
week 8	41.62 b	49.09 a	48.27 a	48.99 a	49.84 a	0.81	<0.001	0.261	0.955
week 12	41.61 b	44.62 a	44.44 a	44.37 a	45.98 a	0.64	<0.001	0.123	0.323
Manganese[5] (mg/L)									
week 4	0.73 d	1.63 a	0.83 d	1.10 c	1.42 b	0.05	<0.001	<0.001	0.680
week 8	1.06 d	1.46 b	1.24 c	1.44 b	1.57 a	0.04	<0.001	<0.001	0.381
week 12	1.14 b	1.43 a	1.41 a	1.48 a	1.47 a	0.03	<0.001	0.119	0.297

a, b, c, d – within a row, means with no common letter were significantly different at P<0.05.

[1]Hens (39-wk-old) had been fed with the basal diet without Zn-Cu-Mn supplementation for 4 weeks before the experimental diet introduction.

[2]NCON = no trace minerals addition (containing 8.82 mg/kg Cu, 24.94 mg/kg Zn, and 16.38 mg/kg Mn); ITM100% = NCON added with 16 mg/kg Cu ($CuSO_4{\cdot}5H_2O$), 80 mg/kg Zn ($ZnSO_4{\cdot}7H_2O$), and 60 mg/kg Mn ($MnSO_4{\cdot}H_2O$); OTM25%, OTM50%, and OTM100% represented NCON added with 4-20-15, 8-40-30, and 16-80-60 mg/kg Cu-Zn-Mn from Zn-bis-(2-hydroxy-4-methylthiobutyrate) (Zn-MHA), Cu-MHA, and Mn-MHA respectively.

[3]SEM = standard error means.

[4]Linear and quadratic tread analysis was performed in a contrast statement to evaluate the effect of different levels of OTM on the various parameters determined.

[5]Means calculated for n = 10 (2 eggs each).

Discussion

Feeding hens with the basal diet without minerals supplementation for 12 weeks did not influence the performance of hens, which indicated the 24.94, 8.82, and 16.38 mg/kg Zn, Cu, and Mn respectively was adequate to meet the requirement for the performance of laying hens (Abdallah et al., 1994). However, in the present study, supplementation of Cu, Zn, and Mn in the corn-soybean basal diet improved the eggshell quality, which also suggested the level of Cu, Zn, and Mn in the corn-soybean basal diet was not adequate for the better eggshell quality (Mabe et al., 2003; Gheisari et al., 2011), and the requirements of minerals for optimal shell quality were higher than those for the performance of hens (Sazzad et al., 1994; Guo et al., 2002). In addition, no significant difference in eggshell weight and thickness between OTM25% and ITM100% suggested that the HMTBA mineral chelate had the higher bioefficiency than sulfate minerals (Li et al., 2005; Abdallah et al., 2009). These results were in line with previous studies that the hens fed the diet supplemented with the lower levels of OTM had similar eggshell thickness and EBS compared to relatively higher level of ITM (Mabe et al., 2003; Fernandes et al., 2008). However, the OTM50% and OTM100% did not further improve eggshell quality as compared to OTM25%, which might be partly due to the antagonistic effects between trace minerals (Li et al., 2005; Huang et al., 2007).

The accumulation of trace minerals in tibia has been accepted by several researchers as an indicator of body mineral status (Berta et al., 2004; Ao et al., 2009; Gajula et al., 2011). The decreased tibia Mn concentration in OTM25% as compared to ITM100% and OTM50%, suggested the lower level of OTM had negative effect on Mn deposition in tibia of hens as described in broilers (Bao et al., 2007; Gajula et al., 2011). Addition of OTM at increasing level had quadratic effect on tibia Cu, Zn, and Mn concentrations with the more retention in OTM50% group, which might be due to the negative feedback mechanism to control excessive minerals deposition in the tissues of animals (Bao et al., 2007).

Egg mineral concentration was variable, and the amount deposited in egg contents might depend on the chemical form of mineral and the amount fed to the hen (Naber, 1979; Dobrzański et al., 2008). The transfer of trace minerals from the hen to the egg contents involves 2 possible routes: 1) via the ovary to the yolk, and 2) via the oviduct to the albumen (Richards, 1997). In this study, OTM25% had similar mineral deposition compared to ITM100%, which also indicated that OTM had the higher bioavailability than ITM for hens. Addition of OTM at increasing level had linear effect on the concentration of Cu in eggs, while not increasing Mn and Zn retention in eggs for hens after 12 weeks feeding. Similarly, Favero et al. (2013) found that there were no differences in Cu, Zn and Mn contents in eggs laid by hens fed with the diet supplemented with 30-30-5 or 60-60-10 mg/kg Zn-Mn-Cu from amino acid minerals complexes. In contrast, some studies found that the supplementation of minerals from OTM increased Zn, and Mn deposition in the egg as compared to ITM (oxidate source) (Kienholz et al., 1961; Naber, 1979). The egg yolk and albumen had 3.2 and 2.2% more Zn in hens fed with the diet supplemented with 60-3-60 mg/kg Zn-Cu-Mn from sulfate plus 40-7-40 mg/kg Zn-Cu-Mn from amino acid minerals complexes, when compared with the addition of 100-10-100 mg/kg Zn-Cu-Mn from sulfates (Favero et al., 2013). The differences in the source ITM and OTM, the level of added minerals, the ratio of trace minerals, and the level of minerals in the basal diet partly explained the discrepancies in these studies.

In conclusion, supplementation of the basal diet with Cu, Zn, and Mn did not increase the performance of laying hens, but improved the eggshell quality in hens from the age of 39 to 51 weeks. Supplementation of Cu, Zn, and Mn from HMTBA mineral chelates at the lower level (OTM25%) had similar eggshell quality compared to relatively high level of minerals (ITM100%) from sulfate minerals in hens from 39 to 51 weeks old, whereas the eggshell quality decreased with increasing level of OTM after 12 weeks feeding. The OTM25% had no negative effect on trace minerals retention in liver and tibia, except decreasing tibia Mn retention, when compared with ITM100% (addition of 16-80-60 mg/kg Cu-Zn-Mn from sulfate minerals). The OTM25% from HMTBA mineral chelates can substitute for ITM100% evaluated by eggshell quality in the diet for hens.

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