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Effect of organic and nano trace minerals at reduced levels in layer diets on egg quality characteristics and its trace mineral content

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ABSTRACT

An experiment was conducted to assess the effect of organic and nano trace minerals (copper, zinc, manganese and iron) at reduced levels in layer diets on various egg quality characteristics and its trace mineral content. 240 Hy-Line W80 layer birds of 29 weeks old were randomly distributed to five treatment groups with four replicates having twelve birds each. Basal diet was supplemented with inorganic trace minerals to meet 100 per cent requirement to form control diet or with organic trace minerals at 75 and 50% of requirement or with nano trace minerals at 50 and 25% of requirement to form four test diets. Each diet was offered to four replicate groups for 84 days which was conveniently divided into three phases of 28 days each. Egg quality parameters, viz. egg weight, egg shape index, albumen index, yolk index, yolk color score, Haugh unit score, egg shell weight, albumen weight and yolk weight among the treatment groups were not affected by source and level of trace minerals. However, egg shell thickness was significantly higher in 25% nano trace mineral group on 28th day. The trace minerals (Zn and Fe) in the yolk were significantly lower at reduced trace mineral levels (50% organic or nano and 25% nano form) when compared to 100% (inorganic) and 75% levels (organic form). It was concluded that the substitution of inorganic trace minerals with organic or nano trace minerals at reduced levels (50 or 25%, respectively) in layer diets does not affect the egg quality parameters, but reduces Zn and Fe content in egg yolk.

Keywords: Egg quality, Egg trace minerals, Organic form, Nano particles, Trace minerals

Trace minerals are vital aspect of poultry diets due to their biochemical processes which are essential for production, reproduction and health of birds. Some trace minerals also play pivotal role in the formation of egg in laying hens. Copper (Cu) is required for egg-shell membrane formation, structure and texture of egg-shell and egg shape (Favero *et al*. 2013). Manganese (Mn) serves as a key activator of enzymes that are involved in the synthesis of mucopolysaccharides and glycoproteins which forms organic matrix of the shell (Georgievski *et al.* 1982). Zinc (Zn) is an essential trace element for enzymes structure and function, bone development and eggshell formation in birds (Nys *et al*. 2004). Iron (Fe) is a vital element required for production of haemoglobin facilitating oxygen and mitochondrial electron transport (Zhao 2010, Gozzelino *et al*. 2010). Conventionally, these trace minerals are incorporated in the form of inorganic salts in animal and poultry diets. However, low bioavailability of element in inorganic salts necessitates using them at higher levels in the diets to meet bird's requirements, which result in increased trace mineral excretion causing environmental pollution (Patra and Lalhriatpuii 2020). Hence, the poultry industry has started using organic form of trace minerals while nano forms are also gaining a place as feed supplements.

The trace minerals in organic form are better bioavailable and also improved productive and reproductive performance, and strengthened immunological response in livestock and poultry (Pal and Gowda 2015). Similarly, nano trace minerals showed better bioavailability due to smaller size, greater specific surface area, high catalytic efficiency and stronger absorbing ability (Tamilvanan *et al*. 2014, Gopi *et al*. 2017). Recent studies have indicated that the inorganic trace minerals can be replaced with organic or nano forms at lower doses with similar or improved performance and egg quality in laying hens (Cufadar *et al*. 2020, Aminullah *et al*. 2021, El-Katcha *et al*. 2022). However, most of the studies attempted to assess individualal nano trace mineral to ascertain their efficacy. In this context, this study aims to evaluate the effects of organic and nano trace minerals (Cu, Zn, Mn and Fe) at reduced dietary levels on the egg quality parameters and egg trace mineral content in laying hens.

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MATERIALS AND METHODS

Experimental birds and management: A total of 240 Hy-Line W80 laying hens of 29 weeks age were weighed and randomly assigned to five treatment groups with four replicates having 12 hens each (48 hens per treatment). All birds were housed in three tier colony cages and four birds were in each cage measuring about 15"×15"×18" and there was one empty cage between two replicates to avoid the access of other diet in adjacent cage. All the birds were provided with drinking water all the times and exposed to 16 h light. The trial lasted for 84 days consisting of three phases of 28 days each. All the procedures employed during the trial have been approved by the Institutional Animal Ethics Committee of Veterinary College, KVAFSU, Hebbal, Bengaluru, Karnataka (VCH/IA/EC/2023/12).

Experimental diets: A practical type layer diet was formulated as per the breeder recommendations without any trace mineral to serve as basal diet (Table 1). Further, the control diet was prepared by supplementing inorganic trace minerals to the basal diet to meet 100% requirement (inorganic TM 100) as specified by breeder manual (Hy-Line W 80 Management Guide 2023). The test diets were prepared by supplementing organic trace minerals to the basal diet to meet 75 (organic TM 75) and 50% requirement (organic TM 50), respectively. Another set of test diets were prepared by supplementing nano trace minerals to the basal diet to meet 50 (nano TM 50) and 25% requirement (nano TM 25). The analysed trace mineral composition of experimental diets is given in Table 2. Each diet was weighed and offered to respective replicates on a daily basis during all the phases, i.e. each bird received 120 g of feed in three doses every day from the 29th to 40th week of age.

Source of trace minerals: The inorganic and organic trace minerals employed were procured from M/s Zeus Biotech Pvt Ltd., Mysuru, Karnataka, India and nano trace minerals from M/s Nano Research Lab, Jamshedpur, Jharkhand, India. The sulphate salts of Cu, Zn, Mn and Fe were employed to prepare organic trace minerals which were proteinate in nature. The oxide forms were employed to prepare nano trace minerals using ball mill. The particle size of nano ZnO, Mn_2O_3 and Fe_2O_3 were ranged from 30 to 50 nm while average particle size of nano CuO was less than 100 nm. All the salts employed were in pure form with purity of 98 to 99.5%.

Egg grading: Two days in a week (Tuesday and Friday), all the eggs produced were weighed and grouped into different grades as small (38-44 g), medium (45-52 g), large (53-59 g), extra large (60-70 g) and jumbo eggs (>70 g) as per the Indian standard.

Egg quality parameters: On first day of experiment and subsequently at every 28-days interval, three eggs were collected from each replicate (12 eggs per treatment), weighed individually with a precision of 0.001 g and labelled for egg quality characteristics evaluation. After external egg quality study, each egg was broken and all of its contents were carefully emptied onto a glass slab for internal egg quality analysis. Egg shape index (ESI) was calculated as: Short axis × 100 / long axis measured using digital Vernier Callipers. Shell thickness was measured at three points (middle, broad and narrow end) of the egg

Table 1. Ingredient and nutrient composition of basal diet

Ingredient	kg	Calculated nutrient	%
Maize	50.00	ME, kcal/kg	2470
Soyabean meal	18.00	Crude protein	16.77
De-oiled rice bran	8.30	Lysine	0.83
Sunflower meal	10.00	Methionine	0.39
Marble Stone	12.00	Methionine + cystine	0.60
Di-Calcium Phosphate	0.800	Threonine	0.63
Common salt	0.200	Ether extract	2.09
Sodium bicarbonate	0.050	Linolic acid	1.20
DL- Methionine	0.150	Calcium	4.23
L-Lysine	0.100	Available Phosphorus	0.40
Choline Chloride	0.060	Sodium	0.17
Phytase	0.015	Chloride	0.16
Enzymes[a]	0.065	Potassium	0.72
Vetshell YO[b]	0.015	Crude fibre	6.01
Chloroteracycline 15 %	0.030	Copper, ppm	5.83
Probiotics[c]	0.020	Iron, ppm	672.17
Exceliv[d]	0.100	Manganese, ppm	23.27
Toxin Binder	0.100	Zinc, ppm	13.21
Vitamin Premix[e]	0.050	Selenium, ppm	0.03
Vitamin B_{12}	0.015	Iodine, ppm	0.00
		C/P ratio	147
Total (kg)	100.00	Cost, INR/kg	30.18

[a]Enzymes (pentosanases, hexosanases, hetero-polysaccharidases, proteases, lipase, phytase); [b]Vetshell YO- vitamin D_3, sodium bicarbonate, Protease, shell matrix modulators, specific amino acid producing probiotics in a base fortified with amino nitrogen, phytase; [c]Probiotics (6000 billion cfu/kg total bacilli count); [d]Exceliv- tricholine citrate, methyl sulfonyl methane, glycine, liver extract, vitamin-E and protein hydrolysate, probiotic, buffer salt, antifungal compound, specific hepato-stimulants and gut stabalizers; [e]Vitamin premix (vitA-25,000,000 IU, vitD_3-6,000,000 IU, niacin-26000 mg, vitE-16000 mg, pantothenic acid-16000 mg, vitB_2-10000 mg, vitB_6-3000 mg, vitk_3-3000 mg, vitB_1-2000 mg, vitB_{12}-16000 mcg, folic acid- 500 mg/kg).

Table 2. Analysed trace mineral content of experimental diets

Element	Inorganic TM 100%	Organic TM 75%	Organic 50%	Nano 50%	Nano 25%
Copper	19.64	17.84	14.24	14.14	11.64
Zinc	98.78	89.56	69.33	66.97	56.44
Manganese	132.00	112.20	97.20	96.60	84.40
Iron	725.90	712.60	694.90	693.40	689.70

Inorganic sources: $CuSO_4$ (25.47% Cu, 98% purity), $ZnSO_4$ (36.50% Zn, 99% purity), $FeSO_4$ (37.0% Fe, 98% purity) and $MnSO_4$ (36.38% Mn, 98.5% purity).Organic sources: Proteinate forms of inorganic salts. Nano sources: CuO (80% Cu, 99% purity), ZnO (78% Zn, 99% purity), Mn_2O_3 (69.6% Mn, 99% purity) and Fe_2O_3 (69.9% Fe, 99.5% purity). TM, Trace minerals.

using a digital screw gauge after removing shell membrane. Yolk index (YI) was calculated as: Yolk height ×100 / yolk diameter measured using Ames Spherometer and Vernier Callipers, respectively. Yolk colour was scored by using Roche yolk colour fan. Albumen index (AI) was calculated as: Albumen height ×100 / albumen diameter. Haugh unit score (HU) was determined by using the formula:

$$HU= 100 \times \log_{10} (H+7.57- 1.7 \times EW^{0.37})$$

Where, H, height of thick albumen (mm) and EW, egg weight (g). The weight of egg components, viz. shell, albumen and yolk were recorded using weighing balance on 28th, 56th and 84th day of the experiment.

Egg trace mineral content: After internal egg quality study, albumen and yolk were separated and stored in container at -20°C for trace mineral estimation. Trace mineral content in egg albumen and yolk was measured by atomic absorption spectrophotometry (Model: Shimadzu AAS-7000).

Statistical analysis: The data was subjected to analysis of variance (ANOVA) using Statistical Package for Social Sciences (SPSS-16). Tukey's post hoc test was applied if differences were found to be significant at $P<0.05$.

RESULTS AND DISCUSSION

The eggs produced under different treatment groups were graded and presented in Table 3. Egg grade values among different treatments were remained unaffected (P>0.05) by the reduced level of organic or nano forms of trace minerals during all the phases of experimental period as well as cumulatively.

The characteristics of eggs at different phases of the experiment are presented in Table 4. The egg weight was not affected (P>0.05) by the source (organic or nano form) or inclusion level of trace minerals in the diets. Similarly, Abedini *et al*. (2018) and Cufadar *et al*. (2020) reported that no significant effect on egg weight when layer diets were supplemented with different levels of Zn (20, 40, 60, 80, 100 and 120 ppm) sourced in different forms (inorganic, nano and organic). Bai *et al*. (2017) and Dong *et al*. (2022) also observed that supplementation of Cu, Zn, Fe and Mn did not affect (P>0.05) egg weight. Contrarily, Aminullah *et al*. (2021) reported that egg weight was significantly (P<0.05) higher in Cu nano particles at 50% inclusion level compared to control (inorganic Cu).

The egg shape index (ESI) values among the different treatment groups were not affected (P>0.05) by source and level of trace minerals in the diets (Table 4). El-Katcha *et al*. (2018) reported that neither organic nor nano Zn supplementation showed a notable impact (P>0.05) on the ESI when compared to inorganic Zn. Yenice *et al*. (2015) also reported that addition of inorganic or organic forms of trace minerals (Cu, Mn, Zn and Cr) to the diet at various levels had no impact on the ESI. Contradictorily, Aminullah *et al*. (2021) reported that the ESI was significantly (P<0.05) lower in 50 % nano Cu fed group when compared to 100 % inorganic, 100 and 75% organic Cu fed groups.

The egg shell thickness (EST) values across the phases were statistically similar among all treatment groups except at 28th day (Table 4). On 28th day, the EST was

Table 3. Egg grades (%) as influenced by different trace mineral sources and levels

Attribute	Inorganic TM 100%	Organic TM 75%	Organic TM 50%	Nano TM 50%	Nano TM 25%	SEM	P-value
Small (38-44 g)				NIL			
Medium (45-52 g)							
1-28 days	2.67	2.90	4.90	4.57	2.08	0.59	0.50
28-56 days	0.28	1.68	2.70	1.10	1.62	0.4	0.46
56-84 days	0	2.24	1.38	0.81	1.37	0.35	0.38
Cumulative	0.95	2.26	2.97	2.14	1.71	0.35	0.48
Large (53-59 g)							
1-28 days	46.87	48.97	48.63	50.84	46.76	1.554	0.937
28-56 days	39.22	38.1	40.82	42.58	36.19	1.559	0.779
56-84 days	32.66	32.13	39.65	43.13	31.97	1.917	0.215
Cumulative	39.46	39.65	42.91	45.42	38.29	1.444	0.547
Extra large (60-70 g)							
1-28 days	50.17	47.83	45.89	44.58	50.88	1.782	0.806
28-56 days	59.97	60.21	56.47	56.03	61.91	1.724	0.820
56-84 days	67.34	65.63	58.42	55.48	66.07	2.025	0.249
Cumulative	59.31	57.98	53.74	52.15	59.62	1.637	0.524
Jumbo (>70 g)							
1-28 days	0.28	0.30	0.57	0	0.28	0.141	0.836
28-56 days	0.53	0	0	0.28	0.28	0.127	0.687
56-84 days	0	0	0.54	0.58	0.57	0.185	0.736
Cumulative	0.27	0.09	0.37	0.28	0.38	0.121	0.961

TM, Trace minerals.

Table 4. Egg characteristics as influenced by different trace mineral sources and levels

Attribute	Inorganic TM 100%	Organic TM 75%	Organic TM 50%	Nano TM 50%	Nano TM 25%	SEM	P-value
Egg weight (kg)							
Day 1	58.34	58.90	58.78	57.60	58.09	0.919	0.994
Day 28	59.98	59.57	59.63	59.65	60.49	0.336	0.908
Day 56	62.21	61.53	61.53	61.51	62.17	0.397	0.958
Day 84	59.43	60.52	61.34	60.32	62.43	0.575	0.554
Cumulative	60.54	60.55	60.83	60.50	61.70	0.264	0.567
Egg shape index							
Day 1	75.16	75.95	74.18	74.25	75.36	0.355	0.493
Day 28	76.65	75.80	76.72	76.63	77.12	0.215	0.417
Day 56	76.64	74.72	74.72	76.11	75.32	0.263	0.072
Day 84	76.95	75.23	74.83	76.70	76.09	0.364	0.287
Cumulative	76.38	75.25	75.42	76.48	76.18	0.167	0.055
Egg shell thickness (mm)							
Day 1	0.372	0.379	0.38	0.385	0.377	0.002	0.192
Day 28	0.365^{b}	0.366^{b}	0.370^{ab}	0.377^{ab}	0.394^{a}	0.003	0.015
Day 56	0.396	0.394	0.4	0.397	0.398	0.002	0.833
Day 84	0.395	0.392	0.393	0.393	0.394	0.001	0.803
Cumulative	0.385	0.383	0.39	0.387	0.389	0.001	0.58
Yolk colour score							
Day 1	7.25	7.50	7.50	7.75	7.75	0.153	0.861
Day 28	7.17	7.00	7.00	7.17	7.17	0.085	0.926
Day 56	6.92	7.17	7.25	7.25	7.33	0.099	0.734
Day 84	6.83	7.00	6.92	7.00	7.08	0.103	0.958
Cumulative	6.97	7.05	7.06	7.13	7.19	0.056	0.759
Yolk index							
Day 1	47.31	47.39	46.91	46.54	46.07	0.468	0.917
Day 28	48.31	46.38	47.09	46.24	45.69	0.354	0.163
Day 56	47.50	47.90	47.36	46.84	46.57	0.302	0.665
Day 84	46.00	45.40	47.48	46.36	45.63	0.255	0.08
Cumulative	47.27	46.43	47.31	46.48	45.96	0.176	0.064
Albumen index							
Day 1	9.464	9.415	9.109	9.278	8.71	0.115	0.243
Day 28	9.897	10.041	9.555	9.383	9.667	0.116	0.401
Day 56	9.646	9.908	9.285	8.84	9.808	0.136	0.073
Day 84	10.501	9.951	10.645	9.742	10.365	0.155	0.313
Cumulative	9.925	9.966	9.828	9.321	9.951	0.081	0.061
Haugh unit							
Day 1	90.89	91.22	89.88	89.13	88.89	0.344	0.107
Day 28	89.40	91.38	88.89	89.4	90.49	0.369	0.201
Day 56	89.09	90.32	87.22	86.67	89.85	0.498	0.073
Day 84	91.56	90.21	91.58	89.84	89.92	0.532	0.716
Cumulative	90.02	90.64	89.23	88.64	90.09	0.277	0.169
Egg shell weight (g)							
Day 28	6.90	6.85	6.86	6.86	6.95	0.039	0.912
Day 56	7.06	6.96	7.02	6.99	7.09	0.421	0.879
Day 84	6.67	6.96	7.08	6.94	7.18	0.662	0.145
Yolk weight (g)							
Day 28	15.89	15.79	15.8	15.81	16.03	0.089	0.908
Day 56	16.41	16.05	16.24	16.28	16.39	0.106	0.841
Day 84	15.97	16.54	16.42	16.65	16.88	0.115	0.137

Table 4 *(Continued...)*

Table 4 *(Concluded)*

Attribute	Inorganic TM 100%	Organic TM 75%	Organic TM 50%	Nano TM 50%	Nano TM 25%	SEM	P-value
Albumen weight (g)							
Day 28	37.19	36.94	36.97	36.99	37.5	0.208	0.909
Day 56	38.75	38.08	38.28	38.23	38.69	0.262	0.913
Day 84	36.80	37.01	37.84	36.73	38.38	0.437	0.717

Mean values bearing different superscripts within the column differ significantly (P≤0.05). TM, Trace minerals.

significantly (P≤0.05) higher in 50% nano trace minerals as compared to control and 75% organic trace minerals, but it was comparable with other treatment groups. Similarly, Aminullah *et al*. (2021) observed that EST showed significant improvement (P≤0.05) at 100 and 75% supplemental level from organic Cu and at 75 and 50% level from nano copper as compared to the control (100 % inorganic Cu). El-Katcha *et al*. (2022) also observed that EST increased in the groups supplemented with 4 and 8 ppm CuO nano particles as well as with higher concentration of organic Cu (P<0.05). Contrarily, Kim *et al*. (2022) and El-Katcha *et al*. (2022) reported that inclusion of inorganic or organic trace minerals in the diets at various levels had no impact on the EST. The improved EST in the present study may be due to the fact that mechanical properties of egg shell may be affected by trace minerals (Zn, Cu and Mn) either by interacting with calcite crystal formation or by their catalytic properties as key enzymes involved in egg shell formation (Mabe *et al*. 2003). Zn as a cofactor of carbonic anhydrase which catalyses carbon dioxide into bicarbonate ions, is closely linked to eggshell formation by affecting the crystal and texture morphologies of the shell (Lai *et al*., 2009). Cu play a role as cofactor of the lysyl-oxidase enzyme that is important in the formation of collagen cross-links present in the eggshell membranes (Chowdhury 1990). Mn is also essential for the synthesis of mucopolysaccharides, which activate the glycosyl transferases, which are constituents of proteoglycans which are involved in eggshell matrix formation (Leach and Gross 1983, Zamani *et al*. 2005).

The yolk index was not affected (P>0.05) by the source (organic or nanoparticles) or inclusion level of trace minerals in the diets (Table 4). The findings are also in consensus with El-Katcha *et al.* (2022) and Elfiky *et al.* (2021) who reported that source and level of trace minerals in laying hen diet had no significant effect on egg yolk index. On contrary, Elsherif *et al*. (2019) reported that significant (P≤0.05) effect on yolk index at 60 and 120 ppm copper sulphate inclusion level in the diet of laying hens.

The egg yolk color score remained unaffected (P>0.05) by the reduced level of organic or nano forms of trace minerals (Table 4). The results are similar to the findings reported that different source (inorganic, organic and nano) and level of trace minerals had no significant effect on egg yolk color of laying hens (Abedini *et al*. 2018, Aminullah *et al*. 2021, Kim *et al*. 2022, Cao *et al*. 2023). Contradictorily, Elfiky *et al.* (2021) found that yolk colour had significantly (P≤0.05) increased by supplementation of nano Se source as compared to control.

The albumen index was not affected by the different source and reduced level of trace minerals in layer diets (Table 4). The results align with the findings of Aminullah *et al*. (2021) who observed that lower supplemental levels of Cu from organic or nanoparticle sources resulted in egg albumen index values similar to those of control group. Similarly, no significant effect of dietary organic and nano trace mineral on albumen index in laying hens was observed (Yenice *et al*. 2015, Saleh *et al*. 2020, El-Katcha *et al*. 2022). On contrary, El-Katcha *et al.* (2018) reported that organic Zn supplementation did not show a notable impact on the average albumen index, whereas nano Zn supplementation significantly decreased the albumen index compared to inorganic Zn.

The present study indicated that both organic and nanoparticle source of trace minerals did not influence (P>0.05) the Haugh unit score (HUS) across the various treatment groups compared to the inorganic source (Table 4). Similarly, Abedini *et al*. (2018) reported that there was no impact observed on HUS in laying hens with dietary supplementation from inorganic, organic and nano trace mineral source. Several studies also indicated that there was no significant effect (P>0.05) of different levels of mineral mixture (Mn, Zn, Cu, I, Fe, Cr, Se) from organic source in the diet of laying hens on Haugh unit (Qiu *et al.* 2020, Kim *et al*. 2022, Chen *et al*. 2022). On contrary, Aminullah *et al*. (2021) observed that the Haugh unit score was significantly decreased (P<0.05) at 15 ppm Cu-nano particle.

The egg exponents, viz. shell, albumen and yolk weight were not affected (P>0.05) by the source and level of trace minerals (Table 4). Similarly, Abedini *et al*. (2018) reported that supplementing the diets with nano ZnO (40, 80, 120 ppm) had no significant effect on egg shell, yolk and albumen weight. Elfiky *et al*. (2021) also reported that both organic and nano Se (0.1, 0.2, 0.3 ppm) did not affect (P>0.05) shell, albumen and yolk weight of eggs. Contrary, nano Zn (60 or 30 ppm) supplementation increased yolk weight compared with same level of Zn from organic source and lower level of nano Zn (30 ppm) and significantly (P<0.05) reduced albumen weight compared with inorganic Zn (El-Katcha *et al*. 2018). Dietary replacement of CuO with nano CuO at 8 ppm significantly increased shell weight (El-Katcha *et al*. 2022).

Egg yolk Zn and Fe content were significantly (P<0.05) affected by the different source and level of trace minerals (Table 5). However, egg yolk Cu and Mn content were

Table 5. Egg trace mineral content (ppm, as such basis) as influenced by different trace mineral sources and levels

Element	Inorganic TM 100%	Organic TM 75%	Organic TM 50%	Nano TM 50%	Nano TM 25%	SEM	P-value
Egg yolk							
Copper	1.62	1.55	1.55	1.52	1.45	0.027	0.458
Zinc	41.67[a]	39.46[ab]	37.50[bc]	36.82[c]	36.49[c]	0.504	0.001
Manganese	1.15	1.06	0.99	1.06	0.96	0.024	0.075
Iron	69.94[a]	65.46[ab]	59.40[bc]	54.93[c]	56.70[c]	1.420	0.001
Egg albumen							
Copper	<0.01	<0.01	<0.01	<0.01	<0.01	-	-
Zinc	<0.01	<0.01	<0.01	<0.01	<0.01	-	-
Manganese	<0.01	<0.01	<0.01	<0.01	<0.01	-	-
Iron	3.28	2.90	2.80	2.64	3.08	0.11	1.00

Mean values bearing different superscripts within the column differ significantly (P≤0.05). TM, Trace minerals.

showed non-significant (P>0.05) difference among the different treatment groups. When compared to control, the egg yolk Zn and Fe content was significantly (P<0.05) decreased with reduced inclusion level of trace minerals sourced through either organic or nano forms however remained comparable to organic 75% group. The trace mineral (Cu, Zn and Mn) content in egg albumen was very much below the detectable level while the Fe content was not affected (P>0.05) by different source and level of trace minerals (Table 5). Similarly, Qiu *et al*. (2020) reported that yolk Fe concentration was significantly higher in inorganic TM (100 %) and organic TM (33%) as compared to inorganic TM (33%) (P<0.05). However, no significant differences (P>0.05) of Cu and Mn levels in yolk among the treatment groups. On contrary, Dong *et al*. (2022) reported that deposition of trace minerals in egg yolk did not differ significantly between control and inorganic TM (100%), but both organic TM (30% and 50%) showed a significant positive effect (P<0.01). Yenice *et al.* (2015) concluded that the organic form of trace mineral mixture significantly increased the Mn, Zn and Cu concentrations in egg.

It was concluded that the substitution of inorganic trace minerals (Cu, Zn, Mn and Fe) in laying hen diet with organic or nano trace minerals at 50 or 25% of requirement, respectively does not affect the egg quality characteristics, while reduced dietary trace minerals levels either through organic or nano forms decreases Zn and Fe content of egg yolk.

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