

# Effect of Dietary Supplementation of Organic Trace Minerals at Reduced Concentrations on Performance, Bone Mineralization, and Antioxidant Variables in Broiler Chicken Reared in Two Different Seasons in a Tropical Region

Rama Rao Savaram [Ven](https://orcid.org/0000-0003-2712-6546)kata<sup>1</sup> (D · Prakash Bhukya<sup>1</sup> D · Mantena Venkata Lakshmi Narasimha Raju<sup>1</sup> D · Rajkumar Ullengala<sup>1</sup>

Received: 21 May 2020 / Accepted: 8 November 2020 / Published online: 20 November 2020  $\odot$  Springer Science+Business Media, LLC, part of Springer Nature 2020

## Abstract

Two experiments were conducted to study the performance, antioxidant activity, and bone mineral variables in broilers fed organic trace minerals (OTM) at lowered concentrations in the diet. In experiment 1, a total of 1500 day-old broiler male chicks were randomly distributed into six groups with 10 replicates of 25 chicks each and housed in floor pens  $(1.90 \times 1.22 \text{ m})$ . One group was fed the maize-soybean meal–based control diet, supplemented with inorganic trace mineral (ITM) premix containing Mn, Zn, Fe, Cu, Se, and Cr at 50, 45, 40, 7.5, 0.30, and 0.25 mg/kg, respectively. The remaining groups of chicks were fed the diets, where the ITM premix was replaced with OTM mix so as to provide the respective minerals at graded levels in the diet (100, 75, 50, 40, and 30% of the control diet). Similarly, in experiment II, a total of 1350 day-old male broiler chicks were divided at random into six groups with 9 replicates of 25 chicks each and fed the maize-soybean meal–based control diet having ITM at levels similar to those of experiment I or the test diets, where the ITM was replaced with OTM so as to provide the minerals at 100, 80, 60, 40, and 20% of the ITM-based control diet. Each diet was fed ad libitum from 0 to 42 days of age. The body weight gain, feed intake, and feed efficiency were not affected by supplementing OTM at 30 and 20% in experiments I and II, respectively. Slaughter variables, activities of glutathione peroxidase and super oxide dismutase, and lipid peroxidation and ferric reducing activity in serum were not affected by supplementing OTM at the lowest level of 20% in the diet. Similarly, bone-breaking strength, ash, and Mn contents were not affected by supplementing OTM at 20% of the mineral concentration in control diet containing ITM. Deposition of Ca, P, Cu, and Fe in tibia ash increased with increased concentration of OTM in the diet. However, Zn content in tibia ash reduced with reduction in OTM level in the diet. Based on the results, it is concluded that trace mineral supplementation in organic form even at 20% of the concentration recommended for ITM may be sufficient in maize-soybean meal–based diet to support the optimum growth, bone ash, bone strength, and serum antioxidant status in commercial broilers.

Keywords Organic trace minerals . Performance . Antioxidants . Broiler chickens

 $\boxtimes$  Prakash Bhukya [drbhukyaprakash@gmail.com](mailto:drbhukyaprakash@gmail.com)

> Rama Rao Savaram Venkata svramarao1@gmail.com

Mantena Venkata Lakshmi Narasimha Raju mvlnraju@gmail.com

Rajkumar Ullengala ullengala@yahoo.com

<sup>1</sup> ICAR-Directorate of Poultry Research, Rajendranagar, Hyderabad, Telangana 500030, India

# Introduction

Trace minerals play a vital role in metabolic processes in the body, which are critical to sustain production and ensure proper skeletal development [[1](#page-6-0)] in chicken. Inadequate intake and interactions among different trace minerals cause mineral deficiency in chicken. Conventionally, inorganic minerals are used in poultry diet for reasons of easy accessibility and low price. However, it has been documented that absorption of inorganic trace minerals (ITM) in chickens is low [[2\]](#page-6-0) and the bioavailability of Cu, Zn, Mn, Se, and Cr from organic source is higher than the ITM [[3](#page-6-0)–[6](#page-6-0)]. The organic trace minerals (OTM) are tagged to small peptides/organic compounds

and have higher bioavailability [[7\]](#page-6-0). The bioavailability of OTM is 1.2 to 1.85 times higher compared to that of ITM [\[8](#page-6-0)]. The performance of chicken fed organic form of Se [[9,](#page-6-0) [10\]](#page-6-0), Cr [\[11](#page-6-0), [12\]](#page-6-0), and other trace (Cu, Zn, Mn) minerals [\[4,](#page-6-0) [5\]](#page-6-0) was reported to be higher or equal when the minerals were included at lower concentrations compared to those fed the recommended levels of ITM in the diet. Organic form of trace minerals (OTM) exerts benefits through modulating (Se) the antioxidant system [[13](#page-6-0)], resistant to antagonisms (Zn vs Cu) in the gastrointestinal tract due to stronger chemical (coordinate-covalent) bonds [[14](#page-6-0)], and also upregulating (Se) transcription of genes encoding energy-associated mitochondrial proteins and protein synthesis [[15\]](#page-6-0).

The trace mineral requirements suggested [[3\]](#page-6-0) for broiler are mostly based on research work conducted using inorganic forms. The bioavailability of minerals from organic source is higher than from inorganic sources [\[4,](#page-6-0) [5,](#page-6-0) [9](#page-6-0), [16\]](#page-6-0). The use of OTM in poultry diet gained wider acceptability due to several advantages associated with their use as mentioned before. Therefore, the OTM are added at a much lower concentration in the diet [[10\]](#page-6-0), without any negative effect on performance. This will further lead to reduced mineral excretion with eventual reduced environmental pollution [[17](#page-6-0)]. Large volume of information is available about the beneficial effects of supplementing single organic trace mineral at a time on different production, immune, and antioxidant variables in broilers. However, limited information is available on feeding of diets supplemented with cocktail of different OTM in broilers during different seasons. In majority of the tropical regions, the broilers are reared in open-sided poultry houses and the variations in environmental temperature and humidity directly impact the physiology and performance of broilers. The broilers have limited ability to dissipate heat at higher ambient temperature and relative humidity. This leads to physiological changes that are accompanied by reduction in feed intake (FI) so as to reduce metabolic heat production [\[18\]](#page-6-0), which results in lowered performance [[19\]](#page-6-0). Heat stress reduces the FI and supplementing the TM at the standard concentration during summer period may not meet the broiler requirement. Hence, the minerals with higher bioavailability (i.e., OTM) could be of great choice to support the performance and immune response, and reduce antioxidant stress in tropical conditions [\[10](#page-6-0)–[12,](#page-6-0) [20\]](#page-6-0).

Considering the higher bioavailability of OTM [[10](#page-6-0), [21,](#page-6-0) [22\]](#page-6-0), it is hypothesized that the requirement of TM may be reduced proportionately to realize the advantage of the higher bioavailability in organic form compared to the suggested mineral requirement. Information regarding the effect of lower levels of OTM complex on immune responses, bone mineralization, and antioxidant responses in broiler is limited. Therefore, two experiments were conducted in two different seasons to investigate the effect of feeding reduced concentrations of OTM on performance, bone strength, mineral deposition, and serum antioxidant responses in broilers reared in a tropical region.

## Materials and Methods

## Experimental Birds, Management, and Diets

Two experiments were conducted on broilers (Cobb 400) to study the hypothesis. A total of 1500 day-old male broiler chicks were randomly distributed into six experimental groups with 10 replicates of 25 chicks each in experiment I, which was conducted during summer season (23.2 to 38.6 °C). Experiment II was conducted during early monsoon season (16.8 to 25.4 °C), where a total of 1350 day-old male broiler chicks were randomly distributed into 6 groups with 9 replicates of 25 chicks each. The chicks of both the experiments were housed in litter floor pens  $(1.90 \times 1.22 \text{ m})$ . The brooder temperature was maintained at about  $36 \pm 0.5$  °C until 7 days of age and gradually decreased to room temperature by 21 days of age. Broilers were vaccinated against Newcastle (6th and 25th days) and infectious bursal diseases (12th and 17th days). The experimental protocols were approved by the Institute Animal Ethics Committee (IAEC/DPR/17/1; 21/10/ 2017).

Maize and soybean meal–based basal diet, devoid of supplemental trace minerals, was prepared to contain 2950 kcal ME/kg and 22.5% CP during starter phase (1 to 21 days) and 3150 kcal ME/kg and 20.0% CP in finisher phase (22 to 42 days) (Table [1\)](#page-2-0) and served as the basal diet.

In experiment 1, a total of 1500 day-old broiler male chicks were randomly distributed into six groups with 10 replicates of 25 chicks each and housed in floor pens  $(1.90 \times 1.22 \text{ m})$ . One group was fed the maize-soybean meal–based control diet, supplemented with ITM containing Mn, Zn, Fe, Cu, Se, and Cr at 50, 45, 40, 7.5, 0.30, and 0.25 mg/kg, respectively. The remaining groups of chicks were fed the diets, where the ITM premix was replaced with OTM mix so as to provide the respective minerals at graded levels in the diet (100, 75, 50, 40, and 30% of the control diet). Similarly, in experiment II, a total of 1350 day-old male broiler chicks were divided at random into six groups with 9 replicates of 25 chicks each and fed the maize-soybean meal–based control diet having ITM at levels similar to those of experiment I or the test diets, where the ITM was replaced with OTM so as to provide the minerals at 100, 80, 60, 40, and 20% of the ITM-based control diet (Table [2\)](#page-2-0). The concentrations of Zn, Mn, Fe, Cu, Se, and Cr in both trace mineral premixes and compounded diets were analyzed with an atomic absorption spectrophotometer (AAnalyst 400, Perkin Elmer, Shelton, CT, USA) and required quantities of the premixes were added in the diets to get the required concentrations of trace minerals in the test diets.

<span id="page-2-0"></span>**Table 1** Ingredient and nutrient composition  $(g/1000 g)$  of broiler starter (1–21 days) and finisher (22–42 days of age) diets



<sup>1</sup> Supplied per kg of diet: retinol acetate 9200 IU, cholecalciferol 1200 ICU, α-tocopherol 10 IU, thiamin 1 mg, pyridoxine 2 mg, cyanocobalamine 0.01 mg, niacin 15 mg, pantothenic acid 10 mg, riboflavin 10 mg, biotin 0.08 mg, menadione 2 mg, choline 650 mg, copper 8 mg, iron 45 mg, manganese 80 mg, zinc 60 mg, selenium 0.18 mg, monensin sodium 50 mg, and hydrated sodium calcium aluminosilicate 800 mg

 $2^2$ Calculated concentrations.  $3^2$ Calculated based on analyzed ingredient composition

#### Performance and Slaughter Parameters

Body weight (BW) and FI were recorded at 14-day intervals (0, 14, 28, and 42 days of age) and feed efficiency (FE) was calculated as the BW gain (BWG) per unit FI. At the end of experiment II, one broiler from each replicate representing the mean BW was slaughtered by cervical dislocation to record slaughter parameters. Ready to cook yield and relative weights of abdominal fat, liver, and breast were recorded and expressed in relation to the pre-slaughter live BW of the respective broiler.

#### Bone Mineralization

The tibia bone was dissected from the carcasses of broiler slaughtered in experiment II and the soft tissue was freed from the bone. The bones were soaked in petroleum ether for 48 h and dried in a hot air oven at 100 °C for 3 h to remove fat. The right tibia of each broiler was used to determine the breaking strength, which was measured using a 3-point method with a universal testing machine (EZ Test, Shimadzu, Japan). The bone was placed on two points with a gap of 50 mm and pressure was applied with pressure sensitive load cell (490 N) at the center of the two points, which coincided with the middle of the bone with a speed of 5 cm per minute. Both the tibias of a broiler were subjected for ashing at  $600 \pm 20$  °C for 6 h.

The bone ash was dissolved in hydrochloric acid (1:10) and filtered through a filter paper (Whatman® Grade No. 1), and the final volume was made to 250 ml with Milli-Q water (Millipore Corporation, Bedford, MA, USA). The mineral aliquot was estimated for P using molybdovanadate reagent [[23\]](#page-6-0), and Ca, Zn, Mn, Fe, and Cu using an atomic absorption spectrophotometer (AAnalyst 400, PerkinElmer, Shelton, CT, USA). The atomic absorption spectrophotometer was calibrated with various concentrations of the Ca, Zn, Mn, Fe, and Cu standards. The standards and samples were positioned and aspirated through a nebulizer. The concentration of each element was retrieved using the software provided with the equipment.

#### Stress Parameters

Blood samples were collected from one broiler per replicate at 42 days of age during experiment II for measuring activities of glutathione peroxidase (GSHPx) and super oxide dismutase (SOD) enzymes. Approximately 3 ml of blood from each broiler was drawn at the 35th day of age from the brachial vein into a centrifuge tube containing citrate (citrate 0.8 g 100 ml) buffer (1.5 ml 10 ml blood) for erythrocyte separation and enzyme estimation. Blood samples were centrifuged at

Table 2 Analyzed and calculated concentrations (mg/kg) of trace minerals in various experimental diets



The values given in parenthesis are analyzed concentrations of the minerals

 $500\times g$  for 15 min at 4 °C to separate the buffy coat and RBC pellet. The RBC was washed thrice with PBS (pH 7.4). The RBC pellet was mixed with an equal volume of PBS and then lysed using distilled water (RBC:distilled water 1:20). The same procedure was applied for quantifying the lipid peroxides [\[8\]](#page-6-0). The degree of peroxidation was expressed as nanomoles of malondialdehyde (MDA)/mg protein. The ferric reducing activity in plasma (FRAP) was estimated through a standard protocol [\[24\]](#page-6-0).

#### Statistical Analysis

Statistical analyses were performed using SAS version 9.2 (SAS Institute Inc., Cary, NC, USA). The variations in the different parameters were analyzed using the oneway analysis of variance procedure, wherein different dietary treatments were designated as the fixed factors and different parameters were taken as the dependent variables. The statistical model of variance analysis used was as follows:

 $Yij = \mu + Ti + eij$ 

where  $\mu$  was the overall mean, Ti was the fixed effect of dietary treatment, and eij was the random error present in the jth observation on the ith treatment. On the detection of overall significant difference, the means of different treatments were separated using Tukey's test.

## Results

The analyzed concentrations of all the trace minerals in compounded diets were higher than the included levels, which could be due to contribution of the minerals from maize and soybean meal of the basal diets.

#### Experiment I

The performance variables (BWG, FI, and FE) were not affected  $(P > 0.05)$  during pre-starter  $(1-14 \text{ days})$  phase by replacing 100% ITM with 30% OTM (Table [3](#page-4-0)). Similarly, BWG and FE were not affected during 0– 28 days of age with inclusion of 30% OTM in place of 100% ITM in the diet. Though the FI  $(0-28$  and 0-42 days) was affected with dietary treatments, the trend was not clear to infer any specific development. The FE  $(0-48$  days) was significantly  $(P = 0.05)$  affected by the dietary treatments during 0 to 42 days of age. The FE in groups fed 100% OTM was significantly higher than in those fed 50% OTM and FE in other groups was intermediate between 100 and 50% OTM.

## Experiment II

#### Slaughter Variables

Replacement of 100% ITM with OTM at reduced concentration of up to 20% did not influence the slaughter variables like ready to cook yield and relative weights of the breast, liver, and abdominal fat (Table [4\)](#page-4-0).

### Bone Mineral Parameters

The total ash content, breaking strength, and Mn concentration in the tibia bone were not affected ( $P > 0.05$ ) by replacing 100% ITM with 20% OTM (Table [4\)](#page-4-0). Deposition of Ca and P in tibia ash increased significantly  $(P < 0.05)$  at the lowest concentration of OTM in the diet. Whereas, the concentrations of Cu and Fe in tibia ash of broilers fed OTM were either similar or higher than of those fed the ITM. The deposition of Zn in tibia ash was lower in broilers fed lower levels (< 80%) of OTM compared to that in those fed 100% ITM or OTM-based diets.

## Antioxidant Responses

Supplementation of OTM at reduced levels (up to 20% of recommended concentration) replacing 100% ITM did not affect  $(P > 0.05)$  lipid peroxidation, FRAP, and the activities of GSHPx and SOD in broilers (Table [5](#page-5-0)).

# **Discussion**

Though the difference was not significant, the FE in broilers fed on 100% OTM-supplemented diets showed an improvement over those fed on the ITM diet in experiment I but not in experiment II, which suggests the potential benefits are perceived by supplementing OTM during heat stress conditions. The previous results from our lab  $[5, 20]$  $[5, 20]$  $[5, 20]$  $[5, 20]$  $[5, 20]$  also indicate an improvement in broiler performance by supplementing the diets with OTM. Similarly, these results agree with the earlier reports in broiler [[25,](#page-6-0) [26](#page-6-0)] and a rural chicken variety (*Vanaraja*) [\[5](#page-6-0)], where significant improvement in chicken performance is reported when diets were supplemented with OTM. In the present study, the performance parameters were not affected with reduction in the levels of OTM in the diet. Similarly, the performance parameters were not affected with OTM supplementation in experiment II, which was conducted during pre-monsoon season (23.2 to 38.6 °C), when the ambient temperature was relatively lower than that of summer season (23.2 to 38.6 °C). The OTM supplementation is reported to improve the chicken performance compared to the inorganic form  $[10-12]$  $[10-12]$  $[10-12]$  $[10-12]$  $[10-12]$  and the response is more prominent during heat stress condition [\[20\]](#page-6-0). It is reported that the organic <span id="page-4-0"></span>Table 3 Performance of commercial broilers fed different concentrations of organic trace

minerals (OTM)



BWG body weight, FI food intake, FE food conversion efficiency, ITM inorganic trace minerals, SEM standard error of mean, N number of replicates

forms of Se, Cr, and Zn are known to enhance performance [\[9](#page-6-0)], antioxidant activity [[5,](#page-6-0) [11](#page-6-0)], and immune response [\[27\]](#page-6-0) in broiler.

Antioxidant and immune responses in broiler were reported to be affected during stress conditions [\[7,](#page-6-0) [28](#page-6-0)]. Furthermore,

heat stress increases mineral excretion [\[29](#page-6-0)] and the numerical improvement in FE observed in 100% OTM fed group could be due to the positive role of OTM compared to ITM. It is reported that the OTM are tagged to small peptides and have higher bioavailability [\[30](#page-6-0)]. The higher net availability of

Table 4 Carcass variables and bone mineral parameters in commercial broilers fed different concentrations of organic trace minerals (OTM) experiment II

Treatment	Slaughter parameters				Tibia parameters							
	<b>RTC</b>	<b>Breast</b> $g/1000$ g pre-slaughter weight	Liver	Abd fat	Ash $\%$	Strength N	Ca g/kg	P	Cu mg/kg	Mn	Zn	Fe
<b>ITM 100</b>	777.0	229.0	20.13	15.92	48.17	136	$336.5^{\circ}$	$167.8^{d}$	$8.66^{bc}$	13.79	$235.1^{ab}$	$255.0^{\circ}$
<b>OTM 100</b>	775.7	239.9	21.34	14.96	48.04	129	$334.6^{\circ}$	$166.4^d$	$10.38^{ab}$	13.11	$240.6^a$	$303.8$ <sup>abc</sup>
OTM <sub>80</sub>	758.3	229.1	19.83	13.66	47.19	134	$340.2^{\circ}$	169.8 <sup>cd</sup>	$8.27^{\circ}$	11.95	$206.5^{\text{cd}}$	$365.2^{\rm a}$
OTM <sub>60</sub>	770.1	233.0	21.69	14.86	47.60	145	$356.5^{b}$	$179.3^{b}$	$10.18^{\text{abc}}$	13.79	$211.3^{\text{cd}}$	$299.6$ <sup>abc</sup>
OTM <sub>40</sub>	765.9	226.4	20.43	15.66	48.10	132	$346.0^{bc}$	$175.3^{bc}$	$10.83^{\rm a}$	12.54	$219.7^{bc}$	$288.0^{bc}$
OTM <sub>20</sub>	775.4	236.4	19.17	16.23	47.62	128	$370.2^a$	186.7 <sup>a</sup>	10.00 <sup>abc</sup>	13.34	$199.7^{\rm d}$	$326.3^{ab}$
$P$ value	0.549	0.672	0.37	0.60	0.547	0.553	0.001	0.001	0.001	0.122	0.001	0.001
$\boldsymbol{n}$	9	9	9	9	9	9	9	9	9	9	9	9
<b>SEM</b>	3.250	2.570	0.360	0.440	0.200	2.7	1.80	1.00	0.190	0.230	2.530	5.900

ITM inorganic trace minerals, OTM organic trace minerals, SEM standard error mean, N number of replicates, RTC ready to cook yield, Abd fat abdominal fat

<span id="page-5-0"></span>Table 5 Antioxidant and immune responses in commercial broilers fed different concentrations of organic trace minerals (OTM) experiment II

Treatment	$FRAP$ ( $\mu$ M)	GSHPx (units/mL)	SOD (units/mL)	$LP$ (nmol MDA/mg protein)
<b>ITM 100</b>	1716	141.4	4.974	2.921
<b>OTM 100</b>	2907	134.6	4.311	2.771
OTM <sub>80</sub>	2507	135.2	3.790	2.440
OTM <sub>60</sub>	2077	138.7	6.529	2.514
OTM <sub>40</sub>	2246	137.8	2.773	2.192
OTM <sub>20</sub>	2363	137.5	4.708	2.610
$\overline{P}$	0.416	0.974	0.184	0.401
N	9	9	9	9
<b>SEM</b>	116.0	2.37	0.45	0.11

FRAP ferric reducing activity in plasma, GSHPx glutathione peroxidase, LP lipid peroxidation, N number of replicates, ITM inorganic trace minerals, OTM organic trace minerals, SEM standard error mean, SOD superoxide dismutase

essential minerals, particularly during stress, might have elicited better response in terms of improved FE during experiment I.

In both the experiments, the BWG and in experiment II the FE were not affected by reducing the TM concentration to 20% in organic form compared to those fed 100% ITM, which suggest the possibility of reducing the dietary levels of TM to about 20% of the standard recommended levels without affecting performance, slaughter variables, and antioxidant variables (lipid peroxidation, activities of GSHPx and SOD) in serum. The higher bioavailability of OTM  $(1.2 \text{ to } 1.85 \text{ times})$  compared to that of ITM [[22](#page-6-0)] might be responsible for sustaining the broiler performance and other parameters even at the sub-optimal levels of their inclusion in diets. The slaughter variables did not differ among the groups fed diets supplemented with graded levels of OTM and ITM. Similar results were reported earlier in broiler [\[31\]](#page-7-0).

However, the concentrations of Ca, P, Cu, and Fe in the bone ash increased in groups fed diets supplemented OTM compared to those in groups fed ITM. The contents of Ca and P in tibia ash were higher in broilers fed diets with lower levels of OTM. Similarly, it has been reported that retention of minerals in organic form is higher compared to their inorganic form [[32](#page-7-0), [33](#page-7-0)], which results in higher retention of minerals in target organs and lower excretion. Furthermore, the higher bioavailability of OTM is reported to reduce the interference and prevent the formation of insoluble complex with other related trace elements in the gut [[33\]](#page-7-0). Also, it was observed that the dietary supplementation of OTM at lower concentrations did not affect the performance and antioxidant responses in rural chicken (Vanaraja) [[5](#page-6-0)]. Increased tibia mineral concentration in chickens fed OTM compared to those fed ITM also confirms the hypothesis that mineral availability is higher in organic form than in inorganic form.

The activities of antioxidant enzymes (GSHPx and SOD) alter naturally for the sustenance and well-being of living organisms. Trace elements are essential components of the antioxidant system in any living being including broiler. In the present study, the activities of GSHPx and SOD, FRAP, and lipid peroxidation did not differ by reducing the concentrations of OTM up to 20%. This indicates that OTM even at the lower level of inclusion in the diet maintained the activities of antioxidant enzymes, ferric reducing ability of plasma, and lipid peroxidation in serum compared to those fed 100% ITM. The absence of negative effects on antioxidant system could be due to the higher bioavailability of OTM [[22](#page-6-0)] and therefore, the absolute availability of the minerals even at lower levels probably met the requirement. Results of the present study revealed increased Ca and P deposition and reduced Zn deposition among the groups fed lower supplemental levels of OTM compared to those groups fed ITM-based diets. Reasons for the reduced Zn deposition in tibia ash with reduction in dietary concentrations of OTM needs further research. Whereas, increased deposition of Ca and P was reported in broilers fed diets with lower levels of organic minerals during starter phase [[34\]](#page-7-0).

# Conclusions

Results of the present study indicate that the supplemental levels of trace minerals can be reduced considerably to 20% (Zn 9.0, Mn 10.0, Cu 1.50, Fe 8.0, I 0.4, Se 0.06, and Cr 0.05 mg/kg and the analyzed values of these minerals in the 20% OTM diet were Zn 19.94, Mn 22.02, Cu 4.11, Fe 25.62, Se 0.13, and Cr

<span id="page-6-0"></span> $0.12 \text{ mg/kg}$  of the recommended concentration when they were supplemented in the form of OTM in maize-soybean meal–based diet for supporting optimum performance and antioxidant activity in commercial broilers. Furthermore, supplementation of OTM at low concentrations significantly improved the feed efficiency of broilers reared during tropical summer season.

## Compliance with Ethical Standards

Conflict of Interest The authors declare that they have no conflict of interest.

# References

- 1. Branca FM, Ferrari M (2002) Impact of micronutrient deficiencies on growth: the stunting syndrome. Ann Nutr Metab 46:8–17
- 2. Van Der Klis JD (1999) Factors affecting the absorption of minerals from the gastro-intestinal tract of broilers. In: Proceedings 8th European Symposium on Poultry Nutrition. WPSA, Bologna, Italy
- 3. NRC (1994) Nutrient requirements of poultry, 9th rev edn. National Academic Science, Washington, DC
- 4. Aksu DS, Aksu T, Özsoy B, Baytok E (2010) The effects of replacing inorganic with a lower level of organically complexed minerals (Cu, Zn and Mn) in broiler diets on lipid peroxidation and antioxidant defense systems. Asian Australas J Anim Sci 8:1066–1072
- 5. Rama Rao SV, Prakash B, Kumari K, Raju MVLN, Panda AK (2013a) Effect of supplementing different concentrations of organic trace minerals on performance, antioxidant activity, and bone mineralization in Vanaraja chickens developed for free range farming. Trop Anim Health Prod. <https://doi.org/10.1007/s11250-013-0384-5>
- 6. Zhao J, Shirley RB, Dibner JJ, Wedekind KJ, Yan F, Fisher P, Hampton TR, Evans JL, Vazquez-Añon M (2016) Superior growth performance in broiler chicks fed chelated compared to inorganic zinc in presence of elevated dietary copper. J Anim Sci Biotechnol 7(13). <https://doi.org/10.1186/s40104-016-0072-1>
- 7. Sahin K, Sahin N, Onderci M, Yaralioglu S, Kucuk O (2001) Protective role of supplemental vitamin E on lipid peroxidation, vitamins E, A and some mineral concentrations of broilers reared under heat stress. Vet Med-Czech 46:140–144
- 8. Ohkawa Y, Ohishi N, Yagi K (1979) Assay for lipid peroxides in animal tissue by thiobarbituric acid reaction. Anal Biochem 95: 351–358
- 9. Prakash B, Rama Rao SV, Raju MVLN, Sreenivasa Reddy C (2019) Effect of supplementing selenized yeast on performance and anti-oxidant responses in Vanaraja and commercial broiler chickens. Indian J Anim Res 53(4):500–504
- 10. Rama Rao SV, Prakash B, Raju MVLN, Panda AK, Poonam S, Krishna Murthy O (2013b) Effect of supplementing organic selenium on performance, carcass traits, oxidative parameters and immune responses in commercial broiler chickens. Asian Australas J Anim Sci 26:247–252
- 11. Rama Rao SV, Raju MVLN, Panda AK, Poonam NS, Krishna Murthy O, Shyam Sunder G (2012) Effect of dietary supplementation of organic chromium on performance, carcass traits, oxidative parameters, and immune responses in commercial broiler chickens. Biol Trace Elem Res 147:135–141
- 12. Rajalekshmi M, Sugumar C, Chirakkal, Rama Rao SV (2014) Influence of chromium propionate on the carcass characteristics and immune response of commercial broiler birds under normal rearing conditions. Poult Sci 93:574–580
- 13. Surai PF, Kochish II (2019) Nutritional modulation of the antioxidant capacities in poultry: the case of selenium. Poult Sci 98(10): 4231–4239. <https://doi.org/10.3382/ps/pey406>
- 14. Richards JD, Fisher P, Evans JL, Wedekind KJ (2015) Greater bioavailability of chelated compared to inorganic zinc in broiler chicks in presence of elevated calcium and phosphorus. Open Access Anim Physiol 7:1–14
- 15. Brennan KM, Crowdus CA, Cantor AH, Pescatore AJ, Barger JL, Horgan K, Xiao R, Power RF, Dawson KA (2011) Effects of organic and inorganic dietary selenium supplementation on gene expression profiles in oviduct tissue from broiler-breeder hens. Anim Reprod Sci 125:180–188
- 16. Kamran S, Shariatmadari F, Karimi MAT, Ahmadi H (2017) Effect of zinc concentration and source on performance, tissue mineral status, activity of superoxide dismutase enzyme and lipid peroxidation of meat in broiler chickens. Anim Prod Sci 58(10):1837–1846. <https://doi.org/10.1071/AN15758>
- 17. Nollet L, van der Klis JD, Lensing M, Spring P (2007) The effect of replacing inorganic with organic trace minerals in broiler diets on productive performance and mineral excretion. J Appl Poult Res 16:592–597
- 18. Teeter RG, Smith MO, Owens FN, Arp SC, Sangiah S, Breazile JE (1985) Chronic heat stress and respiratory alkalosis: occurrence and treatment in broiler chicks. Poult Sci 64:1060–1064
- 19. Geraert PA, Padilha JCF, Guillaumin S (1996) Metabolic and endocrine changes induced by chronic heat exposure in broiler chickens: growth performance, body composition and energy retention. Br J Nutr 75:195–204
- 20. Rama Rao SV, Prakash B, Raju MVLN, Pradeep Reddy E (2016) Effect of supplementing organic forms of zinc, selenium and chromium on performance, anti-oxidant and immune responses in broiler chicken reared in tropical summer. Biol Trace Elem Res 172(2): 511–520
- 21. Yan F, Waldroup PW (2006) Evaluation of Mintrex manganese as a source of manganese for young broilers. Int J Poult Sci 5:708–713
- 22. Wang Z, Cerrate S, Coto C, Yan F, Waldroup PW (2007) Evaluation of Mintrex copper as a source of copper in broiler diets. Int J Poult Sci 5:308–313
- 23. Fiske CH, Subbarow Y (1925) The colorimetric determination of phosphorous. Biol Chem 66:375–400
- 24. Benzie IFF, Strain JJ (1996) The ferric reducing ability of plasma (FRAP) as a measure of 'antioxidant power': the FRAP assay. Anal Biochem 239:70–76
- 25. Saenmahayak B, Bilgili SF, Hess JB, Singh M (2010) Live and processing performance of broiler chickens fed diets supplemented with complexed zinc. J Appl Poult Res 19:334–340
- 26. Zhao J, Shirley RB, Vazquez-Anon M, Dibner JJ, Richards JD, Fisher P, Hampton T, Christensen KD, Allard JP, Giesen AF (2010) Effects of chelated trace minerals on growth performance, breast meat yield, and footpad health in commercial meat broilers. J Appl Poult Res 19:365–372
- 27. Georgieva NV, Gabrashanska M, Koinarski V, Yaneva Z (2011) Zinc Supplementation against Eimeria acervulina-induced oxidative damage in broiler chickens. Vet Med Int. [https://doi.org/10.](https://doi.org/10.4061/2011/647124) [4061/2011/647124](https://doi.org/10.4061/2011/647124)
- 28. Ramnath V, Rekha PS, Sujatha KS (2008) Amelioration of heat stress induced disturbances of antioxidant defense system in chicken by Brahma rasayana. Evid Based Complement Alternat Med 5(1):77–84. <https://doi.org/10.1093/ecam/nel116>
- 29. Siegel HS (1995) Stress, strains and resistance. Br Poult Sci 36:3– 22
- 30. Webb KE, Wong EA, Pan YX, Chen H, Poole CA, Van L, Klang JE (2005) The role of peptides in absorption pathways. In: Taylor-Pickard JA, Tucker LA (eds) Redefining Mineral Nutrition. Nottingham University Press, Nottingham, pp 197–225
- <span id="page-7-0"></span>31. Sheikh A, Tufail B, Gulam AB, Mir S, Mashuq R, Syed S (2011) Response of broiler chicken to dietary supplementation of organic. J Cent Eur Agric 12:498–508
- 32. Bao YM, Choct M, Iji PA, Bruerton K (2007) Effect of organically complexed copper, iron, manganese, and zinc on broiler performance, mineral excretion, and accumulation in tissues. J Appl Poult Res 16:448–455
- 33. Ma WQ, Sun H, Zhou Y, Wu J, Feng J (2012) Effects of iron glycine chelate on growth, tissue mineral concentrations, fecal

mineral excretion, and liver antioxidant enzyme activities in broilers. Biol Trace Elem Res 149:204–211

34. Flaria BD, Silva LM, Junior VRN, Ferreira AH, Rostagno HS, Albino LFT, Hannas MI (2020) Organic trace minerals and calcium levels in broilers' diets to 21 days old. Sci Agric. [https://doi.org/10.](https://doi.org/10.1590/1678-992X-2018-0071) [1590/1678-992X-2018-0071](https://doi.org/10.1590/1678-992X-2018-0071)

Publisher's Note Springer Nature remains neutral with regard to jurisdictional claims in published maps and institutional affiliations.