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# Organic Trace Minerals at Lower Concentrations Can Replace Inorganic Trace Mineral Premix in Broiler Chicken Diet

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## ABSTRACT

An experiment was conducted to study the performance, immune responses, and bone mineral variables in broiler chickens fed organic trace minerals (oTM) at suboptimal concentrations in the diet. A total of 2080 day-old broiler male chicks were randomly distributed into floor pens (6.25 × 4 feet) at the rate of 26 birds per pen. Maize-soybean meal-based control diet was supplemented with inorganic trace mineral premix (iTM; Fe 100, Zn 100, Mn 100, Cu 20, Se 0.50, I 2.5/mg/kg). The iTM premix was replaced in the test diets with oTM to provide minerals at graded levels in the diet (10, 17.5, and 25% of the control diet). Each diet was fed *ad libitum* to a total of 20 replicates of chicks from 1 to 42 days of age. The body weight gain (BWG) was not affected by supplementing oTM at 20% during 1-14 and 1-28 d of age. Both BWG and feed conversion ratio (FCR) were significantly reduced with an increase in oTM $\geq$35%. Both the production variables in groups fed oTM at 20% were similar to those fed the iTM. The relative weight of breast meat was lower ($p<0.05$) and liver weight was higher ($p<0.05$) in groups fed the higher concentration of oTM (50%) than the iTM group. Similarly, tibia breaking strength and P content in tibia ash in oTM 20% were similar to broilers fed on iTM. Tibia ash and Ca content in tibia ash were not affected by dietary variation in the mineral source (iTM vs oTM) and organic mineral concentration. Based on the results, it is concluded that trace mineral supplementation can be reduced to 20% when fed in organic form in broiler chicken diet to support the optimum growth, mineralization, and HI titer to ND vaccine.

**KEYWORDS:** Organic trace minerals, Inorganic trace minerals, Weight gain, Feed efficiency, Bone parameters, Immune response, Broiler chicken

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## INTRODUCTION

Trace minerals are essential in several metabolic processes in the body, which are required to sustain production, skeletal development, cell oxidation, and immune responses (Branca and Ferrari, 2002) in chickens. Trace minerals are traditionally supplemented in inorganic salt form due to economic reasons and ease of procurement but, are less biologically available (Van der Klis, 1999) to the birds and cause environmental pollution. The higher concentration of minerals in poultry excreta can lead to soil and groundwater contamination (Nollet et al., 2007), a scarce resource in the tropical region. Therefore, the research aiming at more bioavailable minerals that enable higher utilization by the poultry and minimize the lesser excretion to the environment by intensive poultry farming is gaining importance worldwide. Another limitation of using inorganic forms of minerals is potential interaction among the trace minerals, which sometimes leads to mineral deficiency in chicken-fed mineral-balanced diets. On the contrary, the mineral availability from organic/ chelated sources of minerals (oTM) is higher than the inorganic trace minerals (iTM) (Aksu et al., 2010, Rama Rao et al., 2013[a], Prakash et al., 2019). Further, the higher availability of oTM leads to reduced excretion of the minerals from birds and minimized contamination of soil and groundwater.

The research data based on inorganic forms of the trace minerals form the basis for arriving at the requirement standards suggested (NRC, 1994) for chicken. Higher inclusion levels of iTM lead to mineral wastage and environmental pollution due to excretion by birds (Leeson, 2003). Considering the advantage of higher bio-availability of oTM (Yan and Waldroup, 2006, Wang et al., 2007; Rama Rao et al., 2013[b]), it is presumed that the requirement of TM can be reduced considerably to realize the advantage of the higher mineral bio-availability from the oTM compared to the standard mineral requirements suggested. Information regarding the effect of lower levels of oTM on immune responses, bone mineralization, and carcass yield in chicken is limited (Rama Rao et al., 2021), which was based on the limited number of replications (Rama Rao et al., 2013[b]). Therefore, the current experiment was conducted using a higher number of replications to find out the effect of feeding the reduced concentrations of oTM on performance, bone mineralization, immune responses, and carcass yield in commercial broiler chickens.

## MATERIALS AND METHODS

### Premix analysis for trace minerals

The concentrations of trace minerals i.e. Zn, Mn, Fe, Cu, Se, and Iodine in inorganic trace mineral premix and organic trace mineral premix were estimated using Atomic Absorption Spectrophotometer (AAnalyst 400, PerkinElmer, Shelton, CT, USA) according to methods suggested by the manufacturer. A specific lamp was used for each mineral and the Atomic Absorption Spectrophotometer was calibrated with various concentrations of the mineral standards.

### Experimental Birds, Management and Diets

Day-old broiler (*Cobb* 430 Y) male chicks (N=2080) were procured from a commercial hatchery (Venkateswara Hatcheries, Pvt. Ltd, Hyderabad, India) and randomly distributed into four treatment groups. Each treatment was allotted to 20 replicates with 26 chicks in each pen (6.5 × 4’). Birds were maintained in floor pens with a floor space of 1.0 $ft^2$ per bird. Brooding was provided with incandescent bulbs and additional heat was provided with coal during the initial 3 weeks of age. The room temperature was maintained at about 37 °C during the first week and subsequently, the temperature was gradually reduced to the ambient temperature at day 21, after which the birds were reared at ambient temperature (20.7+3.17 to 30.8+3.84 °C). The experiment was conducted by following the guidelines of the Institute Animal Ethics Committee (IAEC/DPR/17/1: 21/10/2017).

### Feeding regimen and performance

A three-phase feeding regime (starter: 1 to 14 days), (grower: 15-28 days) and (finisher: 29 to 42 days) was followed. Maize-SBM-meat and bone meal-based basal diets were provided *ad libitum* from 1-42 days. Metabolizable energy and crude protein levels in starter, grower, and finisher diets were 3000, 3100, and 3150 kcal/kg and 22.5, 21, and 19%, respectively (Table 1). The diets were formulated as per the (NRC, 1994) recommendations for all the minerals. The oTM premix was procured from a commercial source (P-Min, Varsha Agro Tech, Bengaluru, India), which contained a mixture of trace minerals in chelated form (Zn 50, Fe 50, Cu 5, Se 0.3, Mn 50, Cr 0.5, and I 3g/kg). The control diet was mixed with a commercial premix containing inorganic trace minerals (Trouw Poultry Min Fe 100, Zn 100, Mn 100, Cu 20, Se 0.50, I 2.5/mg/kg) (Trouw Nutrition). Another three diets were prepared by supplementing the basal diet with 3 concentrations of oTM mix to represent 10 (Fe 10, Zn 10, Mn 10, Cu 2, Se 0.05, I 0.25/mg/kg), 17.5 (Fe 17.5, Zn 17.5, Mn 17.5, Cu 3.5, Se 0.88, I 0..44/mg/kg), and 25% (Fe 25, Zn 25, Mn 25, Cu 5, Se 0.125, I 0.63/mg/kg) of the levels in the iTM mix. Concentrations of metabolizable energy, essential amino acids, calcium, and available phosphorus were maintained uniformly in all the diets in each phase. Each of the diets was randomly assigned to 20 replicate pens (201 × 122 cm, 26 birds/pen) and fed *ad libitum* from day 1 to 42. Feed intake (FI) and body weight (BW) were recorded per pen at day 1, 14, 28, and 42 of age and feed conversion ratio (FCR) was calculated as FI/ body weight gain (BWG). The weight of dead birds was recorded as and when the birds died. The weight of dead birds was used while calculating the FCR of the respective pen. Immunization against Newcastle disease (ND) live attenuated Lasota strain (Lasota) and infectious bursal disease (IBD Intermediate, Venkye’s India Pvt Ltd, Pune, India) live attenuated strain (intermediate) was carried out on days 7, 28, and 14 of age, respectively.

Table 1. Ingredient and nutrient composition (g/1000g) of broiler diets

| Ingredient | Pre starter (1-14d) | Starter (15-28d) | Finisher (29-42d) |
|---|---|---|---|
| Maize | 566.5 | 611.1 | 662.6 |
| oil-veg | 26.1 | 33 | 31.360 |
| Soya DOC 45% | 336.1 | 289.4 | 243.9 |
| Meat cum bone meal | 40.0 | 40.0 | 40.0 |
| Salt | 3.666 | 3.667 | 3.665 |
| Sodium bicarbonate | 1.000 | 1.000 | 1.000 |
| Dicalcium phosphate | 10.44 | 8.51 | 5.94 |
| LSP-powder | 7.351 | 5.338 | 4.349 |
| DL-Methionine | 3.259 | 2.671 | 2.199 |
| L-Lysine HCL | 2.187 | 2.034 | 2.033 |
| L-Threonine | 0.659 | 0.386 | 0.216 |
| Choline Chloride, 75% | 1.000 | 1.000 | 1.000 |
| Vitamin Premix | 0.500 | 0.500 | 0.500 |
| Trace Mineral Mixture | 1.000 | 1.000 | 1.000 |
| Nutrient | | | |
| M.E (Kcal/kg) | 3000 | 3100 | 3150 |
| Protein (%) | 22.59 | 20.77 | 19.09 |
| Dig. Lysine (%) | 1.250 | 1.120 | 1.010 |
| Dig.Methionine(%) | 0.640 | 0.560 | 0.490 |
| Dig TSAA (%) | 0.929 | 0.832 | 0.750 |
| Calcium (%) | 0.880 | 0.760 | 0.660 |
| Available Phosphorus (%) | 0.420 | 0.380 | 0.330 |
| Sodium (%) | 0.180 | 0.180 | 0.180 |
| Dig. Threonine (%) | 0.829 | 0.742 | 0.669 |
| dig. Leucine (%) | 1.734 | 1.630 | 1.536 |
| dig. Iso-leucine (%) | 0.842 | 0.764 | 0.690 |
| dig. Valine | 0.967 | 0.865 | 0.787 |

[1] Supplied per kg of diet: retinol acetate 2.75 mg, cholecalciferol 0.03 mg, á tocopherol 10 mg, 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]calculated concentrations; [3]calculated based on analysed ingredient composition.

### Carcass traits

Carcass traits including ready-to-cook yield (RTC), and relative weights of breast meat, liver, and abdominal fat were recorded by slaughtering 2 birds per replicate at day 43. The birds weighing nearer the mean (±5%) of each replicate were selected for slaughter traits. The RTC includes the edible portion of the carcass with giblet (gizzard, liver, and heart), which was expressed in grams in proportion to the kg live weight of the respective bird.

### Bone mineralization

One bird from each replicate was slaughtered at 43rd day age to study the bone mineral variables of the tibia and sternum (breaking strength, tibia ash, calcium, and phosphorus in tibia ash). The bones were freed of soft tissue and dried at 70 $^{o}$C/3h. The bone samples were defatted by soaking in petroleum ether for 48h. The right tibia and sternum of each bird were used to determine the breaking strength. The strength was measured using a 3-point method with a universal testing machine (EZ Test, Shimadzu, Japan). The bone was rested on two points with a gap of 50 mm and pressure was applied with a pressure-sensitive load cell (10 kg) at the center of the two points, which coincided with the center of the bone at a speed of 5 cm per minute. The tibiae and sternum were ashed independently at 600$\pm$20 $^{0}$C/2h. The bone ash was dissolved in hydrochloric acid (1:10) and the mineral aliquot was estimated for P using molybdovanadate reagent (Fiske and Subbarow, 1925). The bone ash was estimated for Ca using an Atomic Absorption Spectrophotometer (AA Analyst 400, PerkinElmer, Shelton, CT, USA) according to methods suggested by the manufacturer. A specific lamp was used for Ca estimation and the Atomic Absorption Spectrophotometer was calibrated with various concentrations of the mineral standards.

### Immune responses

Cell-mediated immunity (CMI) and humoral immunity (HI) (antibody response against Newcastle disease vaccine) were studied. The broilers were vaccinated against ND by ocular route at 7 and 28 d of age with Lasota strain (ND Lasota Vac-500, Indovax Pvt., Ltd., Hyderabad, India). At 20 d of age, 2 mL of blood was collected from one bird per replicate and the antibody titers in sera against Newcastle disease vaccine were measured (Rama Rao et al., 2021) by haemagglutination test.

### Statistical analysis

The data was checked for normality and equality of variance using the Shapiro-Wilk test and Lavene's test, respectively. The data that did not meet the normality and equality requirements were square root transformed before statistical analysis. The variations in data of different parameters were analyzed using the general linear model procedure of SAS version 9.2 (2008; SAS Institute Inc., Cary, North Carolina, USA). The statistical model of analysis of variance was the following:

$$Yij = \mu + Ti + eij$$

Where µ is the overall mean, Ti is the fixed effect of ith treatment and eij is the random error. Treatment means were compared using Tukey's test.

## RESULTS AND DISCUSSION

### Trace mineral concentration in premixes

The concentrations of trace minerals analysed in oTM were close to the concentrations indicated in the premix. The concentrations of Zn, Fe, Cu, Se, Mn, and I were 53, 59, 5.5, 0.32, 56, and 3.1g/kg, respectively. While the concentrations of the respective minerals in iTM premix were 105, 95, 110, 22, 0.46, 2.3/mg/kg.

Groups fed on the lowest oTM (10%) supplemented diet showed similar performance (BWG and FCR) compared to the iTM 100% fed birds and the broiler performance was significantly reduced in groups fed higher concentrations of oTM (17.5 or 25%) compared to the CD (Table 2). The results suggest that oTM at lower concentrations (10% of iTM) achieved the FCR of 100% iTM, whereas the higher concentrations of oTM (17.5 and 25%)) tested reduced the broiler performance. These results agree with the earlier reports with broilers (Saenmahayak et al., 2010, Zhao et al., 2010) and a rural chicken variety (*Vanaraja*) (Rama Rao et al., 2013[a]), where significant improvement in chicken performance was reported when diets were supplemented with oTM. In the present study, the performance was improved significantly in broilers fed the lower levels of oTM. It is reported that the organic form of Se and Zn are known to enhance performance (Prakash et al., 2019), serum antioxidant variables (Rama Rao et al., 2013[a]), and immune response (Georgieva et al., 2011) in chicken. These results are in line with Bao et al. (2007), who reported improved performance in broilers fed a diet containing moderate concentrations of organic minerals, close to the concentrations used in the present study (Cu 4.0, Fe 40.0, Mn 40.0, and Zn 40 mg/kg), which were lower than those recommended by the NRC, (1994). It can be speculated as the reason for the depressed performance observed in broilers fed the higher concentrations of oTM (17.5 and 25%) than those fed the lower concentrations (10%). The first reason is that even though there is no significant variation in FI among the treatment groups, mineral intake varies due to variable quantities of oTM provided in the test diets which resulted in a higher intake of minerals in broilers fed 17.5 or 25% diets than those fed with

10% oTM. Secondly, absorption, transportation, and deposition of minerals are reported to be higher when they are included in organic form. The higher net availability of essential minerals might have elicited a better response in terms of improved BWG and feed efficiency. The bioavailability of oTM is about 1.2 to 1.85 times higher compared to the iTM (Zhao et al., 2010) in broiler chickens.

Table 2. Performance of broiler chicken fed oTM mix in place of iTM premix

| Treatment | Pre-Starter (1-14d) | | Starter (1-28d) | | Finisher (1-42d) | |
|---|---|---|---|---|---|---|
| | BWG | FCR | BWG | FCR | BWG | FCR |
| iTM | 416.8 | $1.182^{A}$ | 1483 | 1.443 | $2372^{A}$ | $1.666^{C}$ |
| oTM-10 | 424.1 | $1.170^{B}$ | 1486 | 1.440 | $2370^{A}$ | $1.670^{BC}$ |
| oTM-17.5 | 423.7 | $1.173^{AB}$ | 1463 | 1.450 | $2326^{B}$ | $1.686^{AB}$ |
| oTM-25 | 421.0 | $1.178^{AB}$ | 1455 | 1.458 | $2321^{B}$ | $1.690^{A}$ |
| P | 0.660 | 0.071 | 0.470 | 0.199 | 0.025 | 0.014 |
| N | 20 | 20 | 20 | 20 | 20 | 20 |
| SEM | 2.287 | 0.002 | 8.214 | 0.003 | 7.869 | 0.003 |

BWG body weight gain; FI feed intake; P probability; N number of replicates; SEM standard error mean; iTM inorganic trace minerals; oTM organic trace minerals

$^{ABC}$ means having different superscripts in a column differ significantly (P<0.05)

Similar to the current findings, Faria et al. (2020) reported reduced BWG in broilers fed higher concentrations of oTM (920g/ton) compared to those fed the lower concentration (820 g/kg) oTM. The literature and the current data suggest the need to reduce the inclusion levels of TM when included as organic form.

The RTC (ready-to-cook) yield and relative weight of abdominal fat were not affected (P>0.05) by the dietary treatments (Table 3). The breast meat weight decreased, and the relative weight of the liver increased with an increase in the concentration of oTM in the diet. Similar results were reported earlier (Sheikh et al., 2011; Rama Rao et al., 2021).

Table 3. Slaughter variables (g/kg live weight) of broiler chicken fed oTM mix in place of iTM premix

| Treatment | RTC | Breast | Abdfat | Liver |
|---|---|---|---|---|
| iTM | 718.4 | $254.4^{A}$ | 11.05 | $20.47^{B}$ |
| oTM-10 | 708.1 | $257.4^{A}$ | 9.474 | $21.34^{B}$ |
| oTM-17.5 | 697.4 | $242.4^{AB}$ | 9.059 | $22.02^{B}$ |
| oTM-25 | 697.6 | $237.8^{B}$ | 8.932 | $25.07^{A}$ |
| P | 0.138 | 0.026 | 0.528 | 0.017 |
| N | 20 | 20 | 20 | 20 |
| SEM | 3.715 | 2.751 | 0.557 | 0.561 |

RTC ready to cook yield; Abdfat abdominal fat; P probability; N number of replicates; SEM standard error mean; iTM inorganic trace minerals; oTM organic trace minerals

$^{ABC}$ means having different superscripts in a column differ significantly (P<0.05)

The CMI response to PHA-P was not affected by the replacement of iTM with lower levels of oTM in the diet (Table 4). The antibody titer against ND vaccine in groups fed the lowest concentrations of oTM (10% oTM/ton) was similar (P-=0.001) to those fed 100% iTM. However, higher concentrations of oTM (17.5 and 25%) in the broiler diet significantly reduced the humoral immune response compared to the group fed on iTM based diet. Similar to these findings, Shawkat et al. (2018) reported that the relative weight of a lymphoid organ (spleen) in broilers fed the lower concentrations of oTM (375g/kg) was similar to those fed higher levels of TM when included as iTM.

Table 4. Immune responses in broiler chicken fed graded concentrations of oTM in place of iTM premix

| Treatment | CMI, % | ND titre, log 2 |
|---|---|---|
| iTM | 59.25 | 8.20[a] |
| oTM-10 | 64.30 | 7.95[ab] |
| oTM-17.5 | 60.00 | 7.55[b] |
| oTM-25 | 62.00 | 7.00[c] |
| P | 0.761 | 0.001 |
| N | 20 | 20 |
| SEM | 1.793 | 0.103 |

CMI cell mediated immunity; ND Newcastle disease; P probability; N number of replicates; SEM standard error mean; iTM inorganic trace minerals; oTM organic trace minerals
[ABC] means having different superscripts in a column differ significantly ($P<0.05$)

Tibia ash percent and calcium content in tibia ash were not affected ($P>0.05$) by the variation in concentration of oTM in the broiler diet compared to those fed the CD (Table 5). The tibia-breaking strength in broilers fed 20% oTM was statistically similar to those fed the CD. An increase in concentrations of oTM progressively reduced the tibia strength and the strength in groups fed 25% oTM was significantly lower than those fed the CD. Similarly, the P content in tibia ash in broilers fed the lower concentrations of oTM (10 and 17.5%) were similar to those fed 100% iTM in their diet. At the highest concentrations of oTM (25%) the P deposition in tibia ash was significantly reduced than those fed the 10 and 17.5% oTM in the diet. Further, the bone parameters (reduced bone strength and phosphorous content in tibia ash) of the current study (Table 5) indicated the negative effects at higher inclusion levels of oTM in broiler diet, which may partly be due to the higher availability of organic form of TM than the inorganic salts.

Table 5. Tibia bone mineral parameters in broiler chicken fed graded concentrations of oTM in place of iTM premix

| Treatment | Strength (N) | Ash% | Phosphorus, % | Calcium, % |
|---|---|---|---|---|
| iTM | 69.74[ab] | 51.48 | 18.57[ab] | 31.84 |
| oTM-10 | 84.57[a] | 51.72 | 18.79[a] | 32.16 |
| oTM-17.5 | 48.39[bc] | 51.61 | 19.13[a] | 31.60 |
| oTM-25 | 42.40[c] | 52.62 | 17.70[b] | 32.48 |
| P | 0.003 | 0.087 | 0.045 | 0.794 |
| N | 20 | 20 | 20 | 20 |
| SEM | 4.738 | 0.325 | 0.190 | 0.318 |

P probability; N number of replicates; SEM standard error mean; iTM inorganic trace minerals; oTM organic trace minerals
[ABC] means having different superscripts in a column differ significantly ($P<0.05$)

Reduced bone mineral variables (tibia strength and P in tibia ash) variables with an increase in concentrations of oTM contradict our previous work (Rama Rao et al., 2021), where the concentrations of Ca, P, and other trace minerals (Cu, Zn, and Fe) in bone ash increased in groups fed diets supplemented with oTM compared to those fed iTM. It is worth noting that in our previous study, the contents of Ca and P in tibia ash were higher in chickens fed diets with lower levels of oTM compared to those fed higher concentrations of oTM. As reported, the retention of oTM is higher compared to iTM (Bao et al., 2007; Ma et al., 2012), which results in higher retention of minerals even at the lowest levels of inclusion (oTM 100g/ton). The reduced bone mineral variables at higher inclusion levels of oTM in diet (17.5 or 25%) could be due to the higher concentrations of bioavailable elements that might have interacted antagonistically and adversely affected bone mineralization. The higher bio-availability of trace minerals in the organic form (Ma et al., 2012) might have ensured higher/excess availability of the minerals beyond the physiological requirement, which showed some negative responses in bone mineralization, performance, and HI immune response. The similar mineral concentration in birds fed oTM 100g/ton and the CD with 100% iTM also confirms the hypothesis that mineral availability is higher in organic form than in inorganic form. Comprehensively, the data indicated that the TM requirement can be reduced to 10% when supplemented as oTM in commercial broiler diets without compromising the performance, HI titer, bone-breaking strength, and P content in tibia ash.

The data of the current study also indicated that the levels of trace minerals in 10% oTM are optimum for performance as well as bone development. Similarly, Zaho et al. (2010) also found an optimum response with lower concentrations of TM when included as an organic form. As the performance variables in broilers fed 10% oTM were similar to those fed 100% iTM, the concentrations of TM present in 10% oTM (Zn 10.0, Mn 10.0, Cu 1.0, Fe 5, I 0.6, Se 0.06, and Cr 0.01 mg/kg) appears optimum for broiler chicken. The absence of negative effects in broilers fed the lower concentrations of oTM could be due to the higher bioavailability of minerals in organic form (Wang et al., 2007) and therefore, the absolute availability of minerals even at the lower levels of oTM probably met the broiler requirement.

## CONCLUSIONS

The concentrations of oTM can be reduced to 10% of the recommended concentration (Fe 10, Zn 10, Mn 10, Cu 2, Se 0.05, I 0.25/mg/kg) in broiler diet without affecting the performance, bone mineralization and HI response compared to those fed the 100% inorganic TM in the diet (Fe 100, Zn 100, Mn 100, Cu 20, Se 0.50, I 2.5/mg/kg). Higher concentrations of oTM (17.5 and 25%) significantly reduced the broiler performance and bone mineral variables.

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