

## Research Article

**Micro-Encapsulation of All Six Trace Minerals to Reduce Inclusion While Maintaining Performance and Carcass Traits of Broiler**Kevin Liu <sup>1,\*</sup>, Wenbiao LU <sup>2</sup>, Kaiyu Xiao <sup>2</sup>

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\* **Correspondence:** Kevin Liu; E-Mail: [Liu0727@gmail.com](mailto:Liu0727@gmail.com)**Academic Editor:** Małgorzata Starek**Special Issue:** [Dietary Supplements, Food Science, Nutrients and Health](#)*Recent Progress in Nutrition*

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Trace minerals (Cu, Fe, Mn, Zn, I and Se) are essential components of numerous enzymes thus indispensable for metabolism, immunity, growth and reproduction. Inorganic forms of these minerals (ITM) are widely used in the poultry industry often with much in excess in order to cover various uncertainties, resulting in not only oxidative deterioration of feeds and gastrointestinal health, but also large proportions to be excreted into the eco-system, leading to many jurisdictions to set up limits of Cu and Zn usages in feeds. This study investigated feasibility and extent of reduction of supplemental ITM in broiler nutrition, by using 400 newly hatched Ross 308 male chicks, randomly assigned to four treatments with ten floor pen replicates per treatment and 10 birds per pen, to determine the efficacy of encapsulating all six trace minerals into a single micro-granule (Metal Polysaccharides Complex, MPSC, MinCo®) supplemented on descending pattern, on growth performance, digestibility of these minerals and carcass traits of broilers. The control diet was supplemented with ITM at their conventional levels (mg/kg: Cu 15, Fe 40, Mn 100, Zn 100, Se 0.3, and I 1.0) for the entire 42-day of life cycle. Treatments 2-4 tested MPSC in three formulas on descending inclusions. Feed intake, weight gain, feed conversion ratio (FCR) and mortality were measured by phases.



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Concentrations of trace minerals in feces and apparent digestibility were measured on days 33-35 using titanium dioxide. Footpad dermatitis (FPD), wooden breast (WB), white striping (WS), and mineral contents in liver and breast were measured on day 43. Data were analyzed by analysis of variance (ANOVA). The results showed all birds reached expected live weight 3.2 kg by day 42 at FCR 1.55, no significant differences were observed among treatments up to day 35. During the finisher phase, the birds fed on MPSC showed improved growth rate and FCR compared with those fed on ITM ( $P < 0.05$ ). FPD, WB and WS did not differ. Minerals in MPSC showed significantly higher apparent digestibility ( $P < 0.05$ ) than ITM. Calculation on the intakes of trace minerals revealed that the birds fed on ITM consumed 1,255 mg of Cu, Fe, Mn, Zn in total, whilst those on MPSC reduced consumption to 491 mg (Formula 1), 264 mg (Formula 2) and 312 mg (Formula 3). It can be concluded that MPSC encapsulation can significantly increase digestibility and retention of trace minerals which enables reduction of trace minerals usage by approximately 70% without compromising performance and carcass traits.

### Keywords

Trace minerals; encapsulation; broiler; sustainability

## 1. Introduction

Trace minerals, namely copper (Cu), iron (Fe), manganese (Mn), zinc (Zn), iodine (I) and selenium (Se), are indispensable in livestock nutrition for they are the key components in numerous metalloenzymes responsible for antioxidant defense, immune function, connective tissue formation, and energy metabolism. Although these minerals are naturally present in feed ingredients, their concentration and bioavailability are inconsistent and unreliable for the intensive livestock production. Hence, dietary supplementation with exogenous trace minerals has been a universal practice of all commercial farming, typically in the form of inorganic salts such as oxides, sulphates, carbonates (ITM), due mainly to their effectiveness in preventing deficiency, and relatively low cost. However, their bioavailability is generally low. In broilers, their retentions are typically 10-30%, depending on age, dietary concentration, mineral source, phytase supplementation and antagonistic interactions with calcium, phosphorus and phytate [1, 2]. In order to cover the uncertainty, the animal industry generally over-supplies these exogenous minerals to the extent that draws wide concerns on their excretion to the environment [3, 4].

Classical ITM salts are ionically bound and readily dissociate in an aqueous environment, leading to several negative consequences. First, the released ions are chemically reactive, causing antagonistic interactions and oxidation of other nutrients such as enzymes, vitamins, fatty acids, and pigments [5, 6]. As a result, ITMs are the major contributor to oxidative deterioration and loss of nutrients in feeds [7]. Second, supplementing ITMs at recommended levels will introduce 700-1000 g of reactive and corrosive chemicals per metric ton of complete feeds, which impairs palatability and reduces feed intake, especially for piglets [8]. Third, free metal ions may corrode the gastro-intestinal epithelium, impair gut health [9], being a potential cause of wet droppings of the young chicks. Fourth, these ions readily form insoluble complexes with phytate and other polar

molecules in the digesta, reducing their availability to the host animal [10]. Last but not the least, the low retention of the supplemental ITMs in animal body leads to high proportion excreted to the eco-system, and the elevated Cu and Zn concentrations are associated with reduced microbial biomass, inhibition of nitrification, decreased earthworm abundance, impaired root development, phytotoxicity, and increased risks of runoff into surrounding aquatic ecosystems [11]. Such environmental concerns have been growing globally and prompted regulatory authorities to progressively reduce the maximum authorized concentrations of Cu and Zn in complete feeds (EU 2018/1039).

Considerable research has been directed toward identifying more bioavailable mineral sources, including chelates and complexes with amino acids, fatty acids, carbohydrates, soy proteins [12]. Unlike ionic salts, these organic trace mineral (OTM) compounds are of covalent binding, or chelating, chemically more stable, and less likely to release free ions to form indigestible complexes in the gastrointestinal tract. These properties can enhance absorption efficiency and allow lower dietary inclusion levels, particularly in long-lived breeders [13]. However, commercial OTM products vary widely in their chemical composition, binding strength, and stability [14], yet their residual inorganic fractions may still promote oxidative reactions in feeds [3].

Our group [15] described a novel process that encapsulates all six trace minerals in a single polysaccharide matrix under controlled moisture, temperature, and pressure, followed by granulation and air-drying, resulting in *metal polysaccharide complex* (MPSC). The encapsulation effectively holds trace minerals from pre-mature release of ions and direct contact with sensitive feed components, and gradual releases in the gastrointestinal tract will reduce antagonism and oxidative reactions as well as mucosal irritation. Previous studies have shown that MPSC supplementation increased mineral retention compared with both ITM and OTM and can reduce total mineral usage by nearly 70% without compromising broiler performance and carcass parameters [16, 17].

The objective of the present study was to evaluate the efficacy of three MPSC formulations supplemented at descending pattern, on performance and carcass traits of fast-growing broiler, to compare formula and inclusion pattern, towards precision on trace mineral nutrition for sustainable broiler production.

## **2. Materials and Methods**

### **2.1 Animals and Treatments**

Four hundred newly hatched male broiler chicks (Ross 308) were randomly allocated to four dietary treatments, each consisting of 10 floor pen replicates with 10 birds per pen. The sources and levels of trace minerals are shown in Table 1. The inorganic trace mineral (ITM) treatment (Control) represented standard industry practice, with the same levels of supplementation throughout the broiler's life cycle. Three MPSC formulations were evaluated: (1) a formula previously evaluated by our group [16-18]; (2) a formula with a mineral profile close to that described in the Brazilian Feedstuff Table [18] and (3) a formula with elevated levels of iron and selenium in attempt to enhance antioxidant capacity designed for tropical climate. The MPSC formulation and samples (MinCo®) were received from Fujian Syno Biotech Co. Ltd. Their mineral profiles and inclusion levels are shown in Table 2.

**Table 1** Composition and formulations of Metal Polysaccharides Complex (MPSC).

|                 | Control <sup>1</sup> | Encapsulated <sup>2</sup> |                        |                        |
|-----------------|----------------------|---------------------------|------------------------|------------------------|
|                 | ITM                  | Formula 1 <sup>2</sup>    | Formula 2 <sup>2</sup> | Formula 3 <sup>2</sup> |
| Copper, g/kg    | 15                   | 13                        | 18                     | 10                     |
| Iron, g/kg      | 40                   | 140                       | 60                     | 187.5                  |
| Manganese, g/kg | 100                  | 120                       | 100                    | 50                     |
| Zinc, g/kg      | 100                  | 120                       | 90                     | 62.5                   |
| Selenium, g/kg  | 0.3                  | 0.8                       | 1.0                    | 1.25                   |
| Iodine, g/kg    | 1                    | 0.8                       | 0.8                    | 0.5                    |

<sup>1</sup> ITM, inorganic trace minerals: copper sulphate pentahydrate ( $\text{CuSO}_4 \cdot 5\text{H}_2\text{O}$ ); ferrous sulphate monohydrate ( $\text{FeSO}_4 \cdot \text{H}_2\text{O}$ ); manganese sulphate ( $\text{MnSO}_4$ ), zinc oxide ( $\text{ZnO}$ ); potassium iodide (KI); sodium selenite ( $\text{Na}_2\text{SeO}_3$ ).

<sup>2</sup> Metal polysaccharides complex (MinCo<sup>®</sup>), in which Cu as tribasic copper chloride; Fe as ferrous sulphate monohydrate, Mn as tribasic dimanganese chloride; Zn as zinc chloride hydroxide monohydrate; Se as sodium selenite; I as calcium iodate anhydrous. These raw materials are pre-treated, thoroughly mixed, and encapsulated into a carbohydrates matrix to avoid their premature release and oxidation.

**Table 2** Supplemental dosage of trace minerals' premixes, mg/kg final diets.

| Treatment | Minerals  | Day 1-10 | Day 11-21 | Day 22-35 | Day 36-42 |
|-----------|-----------|----------|-----------|-----------|-----------|
| Control   | ITM       | 1000     | 1000      | 1000      | 1000      |
| F1        | Formula 1 | 350      | 300       | 250       | 200       |
| F2        | Formula 2 | 300      | 250       | 200       | 150       |
| F3        | Formula 3 | 300      | 250       | 200       | 150       |

All diets were formulated using locally available ingredients, corn, dehulled soybean meal, cassava, and rice bran, to meet nutrient requirements according to Ross 308 guidelines. The four diets were identical (Table 3) except the trace mineral sources and inclusion levels (Table 2). All diets were supplemented with 1,000 FTU/kg of 6-phytase and contained no antibiotics. Diets were pelleted (3-mm diameter) at 80°C and provided in crumble form for the starter phase (0-10 d) and as pellets thereafter. Four feeding phases were used: starter (0-10 d), grower 1 (11-21 d), grower 2 (22-35 d), and finisher (36-42 d).

**Table 3** Composition and calculated nutrients of basal diets.

|                              | Starter<br>(Day 1-10) | Grower 1<br>(Day 11-20) | Grower 2<br>(Day 21-35) | Finisher<br>(Day 36-42) |
|------------------------------|-----------------------|-------------------------|-------------------------|-------------------------|
| Corn 8%                      | 52.437                | 48.734                  | 45.281                  | 41.511                  |
| Cassava chip                 | 4.000                 | 6.000                   | 8.000                   | 10.000                  |
| SBM (DH) 48%                 | 35.430                | 34.970                  | 34.480                  | 33.506                  |
| Rice bran, full fat          | 4.000                 | 6.000                   | 8.000                   | 10.000                  |
| Soybean oil                  | -                     | 0.710                   | 1.371                   | 2.222                   |
| MCP                          | 0.953                 | 0.648                   | -                       | -                       |
| Limestone                    | 1.046                 | 1.032                   | 1.149                   | 1.151                   |
| Salt                         | 0.278                 | 0.297                   | 0.315                   | 0.321                   |
| DL-Methionine                | 0.347                 | 0.325                   | 0.231                   | 0.295                   |
| L-Lysine HCl                 | 0.248                 | 0.197                   | 0.148                   | 0.114                   |
| L-Threonine                  | 0.111                 | 0.100                   | 0.089                   | -                       |
| Sodium bicarbonate           | 0.276                 | 0.132                   | 0.104                   | 0.090                   |
| Choline Chloride 60%         | 0.104                 | 0.084                   | 0.064                   | 0.069                   |
| Other additives <sup>1</sup> | 0.77                  | 0.77                    | 0.77                    | 0.77                    |
| Total, %                     | 100.00                | 100.00                  | 100.00                  | 100.00                  |
| Nutrients, calculated        |                       |                         |                         |                         |
| ME for poultry, kcal/kg      | 2,900                 | 2,950                   | 3,000                   | 3,050                   |
| Crude protein, %             | 22.07                 | 21.81                   | 21.52                   | 21.02                   |
| Crude fat, %                 | 3.08                  | 3.99                    | 4.86                    | 5.9                     |
| Crude fiber, %               | 3.67                  | 3.8                     | 3.94                    | 4.04                    |
| Ash, %                       | 5.49                  | 5.31                    | 5.05                    | 5.16                    |
| Dig. Lys., %                 | 1.250                 | 1.200                   | 1.150                   | 1.100                   |
| Dig. Met., %                 | 0.647                 | 0.621                   | 0.524                   | 0.581                   |
| Dig. M+C, %                  | 0.930                 | 0.900                   | 0.880                   | 0.850                   |
| Calcium, %                   | 0.70                  | 0.65                    | 0.60                    | 0.60                    |
| Phosphorus-avail., %         | 0.25                  | 0.20                    | 0.09                    | 0.09                    |

<sup>1</sup> Contain multi-vitamins, coccidiostats, pellet binder, mold inhibitor, phytase (1000 FTU/kg).

## 2.2 Housing and Management

The experiment was conducted in an enclosed house with concrete floor pens with rice hull bedding. Each pen was equipped with a tubular feeder and two nipple drinkers, and birds had *ad libitum* access to feed and water. Lighting and management followed the Ross 308 Broiler Management Manual. The birds were vaccinated for Newcastle disease and infectious bronchitis on day 7, and for Gumboro disease on day 14. All management practices followed standard Ross 308 recommendations [19].

### 2.3 Performance

Body weight and feed intake were recorded on pen basis for each growth phase. Mortality and culls were recorded daily, with body weight and cause of death recorded. Weight gain, feed intake, feed conversion ratio (FCR), and mortality were calculated per pen for each phase.

### 2.4 Minerals in Tissues, Feces and Apparent Digestibility

In order to test apparent digestibility, the diets of ITM and MPSC F1 were added 0.3% TiO<sub>2</sub> for Day 32-35, with plastic sheets placed on the pen floors to prevent excreta contamination. Excreta of these two treatments were collected on day 35, on pen basis, dried at 80°C for 24 h, for lab analyses. Feed samples were collected directly from feeders. All feeds and excreta samples were analysed for minerals contents, the apparent digestibility was calculated following below formula:

$$\text{Digestibility coefficient} = 1 - (\text{Marker}_{\text{feces}} / \text{Marker}_{\text{diet}}) \times (\text{Nutrient}_{\text{feces}} / \text{Nutrient}_{\text{diet}})$$

Marker<sub>diet</sub> = concentration of indigestible marker in feed.

Marker<sub>feces</sub> = concentration of indigestible marker in feces.

Nutrient<sub>diet</sub> = nutrient concentration in diet (e.g., Cu, Zn, Mn).

Nutrient<sub>feces</sub> = nutrient concentration in feces.

### 2.5 Footpad Dermatitis, Wooden Breast and White Striping 142

On day 43, one bird per pen was euthanized by CO<sub>2</sub> inhalation, to score footpad dermatitis (FPD) lesion, using a 3-point scale; white striping (WS) on a 4-point scale and wooden breast (WB) using a 5-point scale, with procedure details described in [20].

Following scoring, the liver and breast muscle were sampled from 3 birds per treatment, the samples were dried at 80°C for 24 h, packed, and submitted to Syno Biotech Laboratory for mineral content analysis. Contents of Cu, Fe, Mn, Zn were determined in feed, digesta, liver, and breast muscle samples on dry matter basis using atomic absorption spectrometry [21]. Se concentrations were determined by inductively coupled plasma mass spectrometry (ICP-MS) following the same standard method. Titanium dioxide concentrations in feed and excreta samples were analysed as described in [22].

### 2.6 Statistical Analysis

Data were analyzed by analysis of variance (ANOVA) using a randomized complete block design (RCBD) in SAS (SAS Institute Inc., Cary, NC) [23]. Treatment means were compared using Duncan's multiple range test, with significance declared at P < 0.05.

### 2.7 Ethics Approval

The study was conducted at the NKP Poultry Research Farm, Thailand. Animal procedures were approved under the Guideline of Using Animals for Scientific Purposes by the National Research Council of Thailand (Act B.E. 2558).

### 3. Results and Discussion

#### 3.1 Trace Minerals in Diets and Overall Performance

Concentrations of Cu, Fe, Mn, Zn and Se were analysed for Grower 2 diets (Table 4). The results represented the sums of both supplemental and endogenous minerals. Based on the results, the endogenous or background trace minerals can be estimated as mg/kg: Cu 10, Fe 130, Mn 25, Zn 40 and Se 0.08, suggesting only iron level are above requirement, whilst the rest are insufficient and require exogenous supplementation. For iron, early studies showed naturally occurring iron in plant ingredients is mostly as Fe<sup>3+</sup> trivalent form and much of it is bound to phytate, fiber and polyphenolic compounds with very low bioavailability, approximately 5-20% [2].

**Table 4** Supplemental levels and lab analyses of trace minerals (mg/kg)<sup>1</sup>.

|    |              | ITM   | Formula 1 | Formula 2 | Formula 3 |
|----|--------------|-------|-----------|-----------|-----------|
| Cu | Supplemental | 15    | 3.25      | 4.5       | 2.5       |
|    | Analyzed     | 23.1  | 17.8      | 14.6      | 11.1      |
| Fe | Supplemental | 40    | 35        | 15        | 47        |
|    | Analyzed     | 193.7 | 183.5     | 149.6     | 168.9     |
| Mn | Supplemental | 100   | 30        | 25        | 15.6      |
|    | Analyzed     | 128.8 | 55.5      | 49.5      | 41.3      |
| Zn | Supplemental | 100   | 30        | 22.5      | 15.6      |
|    | Analyzed     | 157.5 | 79.1      | 62.4      | 54.2      |
| Se | Supplemental | 0.30  | 0.20      | 0.25      | 0.31      |
|    | Analyzed     | 0.41  | 0.27      | 0.35      | 0.36      |

<sup>1</sup> The test was conducted on Grower 2 diets for digestibility test. The analyzed levels include endogenous trace minerals.

All day-old chicks arrived in good health condition with average live weight 47 g. The overall performance of the flock (Table 5) exceeded Ross 308 male performance target, i.e. average live weight 3136 g/bird, feed intake 5005 g/bird and FCR 1.596 [19], reflecting adequate nutrition and rearing conditions in this study.

**Table 5** Growth performance of broiler fed on different sources and levels of trace minerals<sup>1, 2, 3</sup>.

| Trt            | Day 1-10     |              |              | Day 11-21    |              |              | Day 22-35    |              |              | Day 36-42         |                   |                    | Day 0-42     |              |              |              | Mortality %  |
|----------------|--------------|--------------|--------------|--------------|--------------|--------------|--------------|--------------|--------------|-------------------|-------------------|--------------------|--------------|--------------|--------------|--------------|--------------|
|                | Wt           | F.           | FCR          | Wt           | F.           | FCR          | Wt           | F.           | FCR          | Wt                | F.                | FCR                | End          | Wt           | F.           | FCR          |              |
|                | gain,        | intake,      |              | gain,        | intake,      |              | gain,        | intake,      |              | wt <sup>1</sup> , | gain,             |                    | intake,      |              |              |              |              |
|                | g            | g            |              | g            | g            |              | g            | g            |              | kg                | kg                |                    | kg           |              |              |              |              |
| ITM            | 260          | 289          | 1.112        | 801          | 989          | 1.234        | 1498         | 2364         | 1.579        | 602 <sup>b</sup>  | 1278 <sup>a</sup> | 2.124 <sup>a</sup> | 3.209        | 3.162        | 4.920        | 1.556        | 0.00         |
| F1             | 250          | 278          | 1.111        | 771          | 952          | 1.235        | 1485         | 2353         | 1.585        | 639 <sup>a</sup>  | 1283 <sup>a</sup> | 2.010 <sup>b</sup> | 3.191        | 3.145        | 4.858        | 1.545        | 3.00         |
| F2             | 258          | 284          | 1.102        | 798          | 989          | 1.240        | 1484         | 2363         | 1.594        | 596 <sup>b</sup>  | 1202 <sup>b</sup> | 2.019 <sup>b</sup> | 3.182        | 3.135        | 4.821        | 1.538        | 5.00         |
| F3             | 255          | 288          | 1.132        | 810          | 994          | 1.228        | 1495         | 2387         | 1.597        | 641 <sup>a</sup>  | 1296 <sup>a</sup> | 2.027 <sup>b</sup> | 3.247        | 3.200        | 4.960        | 1.550        | 1.00         |
| <i>P value</i> | <i>0.120</i> | <i>0.061</i> | <i>0.287</i> | <i>0.857</i> | <i>0.942</i> | <i>0.658</i> | <i>0.857</i> | <i>0.942</i> | <i>0.658</i> | <i>&lt;0.0001</i> | <i>0.014</i>      | <i>0.000</i>       | <i>0.113</i> | <i>0.113</i> | <i>0.088</i> | <i>0.361</i> | <i>0.291</i> |

<sup>1</sup> Start live weight 47 g for all treatments. Feed conversion ratio was corrected for mortality and culls.

<sup>2</sup> ITM, Inorganic trace minerals, dosage at 1000 mg/kg for the entire life cycle; T2-T4, Metal Polysaccharides Complex (MinCo®), dosages for the 4 sequential growing phases in mg/kg feed: F1 350/300/250/200; F2 and F3, 300/250/200/150.

<sup>3</sup> Means within the same column bearing no common superscript differ significantly ( $P < 0.05$ ).

### 3.2 Growth Performance

The growth performance of the broiler flock, as shown in Table 5, such as live weight, feed intake and FCR, exceeded its genetic standard [20], reflecting the overall nutrition program being adequate, including both ITM and MPSC trace minerals. During the first three growing phases (0-35 d), no significant differences ( $P > 0.05$ ) were observed among treatments. Broilers fed on MPSC at 30-35% of the conventional ITM levels achieved comparable performance, suggesting the minerals encapsulation did not restrict early development of the birds. These results are well in line with our previous findings [16, 17], in their evaluation of the same MPSC F1 from Day 1 to Day 35, also in descending inclusion pattern. These results support the encapsulation can largely reduce the inclusion of the trace minerals.

Interestingly, significant performance improvements were observed for birds fed on MPSC during the finisher phase (36-42 d), in terms of weight gain and FCR ( $P < 0.05$ ) comparing with those fed on ITM. The birds fed on MPSC showed better performance (F1 weight gain 687 g; FCR 1.918) and all the three MPSC-fed birds displayed superior FCR to the flock receiving ITM ( $P < 0.05$ ), while no changes on their feed intake, suggesting that the sustained release of the minerals from MPSC may have helped maintain gut health and absorption of nutrients of the birds, leading to improved feed conversion during their late growing stage.

In the present study, throughout the 42-day feeding period, MPSC-fed birds showed numerically higher final live weight (1-2%) and lower FCR (1-2%) than those fed on ITM, even though total supplemental minerals were reduced by two-thirds. Mortality remained low and statistically unaffected, confirming that lower mineral inclusion using MPSC technology provided sufficient trace minerals to support optimal growth performance.

Our earlier study [15] compared MPSC (Formula 1) with ITM and OTM on broiler performance, found the birds receiving MPSC gained significant more weight with improved FCR during the 35-d life cycle. The authors attributed the extra weight gain and better FCR to higher retention of nutrients such as fatty acids and supplemental enzymes and vitamins prior to ingestion by the birds, as significantly higher retention of phytase and NSPase were detected in MPSC diets than the ITM diets after 3 weeks of feed storage. An *in vitro* study [24] showed, through incubation of fresh soy oil with ITM at 40°C, oil oxidation was rapid and drastic, resulting in significant losses of polyunsaturated fatty acids (PUFA). In contrast, MPSC did not cause detectable oxidation during an extended test period. This finding was confirmed by [16] who evaluated the same MPSC (Formula 1) in comparison with ITM and OTM, reported the birds on MPSC gained more weight during the first 10 days of age, due mainly to more efficient feed conversion ( $P < 0.05$ ). The authors assumed the protected and slow-release trace minerals benefited the gut health of the early life.

In terms of MPSC profile and supplemental levels, the results showed no significant impact among the three MPSC formulas. This can be explained, as shown in Table 4, the feed ingredients already carried basic levels of endogenous trace minerals, the alterations in formula profile and inclusion levels among the main trace minerals most likely fell within their buffering zone, which explains the extra iron and selenium in Formula 3 did not produce detectable improvements because the birds were not subjected to any measurable stress. The results clearly demonstrated trace minerals in MPSC form, supplemented at descending pattern can support expected growth

performance, confirming the previous findings and assumption [16, 25] that there is a significant room for reduction of trace minerals supplementation.

### 3.3 Footpad Dermatitis, Wooden Breast, and White Striping

As shown in Table 6, no significant differences were observed among treatments ( $P > 0.05$ ) on footpad dermatitis (FPD), wooden breast (WB), and white striping (WS), which can be attributed to the adequate rearing conditions in this study. Nevertheless, the results indicated that the birds receiving MPSC tended to display lower scores than those on ITM. It is known that trace minerals Zn, Mn, Cu, and Se play important roles in maintaining epithelial and muscular integrity through their antioxidant and collagen-stabilizing functions [9], the improved tissue condition observed on MPSC-fed birds could be attributable to a reduction in free ionic activity and oxidative stress in the gastrointestinal tract, leading to better epithelial health and reduced incidence of myopathies.

**Table 6** Score of footpad dermatitis, wooden breast and white striping of broiler fed on different forms and levels of trace minerals (42 days of age).

| Minerals <sup>1</sup> | FPD <sup>2</sup> | Wooden breast <sup>3</sup> | White striping |
|-----------------------|------------------|----------------------------|----------------|
| ITM                   | 1.80             | 2.30                       | 1.65           |
| F1                    | 1.50             | 2.20                       | 1.50           |
| F2                    | 1.65             | 2.25                       | 1.40           |
| F3                    | 1.70             | 2.20                       | 1.60           |
| P value               | 0.546            | 0.784                      | 0.750          |

<sup>1</sup> The treatment is explained in Table 2.

<sup>2</sup> FPD scoring: 0 = No lesion or minor superficial lesions; 1 = Mild lesion, substantial discolouration of the footpad; 2 = Severe lesion, ulcers or scabs of significant size.

<sup>3</sup> Wooden breast scoring 0 = Normal, no apparent hardened muscle; 1 = 25% of area with hardened muscle; 2 = 50% of area with hardened muscle; 3 = 75% of area with hardened muscle; 4 = almost 100% of area with hardened muscle.

### 3.4 Trace Minerals in Tissues

The concentrations of trace minerals in the breast and liver tissues are shown in Table 7. No significant differences were observed among treatments on breast muscle minerals, despite much lower dietary supply of these trace minerals, indicating muscles can adjust retention levels of the trace minerals to maintain their functionality. These results are in line with earlier reports by [26]. In contrast, mineral contents in the liver revealed more distinct trends. The birds received Cu 15 mg/kg in ITM form retained Cu 28.2 mg/kg whilst those fed on Cu 2.5 mg/kg in MPSC kept (16.9 mg/kg, F3,  $P < 0.05$ ), indicating the birds are able to retain higher proportion of dietary Cu when it is supplied in MPSC form. Similar trends were noted for Fe and Se. In contrast, Mn and Zn concentrations were numerically higher in the livers of birds fed on MPSC, particularly Formula 1. These results are in line with other findings [27, 28] that the breast muscle and liver are able to maintain trace minerals concentrations in a relatively narrow range to reduce the risk of either deficiency or mineral-induced oxidative load. When dietary supply is low, birds increase their

absorption, and organic or encapsulated minerals are more efficient in absorption and deposition [16] due to their stability in the gastro-intestinal tract.

**Table 7** Concentration of trace minerals in breast muscle and liver<sup>1</sup> (mg/kg, dry basis).

| Minerals      | Cu                 | Fe    | Mn   | Zn    | Se                 |
|---------------|--------------------|-------|------|-------|--------------------|
| <b>Breast</b> |                    |       |      |       |                    |
| ITM           | 0.8                | 26.4  | 0.6  | 29.7  | 0.32               |
| F1            | 0.8                | 28.6  | 0.4  | 32.3  | 0.30               |
| F2            | 0.7                | 26.4  | 0.4  | 31.7  | 0.32               |
| F3            | 0.6                | 26.9  | 0.4  | 29.6  | 0.29               |
| <b>Liver</b>  |                    |       |      |       |                    |
| ITM           | 28.2 <sup>a</sup>  | 633.2 | 8.6  | 149.8 | 2.04 <sup>a</sup>  |
| F1            | 28.6 <sup>a</sup>  | 521.2 | 9.1  | 169.5 | 1.75 <sup>ab</sup> |
| F2            | 21.7 <sup>ab</sup> | 552.1 | 9.1  | 119.9 | 1.64 <sup>b</sup>  |
| F3            | 16.9 <sup>b</sup>  | 424.3 | 11.0 | 119.0 | 1.82 <sup>ab</sup> |

<sup>1</sup> The treatment is explained in Table 2. Means within the same column bearing no common superscript differ significantly ( $P < 0.05$ ).

### 3.5 Trace Minerals in Feces and Apparent Digestibility

As shown in Table 8, the concentrations of trace minerals in the fecal samples well reflected their dietary supplementation, for approximately half levels of Cu, Zn, Mn and Se were detected in the feces of the birds fed on MPSC in comparison with samples from ITM fed birds, suggesting reduction of dietary supplementation is the primary and effective means to minimize trace minerals excretion. In this study, high concentrations of iron (553.6 mg/kg and 514.7 mg/kg) were found in both fecal samples, well in line with their dietary concentrations (193.7 mg/kg and 183.5 mg/kg) and supplementation (40 mg/kg and 35 mg/kg).

**Table 8** Trace minerals in dry feces and apparent digestibility<sup>1</sup>.

|    | Feces, mg/kg       |                    | App. digestibility, % |                   |
|----|--------------------|--------------------|-----------------------|-------------------|
|    | ITM <sup>2</sup>   | MPSC <sup>3</sup>  | ITM <sup>2</sup>      | MPSC <sup>3</sup> |
| Cu | 92.7 <sup>a</sup>  | 41.5 <sup>b</sup>  | 16.8 <sup>b</sup>     | 36.6 <sup>a</sup> |
| Zn | 376.2 <sup>a</sup> | 190.9 <sup>b</sup> | 25.2 <sup>a</sup>     | 25.4 <sup>a</sup> |
| Mn | 338.0 <sup>a</sup> | 187.7 <sup>b</sup> | 20.2 <sup>b</sup>     | 30.9 <sup>a</sup> |
| Fe | 553.6 <sup>a</sup> | 514.7 <sup>a</sup> | 12.1 <sup>b</sup>     | 23.4 <sup>a</sup> |
| Se | 1.22 <sup>a</sup>  | 0.54 <sup>b</sup>  | 10.8 <sup>b</sup>     | 38.3 <sup>a</sup> |

<sup>1</sup> Means in the same comparison bearing no common superscript differ significantly ( $P < 0.05$ ).

<sup>2</sup> ITM, inorganic trace minerals, dosage at 1000 mg/kg for the entire life cycle.

<sup>3</sup> MPSC Formula 1, dosages in mg/kg feed: 350/300/250/200 for the four sequential growing phases.

As expected, the encapsulation significantly enhanced apparent digestibility of trace minerals, namely Cu (16.8% vs. 36.6%,  $P < 0.05$ ), Mn (20.2% vs. 30.9%  $P < 0.05$ ), Se (10.8% vs. 38.3%,  $P < 0.05$ ),

and Fe (12.1% vs. 23.4%,  $P < 0.05$ ). For iron, since high level of endogenous Fe coming with feed ingredients, the increase in digestibility is most likely attributable to the portion in MPSC, as higher Fe levels were detected in the breast muscle (Table 7). In this study no increase was observed in Zn digestibility for which reason is unknown. Se digestibility increased around threefold (10.8% vs. 38.3%,  $P < 0.05$ ), which may be explained by both encapsulation and partial reduction of selenite to nano-form Se during the MPSC processing, and it is known that nano-Se has higher absorption and deposition than selenite [29].

In general, the enhanced digestibility can be attributed to the structural stability of MPSC, which minimizes premature ion release and antagonistic interactions with other components, particularly phytate and proteins, in the gastrointestinal tract [6, 10]. The slow and targeted releases of metal ions will facilitate absorption in the small intestines through reduced precipitation in the digesta. Similar improvements were reported with chelated and protein-complexed minerals [15-17, 30, 31], supporting the premise that covalent or matrix-bound trace minerals exhibit superior bioavailability.

### 3.6 Mineral Consumption and Efficiency

Table 9 compares cumulative intakes of the four main trace minerals (Cu, Fe, Mn and Zn) by a single broiler bird growing from day 1 to day 42. The results revealed that a bird consumed a total 1255 mg of the 4 minerals when supplemented in their conventional ITM form and levels following the breeder's recommendation. In contrast, when these minerals were in MPSC form and supplemented on a descending pattern, the total intakes were reduced to 491 mg for F1, 264 mg for F2 and 312 mg for F3, representing drastic reductions in total mineral usage. Birds fed on MPSC consumed only 21% (F2)-39% (F1) of the total minerals mass compared with those fed on the conventional ITM, with no compromise on growth performance nor carcass traits. The improved stability and bioavailability of the MPSC may well explain the higher efficiency, consistent with the earlier findings [15, 16, 27].

**Table 9** Consumption of supplemental trace minerals per broiler bird growing from day 1 to 3.2 kg live weight<sup>1</sup>.

| Minerals <sup>2</sup> | Cu, mg | Fe, mg | Mn, mg | Zn, mg | Total, mg | %    |
|-----------------------|--------|--------|--------|--------|-----------|------|
| ITM                   | 73.8   | 196.8  | 492.0  | 492.0  | 1254.6    | 100  |
| F1                    | 16.2   | 174.9  | 149.9  | 149.9  | 491.0     | 39.1 |
| F2                    | 17.7   | 59.1   | 98.5   | 88.7   | 264.1     | 21.0 |
| F3                    | 10.1   | 188.8  | 50.3   | 62.9   | 312.1     | 24.9 |

<sup>1</sup> The calculation is based on actual supplementation and feed intake (Table 5).

<sup>2</sup> The treatment is explained in Table 2.

Accumulation of trace minerals derived from livestock industry is increasingly drawing public attention, especially Cu and Zn in manure. A long-term study [11] revealed soil Cu concentrations increased from natural background levels of 10-30 mg/kg to 100-300 mg/kg, and Zn from 30-90 mg/kg to 200-500 mg/kg in the intensively manured fields. Excessive accumulation of these trace elements can adversely affect soil microbial activity, reduce earthworm populations, impair plant growth, contaminate surface and ground water through runoff, and increase the risk of heavy metal entry into the food chain. Therefore, minimizing Cu and Zn excretion has become an imperative

objective for the sustainability of the animal industry, which can only be realized through improving their bioavailability with more efficient mineral sources and precision on their inclusion levels.

For the purpose of supplemental precision on trace minerals, the authors proposed a new scheme of trace minerals for broiler production as shown in Table 10, with the numbers derived from our current and previous studies using MPSC, at descending inclusion. The new supplemental scheme represents approximately 30% of conventional ITM inclusion, which can fully support growth performance and carcass traits yet economically viable in that the substantial reduction of inclusion levels can largely offset the expenditure of MPSC production. In practical poultry production, adoption of such scheme (Table 10) would lead to substantial reduction not only in the usage of trace minerals but also their excretion to the eco-system.

**Table 10** Recommendation on supplemental scheme of trace minerals in the form of metal polysaccharides complex (MPSC).

| Phase    | Days of age | Cu, mg/kg | Fe, mg/kg | Mn, mg/kg | Zn, mg/kg |
|----------|-------------|-----------|-----------|-----------|-----------|
| Starter  | 1-10        | 3.0       | 18.0      | 15.0      | 19.0      |
| Grower 1 | 11-24       | 2.5       | 15.0      | 12.5      | 15.6      |
| Grower 2 | 25-34       | 2.0       | 12.0      | 10.0      | 12.5      |
| Finisher | 35-42       | 1.5       | 9.0       | 7.5       | 9.4       |

#### 4. Conclusion

The present study demonstrated feasibility and advantage of encapsulating all six trace minerals into a single metal polysaccharide complex (MPSC). When supplementing at descending pattern, MPSC can effectively substitute the conventional inorganic trace minerals, enabling approximately 70% reduction in usage of trace minerals without compromising growth performance, tissue mineralization and carcass traits. MPSC technology may provide an innovative and viable pathway towards precision and simplicity of minerals nutrition for commercial poultry production. Further research is required to investigate metabolic dynamics and oxidative impact of MPSC in the gastro-intestinal tract.

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#### Author Contributions

Dr Kevin Liu designed the study, analyzed the performance results and prepared the manuscript, Mr. W B Lu set the study objective and secured funding, Mr. K Y Xiao prepared the test samples, supervised laboratory analyses on tissues and feces, and statistics.

#### Competing Interests

All authors declare no conflicts of interest.

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