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Research Article

Effect of Encapsulated Trace Minerals Premix in Comparison with Inorganic and Organic Microminerals on Growth Performance and Mineral Excretion of Broiler

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Abstract

This study was conducted to determine the efficacy of an encapsulated trace mineral premix containing Zn, Cu, Mn, Fe, Se and I on growth performance, bone health and mineral excretion of broiler chickens in comparison with organic and inorganic trace minerals. A total of 640 male Ross 308 broilers were randomly allocated to four dietary treatments with eight pen-replicates (20 birds/pen) each. A common basal diet was produced per growing phase and split into four sub-batches, and supplemented with specific trace mineral premix to create four experimental diets: Treatment 1, inorganic trace minerals (ITM: Zn, Cu, Mn, Fe, Se and I) at the levels recommended by Ross 308 (2019); Treatment 2 diets containing organic trace minerals (OTM) sources of Zn, Cu, Mn and Se, with Zn, Cu and Mn levels being 1/3 of those in ITM; Treatments 3 and 4 diets were supplemented with an encapsulated trace mineral premix (MinCo®, Syno Biotech) at either 250 or 375 mg/kg (M-250 and M-375), of which M-250 provided similar levels of Fe and Zn but lower Cu, I, Mn, Se than the OTM treatment; and M-375 provided slightly greater Fe and Zn, and similar Cu, I, Mn, Se to the OTM treatment. The results showed that the birds reached live weight of approx. 2.5 kg in 35 days of age, at feed conversion ratio (FCR) 1.45. During the starter phase, the birds fed on encapsulated trace mineral premixes had a better FCR than those on ITM, and M-375 had a superior FCR to OTM. During the overall cycle, the birds received encapsulated trace minerals had similar growth performance to those fed on ITM and OTM, but excreted significantly less Cu, Mn and Zn into litter, with no differences on tibia ash and tibia breaking strength.

Key words: Microminerals; Encapsulation; Performance; Excretion; Broiler

1. Introduction

Trace minerals fulfil a central role in many metabolic processes throughout the body and are essential for correct growth and development of all animals. Traditionally inorganic micromineral sources (ITM, such as sulphates and oxides) have been used in poultry diets because they offer a cost-effective solution to meet bird's requirement [1]. However, ITMs are chemically reactive, not only among themselves, but also their interactions with other nutrients in premixes and final diets, such as enzymes, vitamins, fatty acids and pigments, thus reduce the nutritional value of complete diets [2-4]. Several studies have suggested organic trace minerals (OTM, such as chelates and proteinates) may have a higher bioavailability compared to inorganic salts due to their reduced interaction with other dietary components (i.e. phytate, Ca, AA, fiber) and greater absorption [5]. For that reason, OTMs are commonly added at lower inclusion levels that usually reduces mineral excretion. However, their high costs largely restrict their usages in commercial operations. Recent research showed that broiler chickens fed on diets with encapsulated trace minerals performed superior to those fed on ITM despite at much lower levels of minerals supplementation [4]. They attributed the benefits were derived from carbohydrate encapsulation that created a physical separation of the trace minerals from other dietary components until reaching their digestive site in the gastro-intestinal tract, which not only avoided interactions to a very large extent, but also enabled significant reduction of trace minerals usages as well as their excretion to the environment.

The present study was designed to investigate the efficacy of the carbohydrate-encapsulated trace mineral premix at two inclusion levels on growth performance, bone ash and bone strength, mineral excretion in litter and selenium accumulation in muscle of broiler chickens, in comparison with organic and inorganic trace minerals.

2. Materials and Methods

The experimental protocol used in this study was approved by the Institutional Animal Care and Use Committee (Schothorst Feed Research), the Netherlands. The treatment, management, housing, husbandry and slaughtering conditions strictly conformed the European Union Guidelines (European Parliament, 2010). A total of 640 day old male Ross 308 broilers were used. The birds were vaccinated at hatchery against infectious bronchitis. Upon arrival, the birds were randomly allocated to 32 floor pens (20 birds per pen) with wood shavings as bedding material. Each pen had a surface area of 2.2 m^2 and contained one feeder and two drinking nipples. The ambient temperature was gradually decreased from 34.5 °C at arrival to 19.4 °C at 35 days of age. Room temperature and relative humidity were recorded daily. The light was continuously on for the first 24 hours to allow the birds to readily find feed and water. After that, the light schedule was 2D:22L during one day, and then changed to 4D:10L:2D:8L during the remaining period, complying with the EU legislation of a minimum of six hours of darkness from the second day onwards. The experiment had a completely randomized design with four dietary treatments differing by the level and source of trace minerals (Cu, I, Fe, Mn, Se and Zn) as shown in table 1. Two tailor-made micromineral premixes were produced for Treatments 1 and 2. Treatment 1 premix was produced only with inorganic trace minerals, to meet Ross 308 (2019) recommendations of Cu, I, Fe, Mn and Se when added at 0.5% inclusion rate in the final diet. Zinc level was set at 90 mg/kg into the final diet instead of 110 mg/kg (Ross 308 recommendation) to avoid exceeding the maximum allowance limit in the EU of 120 mg/kg (considering 30 mg/kg Zn from dietary ingredients). Treatment 2 premix was produced with organic Zn, Cu, Mn and Se, in which Zn, Mn and Cu were at levels of 1/3 of those in treatment 1, Fe and I were inorganic forms at the same level as treatment 1. Treatments 3 and 4 used MinCo® as ready premix containing all six trace minerals in their inorganic form but encapsulated with selected carbohydrates and was added directly during diet preparation. A separate vitamin premix was prepared and used at the same inclusion rate for all experimental diets. Experimental diets were corn-wheat-soybean meal based (Table 2) and were fed in three phases: Starter (0-10d), Grower (10-28d) and Finisher (28-37d). A common basal diet was produced for each feeding phase and divided into four equal sub-batches. The right amount and type of micromineral premix or MinCo® premix, limestone and filler (diamol) were added in each particular experimental diet. Limestone was used to correct for the Ca content of micromineral premixes. All diets were pelleted with steam addition and the temperatures after leaving the press were below 60°C. The pellet diameter was 2.3 mm for the starter feeds and 3.0 mm for the grower and finisher feeds. The feeds were stored in a cool and dry place prior to feeding. The birds had unrestricted access to a pelleted broiler diet (composition presented in Table 2) and drinking water. All diets met the requirements of nutrients and were supplemented with phytase and NSP enzyme, but no coccidiostat was added. All birds were weighed per pen on D0, D10, D28, and D37. Live weight gain (LWG) was calculated per pen for each growing phase and the entire cycle. Weight of the empty feeders was recorded. Feed added per feeder was weighed and recorded. On feed transition on D10 and D28, the residual feed including the feeder was weighed and recorded. The same procedure was performed on D37, but without feed transition. Feed intake (FI) was calculated per pen for the periods D0-10, D0-28, D0-37, D10-28, and D28-37 and corrected for mortality. Feed conversion ratio (FCR) was calculated per corresponding period. Temperature and relative humidity were monitored daily. All flocks were monitored daily for abnormalities, such as abnormal behavior, clinical signs of illness, and mortality. The European Broiler Index (EBI) was calculated for the overall period (D0-37) according to the following formula:

EBI = ((BWG (g/chick/day) x Viability (%)) / (FCR * 10)

One diet of the starter, grower and finisher phases was analyzed for proximate composition. The analyses were carried out by SFR in duplicate: Moisture was determined after drying at 80°C vacuum to a constant weight (NEN-ISO 6496:1999); Ash was analyzed after ashing the sample at 550 °C for 3 h (NEN-ISO 5984:2003); Crude protein (CP) was determined for total nitrogen content after combustion following Dumas principle (NEN-EN-ISO 16634-1:2008); Crude fat (FAT) was determined after acid hydrolysis step for fat extraction (NEN-ISO 6492:1999); Crude fiber (CF) followed method with intermediate filtration (NEN-EN-ISO 6865:2001). At the end of the trial, 1 kg litter sample was collected from each pen, and 2 birds per pen were euthanized to collect breast muscle (200 g/bird) and tibia samples. Litter, breast muscle and tibia samples were stored at -20°C until lab analyses. Litter samples were analyzed for Zn, Cu, Mn and Fe contents following method NEN-EN 15510:2017. The tibias were dried at 105°C for 24 h and placed in a desiccator, and bone weight was recorded. Tibia breaking strengths (breaking force divided by bone weight expressed as kilogram per gram) were measured using an Instror with 50-kg-load cell at 50-kg-load range with a crosshead speed of 50 mm/min with tibia supported on a 3.35-cm span [6]. Fat-free tibia ash was determined by ashing in tared ceramic crucibles for 24 h at 550℃, based on Regulation EC 152/2009, and calculated by dividing tibia ash weights by tibia dry weight and multiplying by 100 [7]. Se content in breast muscle was analyzed following NEN-EN 13805 for sample digestion and NEN-EN 15763 for Se determination. All data were summarized on pen average basis. Raw data were analyzed for outliers per measurement period. If the residual (fitted – observed value) > 2.5 × standard error on the residuals of the data set, the observation was marked as outlier and excluded from the dataset prior to statistical analyses [8]. The experimental data were analyzed with ANOVA, using Genstat® for Windows (20th version). Treatment means were compared by least significant difference (LSD) after significant effects were confirmed. Values with $P \leq 0.05$ were recognized as statistically significant, whereas $0.05 < P \leq 0.10$ were considered as near-significant trend.

	T1 (ITM[1])		**T2 (OTM[2])**		**T3 (MinCo®-250)[3]**	**T4 (MinCo®-375)[4]**
	mg/kg	Source	mg/kg	Source	mg/kg	mg/kg
Cu	16	$CuSO_4$	5.33	Cu AA (Availa®Cu)	3.75	5.63
Zn	90	$ZnSO_4$	30	Zn AA (Availa®Zn)	32.5	48.75
Mn	120	MnO_2	40	Mn AA (Availa®Mn)	30	45
Fe	20	$FeSO_4$	20	$FeSO_4$	25	37.5
Se	0.3	Na_2SeO_3	0.3	Se (Selisseo®)	0.2	0.3
I	1.25	$Ca(IO_3)_2$	1.25	$Ca\ (IO_3)_2$	0.75	1.125

[1] Ross 308 recommendations 2019; not for Zn that is limited to 90 mg/kg instead of 110 mg/kg to avoid reaching the EU legal limit (120 mg/kg = assuming 30 mg/kg coming from the ingredients).

[2] 1/3 of Ross 308 recommendations (2019) for Cu, Mn and Zn (Zn 1/3 of 90 mg/kg).

[3] Micromineral concentrations delivered with MinCo® 250 mg/kg.

[4] Micromineral concentrations delivered with MinCo® 375 mg/kg.

Table 1: Supplemented level and source of trace minerals of the four dietary treatments

Ingredients (%)	**Starter (Day 0-10)**	**Grower (Day 10-28)**	**Finisher (Day 28-37)**
Corn	30	25	20
Wheat	30.245	37.779	49.26
Soybean meal	29.45	26.415	20.615
Rapeseed meal	3	3	3
Limestone	0.984	0.688	0.459
Monocalcium Phosphate	0.574	0.228	-
Salt	0.18	0.183	0.162
Lysine HCl	0.27	0.265	0.23
DL-Methionine	0.27	0.249	0.163
Threonine	0.068	0.073	0.057
Valine	0.008	-	-
Soybean oil	1.2	2.171	2.285
Palm oil	1.746	2	2
Sodium Bicarbonate	0.224	0.167	0.2
Glucanase-xylanase	0.25	0.25	0.25
Phytase	0.5	0.5	0.386
Vitamin premix	0.5	0.5	0.4
ITM / OTM / MinCo®+ Filler	0.53	0.53	0.53
Total	**100**	**100**	**100**
Calculated Nutrients, g/kg			
Dry Matter	879	879	878
AMEn, kcal/kg	2,850	2950	3000
Crude protein	217	207	187
Crude fat	57.27	68.32	68.4
Crude fiber	26.25	25.93	25.51
Ca	7.41	5.67	4.28
P	5.03	4.15	3.47
Ash	54.24	46.46	38.79
SID Lys	11.84	11.13	9.54
SID M+C	8.49	8.1	6.87
SID Thr	7.23	6.92	6.07
Analyzed nutrients, g/kg			
Dry Matter	908	913	907
Ash	50.7	43.7	38.3
Crude protein	213	212	195
Crude fats	59.2	57.2	56.2
Crude fiber	26.2	26.8	27.8

Table 2: Basal diet composition and nutrients

	ITM	**OTM**	**M-250**	**M-375**	**LSD**	**P value**
Day 0-10						
Weight gain, g	235	238	240	248	13.9	0.27
Feed intake, g	249	250	250	256	13.1	0.67
FCR	1.062^{c}	1.050^{bc}	1.042^{ab}	1.034^{a}	0.0161	**0.01**
Mortality, %	0	2.8	0.6	0	2.73	0.12
Day 10-28						
Weight gain, g	1345	1356	1335	1366	73.8	0.83
Feed intake, g	1778	1799	1765	1786	76.7	0.82
FCR	1.321	1.329	1.323	1.308	0.0252	0.4
Mortality, %	1.9	1.6	1.9	2.6	3.52	0.95
Day 28-37						
Weight gain, g	883	922	912	917	66.5	0.62

Feed intake, g	1575	1610	1567	1612	70.6	0.44
FCR	1.786	1.748	1.724	1.765	0.085	0.49
Mortality, %	3.1	4	1.3	1.7	3.72	0.41
Day 0-37						
Weight gain, g	2463	2515	2487	2530	119.4	0.65
Feed intake, g	3602	3659	3582	3654	117.2	0.45
FCR	1.463	1.456	1.441	1.445	0.0312	0.45
Mortality, %	5	8.5	3.8	4.2	6.58	0.45

*Means within the same row not bearing common letter differ significantly (*P*<0.05).

Table 3: Effect of micromineral source and level on growth performance and mortality*

3. Results

3.1 Birds and diets

The day-old chicks arrived in good health, with average live weight 43.4 g. By the end of the trial (D37) the birds weighed 2525 g, slightly below the expected weight (2592 g) according to Aviagen Performance Guide (2019) for male Ross 308 broiler [9,10]. Measured indoor temperature and relative humidity inside the barn were close to the expected normal parameters. No signs of discomfort of the birds were observed during the entire experiment. The results of the chemical analyses of the experimental diets were in line with the expected values, except for crude fat that was around 11 g/kg below calculated values in Grower and Finisher diets (Table 2).

3.2 Growth performance

Results for body weight, body weight gain (BWG), feed intake (FI), feed conversion ratio (FCR), and mortality in different phases are shown in table 3. The body weight (BW) on days 10, 28 and 37 of age and the EBI between 0 to 37 days of age are presented in table 4. For Starter phase, BWG, FI and mortality were similar among treatments (*P* > 0.10). The birds fed on M-375 grew +5.4% more than those on ITM but statistically not different (*P* > 0.05). The FCR was affected by dietary treatment (*P* = 0.01). The birds receiving ITM showed the worst FCR among the 4 treatments, and no difference was observed between ITM and OTM. The birds fed on M-250 showed significantly lower FCR than those fed on ITM, and those fed on M-375 had a better FCR than those receiving OTM.

During Grower phase (10 to 28 days of age), the experimental diets did not affect growth performance (BWG, FI and FCR), and no differences were observed on mortality. Similarly, growth performance (BWG, FI and FCR) from 0 to 28 days of age was not affected by the different treatments (*P* > 0.10). Similar to the Grower phase, no differences were detected on the growth performance of the birds during the Finisher phase. Likewise, for the entire growth cycle (0 to 37 days of age), the different level and source of trace minerals did not affect overall performance (BWG, FI and FCR).

Table 4 summarizes the BW at the end of each phase and their EBI. The BW of birds on day 10, 28 and 37 and the EBI of the overall growth cycle were not significantly affected by the experimental diets, which is in line with the responses observed on BWG of each growing phase [11].

Treatment	**BW D10, g**	**BW D28, g**	**BW D37, g**	**EBI 0-37d**
Inorganic (ITM)	278	1623	2506	432
Organic (OTM)	281	1637	2558	428
M-250	284	1618	2531	450
M-375	291	1657	2574	453
LSD	14.1	74.9	119.4	40.5
P-value	0.27	0.71	0.65	0.49

Table 4: Effect of micromineral source and level on body weight (BW) on 10, 28 and 37 days of age and EBI

3.3 Bone ash, strength and mineral excretion

The results of tibia ash contents, tibia breaking strength, litter mineral concentration and muscle Se content are shown in table 5. No differences were observed between ITM and M-250 and M-375, whist the OTM treated birds showed significantly lower ($P < 0.05$) ash content compared to the other three treatments. On the other hand, tibia breaking strength was the same for all treatments ($P > 0.05$). The birds received ITM excreted significantly more Cu, Mn and Zn into their litter than the rest of the treatments ($P < 0.05$). The birds on M-250 excreted the lowest level of Mn. Significant differences were also observed in Fe excretion, as OTM treated birds excreted a lower level of Fe while no differences were observed among the rest three treatments. These results generally reflected the level of trace minerals intake. The Se analyses in the breast muscle showed no differences among the birds receiving inorganic Se, whilst the birds received organic Se (OH-SeMet) had significantly higher Se uptake and deposition ($P < 0.05$) than the other three treatments.

	Tibia		**Trace minerals in litter**				**Breast muscle**
	Ash content (g/kg)	Breaking strength (kgf)	Cu (mg/kg)	Fe (mg/kg)	Mn (mg/kg)	Zn (mg/kg)	Se (mg/kg)
ITM	519^{b}	65.4	54.3^{b}	518^{ab}	389^{c}	286^{c}	0.162^{a}
OTM	507^{a}	68.9	30.3^{a}	485^{a}	217^{b}	151^{a}	0.281^{b}
M-250	522^{b}	67.2	27.8^{a}	550^{b}	186^{a}	152^{a}	0.117^{a}
M-375	529^{b}	61.1	30.8^{a}	569^{b}	215^{b}	186^{b}	0.113^{a}
LSD	11.7	11.4	3.64	54.1	22.4	14.9	0.113
P-value	0.006	0.54	<0.001	0.026	<0.001	<0.001	0.024

$^{a\text{-}c}$Means in the same column without a common letter differ significantly ($P<0.05$).

Table 5: The effect of level and source of microminerals on tibia, litter and muscle

4. Discussion

4.1 Evolution of trace minerals

An adequate intake and absorption of trace minerals are important to support multiple metabolic and physiological roles for optimal growth and development. For the large scale of commercial poultry production, only a few (Fe, Mn, Zn, Cu, Se and I) are practically relevant, because their natural concentrations in feed ingredients could be either marginal or deficient. However, the exact requirements of each trace element are difficult to define due to differences in environmental conditions, breeds, feed composition, levels of feed intake and performance. Moreover, the presences of stressors and dietary antagonistic interactions among trace minerals and nutrients (e.g. phytate, calcium), may further complicate the process, such as reduced absorption thus increase their requirements [2]. The maximum authorized micromineral concentrations in animal diets are increasingly restrictive, due mainly to environmental concerns and antimicrobial resistance. Further restrictions can be expected in the future. Since the disadvantages of inorganic trace minerals (sulphates, oxides) are increasingly being realized by the industry, the organic trace minerals (OTM, chelates, proteinates) have been considered as a better solution, for their improved bioavailability as well as reduced interaction with other dietary components (i.e. phytate, Ca). However, recent studies suggest OTM may not be as inert as expected, and physical encapsulation of trace minerals appeared to be a promising technique, in providing trace minerals as essential nutrients while minimizing their interactions with other components in feeds.

4.2 Trace minerals on growth performance

The present study compared three sources of trace minerals, namely inorganic, organic and carbohydrate encapsulated inorganic trace mineral premix, at their commercially relevant doses (Table 1). For treatment 2, the available sources of OTM were Cu, Mn, Zn (Availa® Cu, Mn and Zn) and Se (Selisseo®), whereas I and Fe were inorganic sources due to lack of organic form. Supplemental Cu, Mn and Zn levels in treatment 2 were one third of those in treatment 1. In treatment 3 MinCo® was added at a level of 250 mg/kg, to supply similar (Fe, Zn) and lower (Cu, Mn, I and Se) levels than those in treatment 2 (OTM). Finally, in treatment 4, MinCo® was added at 375 mg/kg to provide similar levels of Cu, I, Mn and Se, and slightly greater (Fe, Zn) levels than those in OTM. All birds showed excellent growth, FCR and EBI, indicating both diets and trace minerals in this study were adequate to support body development. It is worth noting that the birds fed on OTM, M-250 and M-375 had a similar overall (0-28 and 0-37d) performance compared to the birds fed on ITM diet containing greater concentrations of trace minerals. Numerically, M-375 had a superior overall performance to ITM. These results agreed well with an early study by Lu *et al*. [4], the authors who reported the birds fed on MinCo® at 300/250/200 g/mt (respectively for Starter, Grower and Finisher) achieved better growth performance than those fed on ITM, and comparable performance to those received OTM, and the best performance results were obtained from MinCo® dosage of 400/350/300 g/mt. The results showed that during the starter phase (0-10d), the birds fed on the two MinCo® treatments (250 and 375 mg/kg) had a significant better FCR than the birds fed on ITM, and the birds fed on M-375 showed an improved FCR compared to the birds on OTM. The improved FCR is associated with an improved growth (WG) rather than stimulation of feed intake (FI). The improved diet efficiency may be attributable to better availability of the trace minerals in MinCo®, as Lu *et al*. the early study [4] confirmed coating of trace minerals significantly increased absorption and reduced excretion. Moreover, the coating process of the trace mineral premix also protected other essential nutrients (vitamins, enzymes and fatty acids) in diets, which is especially relevant during the Starter phase in which the birds have an immature gastrointestinal system.

4.3 Bone strength, mineral excretion and Se in muscle

The results of tibia ash contents in table 4 suggest no differences among ITM and the two MinCo® treatments, whilst the birds received OTM had lower ash contents ($P<0.05$). Since bone ash is more related to dietary calcium supply and deposition, the reason for lower ash content in the OTM treatment remains unclear. On the other hand, no differences were observed on tibia breaking strength among the 4 groups, suggesting all birds in this experiment developed similar bone strength. Interestingly, the analytical results of Cu, Mn, Zn and Fe concentrations in the litter (Table 5) follow closely with their respective dietary intake. Comparing with Cu excretion from the birds fed on ITM, the OTM and the two MinCo® groups reduced Cu excretion by 44-50%, similar patterns of reduction are also observed on the excretion of Mn and Zn. Likewise, the differences in Fe concentration in the litter sample also reflect dietary Fe supplementation. These results clearly demonstrate that the excretion of trace minerals follows dietary supplementation almost proportionally, and reduction of supplemental trace minerals should be a principal practice in reducing their excretion in litter. Se is a key element in the antioxidant system. For the modern fast growing birds, adequate level of antioxidant capacity is important to maintain their health and cope with various stress factors. Since body Se supply relies largely on dietary intake, the Se reserves in the body mass can reflect the antioxidant status of the flock. As shown in table 5, the Se levels in breast muscle confirmed early findings that organic Se (OH-SeMet) is more efficient in its absorption and deposition into the tissue of the birds, than the Se in the form of sodium selenite. Chemically Se is not a metal thus cannot be chelated. Typical organic form of Se is through chemical binding such as selenomethionine (SeMet) or hydroxy selenomethionine (OH-SeMet) as used in this study. The birds absorb OH-SeMet through their methionine absorption pathway, then convert OH-SeMet to SeMet and deposit into their body proteins, which explains high Se level detected in this study (Table 5).

5. Conclusion and Implication

1) This study demonstrated broiler birds can perform well on diets containing much reduced supplemental trace minerals. Namely, in mg/kg: Cu 3-5, Zn 32-48, Mn 30-45, Fe 20-25, I 0.75-1.12 and Se 0.2-0.3, in either OTM or carbohydrate encapsulated form.
2) The carbohydrate encapsulated trace mineral premix (MinCo®) can support expected growth performance of the broiler birds as well as the organic Zn, Cu, Mn and Se at the similar concentrations. MinCo® premix at 375 g/mt significantly improved FCR during the Starter phase.
3) Reduction of dietary supplemental trace minerals can significantly and proportionally reduce their excretion in litter.

Acknowledgments

The authors declare no conflict of interest.

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