Influence of trace mineral sources on broiler performance, lymphoid organ weights, apparent digestibility, and bone mineralization

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ABSTRACT This experiment was conducted to examine the effect of trace mineral sources on broiler performance, carcass composition, trace mineral digestibility, and tibia bone quality of broiler chickens. A total of 480 Ross 308 male day-old chicks were allocated to 24 pens and assigned to 4 dietary treatments in a completely randomized design. Treatments were as follows: inorganic (I) was basal diet supplemented with 750 g/t inorganic trace mineral premix; organic 1 (O1) and organic 2 (O2) was basal diet supplemented with 375 and 500 g/t organic yeast proteinate trace mineral premix respectively; and hydroxychloride (H) was basal diet supplemented with 1000 g/t salt encrusted trace mineral premix. On day 25, no differences in feed intake (FI), body weight gain (BWG), feed conversion ratio (FCR), or livability (LV) were observed between treatments ($P > 0.05$). On day 38 birds fed O1 and H had higher weight gain ($P < 0.05$) and lower FCR ($P < 0.001$) relative to I. Mineral sources had no impact on FI or LV ($P > 0.05$) on day 38. Spleen percentage of body weight on day 25 was increased in birds fed O1 and H treatments ($P < 0.05$) over the I treatment. Mineral sources had no effect on relative weights of thymus or bursa of Fabricius on day 25, or bone quality and carcass composition on day 39 ($P > 0.05$). Apparent digestibilities of Cu and Zn were greater in birds fed yeast proteinated trace minerals compared to other sources.

Key words: organic trace minerals, digestibility, broiler, bone, hydroxychloride minerals

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INTRODUCTION

Trace minerals are supplemented to avoid deficiencies that cause disturbances in metabolic processes including loss of appetite, lower performance, impaired immune system, and reproductive disorders (Van der Klis and Kemme, 2002). Trace minerals including manganese (Mn), copper (Cu), and zinc (Zn) are essential in various body functions and optimal growth and health (Richards et al., 2010). Trace minerals are involved in several physiological, digestive, and biosynthetic processes within the body. They are cofactors in many enzymes and act as catalysts in enzyme system and participate in immune defense system and hormone secretion pathways (Dieck et al., 2003). Trace minerals influence bone development, feathering, growth, and appetite (Nollet et al., 2007). Traditionally, inorganic trace minerals are supplemented to poultry diets to provide levels of minerals that allow the birds to reach its genetic growth potential and prevent clinical deficiencies (Bao et al., 2007). In commercial diets, inorganic trace minerals are typically used at levels higher than recommended by National Research Council (Inal et al., 2001) due to abundance and low cost. Feeding nutrient and mineral dense diets to broilers to maximize performance may result in excessive mineral excretion (Aksu et al., 2011a). Supplementation with high levels of inorganic trace minerals may be harmful to the environment and wasteful to the cost of operation. High mineral concentrations in manure, when used as fertilizer, can lead high soil concentration that reduce crop yield (Nollet et al., 2007) with surplus minerals filtering through soils and potentially polluting ground water supplies (Jackson et al., 2003). Inorganic minerals such as copper sulfate are hygroscopic and reactive catalyzing lipid oxidation and may react with other feed components. Trace mineral forms with high covalent and low ionic ligand bonding are less reactive and may be more bioavailable. These include minerals complexed with organic compounds and the hydroxychloride forms (Miles et al., 1998; Aksu et al., 2011a). Organic minerals are variety of compounds with polysaccharide or protein ligands (Saripinar-Aksu et al., 2012). Organic minerals can be supplemented at lower concentrations than sulfates and oxides without affecting bird performance. Animal can digest, absorb, and use covalently bound minerals better that ionically bound minerals (Aksu et al., 2011a). Hydroxychloride forms of trace minerals exist naturally as kemite (Mn), atacamite (Cu),

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simonkolleite (Zn), and hibbingite (Fe) (Palache et al., 1944). The hydroxychlorides have been found to be less reactive in feed and more bioavailable than other inorganic forms (Miles et al., 1998; Lu et al., 2010) and have less of an effect on hydrolysis of phytate by phytase (Pang and Applegate, 2006). It has been reported that supplementation with low concentrations of covalently bound organic trace minerals had no negative effect on antioxidant defense systems (Aksu et al., 2010a). The current study was conducted to evaluate the benefits of organic and hydroxychloride trace minerals relative to sulfate, oxide, selenite, and iodate forms on broiler performance, tibia quality and minerals content, carcass characteristics, and the digestibility of trace minerals of broilers reared under floor pen condition.

MATERIAL AND METHODS

The experiment was approved by the Animal Ethics Committee of the University of New England (Approval No: AEC12-030).

Animal Husbandry

A total of 480 day-old feather-sexed male Ross chicks were obtained at hatching from the Baiada hatchery in Tamworth, NSW, Australia. Birds were vaccinated against Newcastle, Marek's, and infectious bronchitis disease at the hatchery according to the commercial vaccination schedule of Baiada. Chicks were assigned to 24 floor pens (75 × 120 cm) randomly allocated to 4 dietary treatments each containing 6 replicates of 17 birds. The study employed a completely randomized design with pen as the unit of measure. Temperature and lighting were adjusted according to husbandry guidelines for the Ross 308 strain. Each pen was equipped with a separate tube feeder and nipple drinkers with water and feed provided ad libitum. At day 10, 2 birds were culled from each pen. Mortality was recorded daily, whereas cumulative pen weight and feed intake were recorded on days 10, 16, 25, and 38.

Diets and Test Mineral Sources

Basal diets for starter and grower were formulated according to Ross 308 nutrient specifications (Table 1). Diets were based on wheat, sorghum, sorghum, soybean meal, meat meal, and canola meal. Ingredients were pre-analyzed prior to formulation. Diets were mixed and pelleted at 65°C. Treatments were as follows: inorganic (I)—the basal diet supplemented with 750 g/t inorganic trace mineral premix; organic1 (O1)—the basal diet supplemented with 375 g/t organic yeast proteinate trace mineral premix; organic2 (O2)—the basal diet supplemented with 500 g/t organic yeast proteinate trace mineral premix; and hydroxycloride (H)—the basal diet supplemented with 1000 g/t salt encrusted trace mineral premix. Dietary treatments varied

Table 1. Composition and nutrient content of diets (kg/t).

Ingredient	Starter 1 to 16 d	Grower 17 to 38 d
Wheat (Aus)	599.92	610.82
SBM (Arg)	233.00	202.00
Meat and bone meal	30.00	30.00
Canola meal	92.00	100.00
Canola oil	19.00	37.00
Limestone	6.00	6.00
Dicalcium phosphate	6.00	3.00
Salt	3.40	1.90
Sodium bicarbonate	2.00	2.00
L-lysine HCL	2.70	2.10
D,L-methionine	2.10	2.00
L-threonine	0.30	0.30
Choline chloride 70%	1.20	0.50
Vitamins[1]	0.50	0.50
Salinomycin 12%	0.50	0.50
Xylanase	0.05	0.05
Zn bacitracin 15%	0.33	0.33
Trace min and wheat	1.00	1.00
Nutrient composition		
ME, Kcal/kg	2950	3100
Protein	227	217
Digestible lysine	12.0	11.0
Digestible methionine	4.44	4.18
Digestible M+C	8.4	8.03
Digestible tryptophan	1.92	1.87
Digestible threonine	7.44	7.04
Digestible arginine	12.60	11.88
Digestible isoleucine	7.80	7.37
Digestible valine	9.24	8.58
Calcium,	8.4	7.4
Non-phytate P	4.0	3.4
Sodium	2.4	1.8
Choline, mg/kg	1850	1450

[1]UNE vitamin concentrate supplied per kg of diet (mg): vitamin AD3, 24.0; vitamin B1, 6.122; vitamin B2 riboflavin, 20.0; vitamin B3, 106.784; vitamin B5, 28.838; vitamin B6, 10.204; vitamin B9, 4.124; vitamin B12, 3.2; vitamin D3, 10.4; vitamin E, 300.0' vitamin H biotin, 20.0; vitamin K3, 13.73; limestone Murgon aglime, 191.083; cereal carrier fines, 100.0; ethoxyquin, 151.515; white oil, 10.0.

in trace minerals as shown in Table 2. The organic and hydroxychloride sources contained chromium, whereas the inorganic source did not. In the grower diet included 0.5% of titanium dioxide as an indigestible marker for the purpose of estimating apparent mineral digestibility in jejunum and ileum. Birds were fed starter to day 16 and grower diet until the end of the study. Residual feed was weighed on days 10, 16, 25, and 38. The inorganic minerals were sulfate, oxide, selenite, and iodate obtained from BEC Company (Brisbane, Australia). Organic minerals were proteinates (Organomin) obtained from Zeus Biotech (Mysore, India). Hydroxychloride minerals (salt encrusted) were obtained from Ritus Chemicals (Chennai, India). The hydroxychloride minerals were produced in a proprietary process involving the drying of salt solutions of various metals and ligands to produce covalently bound hydroxychloride metal complexes.

Sample Collection

On day 11 and 25, 2 birds from each pen were randomly selected, weighed, and euthanized by cervical dislocation. Thymus, bursa, and spleen weights were

Table 2. Supplemental levels of minerals fed to birds on different diets (mg/kg feed).

Treatments	Inorganic[1]	Organic 1[2]	Organic 2[2]	Hydroxychloride[3]
Copper	16.00	3.75	5.00	5.00
Iron	40.00	11.25	15.00	15.00
Iodine	1.25	1.50	2.00	2.00
Selenium	0.30	0.20	0.30	0.30
Manganese	120.00	22.50	30.00	30.00
Zinc	100.00	20.25	30.00	30.00
Chromium	0.00	0.375	0.50	0.50
Analyzed minerals content of grower diet				
Copper	20.04	12.09	18.26	10.80
Iron	113.53	89.38	98.06	83.89
Manganese	173.08	77.15	92.71	75.99
Zinc	156.71	99.07	116.07	62.60

[1]As copper sulfate pentahydrate (25% Cu); as ferrous sulfate monohydrate (30% Fe); as potassium iodide (68% I); as sodium selenite (45.5% Se); as manganous sulfate (31% Mn) and oxide (60% Mn) 2.7:1; as zinc oxide (80% Zn).

[2]As yeast proteinate.

[3]As salt encrusted hydroxychlorides: contains ($Cu_2Cl(OH)_2$, $Zn_5(OH)_8Cl_2{\cdot}H2O$, $Mn_2Cl(OH)_3$, $Fe_2(OH)_3Cl$; $Cr_2(OH)_3Cl_3$) plus various forms of I and Se derived during drying of KI and Na_2SeO_3 in NaCl solution.

taken. On day 25 and 35, digesta samples from jejunum (from duodenum to the Meckel's diverticulum) and ileum (from Meckel's diverticulum to 5 cm above the ileo-cecal junction) were collected and stored in 50 mL plastic containers and frozen directly at −20°C for analysis. On day 38, 4 birds from each pen were randomly selected, weighed, and killed by cervical dislocation. Right tibia sampled were collected and frozen at −20°C for later analysis.

Measurement

Pens and feed were weighed on days 0, 10, 16, 25, and 38. Apparent jejunal and ileal trace mineral digestibility was measured using titanium dioxide (TiO_2) as a marker using the equation.

$$\int \text{trace mineral digestibility} =$$

$$1 - \frac{[\text{trace minerals}/\text{titanium dioxide}]\ \text{digest}}{[\text{trace minerals}/\text{titanium dioxide}]\ \text{diet}}$$

Chemical Analysis

Tibia samples were boiled in deionized water for 10 min, and all soft tissue were removed. Length and width of tibia were measured using a digital calliper (Mitutoyo, Japan). Tibia bone breaking strength was measured using an Instron instrument (Model 1011 Instron Universal Testing Machine, Instron Corp., Canton, USA). Samples were placed on vertical brackets set 40 mm apart, and a 10 mm compression rod was positioned near the center of the bone. The instrument was equipped with a 50-kg-load cell and a crosshead speed of 50 mm/min was used during the breaking strength determination. Tibia bone samples were then dried for 12 h at 105°C and ashed in a Carbolite CWF 1200 chamber furnace (Carbolite, Sheffield, UK) at 550°C for 4 h after starting at 200°C with a 1 h ramp up time.

Digesta samples were freeze dried at −50°C for 1 wk. The dried digesta and tibia ash samples were subjected to digestion in $HClO_4$ (70%) and H_2O_2 (30%) in an Ultrawave Microwave Digestion system (Milestone, Italy) followed by mineral analysis using inductively coupled plasma optical emission spectrometry (ICP-OES, Agilent, Australia) using methods described in Anderson and Henderson (1986). The titanium dioxide concentration in feed samples and freeze-dried digesta was measured as described by Short et al. (1996). The titanium dioxide content was measured by using a Hitachi 150-20 UV spectrophotometer (Hitachi Science System Ltd, Ibaraki, Japan), and the absorbance was measured at 410 nm.

Statistical analysis

The SAS statistical package (PROC GLM) was used to determine significance of differences between treatments (SAS, 2013). Duncan's multiple range test was used to separate individual treatment means when appropriate. Nonparametric analyses were performed on livability data (PROC NPAR1WAY WILCOXON) as data were not normally distributed.

RESULTS

Broiler Performance

Overall bird performance was excellent from 1 to 25 d and 1 to 38 d exceeding Ross 308 performance objectives (Aviagen, 2014). Bird performance results are shown in Table 3. During the period 1 to 10 d and 1 to 16 d, no differences in weight gain, feed intake, or FCR were observed between treatments groups ($P > 0.05$). However, during the 1 to 10 d period, there was a strong tendency ($P = 0.052$) for higher livability of birds in treatments O1 and O2 relative to I. From 1 to 38 d, birds fed treatment diets O1 and H tended to have higher weight gain ($P = 0.052$) when compared to those in treatment I. Lower FCR was observed ($P < 0.001$) in birds fed O1, O2, and H compared to I.

Relative Lymphoid Organ Weights

Table 4 shows no differences ($P > 0.05$) among the treatment groups for relative thymus, spleen, and bursa of Fabricius weight (as percentage of live body weight) on day 11. On day 25, birds fed treatments O1 and H had increased relative spleen weights compared to I. On day 11 and 25, no differences in relative percentage weights were observed among treatments for thymus or bursa of Fabricius.

Table 3. Bird performance on different diets.

Period	Inorganic	Organic 1	Organic 2	Hydroxychloride	P value	Pooled SEM
Initial weight (g/bird)	37.6	37.6	37.8	37.8	0.318	0.058
Body weight gain (g/bird)						
1-10 d	201	209	201	203	0.548	2.531
1-16 d	513	527	526	514	0.713	5.110
1-25 d	1315	1388	1363	1357	0.166	11.772
1-38 d	2824^{b}	3007^{a}	$2942^{a,b}$	2985^{a}	0.052	26.262
Feed intake (g/bird)						
1-10 d	243	248	258	239	0.251	3.508
1-16 d	658	665	678	655	0.580	6.314
1-25 d	1780	1839	1821	1797	0.398	12.826
1-38 d	4134	4221	4147	4177	0.747	28.866
Feed conversion ratio						
1-10 d	1.207	1.191	1.224	1.174	0.242	0.008
1-16 d	1.283	1.264	1.291	1.274	0.187	0.004
1-25 d	1.356	1.325	1.336	1.324	0.097	0.005
1-38 d	1.466^{a}	1.404^{b}	1.409^{b}	1.400^{b}	0.001	0.007
Livability %						
1-10 d	95^{b}	99^{a}	99^{a}	$97^{a,b}$	0.052	0.605
1-16 d	94	98	98	97	0.156	0.706
1-25 d	94	96	96	96	0.743	0.730
1-38 d	94	95	93	94	0.874	0.791

[a,b]means in rows with different superscripts are significantly different ($P < 0.05$).

Table 4. Thymus, spleen, and bursa of Fabricius as percentage from live body weight of birds fed on different diets on day 11 and 25.

	Inorganic	Organic 1	Organic 2	Hydroxychloride	P value	Pooled SEM
Thymus %						
11 d	0.148	0.134	0.140	0.154	0.622	0.005
25 d	0.157	0.164	0.149	0.140	0.731	0.007
Spleen %						
11 d	0.071	0.077	0.077	0.073	0.862	0.003
25 d	0.070^{c}	$0.087^{a,b}$	$0.073^{b,c}$	0.092^{a}	0.016	0.003
Bursa %						
11 d	0.174	0.154	0.189	0.160	0.312	0.007
25 d	0.170	0.179	0.179	0.176	0.971	0.007

[a,b,c]Means in rows with different superscripts are significantly different ($P < 0.05$).

Tibia Bone Quality and Mineral Contents

Table 5 shows tibia quality and tibia mineral content. Dietary treatments had no effect on tibia bone width, length, breaking strength, tibia bone ash percent, or Ca, P, Fe, and Zn concentration ($P > 0.05$). However, Mn concentration in tibia bone of birds fed treatment I was higher ($P < 0.05$) than birds fed O1, O2, and H treatments.

Mineral Digestibility

Apparent digestibilities of Cu, Fe, Mn, and Zn in jejunum and ileum are shown in Table 6. On day 25, the digestibility of Cu in jejunum was higher ($P < 0.05$) in birds fed treatment O2 compared with other treatments. Apparent digestibilities of Mn and Zn in jejunum and ileum were not affected by dietary treatment on day 25 ($P > 0.05$). On day 35, apparent digestibility of Cu in jejunum and ileum was higher ($P < 0.05$) in birds fed O2 relative to I and H. The apparent digestibility of Mn in jejunum and ileum was not affected by dietary treatment ($P > 0.05$). Dietary treatment had no effect on apparent Zn digestibility in jejunum ($P > 0.05$) but birds fed O1 and O2 treatment diets had greater apparent Zn digestibility in the ileum compared to I or H ($P < 0.05$).

DISCUSSION

The use of lower concentrations of hydroxychloride or organically complexed minerals in animal diets has been suggested as a solution to reduce mineral excretion without undesirable effect on performance. The results of the current experiment show equal or higher body weight and lower FCR in birds fed diets with half or less of total levels of Cu, Zn, Fe, and Mn as organic or hydroxychloride compared to sulfate and oxide forms.

Table 5. Tibia bone trace mineral concentration and bone quality of birds fed on different diets on day 38, ash basis.

Parameters	Inorganic	Organic 1	Organic 2	Hydroxychloride	P value	Pooled SEM
Ash %	45.2	45.0	43.9	45.4	0.695	0.5
Ca (%)	37.2	37.4	37.0	37.3	0.564	0.1
P (%)	17.9	18.0	17.7	17.8	0.218	0.6
Cu (mg/kg)	4.7	4.544	4.8	3.9	0.245	0.2
Fe (mg/kg)	443.4	470.3	454.0	412.6	0.598	15.6
Mn (mg/kg)	6.6^{a}	5.2^{b}	5.4^{b}	5.8^{b}	0.007	0.16
Zn (mg/kg)	327.9	320.6	328.8	304.4	0.170	4.6
Bone quality						
Tibia						
Strength (kg)	51.63	48.65	54.92	54.20	0.195	1.099
Weight (g)	17.25	16.21	15.40	16.40	0.382	0.349
Length (mm)	100.27	102.35	100.96	101.17	0.240	0.363
Width (mm)	10.26	10.17	9.99	10.17	0.841	0.104

[a,b]Means in rows with different superscripts are significantly different ($P < 0.05$).

Table 6. Cu, Mn, and Zn apparent digestibility percent in jejunum and ileum of birds fed diets with different trace mineral sources.

Parameters	Inorganic	Organic 1	Organic 2	Hydroxychoride	P value	Pooled SEM
Day 25						
Cu						
Jejunum	0.54^{c}	13.22^{b}	40.11^{a}	13.06^{b}	0.001	3.48
Ileum	3.55	11.63	28.73	13.81	0.071	3.32
Mn						
Jejunum	9.19	10.58	19.06	11.78	0.373	2.11
Ileum	7.74	0.96	5.32	7.32	0.547	3.14
Zn						
Jejunum	18.60	21.05	26.78	16.44	0.312	2.01
Ileum	22.02	26.20	30.54	12.84	0.095	3.78
Day 35						
Cu						
Jejunum	2.28^{b}	$13.85^{a,b}$	30.38^{a}	5.35^{b}	0.005	3.349
Ileum	-2.12^{b}	-1.35^{b}	27.27^{a}	-2.00^{b}	0.0001	2.904
Mn						
Jejunum	6.04	4.33	10.31	11.67	0.649	2.251
Ileum	–4.48	–1.24	6.77	–3.76	0.414	2.335
Zn						
Jejunum	13.91	17.10	21.31	13.83	0.326	1.616
Ileum	9.08^{b}	18.58^{a}	21.23^{a}	5.44^{b}	0.032	2.184

[a,b,c]Means in rows with different superscripts are significantly different ($P < 0.05$).

This is similar to other reports showing that basal diets containing lower levels Zn, Mn, and Cu in organic complexes were similar in weight, feed intake, and FCR than birds fed inorganic minerals as ionic salts of sulfate and oxide (Aksu et al., 2010b, 2011b; Zhao et al., 2010). Bao et al. (2007) reported no significant difference in weight gain or feed intake of birds diets supplemented with organic Cu 4 mg, Fe 40 mg, Mn 40 mg, and Zn 40 mg/kg diet when compared birds fed much higher levels of inorganic forms. This was consistent with current results when low levels of organic or hydroxychloride minerals did not affect performance on day 10, 16, and 25. In the current study on day 38, birds fed O1 and H minerals had higher weight gain and lower FCR when compared to the I treatment. This was in agreement with the report of Rao et al. (2013) showing feed efficiency to be improved significantly with reduction of organic trace minerals in Vanaraja chickens diet compared to those fed 100% inorganic trace minerals. El-Husseiny et al. (2012) reported that body weight gain and FCR were improved by supplementation broilers diet with 50% Zn, 50% Mn, and 50% Cu of their recommendations as organic trace mineral compared with adding 100% of those mineral recommendations as inorganic.

Measurements of absorption and digestion of supplemental trace minerals are generally difficult to make because of the complexity of endogenous mineral excretion. In the current study, on day 25 and 35 apparent digestibility of Cu in the jejunum of birds fed O and H minerals were increased compared to the sulfate form. In the ileum, the highest apparent Cu digestibility was recorded for the higher level of O minerals on day 25 and 35. The apparent digestibility values measured do not take into account the endogenous flow of minerals at different points along the digestive tract. It is of interest that negative apparent digestibility was observed for Cu and Mn in all treatments except the higher level of O minerals in ileum but not jejunum on day 35 but not on day 25. This suggests that endogenous flow is affected by O mineral level, age, and location along the gastrointestinal tract. The high digestibility of O Cu may indicate that inclusion of low level of O Cu is adequate to support or improve a normal body and bone growth. It has been reported that level of Cu has a positive effect on performance of animals (Zhou et al., 1994; Samanta et al., 2011). The digestion results in current study suggest that endogenous flow of Zn is lower than absorption throughout the jejunum and ileum and apparent ileal digestibility may be greater for organic sources than hydroxychloride or oxide. Similar trends were observed by Bao et al. (2010), who suggested that organic Zn had higher digestibility than inorganic sources. The higher Cu and Zn digestibility may indicate that the organic trace minerals have higher bioavailability. It is believed that organic trace minerals may be 1.2 to 1.8 times more bioavailable than inorganic forms (Zhao et al., 2010) and organic minerals may be better absorbed as they do not form the same insoluble complexes as ionically bound trace minerals (Van der Klis and Kemme, 2002).

Although dietary supplementation with different sources of trace minerals did not affect thymus or bursa of Fabricius percentage of body weight at day 11 in the current study, birds fed organic (power level) and hydroxychloride forms of minerals had higher spleen weights as a percentage of body weight to the sulfate and oxide form when measured on day 25. The higher spleen weight may reflect the immune status of the birds. The spleen plays important role in storage, maturation, and generation of lymphocytes and is important in both cellular and humeral immunity (Jeurissen, 1993; Smith and Hunt, 2004). When the humoral immunity increases, the spleen may become larger. It has been reported that there is a positive relationship between spleen mass and immune competence with small spleens less les able to mount an immune response as compared to large ones (Moller and Erritzoe, 2000). In the current study, the fact that the O1, O2, and H treatment premixes contained chromium whereas the I premix did not may have influenced relative spleen weights. Sahin et al. (2003) found dietary chromium tripicolinate supplementation to increase relative spleen weights in heat stressed broilers, but not performance in the current experiment. It is unlikely however that the chromium differences between treatments had any effect on body weight or feed intake. Rajalekshmi et al. (2014) found that dietary chromium propionate supplementation did not affect weight gain, FCR, or feed intake in commercial broilers grown under normal conditions. Thus, the improved performance of birds fed H or O minerals in the current study is likely due to bioavailability or digestibility.

Trace minerals play important role in development, growth, and maintenance of bone. The bone is a complex heterogeneous tissue that supports the musculature, and thus its growth and development are intimately connected with overall body growth (Loveridge, 1993). In the current experiment, the inorganic diet had higher 3.3×, 3.2×, 2.7×, and 4× the levels of added Zn, Cu, Fe, and Mn, respectively, compared to organic (500 mg/kg) and hydroxychloride mineral diets. However, bone quality including breaking strength, tibia weight, tibia length, tibia width and ash, and tibia minerals content was not affected by these low levels of total trace minerals except tibia Mn which was higher in the inorganic (sulfate and oxide) treatment. The trace minerals concentrations in tissue are indicator of mineral status, body storage, and have been used as biomarkers in bioavailability studies (Wang et al., 2007). The results of bone quality and tibia trace minerals content demonstrated that low levels of organic trace minerals were sufficient to support normal bone growth. Same results were found by El-Husseiny et al. (2012), who reported that the birds fed 50% of organic Zn, Mn, and Cu had no effect on tibia weight, length, and diameter compared with those fed 100% inorganic Zn, Mn, and Cu on day 35 of age. The inclusion low levels of organic or hydroxychloride trace minerals in present study did not modify bone strength, demonstrating that the trace minerals as organic or chelated form already met the requirement of chickens for bone growth. Roughead and Lukaski (2003) showed that the Cu and Fe deficiencies are identified to hinder bone growth and bone strength. Rao et al. (2013) found that inclusion of organic Zn, Mn, Cu, Fe, I, Se, and Cr at levels of 50% of recommendations for inorganic levels had no effect on tibia Ca and Fe content. It has been reported that supplementation of organic trace minerals at 66 or 100% of recommended inorganic levels had no effect on tibia Zn and Cu content (Aksu et al., 2011b). Bao et al. (2010) found that dietary supplementation with organic or inorganic Cu, Fe, Mn, and Zn at level 4, 20, 40, and 30 mg/kg, respectively, had no effect on tibia bone contents of Ca, P, Cu, Fe, and Zn when compared to NRC recommended level.

CONCLUSION

Carcass characteristics, tibia bone strength, and minerals content were not affected by lower levels trace minerals in covalent form (O or H) compared to higher levels in ionic (I) form. Further examination of chromium on spleen morphology and immune status is warranted. These results indicate that both covalent bound yeast proteinate and covelent hydroxychloride forms of trace minerals can be used at lower levels than ionic forms such as sulfate and oxide with no negative effect on performance or bone strength. It is concluded that feeding lower levels of minerals in covalent bound form such as proteinates or hydroxychlorides (O or H) may offer performance and economic benefits over ionic bound forms such as sulfates or oxides.

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