



The Digestibility of Organic Trace Minerals along the Small Intestine in Broiler Chickens

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ABSTRACT : An experiment was conducted to evaluate the effects of low concentrations of organic and inorganic dietary trace minerals on broiler performance and trace mineral digestibility along the small intestine of 35-day-old broiler chickens reared under floor-pen conditions. Eight hundred male, day-old Cobb broiler chickens were randomly allocated to 4 dietary treatments (25 birds per pen with 8 replicates per treatment). Broilers fed diets supplemented with 4, 20, 40 and 30 mg/kg, respectively, of Cu, Fe, Mn and Zn from organic chelates and inorganic salts achieved the same body weight gain as those supplemented at the NRC levels (8 mg Cu, 40 mg Fe, 60 mg Mn and 40 mg Zn/kg, respectively) from inorganic salts. However, birds fed a control diet without any supplementation at dietary levels of 7.4-8.8, 60.1-69.2, 14.6-15.4 and 19.1-20.6 mg/kg of Cu, Fe, Mn and Zn, respectively, had decreased feed intake and growth rate. There was no significant difference in the digestibility of Cu in all regions of the small intestine. Throughout the small intestine the apparent absorption of Mn from both organic and inorganic sources was small, whereas the digestibility of Zn seemed to be more complex, exhibiting differences in the apparent absorption due to both mineral source and intestinal site. Therefore, the digestibility of organic Zn was improved ($p < 0.01$) in the ileum compared to inorganic Zn. The digestibility of Zn in the duodenum was smaller ($p < 0.05$) than that in the ileum. (**Key Words** : Organic Trace Minerals, Small Intestine, Digestibility, Broiler Chickens)

INTRODUCTION

In commercial broiler production, a large safety margin used in feed formulation for supplemental inorganic trace minerals results in a high level of mineral excretion, which is harmful to the environment. It is believed that organic trace minerals are better absorbed and utilized than their inorganic counterparts and are protected from interactions that interfere with their bioavailability, thus leading to a reduction in the excretion of minerals (Scott et al., 1982; Leeson, 2003). Theoretically, the organic trace minerals may be able to be supplemented at a much lower level. However, the apparent absorption of Cu, Fe, Mn and Zn based on excreta does not provide a suitable measurement of the bioavailability of trace minerals (Ammerman, 1995). Apart from indigestible trace minerals and cell abrasion, the excreta mainly contain excess trace minerals which can not be absorbed (Underwood and Suttle, 1996b). Most study on

organic trace minerals for broilers have used conventional diets, tending to exceed the bird's requirement (Lee et al., 2001; Paik, 2001) and a negative digestibility of trace minerals. However, using a special control diet (Bao et al., 2007a), which is deficient in Cu, Fe, Mn and Zn, may provide a means for determining the digestibility of organic trace minerals in different parts of the gastro-intestinal tract (GIT) as it becomes possible to avoid trace mineral excess in the GIT.

Thus, the aim of this study was to evaluate the effects of low concentrations of organic and inorganic dietary trace minerals on broiler performance, tibia trace mineral concentrations and the digestibility of trace minerals along the small intestine of 35-day-old broiler chickens reared under floor-pen conditions.

MATERIALS AND METHODS

The experiment was approved by the Animal Ethics Committee of the University of New England (Approval No: AEC 04/147).

Animal husbandry

A total of 800 day-old male Cobb broiler chicks were

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randomly allocated to 32 deep litter pens with 8 replicates of 25 birds per treatment. In the first two weeks, the birds were given starter diets, followed by the finisher diets for 3 weeks. During the finisher period, 5 g Celite (acid insoluble ash (AIA), Celite Corporation, Lompoc, CA)/kg diet was added to all the diets as a marker for digestibility measurement. All the birds were fed *ad libitum* throughout the experiment. For the first three days, the shed temperature was maintained at 35°C and gradually reduced to 23°C at 28 d of age and maintained till the end of the experiment. Body weight and feed intake were recorded weekly for the calculation of body weight gain and FCR corrected for mortality. On d 21 and 35, two birds from each pen were randomly selected, weighed and killed by cervical dislocation. Right tibia from each bird was collected and pooled by pen and then put into plastic bags. On d 35, the body cavity of birds was opened with stainless scissors and the different regions of small intestines was separated. The contents from duodenum, jejunum (from the caudal end of the duodenum to the Meckel's diverticulum) and ileum (from Meckel's diverticulum to 4 cm above ileo-caecal junction) were gently collected and pooled per pen in

plastic containers. All samples were frozen at -20°C and analysed for the trace mineral contents and AIA later.

Dietary treatments

Four diets were formulated with sorghum and isolated soy as the main ingredients, and pelleted to reduce segregation. Diet 1 acted as the control diet (Table 1) with all the nutrients either meeting or exceeding the NRC (1994) nutrient requirements except for Cu, Fe, Mn and Zn, which were maintained at 7.4, 60.1, 14.6 and 19.1 mg/kg for the starter diet and 8.8, 69.2, 15.4 and 20.6 mg/kg for the finisher diet. Three other diets were derived from the control diet with variations only in the source and level of Cu, Fe, Mn and Zn contents; as shown in Table 2. Diet 2 served as the organic diet (Org) and was supplemented with organic sources of Cu, Fe, Mn, Zn at 4, 20, 40 and 30 mg/kg diet, respectively. Diet 3, inorganic diet (Inorg), was supplemented with corresponding contents of Cu, Fe, Mn and Zn from an inorganic source. Diet 4, the NRC diet (NRC) was similar to Inorg diet except that it was supplemented from a feed-grade sulphate source at 8 mg Cu, 40 mg Fe, 60 mg Mn and 40 mg Zn/kg diet. Organic Cu, Fe, Mn and Zn was added to the diets as Bioplex® chelated trace mineral proteinates containing 10% Cu, 10% Mn, 15% Fe and 15% Zn, respectively, supplied by Alltech Biotechnology Pty Ltd (Melbourne, Australia). Inorganic Cu, Fe, Mn and Zn were added to diets as sulphates that contain Zn 35%, Mn 31%, Fe 30% and Cu 25%, respectively, kindly provided by DSM Nutritional Products Australia Pty Ltd (French's Forest, Australia).

Measurements

The apparent absorption of a nutrient can be calculated in any section of the gut provided values for nutrient: AIA ratios in both the diet and gut section are known (Vogtmann et al., 1975). Therefore, Cu, Fe, Mn and Zn digestibility up to the duodenum, ileum and jejunum were estimated from the analysis of AIA and mineral concentrations in feed, freeze-dried digesta from these regions using the equation:

$$\text{Trace Mineral Digestibility} = 1 - \frac{\left(\frac{\text{Trace Minerals}}{\text{AIA}}\right)_{\text{digesta}}}{\left(\frac{\text{Trace Minerals}}{\text{AIA}}\right)_{\text{diet}}}$$

Table 1. The composition of the control diet (g/kg)

	Starter	Finisher
Sorghum	771	819.65
Isolated soy protein	175	120.00
Vegetable oil	16.00	20.00
Calcium carbonate	12.46	11.00
DiCalcium phosphate	18.20	17.00
Sodium chloride	2.50	2.50
Lysine-HCl	1.00	1.00
Celite	0.00	5.00
DL-methionine	2.34	2.35
Vitamin/mineral premix ^a	1.00	1.00
Choline chloride	0.50	0.50
Calculated values (g/kg)		
ME (MJ/kg)	13.06	13.25
CP	225	185
Ca	8.9	8.5
Available P	3.6	3.4
Lysine	13.6	10.2
Analyzed values (mg/kg)		
Cu	7.40	8.80
Fe	60.10	69.2
Mn	14.60	15.4
Zn	19.10	20.6

^a Vitamin-mineral premix supplied the following per kilogram of diet: 10,000 IU vitamin A (retinyl acetate), 2,500 IU vitamin D₃ (cholecalciferol), 50 mg vitamin E (DL-α-tocopheryl acetate), 2 mg thiamine, 10 mg riboflavin, 50 mg niacin, 7 mg D-calcium pantothenate, 7 mg pyridoxine, 25 µg cyanocobalamin, 250 of biotin, 0.3 mg Se, 1 mg I, 0.5 mg molybdenum, and 0.25 mg Co.

Table 2. Supplemental contents of trace minerals fed to birds on different diets (mg/kg diet)

Diet	Added Cu	Added Fe	Added Mn	Added Zn
Control	0	0	0	0
Organic	4	20	40	30
Inorganic	4	20	40	30
NRC	8	40	60	40

Chemical analyses

Feed samples were prepared by grinding them to pass through a 0.5 mm screen in a stainless steel blade grinder. Tibia samples were boiled for approximately 10 min in deionized water and cleaned of all soft tissue. The tibia samples were then dried for 12 h at 105°C in an air-forced convection oven (Qualtex Universal Series 2000, Watson Victor Ltd., Perth, Australia) and then ashed after weighing (550°C for 4 h) in a Carbolite CWF 1200 chamber furnace (Carbolite, Sheffield, UK). The ash samples were ground in a small stainless steel grinder (IKA®-WERKE, Staufen, Germany).

Approximately 0.5 g of feed samples were placed in a Teflon TFM vessel. Eight ml of nitric acid (70%) was added along with 2 ml of hydrogen peroxide (30%). The vessel was closed and introduced to the rotor segment, then tightened using a torque wrench. The segment was inserted into the microwave cavity and the temperature sensor connected. The microwave program was run for 45 min. The rotor was cooled by air until the solution reached room temperature. The vessel was opened and the solution was quantitatively transferred into a 50 ml volumetric flask. The solution was made to 50 ml total volume with deionised water and mixed well for analysis of trace mineral concentration by inductively coupled plasma emission spectroscopy (ICP) (Vista MPX, Melbourne, Australia) (Milestone, 2000).

Trace mineral contents were determined in the tibia bone and digesta samples. Approximately 0.2 g of bone ash or freeze-dried digesta was placed into a Schott bottle in a scrubbed fume cupboard. Two ml of a mixture of HClO₄ (70%) and H₂O₂ (30%) was added to each tube. Each tube was loosely covered with a lid and left overnight. Then 1 ml of H₂O₂ was added and tubes were tightly sealed and placed in an oven set at 80°C for 30 minutes. The bottles were allowed to cool slightly and a further 1 ml H₂O₂ was added before they were capped tightly and digested for 1 h at 80°C. The solution was made to a weight of 25 g and filtered through Whatman No. 1 filter paper for ICP analysis (Aderson and Henderson, 1986).

The concentration of AIA in the feed and in the freeze-dried digesta was determined using the method described by Choct and Annison (1990).

Statistical analysis

Statistical analyses were performed using STATGRAPHICS software (Manngistics, Inc., Rockville, Maryland, USA). The data were analysed using one-way ANOVA with diet as the factor. The significance of difference between means was determined by Duncan's multiple range test. The mortality data were analyzed using Kruskal-Wallis Test and differences were deemed significant when $p \leq 0.05$.

RESULTS

Broiler performance

Bird performance results are shown in Table 3. During the first week, birds on the control diet began to show reduction ($p < 0.001$) in both feed intake and body weight gain. This effect was maintained throughout the 35 days of growth and became more pronounced towards the end of the trial. However, there was no significant difference ($p > 0.05$) in FCR between the control diet and the supplemental diets during the periods of 14-35 d and 1-35 d. There was no significant difference ($p > 0.05$) in mortality rates between treatments, although the birds fed the control diet showed much smaller body weight gain.

Mineral concentrations and ash weight in tibia

Table 4 shows that there were no significant differences among the treatment groups in tibial concentrations of Ca, P, Cu and Fe. However, dietary deficiency in trace minerals strongly affected ($p < 0.001$) Mn and Zn concentrations and total ash weight in the tibia ($p < 0.001$) of both 21 and 35-day-old broiler chickens but there was no significant difference between supplemental treatments. Dietary Zn concentration of 50 mg/kg from both inorganic and organic sources resulted in normal growth and similar levels in tibial zinc concentration.

Trace mineral digestibility

The value of Fe digestibility within the groups varied widely (0.30 vs. -0.005), making it inappropriate to pool the data for statistical analysis.

The effect of the control diet on the digestibility of Cu, Mn and Zn in different intestinal regions when birds fed diets containing different supplemental trace mineral contents are shown in Figure 1. When birds were fed the control diet, there was similar digestibility value of Cu throughout the small intestine. Mn was poorly digested throughout the small intestine and there was no significant difference among different sections. The digestibility of Zn was greatest in the ileum and its digestibility reached 0.50-0.60. There was a significant difference ($p < 0.01$) in the digestibility of Zn between different regions when birds were fed the control diets.

The digestibility of Cu along the small intestine was not affected by variation in supplemental Cu levels and there was no significant difference in its digestibility between treatment groups or between different regions of the small intestine. However, supplementing different levels of Mn significantly increased ($p < 0.05$) Mn digestibility only in the duodenum and there was no significant difference between organic and inorganic sources. Supplementation of Zn did not improve its digestibility in the jejunum but significantly improved ($p < 0.001$) duodenal Zn digestibility. In the ileum,

Table 3. Bird performance on different diets

Period	Control	Organic	Inorganic	NRC	Pooled SEM	p value
Feed intake (g/bird)						
1-7 d	123 ^c	170 ^{ab}	156 ^b	186 ^a	7.9	<0.001
1-14 d	315 ^b	514 ^a	521 ^a	521 ^a	13.1	<0.001
14-35 d	1,283 ^b	2,623 ^a	2,616 ^a	2590 ^a	58.9	<0.001
1-35 d	1,597 ^b	3,137 ^a	3,137 ^a	3111 ^a	62.4	<0.001
Body weight gain (g/bird)						
1-7 d	103 ^b	151 ^a	153 ^a	151 ^a	3.2	<0.001
1-14 d	239 ^b	426 ^a	430 ^a	427 ^a	6.9	<0.001
14-35 d	782 ^b	1,587 ^a	1,607 ^a	1,524 ^a	37.0	<0.001
1-35 d	1,020 ^b	2,013 ^a	2,038 ^a	1,950 ^a	40.9	<0.001
Feed conversion ration						
1-7 d	1.20 ^a	1.13 ^{ab}	1.03 ^b	1.23 ^a	0.042	<0.05
1-14 d	1.32 ^a	1.21 ^b	1.21 ^b	1.22 ^b	0.031	<0.05
14-35 d	1.64	1.71	1.66	1.63	0.049	NS
1-35 d	1.57	1.57	1.54	1.60	0.037	NS
Mortality (%)						
1-7 d	0.5	1.5	0.5	3.0	0.081	NS
1-14 d	1.5	2.5	1.0	4.0	0.012	NS
14-35 d	10.5	5.6	6.1	6.3	0.011	NS
1-35 d	12.5	8.0	7.5	10.5	1.88	NS

Data represent means of eight replicate groups of initially 25 chickens during period 1 to 35 d post-hatching a, b, means within the same row with no common superscript differ significantly ($p < 0.05$ or $p < 0.001$).

The control diet had no exogenous Cu, Fe, Mn and Zn; the organic diet was contained 4 mg Cu, 20 mg Fe, 40 mg Mn and 30 mg Zn from the organic source on the basis of per kg control diet; the inorganic diet had the same levels of Cu, Fe, Mn and Zn as the organic diet but from the inorganic source on the basis of per kg control diet; The NRC diet had 8 mg Cu, 40 mg Fe, 60 mg Mn and 40 mg Zn from an inorganic source on the basis of per kg control diet.

Table 4. The mineral concentrations in dry tibia bones

Parameters	Control	Organic	Inorganic	NRC	SEM	p value
1-21 d						
TTA ^A	0.34 ^b	0.64 ^a	0.67 ^a	0.68 ^a	0.02	<0.001
Ca (mg/g)	140	152	159	162	6.12	NS
P (mg/g)	65 ^b	71 ^{ab}	74 ^a	72 ^a	1.76	<0.01
Cu (µg/g)	1.8	1.6	1.7	1.6	0.29	NS
Fe (µg/g)	65.5	65.0	72.6	82.3	1.82	NS
Mn (µg/g)	1.1 ^b	2.9 ^a	2.8 ^a	2.9 ^a	0.17	<0.001
Zn (µg/g)	73.8 ^b	182.0 ^a	173.5 ^a	181.0 ^a	7.16	<0.001
1-35 d						
Ca (mg/g)	134	138	137	138	4.0	NS
P (mg/g)	63	65	63	64	1.6	NS
Cu (µg/g)	1.6	1.4	1.4	1.5	0.08	NS
Fe (µg/g)	81	77	79	71	6.4	NS
Mn (µg/g)	1.8 ^b	3.2 ^a	3.9 ^a	3.4 ^a	0.2	<0.001
Zn (µg/g)	109 ^b	147 ^a	148 ^a	140 ^a	4.5	<0.001

^A TTA = Total tibia ash: µg/bird.

Data represent means of eight replicates of 2 chicken right tibia bones at age of 21 d and 35 d.

^{a,b} Means within the same row with no common superscript differ significantly ($p < 0.05$).

The control had no exogenous Cu, Fe, Mn and Zn; the organic diet contained 4 mg Cu, 20 mg Fe, 40 mg Mn and 30 mg Zn from the organic sources on the basis of per kg control diet; the inorganic diet had the same levels of Cu, Fe, Mn and Zn as the organic diet but from an inorganic source on the basis of per kg control diet. The NRC diet contained supplemental 8 mg Cu, 40 mg Fe, 60 mg Mn and 40 mg Zn from the inorganic source on the basis of per kg control diet.

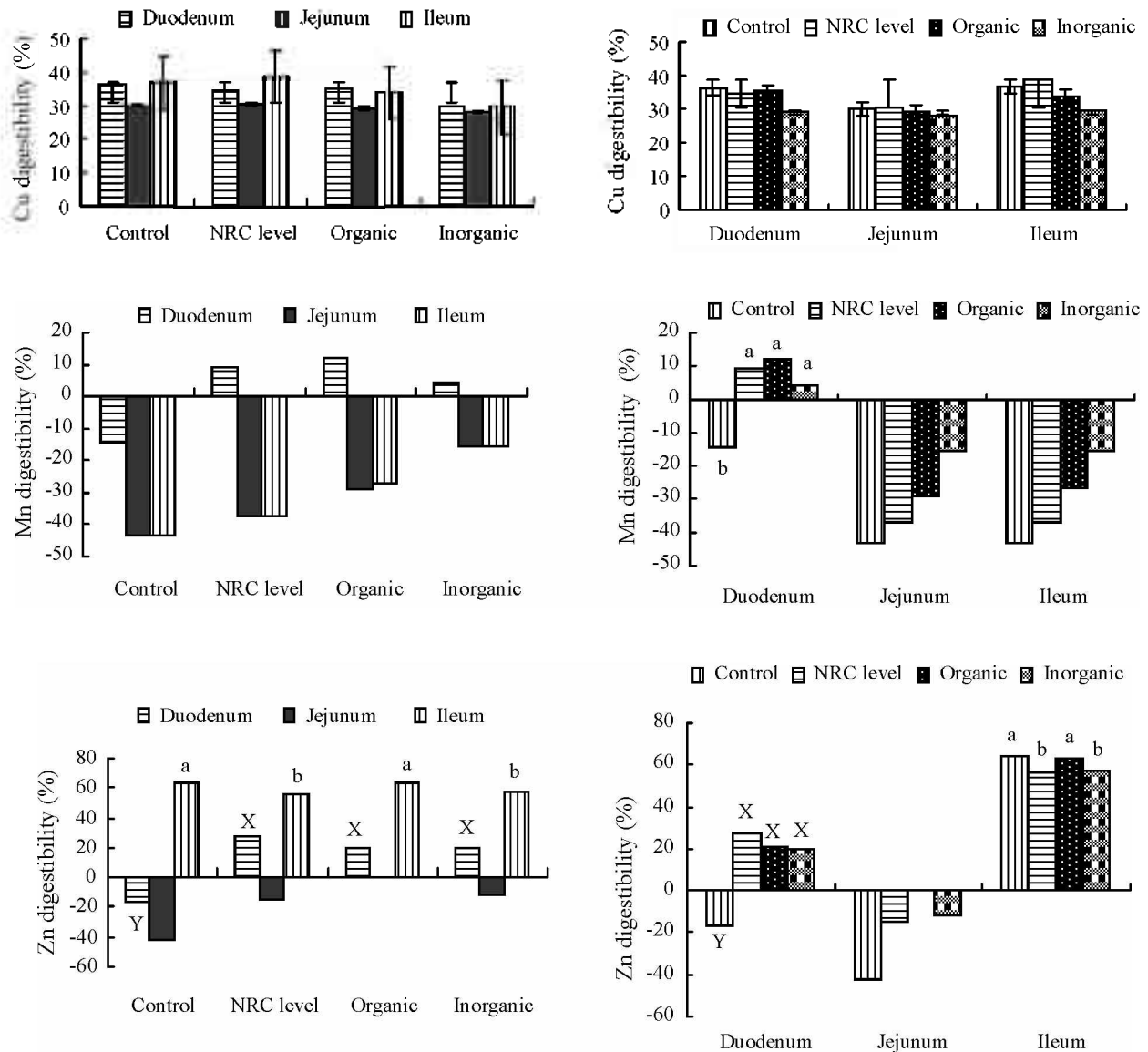


Figure 1. Cu, Mn Zn digestibility in different regions of intestinal tract with different diets (SEM ranged from 0.036 to 0.056, 0.051 to 0.16 and 0.018 to 0.17 in Cu, Mn and Zn digestibility, respectively).

Zn digestibility of the organic group was higher ($p < 0.001$) than that of the inorganic group but similar ($p > 0.05$) to that in the control group.

DISCUSSION

Broiler performance

It has been reported that with a basal diet containing 16 mg Cu and 30 mg Zn/kg diet, there was no difference in growth rate or FCR compared with supplemental Zn treatments from placement to 21 d of age (Burrell et al., 2004). Under floor-pen conditions, with a basal diet containing 7 mg Cu, 250 mg Fe, 22 mg Mn and 30 mg Zn/kg diet, bird performance was not affected by the levels of supplemental trace minerals (Shelton and Southern,

2006). However, in the current experiment, a control diet containing 7.4 mg Cu, 60 mg Fe, 14.6 mg Mn and 20 mg Zn/kg, strongly depressed feed intake from the first week of age. Compared with the NRC (1994) recommendation, the current control diet had much lower Mn and Zn contents. This indicates a primary response to deficiency of trace minerals (Bao et al., 2007b). It is consistent with the effects of dietary Zn deficiency in rats (Shay and Mangian, 2000), where a reduced amount of Zn-adequate diet, equivalent to the amount consumed by similar Zn-deficient rats was provided. Pair-fed control rats experienced depression in growth in an essentially similar fashion to that of the zinc-deficient rats. Therefore, 30 mg/kg dietary Zn might be the minimal requirement to support a normal feed intake for broilers. Thus, in the current experiment, birds fed diets

supplemented with lower level of either organic Zn or inorganic Zn than NRC level did not show any depression in feed intake. This was in agreement with a previous study (Bao et al., 2007b) that the symptoms of trace mineral deficiency as observed here were probably mainly due to dietary Zn deficiency. In contrast to the cage experiment reported previously (Bao et al., 2007a), feed intake of birds fed the control diet in this experiment started to decline as early as during the first week of age. The exact reason is unknown. It would suggest that birds kept under a semi-commercial condition, like the case in this study, were more sensitive to Zn or other trace mineral deficiency.

Although birds fed the control diet numerically showed a higher mortality rate compared with those fed supplemental treatments, the difference was not significant ($p>0.05$). It may indicate that when Zn is inadequate to maintain growth or cellular metabolism, reduced food intake may be a protective mechanism to allow survival (MacDonald, 2000). Thus, Zn deficiency may not result in definite higher mortality.

Surprisingly, during the entire period of the current experiment, there were no significant differences in body weight gain and feed intake between supplemental treatments, indicating that under floor pen conditions, the current low levels of supplementation of trace minerals appear to be enough to support a normal growth rate for broiler chickens.

Mineral concentrations and ash weight in tibia

It was reported that tibia bone ash content and ash concentration were not significantly influenced by tibia bone preparation methods (Kim et al., 2004). Therefore, in this study, tibia bone samples were not defatted. The results of mineral concentrations and ash weight in tibia bones demonstrated that Cu and Fe contents in the control diet were sufficient to support normal bone growth when birds maintained a normal feed intake due to sufficient dietary Zn content. It is in agreement with the findings of a cage experiment (Bao et al., 2009). There was no further effect of additional supplementation, indicating tibial Cu, Fe concentrations may not represent the Cu and Fe status of broilers (Bao et al., 2007a). It has been reported that the breakpoint for Zn supplementation on tibia Zn concentration occurred between 54 and 60 mg/kg diet with organic and inorganic supplements, respectively (Wedekind et al., 1992). In a study using cages, in stead of floor pens, Bao et al. (2007a) found that tibial Zn did not plateau until about 60 mg total Zn content/kg diet. However, in the current experiment under floor-pen conditions, due probably to a relative increase in nutrient recycling, the dietary Zn content of 50 mg/kg from both inorganic and organic sources resulted in normal broiler chicken performance and there was no further effect on tibia Zn

concentration with higher content of supplementation from inorganic salt. This supplemental Zn content (30 mg/kg) plus 20 mg Zn/kg in the control diet was close to that reported by Pimentel et al. (1991) and slightly higher than the NRC recommendation, but it was lower than the plateau level of 75 mg/kg diet reported by Mohanna and Nys (1999). Hence, under the conditions of this experiment, it can be deduced that dietary levels of 12, 80, 55 and 50 mg of Cu, Fe, Mn and Zn per kg, respectively, from either organic or inorganic sources, already meet the requirements of these minerals by broiler chickens up to 35 d of age. Significantly lower concentrations of Zn and Mn in tibia for birds fed on the control diet were due mainly to lower feed intake and thus, poorer growth rate.

Mineral digestibility along the small intestine

There is no satisfactory method to measure the absorption and digestion of trace minerals due to the complexity of endogenous trace mineral excretion in animals. AIA is a popular marker for estimating nutrient digestibility in a wide range of animal species including pigs, poultry and pet species since 1975 (Vogtmann et al., 1975; Choct and Annison, 1990). It has also been proven to be a reliable marker for micro nutrients such as biotin (Bryden, 1989). However, there is no report on this method being used with trace minerals, probably due to high dietary trace mineral concentrations in practice. The current study attempted to avoid excess trace mineral excretion by using a special control diet and lower contents of trace mineral supplementation in order to measure trace mineral digestibility. It is reported that decreased dietary trace mineral intake always results in increased digestibility of trace minerals in humans and rats (Linde, 1996; King et al., 2000). Thus, with a control diet deficient in Cu, Mn and Zn, the different sections of small intestines with the highest digestibility of these trace minerals should represent the major digestion sites along the small intestine.

The current experimental results demonstrate that the digestibility of Cu is similar in all parts of the small intestine. The lower or NRC level of dietary supplementation of Cu had no obvious effect on the digestibility of Cu, compared with the control diet. This finding differs from that observed in rats where Cu is primarily digested in the duodenum (Linde, 1996), indicating that under the current Cu supplemental levels, both organic and inorganic sources of Cu are apparently absorbed efficiently throughout the small intestine. Considering that Cu is generally not an ion that is stored in the body (Underwood and Suttle, 1999a), surplus organic Cu in broiler diets may not contribute to improved bird performance. Manganese was also thought to be digested throughout the length of the small intestine (Keen and Zidenberg-Cherr, 1996) due probably to its relatively

inefficient intestinal absorption in poultry (Collins and Moran, 1999). However, the current findings show that Mn might be digested mainly in the duodenum and the apparent absorption rate is very low. This suggests that Mn digestion is probably dependent on that of other trace minerals or growth rate but not by its dietary concentration.

Usually most of the Fe in excreta represents unabsorbed dietary Fe (Scott et al., 1982). This suggests that birds regulate Fe homeostasis via its absorption. It was not possible to assess Fe apparent absorption in the current study due to large variation in values, indicating that Fe apparent absorption might be affected by other unknown factors or in the control diet, Fe contents might already have exceeded the bird requirement, skewing its absorption results.

The results with the control diet showed that the digestibility of Zn was the highest in the ileum and its digestibility in the jejunum was lower. This is close to the results obtained in the rat where Zn digestibility is much higher in the ileum than that in the duodenum and jejunum (Lönnerdal, 1989), suggesting that the jejunum may be unimportant to Zn digestion for broiler chickens. It was reported that dietary Zn deficiency resulted in morphological and functional changes in the rat jejunum, including shortening and narrowing of jejunal villi (Southon et al., 1985). However, it was clear that Zn deficiency strongly depressed feed intake (King et al., 2000; Reeves, 2003; Bao et al., 2009). The morphological and functional changes in the jejunum were probably due to decreased feed intake but not Zn deficiency per se. Surprisingly, birds on the control diet showed much lower duodenal Zn digestibility than all supplemental groups including NRC level, indicating that duodenal digestibility may be regulated by growth rate or feed intake. In the ileum, it was true that Zn digestibility of the control group was higher than that of the inorganic group but similar to that observed in the organic group due possibly to organic nature of the endogenous Zn content in the control diet. It is worth mentioning that as suggested by Lönnerdal (1989), the mechanisms involved in intestinal digestion of Zn can be complicated by the fact that although the digestibility of Zn in ileum might be higher, it is the duodenum that has the first opportunity to apparently absorb the element from the digesta. This would leave little to be digested in the ileum. Although, compared to that in the ileum, the digestibility of Zn in the duodenum may be lower but has a higher sensitivity towards changes in dietary Zn content. However, in a long term dietary Zn deficient treatment, dietary Zn deficiency may strongly suppress its digestibility in the duodenum. The complexity of Zn digestion results indicates that both organic and inorganic Zn were probably digested efficiently in the duodenum, which might be essential in regulating feed intake, and hence body weight gain.

Improved Zn digestibility in the ileum for organic Zn and control diet might be due to reduced Zn excretion but further study is needed to confirm it. However, it is noticed that this improvement of the Zn digestibility in ileum did not confer any advantage in bird performance over inorganic Zn as shown in the current experiment. The possible reason is that under floor pen conditions, especially during the later period of growth, birds had more access to recycled nutrients, including trace minerals from droppings and bedding material, reducing the efficacy of organic Zn.

It is believed that changes in trace mineral digestibility in the GIT are primary mechanisms for maintaining trace mineral homeostasis (King et al., 2000). For broiler chickens, this homeostasis is probably determined by the bird growth rate and feed intake, which has strong relationship with dietary Zn concentrations (Bao et al., 2009). It is known that Zn is susceptible to hydroxyl-polymerization, which is acid-soluble. In the peri-neutral intestinal environment, Zn readily forms insoluble hydroxide precipitates (Powell et al., 1999). Therefore, it is most likely that the digestion of Zn is regulated in the gizzard by gastric acid output. The gastric acid output, in turn, might be determined primarily by dietary Zn contents, in its regulation of feed intake. Although the current control diet was deficient in Cu, Mn and Zn, the feed intake regulated by dietary Zn contents may be the major factor to influence trace mineral digestibility (Bao et al., 2009). Apart from trace minerals, other ingredients in the current experiment supported a normal growth rate but it is not certain if it is an optimal growth performance, which would have further space for organic Zn to demonstrate its advantage.

IMPLICATIONS

Under semi-commercial, floor-pen conditions, lower supplemental levels of Cu, Fe, Mn and Zn from both organic and inorganic sources than those used in practice supported a normal growth rate and bone development. Copper digestibility was similar in all parts of the small intestine, whereas Mn digestibility was low throughout the small intestine and the main digestion site appears to be the duodenum. Supplemental Zn, on the other hand, was efficiently digested in the duodenum but not in the jejunum. The digestibility of organic Zn was shown to be improved in the ileum but did not contribute to the bird performance, which may be due to other nutrients other than trace minerals.

REFERENCES

- Aderson, D. L. and L. J. Henderson. 1986. Sealed chamber digestion for plant nutrient analysis. *Agron. J.* 78:837-938.

- Ammerman, C. B. 1995. Methods for estimation of mineral bioavailability. In: *Bioavailability of nutrients for animals: Amino acid, minerals, and vitamins* (Ed. C. B. Ammerman, D. H. Baker and A. J. Lewis). pp. 83-94. Academic Press, New York, USA.
- Bao, Y. M., M. Choct, P. A. Iji and K. Bruerton. 2007a. Effect of organically complexed Cu, Fe, Mn and Zn on broiler performance, mineral excretion and accumulation in tissues. *J. Appl. Poult. Res.* 16:448-455.
- Bao, Y. M., M. Choct, P. A. Iji and K. Bruerton. 2007b. Interactions between Zn and other trace minerals in broiler chickens. *Recent Advances in Animal Nutrition in Australia*, Armidale, Australia, p. 248.
- Bao, Y. M., M. Choct, P. A. Iji and K. Bruerton. 2009. Optimal dietary inclusion of organically complexed zinc for broiler chickens. *Br. Poult. Sci.* 50:95-102.
- Bryden, W. L. 1989. Intestinal distribution and absorption of biotin in the chicken. *Br. J. Nutr.* 62:389-398.
- Burrell, A. L., W. A. Dozier, A. J. Davis, M. M. Compton, M. E. Freeman, P. E. Vendrell and H. L. Ward. 2004. Responses of broilers to dietary zinc concentrations and sources in relation to environmental implications. *Br. Poult. Sci.* 45:255-263.
- Choct, M. and G. Annison, 1990. Anti-nutritive activity of wheat pentosans in broiler diets. *Br. Poult. Sci.* 31:811-821.
- Collins, N. E. and E. T. J. Moran. 1999. Influence of supplemental manganese and zinc on live performance and carcass quality of broilers. *J. Appl. Poult. Res.* 8:222-227.
- Keen, C. L. and S. Zidenberg-Cherr. 1996. Manganese. In: *Present knowledge of nutrition* (Ed. E. E. Ziegler and L. J. J. Filer). pp. 334-343. ILSI Press, Washington, DC, USA.
- Kim, W. K., L. M. Donalson, P. Herrera, C. L. Woodward, L. F. Kubena, D. J. Nisbet and S. C. Ricke. 2004. Effects of different bone preparation method (fresh, dry, and fat-free dry) on bone parameters and the correlations between bone breaking strength and the other bone parameters. *Poult. Sci.* 83:1663-1666.
- King, J. C., D. M. Shames and L. R. Woodhouse. 2000. Zinc homeostasis in humans. *J. Nutr.* 130:1360S-1366S.
- Lee, S. H., S. C. Choi, B. J. Chae, J. K. Lee and S. P. Acda. 2001. Evaluation of metal-amino acid chelates and complexes at various levels of copper and zinc in weaning pigs and broiler chicks. *Asian-Aust. J. Anim. Sci.* 14:1734-1740.
- Leeson, S. 2003. A new look at trace mineral nutrition of poultry: Can we reduce the environmental burden of poultry manure. In: *Nutritional biotechnology in the feed and food industries, Proceedings of Alltech's Nineteenth annual Symposium* (Ed. T. P. Lyons and K. A. Jacques). pp. 125-129. Nottingham University Press, Nottingham, UK.
- Linde, M. C. 1996. Copper. In: *Present knowledge in nutrition* (Ed. E. E. Ziegler and L. J. J. Filer). pp. 307-319. ILSI Press, Washington, DC, USA.
- LÖnnerdal, B. 1989. Intestinal absorption of zinc. In: *Zinc in human biology* (Ed. C. F. Mills). pp. 33-53. Springer-Verlag Berlin Heidelberg, London, UK.
- MacDonald, R. S. 2000. The role of Zinc in growth and cell proliferation. *J. Nutr.* 130:1500S-1508.
- Milestone, I. 2000. Application field food/feed June 2000 Ethos Plus, Milestone Inc., Monroe, USA.
- Paik, I. K. 2001. Application of chelated minerals in animal production. *Asian-Aust. J. Anim. Sci.* 14:191-198.
- Powell, J. J., R. Jugdaohsingh and R. P. H. Thompsson. 1999. The regulation of mineral absorption in the gastrointestinal tract. *Proc. Nutr. Soc.* 58:147-153.
- Reeves, P. G. 2003. Patterns of food intake and self-selection of macronutrients in rats during short-term deprivation of dietary zinc. *J. Nutr. Biochem.* 14:232-243.
- Scott, M. L., M. C. Nesheim and R. J. Young. 1982. Essential inorganic elements. In: *Nutrition of the chicken* (Ed. M. L. Scott, M. C. Nesheim and R. J. Young). pp. 277-382. M.L.Scott & Associates, New York, USA.
- Shay, N. F. and H. F. Mangian. 2000. Neurobiology of zinc-influenced eating behaviour. *J. Nutr.* 130:1493S-1499S.
- Shelton, J. L. and L. L. Southern. 2006. Effects of phytase addition with or without a trace mineral premix on growth performance, bone response variables, and tissue mineral concentrations in commercial broilers. *J. Appl. Poult. Res.* 15:94-102.
- Southon, S., J. M. Gee, C. E. Bayliss, G. M. Wyatt, N. Horn and I. T. Johnson. 1985. Intestinal microflora, morphology and enzyme activity in zinc-deficient and zinc-supplemented rats. *Br. J. Nutr.* 55:603-611.
- Underwood, E. J. and N. F. Suttle. 1999a. Copper. In: *The mineral nutrition of livestock* (Ed. E. J. Underwood and N. F. Suttle). pp. 283-342. CABI Publishing, Wallingford, UK.