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Effects of Supplementing with Single or Multiple Trace Minerals on Growth Performance, Fecal Mineral Excretion and Nutrient Utilization in Pullets from 1 to 18 Weeks of Age

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ABSTRACT: This study investigated whether supplemental Cu, Fe, Zn, and Mn are needed in a practical diet for pullets. Four hundred and twenty females of an egg-laying strain (1-d-old, Lohmann Brown Layer) were randomly distributed into 4 groups, consisting of 7 replicates of 15 birds each. During the 18-week experimental period, chicks were given three basal diets in sequence, each with single or multiple Mn, Zn and Cu supplementation to improve the mineral balance gradually. In the Control, no Mn, Zn, and Cu were added; in the single Mn supplemented group (sMn) Mn was added to 120, 60, and 60 mg/kg for 1-6, 7-12, and 13-18 weeks of age, respectively; in the multiple Mn and Zn supplemented group (mMnZn), Mn was added to 180, 90, and 90 mg/kg and Zn was added to 120, 105, and 105 mg/kg for 1-6, 7-12, and 13-18 weeks of age, respectively; in the multiple Mn, Zn, Cu supplemented group (mMnZnCu), Mn, Zn, and Cu were added to the same multiple of basal Fe concentration relative to NRC (1994) recommendations. Energy and protein metabolizability were determined by subtracting energy/protein intake by energy/protein excretion (from both feces and urine) and dividing by energy/protein intake. There were no statistically significant differences between groups in terms of feed intake, final body weight or tibia length throughout the experiment. Optimal growth performance was observed in the Control, while adding trace minerals to basal diets tended to result in decreased productive performance. Protein metabolizability was increased by mMnZn and mMnZnCu treatments, but energy metabolizability was not affected. Concentrations of Mn, Zn, Cu in excreta varied greatly related to dietary content, and the retentions of Cu, Fe, Zn and Mn were all increased due to the improvement of mineral balance. Based on these results, it is suggested that the concentrations of Cu, Fe, Zn and Mn in typical basal diets used in this study were adequate for normal growth for pullets from 1 to 18 weeks of age. (Key Words: Trace Mineral, Pullet, Mineral Excretion, Growth Performance)

INTRODUCTION

Trace minerals play an important role in numerous biochemical functions in avian and mammalian species, being essential components of many enzymes (Vallee and Auld, 1990), and have both structural and catalytic functions in metalloenzymes (O'Dell, 1992). For example, copper is an indispensable component of uricase, ascorbic acid oxidase and ceruloplasmin, and zinc is essential for carbolic anhydrase and carboxypeptidase (Wang et al., 2002). Furthermore, trace mineral is required for normal immune function (Goswami, et al., 2005), as well as proper skeletal development and maintenance (Brandeo-Neto et al., 1995). When there are trace mineral deficiencies, thymic

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atrophy (Chandra and Au, 1980) and skeletal malformation (Blamberg et al., 1960) may occur for mice and laying hens, respectively. Thus, trace mineral premix is widely included in commercial diet formulation.

The requirement of trace minerals can vary widely due to environmental conditions such as temperature, humidity, management and severity of stress, and physiological status of animals (Tian et al., 2001). Therefore, it is not easy to determine the optimum supplementation of trace minerals in commercial diet. Trace mineral requirements of poultry (NRC, 1994) have been defined under laboratory-type conditions where animals are well cared for and the environmental conditions are maintained as close to optimal as possible. However, under practical conditions, productive performance have been greatly improved by domestic breeders, and animals are exposed to various environmental and disease challenges. Thus, trace minerals suggested by NRC may not adequate for commercial animals to growth optimally and keep healthy.

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Table 1. Composition and nutrient levels of basal diets provided to pullets from 1 to 18 weeks of age

Ingredients (g/kg)	Week 1-6	Week 7-12	Week 13-18
Corn	673.0	655.0	580.0
Wheat bran	50.0	120.0	47.0
Soybean meal	146.0	75.0	20.0
Cotton seed meal	30.0	30.0	70.0
Rape seed meal	30.0	30.0	70.0
DDGS ¹	-	-	50.0
Rice bran	-	-	80.0
Fish meal	30.0	30.0	-
Meat and bone meal	27.0	23.0	30.0
Limestone	8.2	10.0	7.2
Sodium Chloride	2.3	2.4	1.1
Vitamin-mineral premix ²	0.3	0.3	0.2
L-lysine sulfate	1.5	1.0	1.3
Methionine	0.4	0.8	0.2
Flavomycin ³	0.1	-	-
Aureomycin ³	-	0.5	-
Zeolite powder	-	21.0	42.0
Analyzed composition (g/kg	g)		
Crude protein	177.0	155.0	150.0
$ME^3(MJ/kg)$	11.84	11.76	10.00
Lysine	8.7	7.0	5.8
Methionine	3.5	3.5	2.5
Met+cys	6.0	5.7	4.9
Tryptophan	6.6	5.5	5.0
Total phosphorus	6.5	6.3	6.0
Available phosphorus ⁴	4.3	4.0	4.0
Calcium	10.4	10.0	10.0

¹DDGS = Distiller's dried grain soluble.

To minimize the possibility of trace mineral deficiencies, a common practice is to formulate the diet with trace mineral concentrations double or even higher than NRC (1994) recommendations, without considering their contents in the natural ingredients of the diet. When trace minerals are fed in excess of animal requirements, more are excreted in waste because of the homeostatic mechanisms that serve to regulate tissue concentrations of minerals (Spears, 1996). Soil that has received repeated applications of domestic manure might even accumulate excessive levels of trace minerals, especially Cu and Zn (Kingery et al., 1994; Mueller et al., 1994), which are toxic to many plants and some foraging animals. For example, Zn and/or Cu accumulation in soil has been implicated in reduced crop yields (Tucker, 1997). Additionally, because of the interactions that occur between various minerals, excessive concentrations of one element may result in a deficiency in

the amount available to the bird of some other element. From the point of view of the environment, formulating diets with mineral concentrations close to requirements would seem to be an appropriate way to reduce the concentrations of minerals in the waste without affecting animal performance (Greech et al., 2004). Therefore, determining trace mineral requirement of poultry with the prevention of heavy metal environmental contamination has been challenging.

The requirement for trace minerals are often fulfilled by concentrations present in conventional feed ingredients (NRC, 1994). Soils vary, however, in their content of trace minerals, and plants vary in their uptake of minerals. Consequently, feedstuffs grown in certain geographic areas may be rich or deficient in specific elements. Thus, supplementation of trace minerals in poultry diet should be completed according to the concentrations of these minerals from natural ingredients (Yao et al., 2003). Kim et al. (1997) and Mavromichalis et al. (1999) have indicated that the trace mineral premix could be deleted in the diets for finishing pigs with no negative effects on growth or carcass traits or quality. There appears to have been little relevant research conduct, and the objective of the present study was to determine whether or what level of supplemental Cu, Fe, Zn and Mn are needed in practical diets for pullet from 1 to 18 weeks of age.

MATERIALS AND METHODS

Chicken management

The animal protocol for this research was approved by the Northwest A & F University Animal Care and Use Committee. A total of 420 females of an egg-laying strain (1-d-old, Lohmann Brown Layer) were randomly divided into 4 groups, consisting of 7 replicates of 15 birds each. Chickens were allowed to free access to feed and water.

Dietary treatments

Three basal diets were provided for three stages of the experiment and the analyzed nutrient levels of each are included in Table 1. All diets were formulated to meet NRC (1994) nutrient recommendations. Concentrations of all the four trace minerals in basal diets (Control) were found to exceed NRC recommendations (Table 2), of which Fe was proportionately the most in excess, followed by Cu, Zn and Mn, in descending order. Taking the balance among minerals into account, in the single Mn supplemented group (sMn), Mn was added to 120, 60, and 60 mg/kg for 1-6, 7-12 and 13-18 weeks of age, respectively; in the multiple Mn and Zn supplemented group (mMnZn), Mn was added to 180, 90, and 90 mg/kg and Zn was added to 120, 105, and 105 mg/kg for 1-6, 7-12 and 13-18 weeks of age, respectively; in the multiple Mn, Zn, Cu supplemented

² Supplied the following per kilogram of complete diet: vitamin A, 15,000 IU; vitamin D₃, 3,750 IU; vitamin E, 8.3 mg; vitamin K, 2.1 mg; vitamin B₁₂, 0.006 mg; thiamine, 1.5 mg; riboflavin, 7.2 mg; pyridoxine, 4.5 mg; folic acid, 0.6 mg; pantothenic acid, 12 mg; biotin, 0.027 mg; Se (as NaSeO₃) 0.3 mg; and I (as KIO₃), 0.4 mg.

³ 0.1 g/kg flavomycin contained the active ingredient of 5 mg/kg, and 0.5 g/kg aureomycin contained 10 mg/kg.

⁴ Calculated values, all the nutrtient values are presented on an "as fed" basia.

Table 2. Mean of concentrations of Cu, Fe, Zn and Mn in experimental diets (mg/kg)

Diotory treatment	Supplement (mg/kg)				De	Determined concentration (mg/kg)		
Dietary treatment	Mn	Zn	Cu	Fe	Mn	Zn	Cu	Fe
Week 1-6								
Control	-	-	-	-	94.7	103.3	20.6	382.8
sMn	25.3	-	-	-	122.3	101.7	22.2	379.4
mMnZn	85.3	16.7	-	-	179.2	121.0	21.5	380.9
mMnZnCu	189.9	87.1	3.2	-	284.6	192.5	24.2	381.1
Week 7-12								
Control	-	-	-	-	34.2	73.5	15.7	330.2
sMn	25.8	-	-	-	59.2	70.2	16.9	335.5
mMnZn	55.8	31.5	-	-	91.8	105.8	17.2	333.9
mMnZnCu	131.7	120.0	6.5	-	168.9	193.5	22.1	332.1
Week 13-18								
Control	-	-	-	-	55.4	71.0	14.9	506.7
sMn	4.6	-	-	-	61.2	72.9	13.2	510.4
mMnZn	34.6	34.0	-	-	90.6	105.8	16.7	504.6
mMnZnCu	199.0	225.8	19.0	-	251.8	296.4	32.7	505.1

Table 3. Effects of dietary treatments on the performance in pullets

Item		Treatn	SEM	Probability		
item	Control	sMn	mMnZn	mMnZnCu	SEM	Probability
Week 1-6						
Final body weight (g/bird d)	439.4	419.9	434.8	423.4	13.48	NS
Daily feed intake (g/bird d)	29.0	28.6	29.8	29.1	1.52	NS
DWG (g/bird d)	10.7 ^a	10.2 ^b	10.5 ab	10.4 ^b	0.58	p<0.05
Feed conversion (gain/feed)	0.371 ^b	0.357^{a}	0.356^{a}	0.357 a	0.007	p<0.05
Week 7-12						
Final body weight (g/bird d)	1197.0	1193.0	1172.3	1170.4	1.24	NS
Daily feed intake (g/bird d)	49.7	49.7	50.1	49.9	0.78	NS
DWG (g/bird d)	14.7	14.7	14.4	14.4	0.69	NS
Feed conversion (gain/feed)	0.294	0.294	0.289	0.286	0.005	NS
Week 13-18						
Final body weight (g/bird d)	1618.4	1591.7	1574.6	1610.1	1.44	NS
Daily feed intake (g/bird d)	88.5	89.4	88.8	89.1	1.03	NS
DWG (g/bird d)	10.1 ^{ab}	9.5 ^b	9.6 ^b	10.4^{a}	1.24	p<0.05
Feed conversion (gain/feed)	0.114^{ab}	0.105^{a}	0.108^{a}	0.116^{b}	0.008	p<0.05
Week 1-18						
Daily feed intake (g/bird d)	56.0	56.2	55.7	56.0	1.09	NS
DWG (g/bird d)	21.8	21.6	21.2	21.8	0.31	NS
Feed conversion (gain/feed)	0.200 ^b	0.189^{ab}	0.189^{ab}	0.187^{a}	0.006	p<0.05
Tibia length in 14 weeks (cm)	10.00	10.14	9.99	10.02	0.71	NS

¹ Means within rows with no common superscript differ significantly (p<0.05).

group (mMnZnCu), Mn, Zn, and Cu were added to reach the same multiple of basal Fe concentration relative to NRC (1994) recommendations. All minerals were supplemented in the form of sulfate, and analyzed concentrations in the experimental diets are shown in Table 2.

Sample collection and analytical procedures

Feed intakes and body weights were recorded weekly, and these were used to calculate the daily feed intake, daily weight gain (DWG) and feed conversion. Excreta were collected from d 63 to 67 of the experiment at 2 h intervals after a 3-d adoption adaptation period, from a droppings

belt under the cage. Excreta were weighted and frozen at -20°C for future analysis. Crude protein, energy, calcium and phosphorus values of the experimental diets or excreta were determined by chemical analysis (AOAC, 1996).

Feed and excreta samples were dried, then ground to pass a 1-mm screen and prepared for mineral analysis by wet ashing using a microwave digestion system (model MDS-81D, CEM Corp., Matthews, NC), then analyzed for Cu, Fe, Zn, and Mn concentrations via flame atomic absorption spectrophotometry followed the methods presented by Greech et al. (2004).

Protein metabolizability was calculated by subtracting

² Values represent the mean of 7 replicate pens per treatment each having 15 pullets.

 $^{^{3}}$ NS = p>0.05.

protein intake by protein excretion (from both feces and urine) and dividing by protein intake, and the same was done to calculated energy metabolizability. Trace mineral retention was calculated by the methods which was described by Burrell et al. (2004) for determining zinc retention.

At the end of week 14, two birds of each replicate were sacrificed after electric stunning, and the length of left tibia was determined by vernier calipers.

Statistical analysis

The data were analyzed using the GLM procedure of SAS (SAS Institute, 1999), and significant differences were further analyzed by Duncan's multiple range test. Statistical significance was established at p<0.05.

RESULTS

The data demonstrated that neither final body weight, feed intake, DWG from 7 to 12 weeks of age nor tibia length at the end of 14 weeks of age was significantly

affected by treatments. The lowest value (10.2) for DWG from 1 to 6 weeks of age resulted from the sMn treatment, while the highest (10.7) was from the Control (Table 3). DWG for chicks fed the mMnCuZn diet was higher than that in sMn and mMnZn but similar to that in Control from 13 to 18 weeks of age. However, for the whole experimental period, no statistically significant difference of DWG was observed between treatments. Feed conversion was improved for pullets fed with the basal diet from 1 to 6, 13 to 18, and 1 to 18 week of age, but there was no effect in the 7 to 12 period.

There was no significant difference of fecal moisture percentage between the four experimental treatments. Concentrations of Mn, Zn, and Cu on the dry matter basis in excreta varied greatly and were related to their dietary content (Table 4). Compared with the Control, chickens fed the sMn diet had a lower fecal Cu concentration, but fed the mMnZn diet had a higher fecal Cu concentration, suggesting that Zn might regulate Cu metabolism. Fecal iron concentration was decreased by adding Mn and Zn (mMnZn group) but not affected by adding Mn, Zn and Cu

Table 4. Fecal Cu, Fe, Zn, and Mn concentration and fecal moisture percentage by treatments

Item		Treatr	SEM	Probability		
	Control	sMn	mMnZn	mMnZnCu	SEM	Tiobability
Cu (mg/kg)	27°	23 ^d	34 ^b	59 ^a	0.74	p<0.05
Fe (mg/kg)	1,161 ^a	$1,188^{a}$	971 ^b	$1,059^{ab}$	1.21	p<0.05
Zn (mg/kg)	164 ^d	241°	325 ^b	595 ^a	1.06	p<0.05
Mn (mg/kg)	138 ^e	216 ^c	318 ^b	602 ^a	1.19	p<0.05
Fecal moisture percentage (%)	74.69	74.41	72.78	72.74	1.20	NS
Protein metabolizability ⁵ (%)	55.44 ^b	63.49 ^a	62.77 ^a	64.58 ^a	1.17	p<0.05
Energy metabolizability ⁵ (%)	82.69	84.48	83.36	84.13	1.72	NS

¹ Means within rows with no common superscript differ significantly (p<0.05).

Table 5. Trace mineral balance of pullets fed on a basal diet supplemented with single or multiple trace minerals from 1 to 18 weeks of age

Item			SEM	Probability			
пеш		Control	sMn	mMnZn	mMnZnCu	SEM	Fiooability
Cu	Intake (mg/d bird)	1.0	1.1	1.1	1.2	0.17	NS
	Excretion (mg/d bird)	0.8	0.7	0.7	0.7	0.09	NS
	Retention ⁴ (%)	$20^{\rm c}$	36 ^a	36 ^{ab}	42 ^a	1.76	p<0.05
Fe	Intake (mg/d bird)	19.0	18.9	19.0	19.0	1.53	NS
	Excretion (mg/d.bird)	15.7 ^a	14.2 ^a	13.1 ^{ab}	12.5 ^b	0.86	p<0.05
	Retention (%)	17 ^b	25 ^{ab}	31^{ab}	34 ^a	2.35	p<0.05
Zn	Intake (mg/d bird)	5.1	5.1	6.1	9.6	0.92	p<0.05
	Excretion (mg/d bird)	4.74 ^b	4.00^{b}	4.25 ^b	6.51 ^a	0.58	p<0.05
	Retention (%)	7 ^c	22^{bc}	30^{ab}	32. ^a	1.21	p<0.05
Mn	Intake (mg/d bird)	4.7^{d}	6.1°	9.0^{b}	14.2^{a}	0.74	p<0.05
	Excretion (mg/d bird)	4.3°	$5.0^{\rm c}$	6.3 ^b	11.7 ^a	0.31	p<0.05
	Retention (%)	9^{d}	18 ^{ab}	30^{a}	18 ^{ab}	0.09	p<0.05

¹ Means within rows with no common superscript differ significantly (p<0.05).

² Values represent the mean of 7 replicate pens per treatment each having 15 pullets.

 $^{^{3}}$ NS = p>0.05.

⁴ Fecal concentrations of Cu, Fe, Zn and Mn were described on dry matter basis.

⁵ Protein or Energy metabolizability was calculated by subtracting protein (energy) intake by protein (energy) excretion and dividing by protein (energy) intake

² Values represent the mean of 7 replicate pens per treatment each having 15 pullets.

 $^{^{3}}$ NS = p>0.05.

(mMnZnCu), implying that Cu might influence Fe metabolism. Additionally, protein metabolizability was improved by trace mineral supplementation, but that for energy was not affected.

Trace mineral balance of pullet provided with diets containing gradient concentrations of Mn, Zn, and Cu was shown in Table 5. Adding 25 mg/kg Mn (sMn) to the basal diet increased the retentions of Cu and Mn, not affected Fe and Zn retentions. When a combination of 55 mg/kg Mn and 30 mg/kg Zn (mMnZn) was supplemented, the retention ratio for Cu, Zn, and Mn was increased by 80%, 329% and 233%, respectively, but that for Fe was not affected. In mMnZnCu treatment, retention ratios of the four minerals were all higher than that in the Control.

DISCUSSION

Pollution of the environment by minerals from the poultry industry has increasingly attracted public attention. This experiment was carried out to determine whether it is necessary to supplement mineral elements in basal diets for pullet to achieve optimal performance. Chemical analysis showed that concentrations of Cu, Fe, Zn, and Mn in basal diets present all have exceeded NRC recommendations, of which Fe was proportionately the most in excess, followed by Cu, Zn and Mn, in descending order. Richard et al. (2000) demonstrated that common feedstuffs contain trace mineral essential for animals, but these trace elements are often in a form which render them unavailable to the animal. The knowledge of bioavailability of trace minerals within feedstuffs is of great importance in estimating trace mineral need, but available information on this topic is limiting. O'dell et al. (1972) and Nwokolo and Bragg (1980) showed 40-70% available of Mn, Cu, and Zn in Canolo meal and 50-78% available in soybean meal. Some ingredients can contain very high levels of trace minerals. For example, it has been reported that defluorinated phosphates contain up to 10,000 mg/kg Fe, and that is up to 50% available (Henry et al., 1992). At this level, 1% dietary phosphate will supply 50 mg/kg Fe, which is close to the requirement for most classes of poultry. The present study showed 7-20% available for Cu, Fe, Zn, Mn in basal diet (Table 5, Control treatment), much lower than the previous values from a single ingredient.

The results demonstrated that growth performance was not improved or even adversely affected by supplementation of practical basal diets with Cu, Zn and Mn. This suggests that concentrations of these trace minerals contained in basal diets were adequate enough to support chicks' growth. Some previous studies have indicated that growth performance and carcass quality were not affected in growing pigs when dietary trace mineral

concentration are provided in excess of NRC (1998) recommendations (Kim et al., 1997; Mavromichalis et al., 1999). At no stage in the experiment was feed intake affected by treatments, which agrees with our previous study (Yao et al., 2003). High dietary trace mineral concentration has been shown to decreased DWG of broilers (Ruiz et al., 2000; Marron et al., 2001) and the present study also found chicks fed the diet with gradient supplemental levels of Mn, Zn, and Cu resulted in both lower DWG and feed conversion from 1 to 6 and 13 to 18 weeks of age, which may result from the potential toxicity by high concentration of minerals.

Burrell et al. (2004) showed that optimum body weight gain was achieved at 80 mg/kg supplemental Zn, which exceeds the NRC recommendation of 40 mg/kg. However, no improvement of performance was observed by the maximal supplementation of 225 mg/kg of Zn (for 13 to 18 weeks in mMnZnCu group) in this study. This contradiction may be related to the high Fe concentrations in the basal diet, and any antagonistic interactions between Fe and Zn that might occur. When dietary Mn content is deficient, tibia maldevelopment may occur in chickens (Sands and Smith, 1999). In this study tibia length of chickens fed the basal diet was not adversely affected, indicating that basal diets commonly applied in poultry industry in northwest China contained adequate Mn to meet the requirements of pullets.

The present study showed that trace mineral supplementation increased protein metabolizability but did not affect the energy metabolizability. This phenomenon was also observed in our previous study (Yao et al., 2003). The increment of protein metabolizability may be related to the stimulation by supplemental trace minerals on the activity of digestive enzyme because those are cofactors of numerous enzymes. The reason for the lack of response in energy metabolizability to dietary mineral concentration is not clear. The improvement of protein retention did not result in increased growth performance in this study. This may be explained by the fact that energy rather than protein was most likely to be limiting based on NRC (1994) recommendations and analyzed nutrient concentrations in basal diets (Table 2).

The results demonstrated that growth performance was not improved or even adversely affected by supplementation of practical basal diets with Cu, Zn and Mn. This suggests that concentrations of these trace minerals used in this study were adequate enough to support chicks' growth. Some previous studies have indicated that growth performance and carcass quality were not affected in growing pigs when dietary trace mineral concentration are provided in excess of NRC (1998) recommendations (Kim et al., 1997; Mavromichalis et al., 1999). At no stage

in the experiment was feed intake affected by treatments, which agrees with our previous study (Yao et al., 2003). High dietary trace mineral concentration has been shown to decreased DWG of broilers (Ruiz et al., 2000; Marron et al., 2001) and the present study also found chicks fed the diet with gradient supplemental levels of Mn, Zn, and Cu resulted in both lower DWG and feed conversion from 1 to 6 and 13 to 18 weeks of age, which may partly result from the toxicity by high concentration of minerals.

Greech et al. (2004) have reported that fecal concentrations of Cu, Zn and Mn were lower (p<0.05) for pigs fed reduced trace minerals diets. When trace minerals are fed in excess of requirement, more is excreted in waste (Spears, 1996). In the current study, supplementing 120 and 135 mg/kg Zn and Mn increased fecal concentrations of Zn and Mn from 164 and 138 mg/kg to 595 and 602 mg/kg, respectively. In all stages of the experiment, no Fe was added, but fecal Fe concentration also varied, which may be explained by the interaction between Fe and Mn, Zn, or Cu. In the mMnZn treatment, fecal Cu content was lower than that in Control, which may be due to Zn and/or Mn improved Cu utilization, a phenomenon also observed by O'Dell (1997).

The balance among minerals, in regard to dietary concentrations relative to animal requirement, is an important factor affecting mineral utilization (Greech et al., 2004). As our expectations, retentions for Cu, Fe, Zn, and Mn were increased by improving the balance among the four mineral with Mn, Zn or Cu added. In this experiment, trace mineral concentrations were gradually added to be closer to the ratios of Cu, Fe, Zn, and Mn based on NRC (1994) standards. However, NRC recommendations were mainly derived from researches conducted to determine a single nutrient requirement, but because antagonistic interactions occurred, it is difficult to express the requirement for one element without consideration of the quantity of the other. Therefore, the optimal ratios among trace minerals need for further study.

Chemical analysis of the diet does not indicate the biological effectiveness of trace mineral absorption. There are many factors that influence the bioavailability of minerals, including chemical form, interaction with other minerals, chelators, inhibitors, species and physiological state of animals and processing conditions to the individual or complete diet. To estimate accurately trace mineral requirements for domestic animals, presenting them in the form of available mineral concentrations, similar that normally adopted for available phosphorus, may be more effective than in the form of total trace element concentrations.

It is concluded that the concentrations of Cu, Fe, Zn and Mn in the typical commercial diet in northwest of China were adequate for normal growth in pullets from 1 to 18

weeks of age. For good performance and minimum mineral excretions, it appears that Cu, Fe, Zn and Mn supplementation are not necessary with the type of diet used in this study. As the concentration of iron in the present study was rather high, the optimal ratios between copper, iron, zinc and manganese need further study.

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REFERENCES

AOAC. 1996. Official Method of Analysis of AOAC International (16th Ed), Gathersburg, MD, AOAC International.

Blamberg, D. L., U. B. Blackwood, W. C. Supplee and G. F. Combs. 1960. effect of zinc deficiency inheson hatchability and embryonic development. Processing of the Society for Experimental Biology and Medicine 104:217-220.

Brandeo-Neto, J., V. Stefan, B. Mendonga, V. Bloise and A. Castro. 1995. The essential role of zinc in growth. Nurt. Res. 15:335-358.

Burrell, A. L., W. A. Dozier, A. J. Dabis and T. L. Ward. 2004. Response of broilers to dietary zinc concentrations and sources in relation to environmental implications. Br. Poult. Sci. 45:255-263.

Chandra, R. A. and B. Au. 1980. single nutrient deficiency and cell-mediated immune responses. 1. zinc. American Journal of Clinical Nutrition, 33:736-738.

Goswami, T. K., R. Bhar, S. E. Jadhav, S. N. Joardar and G. C. Ram. 2005. Role of dietary zinc as a nutritional immunomodulator. Asian-Aust. J. Anim. Sci. 18:439-452.

Greech, B. L., J. W. Spears, W. L. Flowers, G. M. Hill, K. E. Lioyd, T. A. Armstrong and T. E. Engle. 2004. Effect of dietary mineral concentration and source on performance, mineral status, and fecal mineral excretion in pigs. J. Anim. Sci. 82:2140-2147.

Henry, P. R., C. B. Ammerman, R. D. Miles and R. C. Littell. 1992. Relative bioavailability of iron in feed grade phosphates for chicks. J. Anim. Sci. 70:228-233.

Kim, I. H., J. D., J. H. Hancock, J. S. Lee, H. H. Park, C. S. Kropf, J. O. Kim and R. H. Hines. 1997. Effects of removing vitamin and trace mineral premixes from diet on growth performance, carcass characteristics, and meat quality in finishing pigs. J. Anim. Nutr. Feed (Korean), 21 489-496.

Kingery, W. L., C. W. Wood, D. P. Delaney, J. C. Williams and G. L. Mullins. 1994. Impact of long-term land application of broiler litter on environmental related soil properties. J. Envir. Qua. 23:139-147.

Marron, L., M. R. Belford and K. J. McCracken. 2001. The effects of adding xylanase, vitamin C and copper sulfate to wheat-based diets on broiler performance. Br. Poult. Sci. 42:493-500.

Mavromichalis, I., J. D. Hancock and I. H. Kim. 1999. Effects of

- omitting vitamin and trace mineral premixes and reducing inorganic phosphorus additions on growth performance, carcass characteristics and muscle quality in finishing pigs. J. Anim. Sci. 77:2700-2708.
- Mueller, J. P., J. P. Zublena, M. H. Pooer, J. C. Barker and J. T. Green. 1994. Managing Pasture and Hay Fields Receiving Nutrients From Anaerobic Swine Waste Lagoons. NC coop. Ext. Service, AG-506.
- NRC. 1994. Nutrient Requirements of Poultry (9th Ed.) National Academic Press. Washington, DC.
- Nwokolo, E. and Bragg D. B. Bragg. 1980. Biological availability of minerals in rapeseed meal. Poult. Sci. 59:155-158.
- O'Dell, B. L., C. E. Burpo and J. E. Savage. 1972. Evaluation of zinc availability in foodstuffs of plant and animal origin. J. Nutr. 102:653-660.
- O'Dell, B. L. 1992. Zinc plays both structural and catalytic roles in metalloproteins. Nutr. Rev. 50: 48-50.
- O'Dell, B. L. 1997. Mineral-iron interaction as assessed by bioavailability and iron channel function. Pages 641-659 in Handbook of Nutritionally Essential Mineral Elements (Ed. B. L.'Dell and R. A. Sunde) Marcel Dekker Inc., New York.
- Richard, D. Miles and Peter R. henry. 2000. Relative bioavailability of trace mineral. Ciência Animal Brasilerral (2):73-93.
- Ruiz, J. A., A. M., Perez-vendell and E. Estevan-Garcia. 2000. Effect of dietary iron and copper on performance and oxidative stability in broiler leg meat. Br. Poult. Sci. 41:163-167.

- Sands, J. S. and M. O. Smith. 1999. Broiler in heat stress condition: effect of dietary manganese proteinate or picollinate supplementation. J. Appl. Poult. Sci. 8:280-287.
- SAS Institute. 1999. SAS User's Guide: Version 6.12 SAS Institute inc., Carry, NC.
- Spears, J. W. 1996. Optimizing mineral levels and sources for farm animals, in: Nutrient management of animals to enhance and protect the Environment, pp. 259-275 (CRC Press).
- Tian, J. Z., J. H. Lee, J. D. Kim, Y. K. Han, K. M. Park and In K. Han. 2001. Effects of different levels of vitamin-mineral premixes on growth performance, nutrient digestibility, carcass characteristics and meat quality of growing-finishing pigs. Asian-Aust. J. Anim. Sci. 14:515-524.
- Tucker, M. R. 1997. Experiences with metal toxicities in North Carolina, in: Soil Science., pp. 97-100.
- Vallee, B. L. and D. S. Auld. 1990. The metallobiochemistry of zinc enzymes, in: (Ed. A. Meiter) Advances in Enzymology, pp. 283-429 (New York, John Wiley).
- Wang, J. Y., S. G. Zhu and F. C. Xu. 2002. The important role of trace mineral in coenzymes in: Biochemistry. High education Prsee, pp. 456-468 (Chinese).
- Yao, J. H., J. Y. Zhang, T. Zhang, N. T. Zhang and J. X. Zhang. 2003. Effect of dietary contents of trace minerals on the performance and apparent metabolic energy of laying hens. J. Northwest Science and Technology University of Agriculture and Forestry (Chinese), 31(4):36-40.