Effect of High Dietary Copper on the Morphology of Gastro-Intestinal Tract in Broiler Chickens

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ABSTRACT : An experiment was conducted to study the effects of high dietary copper supplementation on the gastro-intestinal tract morphology of broiler chickens. Eighty 3-week-old broiler chicks were divided randomly into eight groups of four dietary treatments and over three week were fed isoenergetic and isonitrogenous diets that contained 0, 100, 250, or 500 mg/kg of supplemental copper from cupric sulfate. The copper supplementation in the broiler diet up to 250 mg/kg did not significantly influence broilers' performance. A high dietary copper supplementation of 500 mg/kg did significantly depress growth and feed conversion in the broilers ($p<0.05$). Copper supplementation more than 250 mg/kg in the broiler diet significantly influenced the morphology of the GI tract, as shown by severe oral lesions and gizzard erosion. It also significantly depressed the villi height and significantly thickened the muscular layer in the duodenum ($p<0.05$). The severely damaged villi were observed by scanning electronic microscope from the duodenum samples of broilers fed a 500 mg/kg copper supplemented diet. The 500 mg/kg copper supplemented diet also significantly influenced the plasma constituents. Plasma glucose concentration was significantly depressed ($p<0.05$) (*Asian-Aus. J. Anim. Sci. 1999. Vol. 12, No. 4 : 548-553*)

Key Words : Copper, GI Tract, Morphology, Aspartate Aminotransferase, Alanine Aminotransferase, Creatine Kinase, Blood Constituents

INTRODUCTION

Copper is an essential nutrient for poultry (NRC, 1994), but excess amounts of copper will have adverse affects on birds. Inclusion of up to 250 mg/kg of copper in the diet depressed growth and feed efficiency in broilers (Leach et al., 1990; Funk and Baker, 1991), as well as damaged gizzards (Popoulis and Jensen, 1976; Robbins and Baker, 1980ab). Increased amounts of copper up to 500 mg/kg in the broiler diet produces severe lesion mucosa in the oral cavity and gizzard (Jensen et al., 1991) and depressed growth. It also changed the GI morphology, depressed villi height and chicken muscular layer (Chen et al., 1997ab), and damaged liver function and growth performance of young chicks both in layer pullets and Taiwan country chickens (Chen et al., 1996. 1997ab). The high dose of copper inclusion depressed intake of feed and live-weight gains of chicks. It is not clear whether the low feed-intake caused low live-weight gain, or high copper damaged the GI tract that caused malabsorption of nutrients.

Since villi and crypts of the mucosal cells are the functional units of the small intestine. Therefore, the morphology of the villi, crypts and the muscle layer will reflect their function, secretion of digestive enzymes and absorption of nutrients. Factors that influence growth rate and turnover of mucosal cells will also influence nutrient absorption. This experiment is therefore to study the effects of high dietary copper supplementation on GI tract morphology of the broiler chickens.

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MATERIALS AND METHODS

Eight health of three weeks old broilers with mean live-weight of 706 g were selected from the battery incubator, tagged with wing numbers, and randomly allocated into eight cage groups that were raised 50 cm from the ground to prevent the incidence of intestinal coccidiosis. Broiler groups were then allocated into four dietary treatments and were fed diets that contained 0, 100, 250, or 500 mg/kg of supplemental copper from cupric sulfate ($CuSO_4 \cdot 5H_2O$) for three weeks. The basal corn-soybean ration was formulated according to the NRC (1994) nutrient requirements, as presented in table 1.

During the experimental feeding period, feed consumption and live-weight of broilers were measured weekly to calculate feed efficiency and live-weight gain. At the end of the experiment, blood was withdrawn from wing vein of all experimental chickens after 12 h fasting to measure the blood glucose, hemoglobin, and hematocrite. Eight chickens from each replicate were scarified; The intestine segments were cut open longitudinally and rinsed with saline solution to clean the contents; excess fat was removed, and the excess water of the intestine samples was then dried with tissue paper. Intestinal samples of 5 cm length were taken from every segment of the intestine: duodenum (between the post gizzard and the duodenal loop), jejunum (15 cm anterior to Meckel's diverticulum) and ileum (between Meckel's diverticulum and ileocecalic junction).

Samples were rinsed first with 0.4 M KCl solution and were then placed into a 10% buffered neutral formalin solution (pH 7.2-7.4). All samples were gradually dehydrated through an increasing concentration of ethyl alcohol (50%-100%). These specimens were first embedded in paraffin, then prepared by sectioning at 6

μm and staining with hematoxylin and eosin. The intestinal histology was measured according to Hampson (1986). The villi height was measured by averaging the height of the ten intact villi. A crypt depth was measured by averaged of 30 measurements. Muscle layer thickness was calculated by averaging the thickness at 20 locations.

Table 1. Composition and calculated analysis of the basal diet

	g/kg
Ingredients	
Yellow corn meal	555.8
Soybean meal, 44%	320.0
Fish meal, 65%	25.0
Soybean oil	65.0
Dicalcium phosphate	12.0
Limestone	12.0
Iodized salt	3.5
Vitamin premix[a]	0.5
Mineral premix[b]	2.0
Choline chloride, 50%	1.0
DL-Methionine	1.0
Total	1000.0
Calculated value	
Crude protein	202.6
Metabolizable energy, MJ/kg	13.46
Calcium	9.0
Methionine	4.6

[a] Vitamin premix supplied the following per kg diet: vitamin A, 25,000 IU, vitamin D_3, 3125 ICU; vitamin E, 37.5 IU; vitamin K_3, 6.25 mg; vitamin B_1, 3.75 mg; vitamin B_2, 12.5 mg; vitamin B_6, 10.0 mg; Ca pantothenate, 18.8 mg; niacin, 50 mg, biotin, 0.06 mg; folic acid, 1.25 mg; vitamin B_{12}, 0.05 mg.

[b] Mineral premix supplied the following (kg^{-1} diet): Cu ($CuSO_4 \cdot 5H_2O$, 25.45% Cu), 6 mg; Fe ($FeSO_4 \cdot 7H_2O$, 20.09% Fe), 50 mg; Mn ($MnSO_4 \cdot H_2O$, 32.49% Mn), 60 mg; Se ($NaSeO_3$, 45.56% Se), 0.075 mg.

Samples were perpared for the scanning eletronic microscope (SEM) according to method of Paulini et al. (1987), as modified by Moore et al. (1988). All gut samples were fixed initially in 10% buffered neutral formalin, then fixed again by placing in 0.1 M phosphate buffer of pH 7.3 for three times at 10 min each, then placed in 1% osmium tetroxide overnight. These samples were then rinsed in a phosphate buffered solution four times for 15 min each, and gradually dehydrated by increasing alcohol concentrations: 50, 70, 80, 90, and 95% for 10-15 min. The concentration was then increased to 100% three times for dehydration, the samples were then dried, mounted on aluminum stubs and coated with gold for 30 min, and then placed in the SEM (Bausch & Lomb Ltd., Hitachi 2400) for scanning.

Blood sera were analyzed for total protein, albumin, and the activity of the serum enzymes, α-amylase, alkaline phosphatase, aspartate aminotransferase (AST, EC 2.6.1.1), alanine aminotransferase (AAT, EC 2.6.1.2), and creatine kinase (CK, EC 2.7.3.2.). These analysis were according to Bergmeyer (1983) using an automatic blood chemical analyzer (Gilford system 103) with Roche testing kits (Roche COBAS MIRA PLUS) which measured NADH at the wavelength of 340 nm.

All data were analyzed by using the General Linear Models (GLM procedure) of statistical analysis system (SAS, 1985). Significant differences among treatments were determined by Duncan's New Multiple Range Test.

RESULTS AND DISCUSSION

Effect on performance

The effect of levels of dietary copper on the performance of the broilers is presented in table 2. Inclusion of copper up to 250 mg/kg did not showed a significant effect, but inclusion of 500 mg/kg significantly depressed feed intake and live-weight gains of the three to six weeks old broiler chickens ($p<0.05$). This result is similar to the results of Chen et al. (1996, 1997ab), where feed intake and body weight gains were significantly reduced by the supplementation of 500 mg/kg of copper in the diet of Taiwan country chickens and the layer pullets. Feed efficiency in the present study however did not show a significant impact from the inclusion of a high dose of copper in the diet.

Table 2. Effect of different levels of copper in the diet on growth performance of 3-6 weeks old broiler chickens

	Level of copper inclusion (mg/kg)			
	0	100	250	500
Feed intake (g/bird/day)	114.5 ± 9.1^a	114.4 ± 6.3^a	113.5 ± 2.1^a	103.5 ± 3.5^b
Live-weight gain (g/day)	61.0 ± 2.8^a	61.5 ± 2.1^a	59.0 ± 1.4^a	53.0 ± 1.0^b
Feed/gain (g/g)	1.88 ± 0.06	1.85 ± 0.04	1.92 ± 0.08	1.95 ± 0.07

Each value represents Mean $\pm$ SD.

[a,b] Means of the same row with the different superscript are significantly different ($p<0.05$).

Effect on necropsy of GI tract

Gross observation of the GI tract, including oral cavity, esophagus, gizzard, proventricular, small intestine and large intestine, showed obvious lesions in the oral cavity, tongue and pharynx from those chickens that were fed a diet with including 250 mg/kg copper, as shown in figure 1. The GI tract showed severe oral lesions and gizzard erosion from the inclusion of 500 mg/kg copper. Since copper is a strong antioxidant, inclusion of excess amount of copper in the diet damaged not only mucosa but also muscular layer of the intestinal tract (Jensen et al., 1991). However there was not observed any gross damage in crop and esophagus or any distended or pathological changes in proventricular, as shown in figure 2. This is different from the observation of Jensen et al. (1991), that diets

supplemented with more than 169 mg/kg copper caused oral lesion and damage to the lining of gizzard.

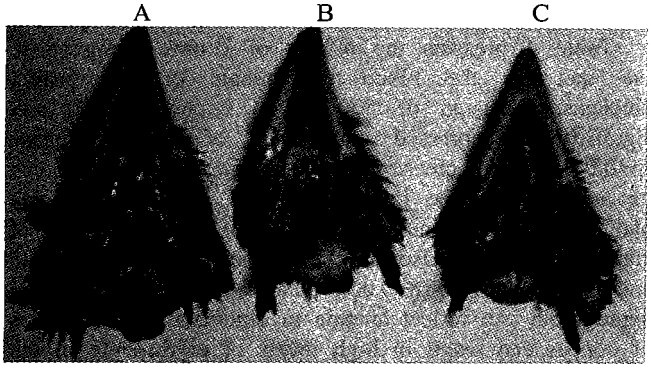

Figure 1. The effect of levels of dietary copper inclusion on oral lessons. (A) Control, (B) 250 mg/kg inclusion, (C) 500 mg/kg inclusion

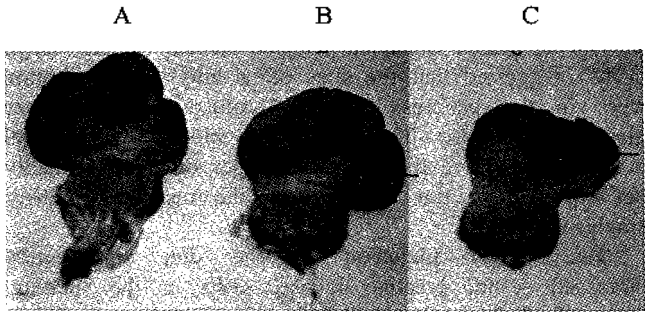

Figure 2. The effect of levels of dietary copper inclusion on oral lessons. (A) Control, (B) 250 mg/kg inclusion, (C) 500 mg/kg inclusion

Effects on GI histology

Figure 3 presents the normal histology of GI tracts of broiler chickens that were fed the control basal diet.

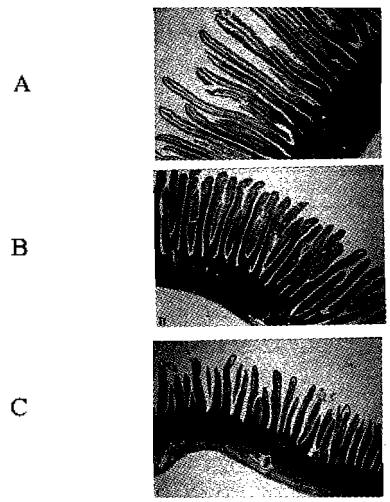

Figure 3. The normal mucosal histology of broiler chickens. (A) Duodenum, (B) Jejunum, (C) Ileum

Table 3 presents the effects of dietary copper in the intestinal histology in different GI tract segments. The mean villi height and crypt depth were 1,109 and 99 μm, 895 and 94, and 698 and 84 in duodenum, jejunum and ileum, respectively. This indicated a decline in villi height and crypt depth from duodenum, to jejunum and ileum. This agrees with the results of Turk (1982) and Chen et al. (1997b) where it was shown that in 6-week-old growers the villi height and crypt depth decreased along the intestine from duodenum to ileum. The longer villi in broilers than pullet chicks may contribute to thier faster growth due to the larger surface area for nutrient absorption (Yamauchi and Isshiki, 1991). Low copper inclusion (100 mg/kg) in the diet did not significantly influence the villi height, crypt depth and the thickness of muscle layer in the duodenum, jejunum and ileum of the broiler chickens. Inclusion 250 mg/kg significantly depressed villi height and thickened the muscle layer in the duodenum ($p<0.05$). High levels of copper supplementation up to 500 mg/kg severly depressed villi height, not only in the duodenum but also in the jejunum ($p<0.05$). These results agree with Chen et al. (1997b) for the 6-week-old growers that were fed a diet supplemented with 500 mg/kg copper.

Table 3. Effect of different levels of copper in the diet on intestinal histology of 6 weeks old broiler chickens, μm

Items	Level of copper inclusion (mg/kg)			
	0	100	250	500
Duodenum				
Villus height	1109 ± 143^{a}	1099 ± 149^{a}	989 ± 44^{b}	753 ± 149^{c}
Crypt depth	99 ± 15	95 ± 10	93 ± 12	104 ± 10
Muscle layer thickness	207 ± 11^{b}	216 ± 13^{ab}	218 ± 11^{a}	220 ± 13^{a}
Jejunum				
Villus height	895 ± 91^{a}	880 ± 92^{a}	872 ± 147^{a}	749 ± 116^{b}
Crypt depth	94 ± 13	98 ± 11	95 ± 12	90 ± 12
Muscle layer thickness	213 ± 14	219 ± 11	215 ± 10	217 ± 10
Ileum				
Villus height	698 ± 11	689 ± 117	634 ± 101	625 ± 101
Crypt depth	84 ± 10	86 ± 7	89 ± 12	90 ± 14
Muscle layer thickness	214 ± 15	204 ± 26	217 ± 10	214 ± 12

Each value represents Mean $\pm$ SD of 16 broiler chickens.
[a,b,c] Means of the same row with the different superscript are significantly different ($p<0.05$).

Since the GI epithelium has protection, secretion and absorption functions (Gibson et al., 1989), therefore changes in GI environment, i.e., diet, pH and microflora, parasites and disease, influence the rate of the mucosal cells turnover (Vahouny et al., 1985). Normal life cycle for enterocytic cells of villi is approximately 48 to 96 hours for broiler chickens. Since mature villi cells are responsible for the absorptive function, increased rate of turnover of the enterocytic cells will decrease the

maturity of cells and reduce the rate of absorption (Fernando and McCraw, 1973). Since copper stimulates villi cells in chickens, this attributes to the shortening villi and decreasing area of absorption. However this is not in the case in weaning piglets. As Radecki et al. (1992) reported, the inclusion of 250 mg/kg copper in the diet promoted growth performance of the piglets. They attributed this to a decrease of mucosal cell turnover rate in the jejunum, hence a decrease of the energy requirement for tissue maintenance.

Effects of copper inclusion in the diet did not significantly influence crypt depth in the various segments of the small intestine in broiler chickens ($p>0.05$). These results also agree with Chen et al. (1997b), that copper inclusion did not impact the crypt depth of the 6-week-old growers. Factors that change the intestinal environment will influence enterocytic cells. The increase of crypt depth highly correlates to the decrease of enzymes activity on the brush border of piglets (Hampson, 1986). Our results also showed indirect evidence of a correlation between crypt depth and the enzyme activity in the blood serum of broiler chickens, since the sera levels of α-amylase and alkaline phosphatase which may reflect secretion functions of the mucosa were not increased by the inclusion of 500 mg/kg copper in the diet (table 3).

The primary effect of the inclusion of 250 mg/kg copper in the diet was to significantly thicken the muscle layer in the duodenum ($p<0.05$). Thickened muscle layer and shorten duodenal villi may result in an increase in peristaltic movement of the intestine. This may increase passage rate of digesta, decrease retention of the digesta, hence decrease nutrient absorption, and decreased nutrient absorption generally results in depressed growth performance. Our results did not show the same trend of the decrease in growth performance from the inclusion of 250 mg/kg copper (table 2). There was, however, evidence of the damaged muscle, as shown by the oral lesions, the gizzard erosions and the significant increase of the muscular specific enzyme, creatine kinase in the serum of the broiler chickens whose diet was supplemented by 250 mg/kg copper (table 4).

Effect on the blood constituents

Table 4 presents the effects of copper inclusion in the diet on the blood constituents of the broiler chickens. Inclusion of 500 mg/kg copper in the diet significantly depressed the level of blood glucose ($p<0.05$). This decrease in the glucose concentration can be attributed to the low feed intake in the group that had high copper inclusion (table 2). High dosage of copper in the diet also significantly increased hematocrite and hemoglobin ($p<0.05$). Pact cell volume and hemoglobin can reflect anemia in chickens. High dosages of copper can result in high levels of erythrocyte in the blood since copper is a major cofactor in hematogenesis.

The inclusion of copper in the diet for three weeks did not significantly influence the serum α-amylase activity in the broiler chickens ($p>0.05$). This agreed with the observation of Chen et al. (1996) that inclusion of 500 mg/kg copper in the diet did not influence the activity of starch digestive enzyme in the pullets. Boyd (1988) suggested that the sites of α-amylase distribution were in the pancreas, intestinal mucosa and salivary glands of chickens. Therefore lower blood glucose level in chickens with a high dietary copper diet can probably be attributed to the low feed intake instead of lower amylase activity.

The copper inclusion in the diet significantly increased serum creatine kinase, even in the medium dose of 250 mg/kg inclusion in the diet ($p<0.05$). Creatine kinase is a muscular specific enzyme (Wang, 1992), and high serum levels of this enzyme reflect release of the enzyme from muscular damage. Data from this trial indicate that adding more than 250 mg/kg copper to the diet damages muscle layer in the intestines of broiler chickens.

Aspartate aminotransferase and alanine aminotransferase are enzymes widely distributed in the liver, heart and skeleton muscles in pigeon, layer and broiler chickens. Inclusion of copper in the diet did not significantly

Table 4. Effect of different levels of copper in the diet on blood constituents of 6 weeks old broiler chickens

Items	Level of copper inclusion (mg/kg)			
	0	100	250	500
Glucose (mg/dL)	156.5 ±18.0^{a}	149.3 ±14.8^{ab}	151.5 ±15.6^{ab}	41.0 ±16.0^{b}
Pact cell Volume (%)	29.8 ±1.3^{b}	31.9 ±2.1^{ab}	30.1 ±1.5^{b}	32.7 ±2.7^{a}
Hemoglobin (mg/dL)	6.35 ±0.54^{c}	7.91 ±0.74^{b}	8.29 ±0.73^{a}	8.99 ±0.74^{a}
α-Amylase (U/L)	722 ±100^{a}	697 ±85^{c}	739 ±97^{a}	726 ±115^{a}
Alkaline phosphatase (U/L)	4,496 ±1844^{ab}	5,269 ±1,160^{a}	4,742 ±1,402^{a}	3,690 ±1,075^{c}
Creatine kinase (U/L)	1,137 ±157^{c}	1,202 ±256^{c}	1,889 ±402^{b}	2,169 ±275^{a}
AST (U/L)	190 ±19^{b}	202 ±18^{b}	205 ±23^{b}	362 ±104^{a}
AAT (U/L)	7.2 ±1.7	8.0 ±1.8	8.1 ±2.0	8.3 ±3.9
Total protein (g/dL)	3.26 ±0.40	3.23 ±0.24	3.36 ±0.47	3.50 ±0.25
Albumin (g/dL)	1.26 ±0.11	1.23 ±0.10	1.26 ±0.11	1.30 ±0.12

Each value represents Mean±SD. of 16 broiler chickens.
^{a,b,c} Means of the same row with the different superscriot are significantly different ($p<0.05$).

influence the serum level of AAT ($p>0.05$). However high doses of copper (500 mg/kg) significantly increased serum AST ($p<0.05$), resulting in damaged liver and muscular cells of the broiler chickens. This observation agrees with the results of Chiou et al. (1997) in their layer study, that a high serum AST level and damage liver results from a diet including 600 mg/kg copper. Chen et al. (1996, 1997b) also observed an increase in the enzyme level as a result of increase level of dietary copper in the Taiwan country chickens and the pullet chickens.

Alkaline phosphatase is widely distributed in all tissue cells in the body, but concentrated more in the oesteoblast cells, liver and the enterocytic cells (Bogin, 1992). During the fast growing period, the oesteoblast cells rupture and release the enzyme into the blood stream. This usually contributes to the increase of serum alkaline phosphatase activity in young chickens. High doses of copper (500 mg/kg) in the diet, however, significantly depressed the alkaline phosphatase in the blood serum ($p<0.05$). This low level of the enzyme activity may result in low feed intake and poor performance of broiler chickens that were fed a high-copper diet (table 2).

Copper inclusion did not significantly influence sera total protein and albumin in this trial, indicating that inclusion of 500 mg/kg copper in the diet did not disturb normal protein metabolism of the broiler chickens.

Effect on morphology of the GI tract

Figure 4 presents the effects of levels of dietary copper inclusion on the morphology of the GI tract. The SEM micrograph shows the different shapes of villi; plate-like in the duodenum, fold shape in the jejunum and tongue shape in the ileum.

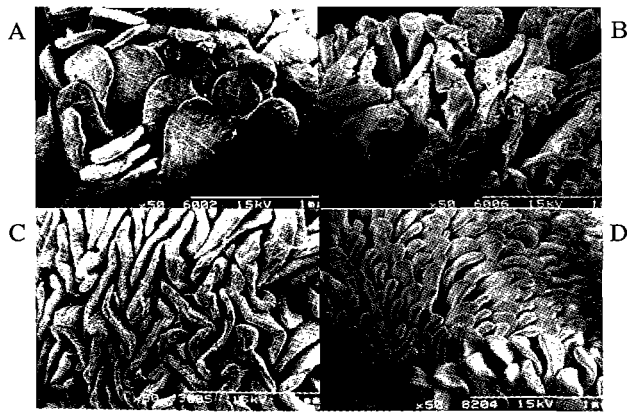

Figure 4. The mucosal morphology of broiler chickens fed diet with different copper inclusion. (A) Normal duodenum, (B) Damage duodenum with 500 mg/kg copper inclusion, (C) Normal jejunum, (D) Normal ileum

This result is in agreement with the observation of the broiler and pullet chickens by Yamauchi and Isshiki (1991). They concluded that the plate-like villi have a better nutrient absorption. From the villi SEM micrograph of the 500 mg/kg copper inclusion group, damaged villi can be prominently seen. The shorter villi in this trial can also indicate villi damage (table 3). Copper damage to the muscle layer can also be noted from the significantly increase of the specific enzyme activity of muscular cells, creatine kinase, and the activity of aspartate aminotransferase in the blood serum from the high copper inclusion group (500 mg/kg) (table 4). Any damage to the digestive tract will extend from oral cavity down to the duodenum, since the major site of copper absorption is in duodenum (Starcher, 1969), with less copper damage after the duodenum. The normal jejunal and ileal villi in this region can also be observed from the SEM micrograph.

It appears that a high copper (500 mg/kg) diet will damage duodenal villi, impact nutrient absorption, depress intake of feed, thus resulting in poor growth performance of broiler chickens.

REFERENCE

Bergmeyer, H. U. 1983. Methods of enzymatic analysis. Vol. III. Enzymes 1: Oxidoreductase, Transferase. Verlay Chemie, Deerfield Beach. FL, pp. 126, 416, 510.

Bogin, E. 1992. I. Fundamentals of clinical chemistry. pp. 9-15. In: Handbook of animal clinical chemistry. Rochester:; Kodak Publ. Inc. USA.

Boyd, J. W. 1988. Serum enzymes in the diagnosis of diseases in man and animals. J. Comp. Pathol. 98:381-404.

Chen, K. L., C. P. Wu and J. J. Lu. 1996. Effects of dietary copper levels on performance, tissues and serochemistry value of Taiwan country chicken. J. Biomass Energy Soc. Chin. 15(1,2): 70-75 (in Chinese).

Chen, K. L. C. L. Chen, T. F. Lien and Y. M. Horng. 1997a. Effect of dietary levles of copper on tissue residue and serochemistry value of growers. Taiwan. J. Vet. Med. Anim. Husb. 67:45-50 (in Chinese).

Chen, K. L., C. P. Wu and C. L. Chen. 1997b. Effects of dietary levels of copper on performance and intestinal structure in growers. J. Chia-Yi Inst. Agric. Technol. 50:31-39 (in Chinese).

Chiou, P. W. S., K. L. Chen and B. Yu. 1997. Toxicity, tissue accumulation and residue in egg and excreta of copper in laying hens. Anim. Feed Sci. Technol. 67:49-60.

Fernando, M. A. and B. M. McCraw. 1973. Mucosal morphology and cellular renewal in the intestinal of chickens following a single infection of Eimeria acerunlina. J. Parasitol. 59:793-501.

Funk, M. A. and D. H. Baker. 1991. Toxicity and tissue accumulation of copper in chicks fed casein and soy-based diets. J. Anim. Sci. 69:4505-4511.

Gibson, P. R., E. Van De Pol, L. E. Maxwall and W. Doe. 1989. Isolation of colonic crypts that maintain structural and metabolic viability in vitro. Gastroenterol. 96:283-291.

Hampson, D. J. 1986. Alteration in piglet small intestinal structure at weaning. Res. Vet. Sci. 40:32-40.

Jensen, L. S., P. A. Runn and K. N. Dolson. 1991. Induction of oral lesion in broiler chicks by supplementation the diets with copper. Avian Dis. 35:963-973.

Leach, R. M., C. I. Rosenblum, M. J. Amman and J. Burdette. 1990. Broiler chicks fed low-calcium diets. 2. Increased

sensitivity to copper toxicity. Poult. Sci. 69: 1905-1910.
Moore, R. J., E. T. Kornegay, R. L. Grayson and M. D. Lindemann. 1988. Growth, nutrient utilization and intestinal morphology of pigs fed high fiber diets. J. Anim. Sci. 66:1570-1579.
National Research Council. 1994. Nutrient Requirement of Poultry. National Academy Press. Washington, D. C.
Paulini, I., T. Metha and A. Hargis. 1987. Intestinal structural changes in African green monkeys after long term psyllium or cellulose feeding. J. Nutr. 117:253-266.
Popoulis, C. and L. S. Jensen. 1976. Effect of high dietary copper on gizzard integrity of the chick. Poult. Sci. 55:113-121.
Robbins, K. S. and D. H. Baker 1980. Effect of high-level copper feeding on the sulfur amino acid need of chicks fed corn-soybean meal and purified crystalline amino acid diets. Poult. Sci. 59:1099-1108.
Radecki, S. V., P. K. Ku, M. R. Bennink, M. T. Yokoyama and E. R. Miller. 1992. Effect of dietary copper on intestinal mucosa enzyme activity, morphology and turnover rate in weanling pigs. J. Anim. Aci. 70:1424-1431.
Robbins, K. S. and D. H. Baker. 1980b. Effect of sulfur amino acid level and source on the performance of chicks fed high levels of copper. Poult. Sci. 59:1246-1253.
Statistical Analysis System. 1985. SAS user's guide, 5yh ed. version, Statistical Analysis System Institute Inc., Cary, NC.
Starcher, H. 1969. Studied on the mechanism of copper absorption in the chick. J. Nutr. 97:321-326.
Turk, D. E. 1982. The avian gastrointestinal tract and digestion. Poult. Sci. 61:1225-1224.
Vahouny, G. V., T. Le, I. Ifrim, S. Satchithanadam and M. W. Cassidy. 1985. Stimulation of intestinal cytokinetics and mucin turn over in rats fed wheat bran or cellulose. Am. J. Clin. Nutr. 41:895-900.
Wang, C. H. 1992. Plasma and tissue enzyme activities in young chickens. J. Chin. Soc. Vet. Sci. 18(3):132-138.
Yamauchi, K. E. and Y. Isshiki. 1991. Scanning electron microscopic observations on the intestinal villi in growing white leghorn and broiler chickens from 1 to 30 days of age. Br. Poult. Sci. 32:67-78.