ISSN 1819-1878

Asian Journal of Animal Sciences

http://knowledgiascientific.com

Knowledgia SCIENTIFIC
A Place to Publish Outstanding Research

Asian Journal of Animal Sciences 4 (3): 96-106, 2010
ISSN 1819-1878

Growth Performance and Histological Alterations of Intestinal Villi in Broilers Fed Dietary Mixed Minerals

J. Ruttanavut and K. Yamauchi
Laboratory of Animal Science, Kagawa University, Kagawa-ken, Japan

Abstract: To investigate the growth performance and histological intestinal alterations of broilers fed on dietary mixed minerals (MM), 7 day old broiler chicks were divided into 4 groups, with 4 replicates of 12 male chicks. They were fed *ad libitum* for 49 days with a basal commercial mash diet supplemented with MM at 0, 0.5 and 1 g kg^{-1} throughout the experiment (control, 0.5 g kg^{-1} MMS, 1 g kg^{-1} MMS) and 0.5 g kg^{-1} during the finisher period (0.5 g kg^{-1} MMF). Mineral supplementation did not influence the growth performance of broilers during the first 3 weeks, but tended to show higher growth and feed efficiency for the MM groups than for the control group at week 7. In light microscopic observation, values of the intestinal villus height, villus area and cell mitosis numbers were higher ($p<0.05$) in the 0.5 g kg^{-1} MMS and 0.5 g kg^{-1} MMF groups than those of the control in the duodenal segment. In scanning electron microscopic observation, all MM groups showed more protuberant epithelial cells on the villus apical surface of the duodenum. The present histological intestinal alterations in broilers fed the MM diet demonstrate that intestinal function could be stimulated in the duodenum, resulting in improved growth performance. The results obtained in this study have revealed that minerals may need to be supplemented during the finisher period of broiler production at a higher content than the feed manufacturer recommendation, to allow optimal performance.

Key words: Mixed minerals, chicken, intestinal histology, epithelial cell

INTRODUCTION

The benefits of mineral supplementation on the production performance of animals have been known for many years. Minerals have important biological functions and must be provided in adequate amounts. Inadequate mineral intake may affect hormonal secretion, enzyme activity, muscle function, bone mineral content, other body mineral functions (Peters and Mahan, 2008), reduced productivity and loss of resistance to diseases (Inal *et al*., 2001). The most-needed minerals for supplementing a basal diet composed of grain (oilseed meal) are Ca, P, Na, Cl, Fe, Cu, I, Mn, Se and Zn (McDowell, 1992). Of all the minerals, the requirement for calcium is the most variable, both between species and within a species, depending upon a bird's physiological state; whereas variations in trace mineral requirements are known to vary a few-fold between species or within a species due to physiological state (Klasing, 1998). Usually, mineral deficiencies are not commonly observed because feed manufacturers use much higher concentrations than those specified

Corresponding Author: Dr. Koh-en Yamauchi, Laboratory of Animal Science, Faculty of Agriculture, Kagawa University, Miki-cho, Kagawa-ken, 761-0795, Japan Tel/Fax: +81-87-891-3053

by requirements (with the exception of a few minerals). This is done to provide a safety margin that accounts for factors in commercial conditions that might lead to higher dietary mineral needs. However, continued changes in management of livestock, variation in feedstuff composition, instability due to storage conditions and methods of feed processing may increase the need for supplemental minerals. In addition, the modern broiler has been intensively selected for higher growth rates and increased feed conversion. Today, broilers are ready for market at 6 weeks of age with a body weight of 2.6 kg (Santose *et al*., 2005). Unfortunately, the mineral requirements of broiler chickens, as determined by several organizations 10 to 20 years ago, may not support optimal chicken performance in today's strains. Optimal mineral allowances are needed to permit animals to achieve their full genetic potential for optimal performance (McDowell, 1992). Therefore, it is necessary to add an increasing number of minerals to properly fortify broiler diets. Growth response in broiler chickens has been used as the primary criterion for determining requirements of minerals because broilers are ideal assay animals with a limited nutrient store, high nutrient demand and rapid growth rate (Bao *et al*., 2009). In our earlier study, improved growth performance due to elevation of the intestinal function was reported in Aigamo ducks (Khambualai *et al*., 2009) and chickens (Incharoen *et al*., 2009) after feeding zeolite including plant extract and in pigs fed wood charcoal powder including vinegar liquid (Mekbungwan *et al*., 2008). Since, the small intestine is the site of nutritional absorption from the intestinal lumen through the mucosal epithelial cells into the blood or lymphatic system and as the histology of the intestinal villi and epithelial cells on the villus apical surface is affected by dietary feed components, increasing mineral levels may also affect intestinal function. In this study, the effects of mixed mineral supplementation on feed intake, body weight gain and feed efficiency were examined in broiler. Then, light microscopic observations of villus height, villus area, cell area and cell mitosis number and scanning electron microscopic observations of villus apical surface were compared in each intestinal segment of each group.

MATERIALS AND METHODS

Animals and Diets

All experimental treatments were performed according to the humane care guidelines for the use of animals for experimentation as provided by Kagawa University, Japan (Kagawa University, 2006). In a preliminary experiment, we observed the effect of gradients of Mixed Minerals (MM) (0.5, 1 and 2 g kg^{-1}) on the growth performance of broilers and found that at a dose of 0.5 g kg^{-1}, MM improved body weight gain and feed efficiency, whereas at other concentrations, MM failed to increase the growth performance of broilers. Accordingly, in the current study, 0.5 g kg^{-1} MM was selected, together with a 1 g kg^{-1} MM group to observe clearer effects on growth performance and histological alteration in the intestine.

A total of 48 one-day-old male Marshall Chunky broilers were obtained from a commercial farm. They were housed in electrically heated brooder cages and had *ad libitum* access to water and were fed conventional starter mash diet (CP: 220 g kg^{-1}, ME: 12.8 MJ kg^{-1}) for one week. At 7 day of age, the birds were weighed individually and randomly divided into 4 groups with 4 replicates of 3 birds on a similar body weight basis. The birds were housed in 45×90 cm pens (3 birds in each pen) in an environmentally controlled room with continuous light.

The basal diets (Table 1) were supplemented with MM in the form of hydroxides (Core®; 300 mg Calcium, 150 mg Magnesium, 25 mg Zinc, 15 mg Ferrous, 4 mg Manganese

Table 1: Feed formulations and nutrient composition of commercial broiler starter and finisher mash diets (g kg^{-1})

Item	Starter 1 to 21 day	Finisher 22 to 49 day
Ingredients		
Corn, Milo	610.0	640.0
Corn gluten meal	290.0	-
Soybean meal	-	270.0
Rice bran	23.0	52.0
Fish meal	70.0	30.0
Tallow	5.0	6.0
Concentrate mixture[1]	2.0	2.0
Nutrient composition		
Crude protein	220.0	180.0
Metabolizable energy (MJ kg^{-1})	12.8	13.6
Crude fiber	40.0	40.0
Crude fat	40.0	60.0
Calcium	10.0	8.0
Phosphorus, available	4.0	3.0
Magnesium g kg^{-1} (as fed, analysed)	1.9	1.8
Zinc mg kg^{-1} (as fed, analysed)	107.5	100.1
Ferrous mg kg^{-1} (as fed, analysed)	147.7	135.0
Manganese mg kg^{-1} (as fed, analysed)	123.3	130.6
Copper mg kg^{-1} (as fed, analysed)	28.9	30.7

[1]Concentrate mixture including (per kg of diet): vitamin A 9,600 IU, vitamin D_3 1,920 IU, vitamin E 35 mg, vitamin K 2.6 mg, vitamin B_1 5.8 mg, vitamin B_2 7.3 mg, vitamin B_6 0.4 mg, vitamin B_{12} 2.6 μg, biotin 0.2 mg, pantothenic acid 16.1 mg, folic acid 1.0 mg, nicotinic acid 69.1 mg, choline 1,400 mg, zinc 79.9 mg, copper 12.8 mg, manganese 92.4 mg

Table 2: Supplemental levels of mixed minerals fed to birds on different diets (mg kg^{-1})

Diets	Added Ca	Added Mg	Added Zn	Added Fe	Added Mn	Added Cu
Control	-	-	-	-	-	-
0.5 g kg^{-1} MMS[1]	150	75	12.5	7.5	2	1
1.0 g kg^{-1} MMS[2]	300	150	25.0	15.0	4	2
0.5 g kg^{-1} MMF[3]	150	75	12.5	7.5	2	1

[1]Basal diet with mixed minerals during 7-49 days of age. [2]Basal diet with mixed minerals during 7-49 days of age. [3]Basal diet with mixed minerals during 22-49 days of age.

and 2 mg Copper g^{-1} MM) at 0, 0.5 and 1 g kg^{-1} throughout the experiment (control, 0.5 g kg^{-1} MMS, 1 g kg^{-1} MMS) and 0.5 g kg^{-1} during the finisher period (0.5 g kg^{-1} MMF) in order to increase concentrations of Ca, Mg, Fe, Zn, Mn and Cu (Table 2). A conventional starter mash diet was fed to birds up to 21 days of age and then the diet was changed to the finisher mash diet (CP: 180 g kg^{-1}, ME: 13.6 MJ kg^{-1}) until 49 days old. Each bird had free access to water and feed throughout the experiment. Feed intake and body weight were measured every week.

Microscopic Examinations

At the end of feeding period, 4 birds with similar body weight were selected from each treatment and killed by decapitation under light anesthesia with diethyl ether. In these birds, the entire small intestine was quickly excised and placed in a beaker with a mixture of 3% glutaraldehyde and 4% paraformaldehyde fixative solution in 0.1 M cacodylate buffer (pH 7.4). The same fixative solution was also injected into the intestinal lumen. Immediately after finishing this injection step, each intestinal segment was prepared for light and scanning electron microscopy. For each intestinal segment, the segment from gizzard to pancreatic and bile ducts was regarded as duodenum; for jejunum, the area included segment from the ducts to Meckel's diverticulum; and for ileum, the area included segment from the diverticulum to ileo-ceco-colic junction. All tissue samples from each of those birds were taken at the middle of each part.

Light Microscopic Examination

A 3 cm length of each intestinal segment was transversally cut and fixed in Bouin's fixative solution. The tissue samples were dehydrated with graded ethanol (70, 80, 90, 95, 99 and 100% by turns), then embedded in paraffin and subsequently cut and placed onto slides with 4 μm thickness. The tissues were stained with hematoxylin-eosin for light microscope measurement of villus height, villus area, epithelial cell area and cell mitosis number. These values were measured using an image analyzer (Nikon Labophot-2, Tokyo, Japan).

Measurement of Villus Height

The highest 2 villi having the lamina propria were randomly selected per transverse section. The villus height was measured from the villus tip to the bottom. The mean villus heights from 4 birds (16 villi from 8 difference sections in each segment per bird) were expressed as a mean villus height for one group.

Measurement of Villus Area

The width of villus was measured at the basal and apical parts and two villi were selected from each section. Villus area was calculated from the villus height, basal width and apical width. A total 16 calculations of the villus area were made for each bird. The average of these was expressed as the mean for each bird. Finally, the 4 bird means were expressed as a mean villus area for one group.

Measurement of Epithelial Cell Area

The area of epithelial cell layer was randomly measured at the middle part of the villus and then the cell nuclei within this measured epithelial cell layer were then counted. Finally, the area of the layer was divided by the number of cell nuclei to obtain an epithelial cell area. A total of 16 samples per bird were counted in each group.

Measurement of Cell Mitosis Number

Mitotic cells having homogenous, intensely stained basophilic nuclei with hematoxylin in one transverse section were counted. Total mitosis numbers were counted from 4 different sections for each bird and these 4 values were used to calculate the mean for one bird. Finally, these 4 means from 4 birds were expressed as mean cell mitosis in one group.

Scanning Electron Microscopy

A 2 cm tissue sample of each intestinal segment lying next to the light microscopy was transversely cut, slit longitudinally, opened and washed with 0.1 M phosphate buffered saline (pH 7.4). To prevent curling, the edges of tissue sample were pinned flat to the paraffin-covered bottom of a Petri dish containing a mixture of 3% glutaraldehyde and 4% paraformaldehyde fixative solution in a 0.1 M cacodylate buffer (pH 7.4) at room temperature for 2 h. Then, samples were cut into 4×7 mm^2 squares, washed with a 0.1 M cacodylate buffer and postfixed with 1% osmium tetroxide for 2 h. The specimens were washed in distilled deionised water, dehydrated in ethanol of increasing concentration (45, 70, 80, 90 and 100%). These specimens were freeze-dried in a critical point drying apparatus (Hitachi Freeze Dryer, Tokyo, Japan), sputter coated with platinum (Hitachi E-1030 Ion Sputter, Hitachi Ltd, Tokyo, Japan) and observed with a scanning electron microscope (Hitachi S-4300SE/N, Hitachi Ltd, Tokyo, Japan).

Statistical Analysis

Growth performance and all light microscopic data obtained in the experiments were statistically analyzed using one-way ANOVA and significant differences among the treatments were determined with Duncan's multiple range test using the SAS program (SAS, 2000). The results were expressed as means and standard errors. Differences at $p<0.05$ were considered as significant.

RESULTS AND DISCUSSION

Animal Performance

The results of growth performance for birds during the 3 and 7 week periods are shown in Table 3, in the first 3 week, feed intake of birds was similar across diets (961.66, 952.91, 941.42, 970.08 g for control, 0.5 g kg^{-1} MMS, 1 g kg^{-1} MMS and 0.5 g kg^{-1} MMF groups, respectively). However, the overall feed intake (7 to 49 day) was higher for the experimental groups (4412.50, 4270.83, 4342.08 g for 0.5 g kg^{-1} MMS, 1 g kg^{-1} MMS and 0.5 g kg^{-1} MMF groups, respectively) than in the control group (4410.42 g).

At the end of the starter period of the experiment (7 to 21 d), the body weight gains of birds fed 0.5 g kg^{-1} MMS (665.0 g), 1 g kg^{-1} MMS (658.75 g) and 0.5 g kg^{-1} MMF (662.08 g) diets were similar to the control birds (659.75 g). However, the overall body weight gains of birds (7 to 49 day) fed MM diets were greater than those of birds fed the control diet (2479.16, 2446.66, 2498.33, 2408.12 g for 0.5 g kg^{-1} MMS, 1 g kg^{-1} MMS, 0.5 g kg^{-1} MMF and control groups, respectively).

During the starter period, all birds fed the four diets had similar feed efficiency. However, the overall feed efficiency (7 to 49 day) for birds fed MM diets (0.561, 0.573, 0.575 for 0.5 g kg^{-1} MMS, 1 g kg^{-1} MMS and 0.5 g kg^{-1} MMF groups, respectively) were greater than those of birds fed the control diet (0.546).

Light Microscopic Parameters

The villus height, villus area, cell area and cell mitosis of the control and experimental groups are shown in Fig. 1a-d, most values of these light microscopic parameters were higher than those of the control in the duodenum. The duodenal villus height and villus area were higher ($p<0.05$) in chickens fed 0.5 g kg^{-1} MMS and 0.5 g kg^{-1} MMF diets. Cell areas were not different among the groups. Duodenal cell mitosis were greater ($p<0.05$) in the 0.5 g kg^{-1} MMF group than other groups.

Scanning Electron Microscopic Observations of Epithelial Cells on the Villus Tip

On the duodenal villus apical surface of the control group (Fig. 2a), flat cell areas (small arrow) and faintly protuberated cells (large arrow) were observed. In the 0.5 g kg^{-1} MMS and

Table 3: Feed intake, body weight gain and feed efficiency in broilers fed on the control diet, the dietary 0.5 g kg^{-1} MMS, 1 g kg^{-1} MMS and 0.5 g kg^{-1} MMF diets at 3 and 7 weeks of age

	Diet			
Parameters	Control	0.5 g kg^{-1} MMS	1 g kg^{-1} MMS	0.5 g kg^{-1} MMF
Feed intake (g)				
3 weeks	961.66±10.31	952.91±16.60	941.42±13.96	970.08±8.63
7 weeks	4410.42±47.15	4412.50±27.94	4270.83±33.59	4342.08±55.94
Body weight gain (g)				
3 weeks	659.75±28.67	665.00±15.10	658.75±21.92	662.08±13.95
7 weeks	2408.12±39.25	2479.16±40.79	2446.66±53.33	2498.33±45.49
Feed efficiency				
3 weeks	0.686±0.02	0.697±0.02	0.699±0.01	0.682±0.01
7 weeks	0.546±0.01	0.561±0.01	0.573±0.02	0.575±0.01

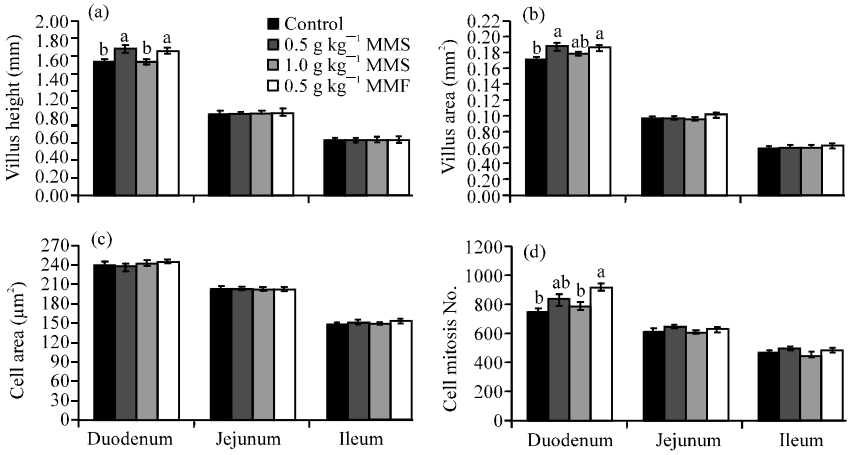

Fig. 1: (a) Villus height, (b) villus area, (c) cell area and (d) cell mitosis number in each intestinal segment of broilers fed the control diet, the dietary 0.5 g kg^{-1} MMS, 1 g kg^{-1} MMS and 0.5 g kg^{-1} MMF diets (Mean±SE; n = 4). Means with different letter(s) are significantly different from each other ($p<0.05$)

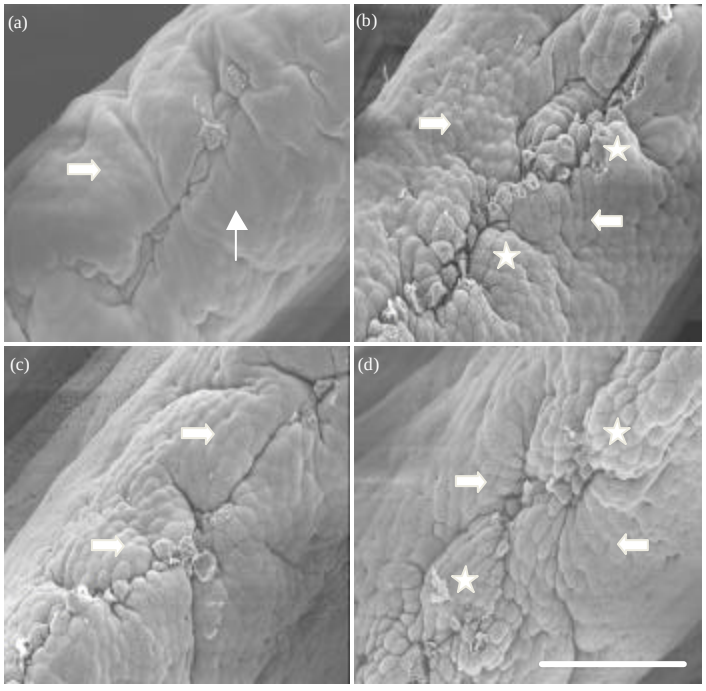

Fig. 2: Duodenal villus apical surface in broilers fed on the (a) control (Small arrow: Flat cells; Large arrow: Faintly protuberated cells), (b) the dietary 0.5 g kg^{-1} MMS (Arrows: Protuberated cells. Stars: Cell clusters), (c) 1 g kg^{-1} MMS (Arrows: Protuberated cells) and (d) 0.5 g kg^{-1} MMF diets (Arrows: Protuberated cells; Stars: Cell clusters). Scale bar = 50 μm

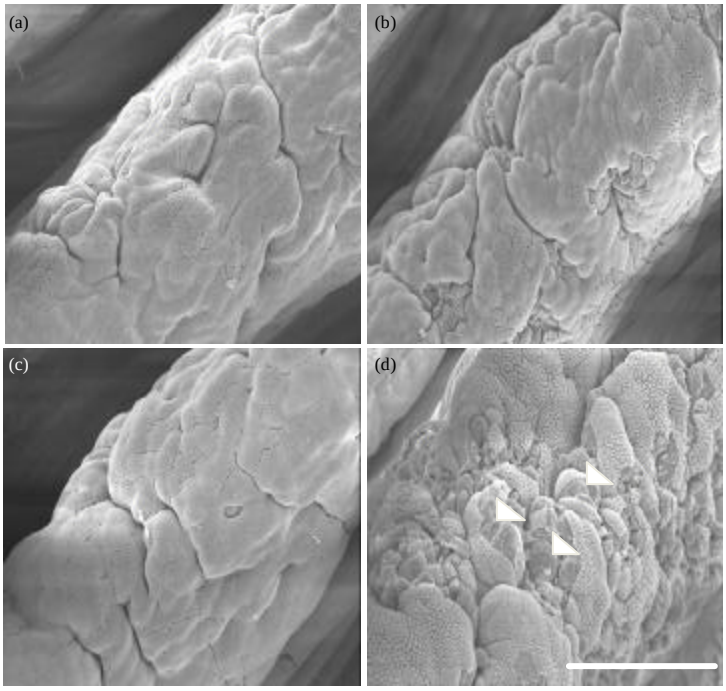

Fig. 3: Jejunal villus apical surface in broilers fed on the (a) control, (b) the dietary 0.5 g kg^{-1} MMS, (c) 1 g kg^{-1} MMS and (d) 0.5 g kg^{-1} MMF diets (Arrowheads: Cells having no microvilli). Scale bar = 50 μm

0.5 g kg^{-1} MMF groups (Fig. 2b, d), more protuberated cells (arrows) and cell clusters (stars) around the central sulcus were observed. In the 1 g kg^{-1} MMS group (Fig. 2c), the villus surface showed slightly protuberated cells (arrows).

In the jejunum, the villus apical surface of the 0.5 g kg^{-1} MMS and 1 g kg^{-1} MMS groups (Fig. 3b, c) showed morphology similar to the control (Fig. 3a). In the 0.5 g kg^{-1} MMF group (Fig. 3d), cells having no microvilli (arrowheads) were frequently observed.

In the ileum, the villus apical surface of the control (Fig. 4a) and the experimental groups (Fig. 4b-d) showed a similar morphology.

Poultry confined within intensive production systems are particularly susceptible to mineral deficiencies. McDowell (1992) reported that optimum animal performance required under modern commercial conditions cannot be obtained by fortifying diets just to meet minimum requirements. Therefore, adequate margins of safety must provide for several factors that may increase certain dietary mineral requirements. In the present study, it was observed that birds fed MM had no beneficial effects on growth performance during the first three weeks. However, the addition of MM to the basal diet resulted in improved growth performance compared with the control group at 7 weeks. These results are in consistent with Bao *et al.* (2009), who reported that modern broiler chickens need more minerals in the 14-35 day period than they do in the 1-14 day period and that Ca, Cu, Fe, Mn or Zn are also required for the growth, development and maintenance of healthy bone. This indicates that the industry-recommended level of minerals shown in Table 1 would not be sufficient for the finisher phase of broiler chickens, but had already met the requirement for starter phase. The

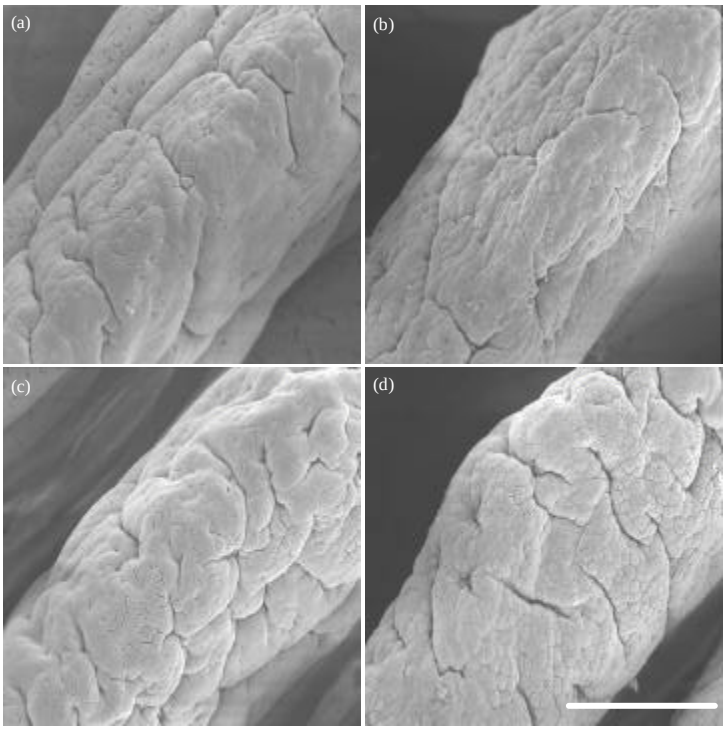

Fig. 4: Ileal villus apical surface in broilers fed on the (a) control, (b) the dietary 0.5 g kg^{-1} MMS, (c) 1 g kg^{-1} MMS and (d) 0.5 g kg^{-1} MMF diets. All groups show a similar morphology. Scale bar = 50 μm

main component of MM is calcium. Calcium is important in the skeletal and cellular functions of chickens (Ziaei *et al*., 2008). It was reported that increasing the dietary Ca level from 6.5 to 10 g kg^{-1} (Elliot *et al*., 1995) or from 5.4 to 12.4 g kg^{-1} (Boling-Frankenbach *et al*., 2001) quadratically increased growth performance in broilers. This is in agreement with the present result that supplementing mixed minerals to basal diet had a tendency to increase body weight gain and feed efficiency. In general, trace minerals function as catalytic or structural cofactors in metal-containing enzymes and proteins that are contained in the cells (Richards, 1997). Dozier *et al*. (2003) reported that a high concentration of trace minerals is sometimes used because it is thought to elicit growth-promoting activity. In addition to growth promoting effect, minerals play a major role in the immune response, the body's defense system against infectious disease and mineral supplementation above requirements is required for optimal immune responses (McDowell, 1992).

Although, mineral accumulation into ground and surface waters from poultry manure has generated environmental concerns, reducing dietary minerals to the minimum requirements may not allow for a margin of safety and could potentially adversely affect performance if less bioavailable sources of minerals are used. In the present study, the weight gain of birds fed the lower level of MM supplementation were greater than the gain of birds fed diets with a higher level of MM. These results show that MM increased weight gain in broilers when fed at an appropriate level, but was less effective when higher levels were fed. These results are supported by findings of Peters and Mahan (2008), who reported that possible chelating

interactions may occur between macro- and micro-minerals in the lumen of the digestive tract that may be more pronounced when greater mineral concentrations are fed. This could possibly affect the absorption and biological function of minerals (Ammerman *et al*., 1998). With some interactions, high levels of one mineral increase the requirement for one or more other minerals, as high calcium increases the need for phosphorus, magnesium, iron, manganese and zinc; excessive concentrations of one element may result in a deficiency in the amount of some other element available to the bird (Klasing, 1998).

In the present birds fed the MM diets, body weight gain was greater than that of the control birds. In the MM-fed birds, most values of intestinal villus height, villus area and cell mitosis were higher than those of the control in the duodenal segment. In addition, protuberated cells were also observed in this part of the MM groups. This suggested that the ingested feed might be effectively absorbed from the duodenal epithelial cells. The aim of the present study was to observe the effects of dietary MM on the villus morphological changes in these birds, because a close relationship between morphological change and the features of intestinal function is well known. Intestinal morphology was markedly affected by the fed diets (Langhout *et al*., 1999; Yasar and Forbes, 1999) and the micronutrient content in feed also influenced the morphology of intestines (Iji *et al*., 2001). According to our results, increasing mineral levels induced the histological alterations of intestinal villi. Histologically, it has been suggested that long villi result in an increased surface area that is capable of greater absorption of available nutrients (Caspary, 1992). Increased villus size has been associated with activation of cell proliferation (Lauronen *et al*., 1998). Langhout *et al*. (1999) reported that the increased villus height, cell area and cell mitosis numbers in the intestine are indicators that the function of the intestinal villi is activated. These reports correspond with our observation that light microscopic parameters were greater in the MM-fed birds, indicating a higher function of the intestinal villi. Sun *et al*. (2005) also reported that micronutrients may have an effect on the development of the intestinal structure of broiler chickens after 35 days of age. The histological and morphometrical results of this study revealed that the intestinal response to the MM diets varies according to the region of the small intestine. When MM birds were compared with control birds, the most remarkable differences in the intestinal structure were seen in the duodenum. This indicates that dietary MM seems to be more effectively absorbed from the intestinal epithelial cells of the duodenum than from other parts. According to Isshiki *et al*. (1989), who reported that in the chickens fed a conventional diet, most of the ingested nutrients were absorbed in the upper part of the intestine. Klasing (1998) also reported similar results that most of the dietary minerals are absorbed from the duodenum and jejunum parts. Minerals serve a wide variety of structural and functional purposes: some minerals are primary regulators of cellular processes, some serve as activators or catalysts of enzymes, some are necessary components of deoxyribonucleic acid (DNA) and ribonucleic acid (RNA) (Klasing, 1998). Thus, increased mineral supplementation may also promote intestinal functions.

Also, in the present study, more protuberated cells were observed on the duodenal villus apical surface of the MM groups. Such cells were reported in chickens (Yamauchi *et al*., 2006), Aigamo ducks (Ruttanavut *et al*., 2009) and piglets (Mekbungwan *et al*., 2008) showing heavy body weight gain. Cell protuberances on the villus apical surface have been demonstrated to show an activated absorptive function of the villi (Yamauchi *et al*., 2006). Moreover, cells having no microvilli were observed on the jejunal villus apical surface in the 0.5 g kg^{-1} MMF group. Such areas having cells with no microvilli might be induced by activated cell mitosis, resulting in a quicker cell turnover

(Yamauchi *et al*., 2006). From these studies, the present increased light microscopic parameters and more protuberated cells suggest that the function of villi and epithelial cells might be stimulated after feeding dietary MM.

CONCLUSION

The present histological intestinal alterations in broilers fed the MM diet demonstrate that intestinal function could be stimulated in the duodenum, resulting in improved growth performance. The results obtained in this study have revealed that minerals may need to be supplemented during the finisher period of broiler production at a higher content than feed manufacturer recommendation, to allow optimal performance.

REFERENCES

Ammerman, C.B., P.R. Henry and R.D. Mile, 1998. Supplemental Organically Bound Mineral Compounds in Livestock Nutrition. Nottingham University Press, Nottingham.

Bao, Y.M., M. Choct, P.A. Iji and K. Bruerton, 2009. Optimum dietary inclusion of organically complexed zinc for broiler chickens. Br. Poult. Sci., 50: 95-102.

Boling-Frankenbach, S.D., J.L. Snow, C.M. Parsons and D.H. Baker, 2001. The effect of citric acid on the calcium and phosphorus requirements of chicks fed corn-soybean meal diets. Poult. Sci., 80: 783-788.

Caspary, W.F., 1992. Physiology and pathophysiology of intestinal absorption. Am. J. Clin. Nutr., 55: 299-308.

Dozier, W.A., A.J. Davis, M.E. Freemann and T.L. Ward, 2003. Early growth and environmental implications of dietary zinc and copper concentration and sources of broiler chicks. Br. Poult. Sci., 44: 726-731.

Elliot, M.A., K.D. Roberson, G.N. Rowland and H.M. Edwards, 1995. Effect of dietary calcium and 1,25-dihydroxycholecalciferol on the development of tibial dyschondroplasia in broilers during the starter and grower periods. Poult. Sci., 74: 1495-1505.

Iji, P.A., A. Saki and D.R. Tivey, 2001. Body and intestinal growth of broiler chicks on a commercial starter diet. 1. Intestinal weight and mucosal development. Br. Poult. Sci., 42: 505-513.

Inal, F., B. Coskun, N. Gulsen and V. Kurtoglu, 2001. The effects of withdrawal of vitamin and trace mineral supplements from layer diets on egg yield and trace mineral composition. Br. Poult. Sci., 42: 77-80.

Incharoen, T., O. Khambualai and K. Yamauchi, 2009. Performance and histological changes of the intestinal villi in chickens fed dietary natural zeolite including plant extract. Asian J. Poult. Sci., 3: 42-50.

Isshiki, Y., Y. Nakahiro and Z.X. Zhou, 1989. Feed digestibility in different intestinal parts of chickens. Jpn. J. Zootech. Sci., 60: 1082-1092.

Kagawa University, 2006. Rules of Animal Experiment in Kagawa University. Academic Press, Kagawa.

Khambualai, O., J. Ruttanavut, M. Kitabatake, H. Goto, T. Erikawa and K. Yamauchi, 2009. Effects of dietary natural zeolite including plant extract on growth performance and intestinal histology in Aigamo ducks. Br. Poult. Sci., 50: 123-130.

Klasing, K.C., 1998. Comparative Avian Nutrition. University Press, Cambridge, pp: 277-299.

Langhout, D.J., J.B. Schutte, P. van Leeuwen, J. Wiebenga and S. Tamminga, 1999. Effect of dietary high and low methyllated citrus pectin on the activity of the ileal microflora and morphology of the small intestinal wall of broiler chickens. Br. Poult. Sci., 40: 340-347.

Lauronen, J., M.P. Pakarinen, P. Kuusanmakai, E. Savilahti, P. Vento, T. Paavonen and J. Halttunen, 1998. Intestinal adaptation after massive proximal small-bowel resection in the pig. Scand. J. Gastroenterol., 33: 152-158.

McDowell, L.R., 1992. Minerals in Animal and Human Nutrition. Academic Press, San Diego, California.

Mekbungwan, A., K. Yamauchi, T. Sakaida and T. Buwjoom, 2008. Effects of a charcoal powder-wood vinegar compound solution in piglets for raw pigeon pea seed meal. Animal, 2: 366-374.

Peters, J.C. and D.C. Mahan, 2008. Effects of dietary organic and inorganic trace mineral levels on sow reproductive performances and daily mineral intakes over six parities. J. Anim. Sci., 86: 2247-2260.

Richards, M.P., 1997. Trace mineral metabolism in the avian embryo. Poult. Sci., 76: 152-164.

Ruttanavut, J., K. Yamauchi, H. Goto and T. Erikawa, 2009. Effects of dietary bamboo charcoal powder including vinegar liquid on growth performance and histological intestinal change in aigamo ducks. Int. J. Poult. Sci., 8: 229-236.

Santose, F.S., M.B. Farnell, G. Tellez, J.M. Balog and N.B. Anthony *et al*., 2005. Effect of prebiotic on gut development and ascites incidence of broilers reared in a hypoxic environment. J. Poult. Sci., 84: 1092-1100.

SAS, 2000. SAS User's Guide: Statistics. SAS Institute, Cary, NC.

Sun, X., A. McElroy, K.E. Jr. Webb, A.E. Sefton and C. Novak, 2005. Broiler performance and intestinal alterations when fed drug-free diets. Poult. Sci., 84: 1294-1302.

Yamauchi, K., T. Buwjoom, K. Koge and T. Ebashi, 2006. Histological alterations of the intestinal villi and epithelial cells in chickens fed dietary sugar cane extract. Br. Poult. Sci., 47: 544-553.

Yasar, S. and J. M. Forbes, 1999. Performance and gastrointestinal response of broiler chicks fed on cereal gain-based foods soaked in water. Br. Poult. Sci., 40: 65-76.

Ziaei, N., J.H. Guy, S.A. Edwards, P. Blanchard, J. Ward and D. Feuerstein, 2008. Effect of reducing dietary mineral content on growth performance, excreta dry matter content and blood parameters of broilers. Br. Poult. Sci., 49: 195-201.