Effects of Cu (II)-exchanged Montmorillonite on Growth Performance, Intestinal Microflora, Bacterial Enzyme Activities and Morphology of Broilers

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ABSTRACT : Two hundred forty 1-d-old Arbor Acres broiler chicks were used to investigate the effects of Cu (II)-exchanged montmorillonite (CEM) or montmorillonite on the growth performance, intestinal microflora, bacterial enzyme activities and morphology of broilers. The chicks were assigned randomly into three groups with 80 chicks per treatment. The three dietary treatments were basal diet only (control group), basal diet +1 g kg^{-1} montmorillonite, and basal diet +1 g kg^{-1} CEM. The results showed that the addition of CEM to the diet increased significantly the body weight and feed efficiency, but a similarly significant increase was not found in broilers fed the diet containing montmorillonite. Supplementing the CEM in the diet of broilers also decreased the numbers of *Clostridium perfringens* and *Escherichia coli* in the small intestine and cecum. The addition of either CEM or montmorillonite to the diet depressed the activities of β-glucosidase and β-glucuronidase in the small intestinal and cecal contents. Data of villus height and crypt depth for duodenum, jejunum and ileum indicated that dietary addition of CEM or montmorillonite improved the small intestinal mucosal morphology. ***(Asian-Aust. J. Anim. Sci. 2003. Vol 16, No. 11 : 1673-1679)***

Key Words : Growth Performance, Intestinal Microflora, Bacterial Enzyme, Intestinal Morphology, Montmorillonite, Broiler

INTRODUCTION

Montmorillonite, one of aluminosilicate clay, has a 2:1 layer structure (Borchardt, 1989). The inner layer is composed of an octahedral sheet, which is situated between SiO_4 tetrahedral sheets. The replacement of Al^{3-} for Si^{4-} in the tetrahedral layer and Mg^{2+} or Zn^{2-} for Al^{3-} in the octahedral layer results in a net negative charge on the clay surfaces. This charge imbalance is offset by interlayer hydrated cations, the predominant ones being Na^{+} and Ca^{2-}. These interlaminar cations can be exchanged with other metal cations such as Ag^{-}, Cu^{2-} or Zn^{2+}. It is well known that montmorillonite has a greater adsorption, which is attributed to its larger specific surface area and higher cation exchange capacity (Ramos and Hernandez, 1996; He et al., 1999; Hu et al., 2002). Dale et al. (1991) reported that montmorillonite could adsorb organic substances either on its external surfaces or within its interlaminar spaces, by the interaction with or substitution of the exchange cations present in these spaces. The in vitro study showed that montmorillonite could adsorb *Escherichia coli* and *Staphylococcus aureus*, but its antibacterial effect was not observed (Hu et al., 2002). On the other hand, montmorillonite effectively improves gastrointestinal mucus resistance to various aggressions by interacting closely with the mucous glucoproteins (Droy-Lefaix et al., 1985).

Copper has many functions in biochemical and biological cycles (Kaim and Schwederski, 1994). It is known that copper belongs to important essential element necessary for plants and animals. Moreover, copper has antimicrobial activity.

In the present work, Cu^{2-}-exchanged montmorillonite (CEM) was prepared by an exchange reaction. The aim is to investigate the effect of CEM on growth performance, intestinal microflora, bacterial enzyme activities and small intestinal morphology in broiler.

MATERIALS AND METHODS

Montmorillonite

The montmorillonite sample used in this study was collected from Mongolia, China. Its structural formula determined from chemical analysis is $[Na_{0.07}\ K_{0.06}\ Ca_{0.19}]\ [Fe^{2-}_{0.01}\ Fe^{3+}_{0.15}\ Mg_{0.20}\ Al_{1.65}]\ [Si_{3.76}\ Al_{0.24}]\ O_{10}\ (OH)_2 \cdot n\ H_2O$. The cation exchange capacity of the montmorillonite was 105 mmol/100 g, determined by leaching with 1 M ammonium acetate at pH 7, washing with 90% ethanol, displacing the NH_4^{-} with 1 M NaCl and measuring the amount displaced with an autoanalyzer (Theng et al., 1997). The specific surface area was 90.4 m^2/g, measured on NOVA vs. 3.70 n by N_2 adsorption at 77 K and application of BET equation (Stadler and Schindler, 1993).

Preparation of CEM

Montmorillonite was ground and washed in deionized water at a ratio of 10 g clay: 100 ml water for 24 h under agitation. The resulting clay suspension was centrifuged and the wash water discarded. Clay was rehydrated with 100 ml water to which Cu^{2+} ($CuSO_4 \cdot 5H_2O$, analytical grade) was

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Table 1. The composition of basal diet

Ingredient (%)	1 to 21 d	22 to 42 d	Composition by analysis (%)	1 to 21 d	22 to 42 d
Yellow corn	56.17	60.15	ME[3] (MJ/kg)	12.96	13.01
Soybean meal (44.1%, CP)	32.00	30.00	CP (N×6.25)	21.93	20.18
Fishmeal	5.00	3.00	Calcium	1.05	0.92
Rapeseed oil	3.00	3.00	Total phosphorus	0.71	0.66
Dicalcium phosphate	1.40	1.40	Lysine	1.12	0.96
Limestone	0.90	1.00	Methionine	0.47	0.38
Salt (NaCl)	0.28	0.31	Methionine+cystine	0.90	0.71
DL-methionine	0.12	0.05	Copper (mg/kg)	22.96	21.58
Choline chloride (50%)	0.13	0.09			
Mineral premix[1]	0.50	0.50			
Vitamin premix[2]	0.50	0.50			

[1] The mineral premix supplied the followings per kilogram of complete feed: copper ($CuSO_4$) 6 mg; zinc ($ZnSO_4$) 80 mg; iron ($FeSO_4$) 80 mg; manganese ($MnSO_4$) 100 mg; iodine (KI) 0.35 mg; selenium (Na_2SeO_3) 0.25 mg; cobalt ($CoCl_2$) 0.4 mg.

[2] The vitamin premix supplied the followings per kilogram of complete feed: vitamin A (retinyl acetate) 8,800 IU; cholecalciferol 3,000 IU; vitamin E (dl-α-tocopheryl acetate) 30 IU; menadione 1.6 mg; thiamin 1.1 mg; riboflavin 6.6 mg; pyridoxine 4.4 mg; vitamin B_{12} 0.02 mg; pantothenic acid 11 mg; niacin 66 mg; folic acid 1 mg; biotin 0.2 mg. [3] Calculated composition.

added at an amount of ≈1.5 times the cation exchange capacity of the clay. The resulting mixture was then agitated for 24 h. The CEM was then separated by centrifugation and washed under agitation with 100ml deionized water. The washed material was dried at 60°C, and then ground. The supernatant was diluted properly and then copper concentration was measured using an atomic absorption spectrophotometer (AA-6501, Japan). The amount of copper in CEM was 60.94 mmol/100 g according to calculating as the difference between the copper concentration in a control sample prepared without montmorillonite and the sample supernatant concentration.

Animals and diets

All procedures were approved by the University of Zhejiang Institutional Animal Care and Use Committee. Two hundred forty 1-d-old Arbor Acres broiler chicks were randomly divided into three groups, each with 80 chicks. The chicks were raised in wire cages (length×width×height, 100×80×50 cm) in a house. Each cage was provided with a plastic feeder and a water trough. Ten chicks were kept in each cage and eight cages were used for each treatment. Chicks in Group 1 were fed basal diet only (control). Group 2 was fed basal diet +1 g kg^{-1} montmorillonite, and Group 3 was fed basal diet +1 g kg^{-1} CEM. Compositions of the basal diet and nutrient levels for starter (1 to 21 d) and grower (22 to 42 d) are presented in Table 1. The diets were provided *ad libitum* in mash form throughout the experimental period. Heating lamps of 100 W were used to supply brooding heat for 10 d. Chickens were weighed on a cage basis at 1, 21 and 42 d of age to determine weight gain. Feed consumed on cage basis was recorded daily, the uneaten discarded and dead broiler were weighed to calculate feed efficiencies. All birds' management was in accordance with the guidelines of raising Arbor Acres broiler (Wang, 2000).

Sampling procedure

Intestinal samples (digesta) were collected at 21 and 42 d of age. Twelve chickens were randomly selected from each treatment and fasted overnight (20:00 h to 08:00 h) before sampling. The chickens were euthanatized by severing the jugular vein. The carcasses were immediately opened and the entire intestine removed aseptically.

Eight chickens were used to sample the intestinal contents. To ease the sampling process, the mesentery was cut to uncoil the tract. The contents taken from the small intestine (from the distal end of the duodenum to the ileo-caecal junction) and cecum were massaged in tract from both ends. Before cutting intestine, the small intestinal and cecal sections containing digesta were ligated from both sides with nylon threads. The cutted small intestinal and cecal sections were used immediately to count bacteria. The residual small intestinal section containing digesta was cut open, and the contents removed with a spatula, with care taken not to damage the mucosa. The digesta samples were frozen immediately in liquid N_2 and stored at -70°C until used.

Four chickens were used to sample intestinal tissue for light microscopic observations. The specimens from the middle part of duodenum, jejunum and ileum segment were excised, and rinsed in physiological saline, respectively. Samples were preserved in 10% formalin.

Bacterial enumeration

Approximately 1 g of the contents from small intestine or cecum were blended under CO_2 in 9 ml of anaerobic dilution (ADS, Bryant and Allison, 1961), respectively, and then homogenized for 3 min. From the initial 10^{-1} dilution, 10-fold serial dilutions were subsequently made in ADS for anaerobic bacterial enumeration (Bryant, 1972). The initial dilution in ADS was also used as a source for serial dilutions in PBS for enumeration of aerobic bacterial

Table 2. Body weight and feed efficiency of broilers fed basal diet only or with either montmorillonite or CEM[1]

Treatment	Body weight (g)			Feed to gain ratio	
	1 d	21 d	42 d	1 to 21 d	1 to 42 d
Control	46.3	645.7 [a]	1808.8 [a]	1.58 [a]	1.97[a]
Montmorillonite	46.4	661.6 [ab]	1846.9 [ab]	1.55 [ab]	1.92[a]
CEM	46.2	681.4 [b]	1881.9 [b]	1.50 [b]	1.85[b]
SEM2	0.36	6.9	16.8	0.026	0.023

[1] Values are presented as means; n=8 per treatment. Means in a column with no common superscript differ significantly ($p<0.05$). [2] Standard error of the mean.

populations. Triplicate plates were then inoculated with 0.1 ml samples and incubated at 37°C aerobically or anaerobically as appropriate. Three dilutions were plated for each medium. The plate media used were Wilkins Chalgren agar (Oxoid, England) for total anaerobes. Brain Heart Infusion agar (Oxoid, England) for total aerobes. MRS agar (Difco; USA) for *Lactobacilli*, Reinforced Clostridial Agar plus supplements for *Bifidobacteria* (Munoa and Pares, 1988). MacConkay's No.2 (Oxoid, England) for *Escherichia coli*, and Tryptose Sulphite Cycloserine agar for *Clostridium perfringens* (Nordic Committee on Food Analysis, 1997). Total numbers of bacterial colonies were counted at the end of each incubation period. Single colonies were removed from selective media plates and grown in peptone yeast glucose broth. Subsequently, the bacteria were characterized to genus level on the basis of colonial appearance, Gram reaction, spore production, cell morphology and fermentation end-product formation (Holdeman et al., 1977).

Bacterial enzyme assay

β-Glucosidase (EC 3.2.1.21) and β-glucuronidase (EC 3.2.1.31) activities were analyzed under anaerobic conditions by using the method of Jin et al. (2000). β-Glucosidase activity unit was expressed as micromole of p-nitrophenol released from p-nitrophenyl-β-D-glucopyranoside per hour per gram of intestinal digesta protein. The amount of nitrophenol released was determined by comparison with a standard nitrophenol curve. One β-glucuronidase activity unit will liberate a micromole of phenolphthalein from phenolphthalein glucuronic acid per hour per gram of intestinal digesta protein. The amount of phenolphthalein released was determined by comparison with a standard phenolphthalein curve. All the chemicals were acquired in a kit (No.325 (Sigma Chemical Co., St. Louis, MO.)).

Protein determination

The intestinal digesta protein concentrations were determined by the method of Lowry et al. (1951). Bovine serum albumin was used as a standard. All chemicals were purchased in a kit (No. 690).

Light microscopy

The fixed samples were embedded in paraffin. Transverse sections were cut into 6 μm samples, every 10th section was collected, and stained with hematoxylin-eosin. Villus height and crypt depth were measured using image processing and analysis system (Version 1, Leica Systems Ltd., Cambridge, England). 10 villi and 10 crypts were selected per section. A total of eight sections were counted per bird. An average of these eight sections per bird was expressed as a mean villus height or crypt depth for each bird, respectively. Finally, these four mean villus heights or crypt depths from four birds were expressed as a mean villus height or crypt depth for one treatment group.

Statistical analysis

One-way analysis of variance was performed using the General Linear Models Procedures of the SAS software (1989). Differences among means were tested using Duncan's multiple range tests. A significant level of 0.05 was used.

RESULTS

Growth performance

The body weight, cumulative feed to gain ratio of broilers from 1 to 42 d of age fed diets without or with either montmorillonite or CEM are summarized in Table 2. The dietary addition of CEM significantly ($p<0.05$) increased body weight and feed efficiency of broilers after 21 and 42 days of feeding. However, similarly significant increases in body weight and feed efficiency were not found in broilers fed the diet treated with montmorillonite, but broilers receiving montmorillonite had slightly greater body weight and feed efficiency than the control. On the other hand, birds fed the CEM-supplemented diet had higher ($p<0.05$) feed efficiency than those fed on montmorillonite after 42 days of feeding, but not after 21 days of age. There was no significant difference between the body weight of broilers fed the diet with CEM or montmorillonite.

Intestinal microflora

The result on the intestinal microbial populations is presented in Table 3 and Table 4. The counts of *C. perfringens* in the small intestine of broilers fed on diet with CEM were decreased significantly ($p<0.05$) as compared

Table 3. Counts of microflora in the small intestinal and cecal contents of broilers fed basal diet only or with either montmorillonite or CEM at 21 d of age[1,2]

Organ	Total aerobes	Total anaerobes	*Lactobacilli*	*Bifidobacteria*	*C. perfringens*	*E. coli*
Small intestine						
Control	7.15	8.23	7.83	8.52	5.06[a]	7.07[a]
Montmorillonite	7.07	8.25	8.22	8.43	4.89[a]	6.55[ab]
CEM	6.64	8.02	8.00	8.42	4.08[b]	6.22[b]
SEM[3]	0.19	0.31	0.26	0.30	0.21	0.28
Cecum						
Control	8.21	9.78	8.87	9.77	5.59	7.93[a]
Montmorillonite	8.03	9.92	8.75	9.84	5.63	7.79[ab]
CEM	7.81	9.67	9.08	9.62	4.95	7.15[b]
SEM	0.22	0.22	0.23	0.31	0.24	0.25

[1] Bacterial numbers are expressed as $\log_{10}$ colony-forming units per gram of DM.
[2] Values are presented as means; n=8 per treatment. Means in a column with no common superscript differ significantly ($p<0.05$).
[3] Standard error of the mean.

Table 4. Counts of microflora in the small intestinal and cecal contents of broilers fed basal diet only or with either montmorillonite or CEM at 42 d of age[1,2]

Organ	Total aerobes	Total anaerobes	*Lactobacilli*	*Bifidobacteria*	*C. perfringens*	*E. coli*
Small intestine						
Control	7.16	8.04	8.01	8.34	4.85[a]	6.87
Montmorillonite	7.00	8.15	8.01	8.60	4.56[ab]	6.94
CEM	6.97	7.83	8.29	8.24	4.03[b]	6.43
SEM[3]	0.20	0.21	0.27	0.27	0.22	0.21
Cecum						
Control	8.03	9.57	8.52	9.54	5.47	7.87
Montmorillonite	8.03	9.78	9.06	9.68	5.42	7.72
CEM	7.79	9.62	8.87	9.51	5.02	7.52
SEM	0.20	0.24	0.28	0.26	0.23	0.32

[1] Bacterial numbers are expressed as $\log_{10}$ colony-forming units per gram of DM.
[2] Values are presented as means; n=8 per treatment. Means in a column with no common superscript differ significantly ($p<0.05$).
[3] Standard error of the mean.

Table 5. β-Glucosidase and β-glucuronidase activities in the intestinal and cecal contents of broilers fed basal diet only or with either montmorillonite or CEM[1]

Organ	β-glucosidase activity (μmol/h per g protein)		β-glucuronidase activity (μmol/h per g protein)	
	21 d	42 d	21 d	42 d
Small intestine				
Control,	39.30[a]	50.25[a]	61.29[a]	84.78[a]
Montmorillonite	33.75[ab]	33.45[b]	51.19[ab]	53.71[b]
CEM	30.72[b]	25.06[b]	46.75[b]	49.35[b]
SEM[2]	2.6	3.0	3.5	5.6
Cecum				
Control	28.05[a]	35.95	93.96	105.34
Montmorillonite	26.64[ab]	34.42	84.87	98.10
CEM	22.56[b]	32.24	86.95	94.35
SEM	1.8	2.0	3.7	4.6

[1] Values are presented as means; n=8 per treatment. Means in a column with no common superscript differ significantly ($p<0.05$).
[2] Standard error of the mean.

with the control, but a similarly significant reduction in the cecum was not observed. However, there was a tendency for the *C. perfringens* population to be slightly lower in the cecum of birds fed the diet treated with CEM than the control. The addition of montmorillonite to the diet had no significant influence on the numbers of *C. perfringens* in the small intestine and cecum.

The counts of *E. coli* were reduced significantly ($p<0.05$) in the small intestine and cecum of broilers fed the diet including CEM at 21 d of age, but not at 42 d of age. The treatment with montmorillonite of feed did not produce a significant difference in *E. coli* populations in the small intestine and cecum of broilers at either 21 or 42 d of age as compared with the control or the treatment with CEM.

There were no significant differences in the total aerobes, total anaerobes, *lactobacilli* and *bifidobacteria* in the small intestine and cecum of broilers fed basal diet only or with either montmorillonite or CEM after 21 and 42 days of feeding.

Bacterial enzymes

The effects of montmorillonite or CEM on the activities of β-glucosidase and β-glucuronidase in the small intestinal and cecal contents are shown in Table 5. The activities of β-glucosidase and β-Glucuronidase in the small intestine of birds fed on diet supplemented with CEM were significantly ($p<0.05$) lower than the control. Similarly, a significant ($p<0.05$) decrease in the β-glucosidase activity

Table 6. Morphology of the small intestinal mucosa of broilers fed basal diet only or with either montmorillonite or CEM[1]

Organ	Villus height (μm)		Crypt depth (μm)	
	21 d	42 d	21 d	42 d
Duodenum				
Control	1,072.2^{a}	1,102.2^{a}	211.8^{a}	204.0^{a}
Montmorillonite	1,148.7^{b}	1,190.4^{b}	189.9^{b}	192.9^{ab}
CEM	1,160.2^{b}	1,201.0^{b}	191.3^{b}	188.6^{b}
SEM[2]	23.8	27.6	5.9	4.7
Jejunum				
Control	913.6^{a}	923.3^{a}	165.1^{a}	160.5
Montmorillonite	984.5^{b}	977.9^{ab}	150.3^{b}	150.2
CEM	996.4^{b}	1,000.8^{b}	146.2^{b}	151.4
SEM	22.2	22.1	4.3	4.1
Ileum				
Control	561.2^{a}	600.2	127.1	121.9
Montmorillonite	587.9^{ab}	620.3	130.4	115.6
CEM	616.6^{b}	615.2	117.4	116.9
SEM	16.7	12.7	4.9	5.4

[1] Values are presented as means; n=4 per treatment. Means in a column with no common superscript differ significantly ($p<0.05$).
[2] Standard error of the mean.

was also observed in the cecum at 21 d of age, but not at 42 d of age. The cecal β-glucuronidase activity was depressed slightly as compared to the control.

The addition of montmorillonite to the diet depressed significantly ($p<0.05$) the activities of β-glucosidase and β-glucuronidase in the small intestine at 42 d of age. However, there were no significant differences between the control and the montmorillonite group in the activities of β-glucosidase and β-glucuronidase in the small intestine at 21 d of age and in the cecum at 21 or 42 d of age, but broilers treated with montmorillonite had slightly lower bacterial enzyme activities.

Morphological measurement of the small intestinal mucosa

Small intestinal villus height and crypt depth in each intestinal segment of chickens are presented in Table 6. Compared with the control, villus heights in all small intestinal parts of chickens fed the CEM-supplemented diet showed to be longer ($p<0.05$) after 21 days of feeding, but only duodenal and jejunal villi were longer ($p<0.05$) in chickens fed on the diet containing montmorillonite. At 42 d of age, villus heights in duodenum and jejunum of chickens raised on CEM-supplemented diet were longer ($p<0.05$) than the control. Similarly, longer ($p<0.05$) duodenal villi were also observed in chickens fed the diet treated with montmorillonite.

Control chickens had deeper ($p<0.05$) crypt depths in duodenum and jejunum than those treated with either montmorillonite or CEM after 21 days of feeding. At 42 d of age, chickens receiving CEM had shallower ($p<0.05$) duodenal crypts as compared to the control.

DISCUSSION

Effect of dietary montmorillonite or CEM on growth performance of broiler

Clays are widely applied in many fields of technology and science. Such a wide usefulness of clays is a result of their high specific surface area, high chemical and mechanical stability, and a variety of surface and structural properties. Traditionally, clays have been incorporated in animal diets as a technological additive to improve feed manufacture (Angulo et al., 1995). But the results of previous experiments on the effects of clays on animal performance were generally inconsistent (Poulsen and Oksbjerg, 1995; Ouhida et al., 2000). On the present results, the addition of CEM to the diet significantly increased the body weight and feed efficiency of chickens after either 21 or 42 days of feeding. But a similarly significant improvement was not found in broilers fed the diet adding montmorillonite. These results indicate that montmorillonite, which managed by ion exchange with Cu^{2-}, has an ability to increase the growth performance of broiler.

Effect of dietary montmorillonite or CEM on intestinal microflora of broiler

There are many *Clostridia* in intestinal tract of broiler. But some are harmful to animal health. The previous investigations showed that *C. perfringens*, one of pathogenic *Clostridia*, was suspected to be responsible for chicken growth depression (Fuller et al., 1979; Stutz and Lawton, 1984). Elam et al. (1953) demonstrated that antibiotic growth promoters were mainly related to an inhibiting effect on *C. perfringens*. In the present study, CEM supplementation significantly decreased the number of *C. perfringens* in the small intestine of chicken. In the cecum, although there was no significant difference between the *C. perfringens* populations in broilers fed diets with or without CEM, broiler treated with CEM had slightly lower count of *C. perfringens*. However, dietary addition of montmorillonite had no significant influence on small intestinal and cecal *C. perfringens* populations. The present results suggest that the growth-promoting effect of CEM is probably involved in its action against *C. perfringens*.

The experimental birds receiving CEM showed lower numbers of *E. coli* in the small intestine and cecum than the control birds, but montmorillonite had no effective action against *E. coli*. These results were in agreement with the findings *in vitro* of Hu et al. (2002). Cik et al. (2001) reported that zeolite, one of aluminosilicate clays, which accomplished by the ion-exchange reaction of Na^{+} for Cu^{2+} ions, effectively inhibited the growth of *E. coli*.

Lactobacillus and *Bifidobacterium* are known to benefit the animal health, which are probably attributed to their strong ability to attach to the animal intestine, their

antagonism towards pathogenic bacteria and their ability to competitively exclude some pathogenic bacteria. The results in the present study demonstrated that broilers given feeds containing montmorillonite or CEM had no significant effects on the numbers of *Lactobacilli* and *Bifidobacteria*.

In agreement with the results of Salanitro et al. (1978) and Jin et al. (1997), the present study showed that microbial numbers, irrespective of montmorillonite or CEM additions, were higher in the cecum than in the small intestine.

Effect of dietary montmorillonite or CEM on the bacterial enzyme activities in the intestine of broiler

The results in the present study demonstrated that dietary addition of CEM depressed the activities of β-glycosidase and β-glucuronidase in small intestine and cecum. β-Glycosidase is involved in the carcinogenicity of the naturally occurring substance. It has been postulated that amygdaline is hydrolyzed in the gut by bacterial β-glucosidase to yield mandelonitrile, which is unstable and is readily hydrolyzed to release toxic cyanide (Goldin and Gorbach, 1976). β-Glucuronidase is believed to be largely responsible for the hydrolysis of glucuronides in the lumen of the gut. This reaction is potentially important in the generation of toxic and carcinogenic substances as many compounds are detoxified by glucuronide formation in the liver and subsequently enter the bowel via the bile. In this manner, toxic aglycones can be regenerated in the bowel by bacterial β-glucuronidase (Nalini et al., 1998; Jin et al., 2000). Although these potential harmful metabolites may not cause disease to chickens, they may hinder the growth performance or reduce feed efficiency.

The reduction of β-glucosidase and β-glucuronidase activities in chickens fed CEM may be attributed to the lower numbers of *C. perfringens* and *E. coli* in the intestine. Hawksworth et al. (1971) reported that *E. coli* produced 15.4 times more β–glucuronidase per strain than any other genera tested. Furthermore, over 90% of *E. coli* strains are able to produce β-glucuronidase, whereas only less than 40% of *Lactobacillus* strains show an ability to produce glucosidase (Drasar and Hill, 1974).

The addition of montmorillonite to the diet of broiler also reduced bacterial β-glucosidase and β-glucuronidase activities in the intestine, especially in the small intestine after 42 days of feeding, but the decreased degree was less than the treatment with CEM, except cecal β-glucuronidase activity after 21 days of feeding. However, the present results of bacterial enumeration showed that montmorillonite had no effective inhibition on the growth of intestinal microflora, indicating that the observed lower activities of intestinal bacterial enzymes associated with the dietary addition of montmorillonite might not be result from the decrease of certain bacterial populations. The active mode of montmorillonite against bacterial enzyme activity has been unclear.

The present results showed that CEM had different influence on the activities of bacterial enzymes in different intestinal part. On the other hand, compared with the small intestine, β-glucuronidase activity, irrespective of montmorillonite or CEM additions, was higher in the cecum, while β-glucosidase activity was lower except those cecum of broilers fed the diet containing CEM or montmorillonite after 42 days of feeding.

Effect of dietary montmorillonite or CEM on the change in small intestinal morphology

The structure of the intestinal mucosa can reveal some information on gut health. A shortening of the villus decreases the surface area for nutrient absorption. The crypt can be regarded as the villus factory, and a large crypt indicates fast tissue turnover and a high demand for new tissue. Such a change in intestinal morphology can lead to poor nutrient absorption, increased secretion in the gastrointestinal tract, diarrhoea, reduced disease resistance and lower overall performance (Xu et al., 2002).

In the present study, the increase in villus height and the decrease in crypt depth in the small intestinal mucosa of broilers fed on diet including CEM were observed. This result indicates that the dietary addition CEM may improve the small intestinal mucosal morphology, but the beneficial effects on different intestinal parts are different. Such changes in villus height and crypt depth in the presence of CEM may explain the lower numbers of *C. perfringens* and *E. coli* and the lower activities of bacterial enzymes in the small intestine.

However, the addition of montmorillonite to the diet also produced a positive effect on small intestinal mucosa. This improvement may, on the one hand, be due to the lower activities of bacterial enzymes in the small intestine. On the other hand, it is reported that montmorillonite, a mucus stabilizer, effectively acts by attaching to the mucus to preserve the mucosa from the toxic effects of drugs and toxins (Albengres et al., 1985).

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