Effects of Copper-bearing Montmorillonite on Growth Performance and Digestive Function of Growing Pigs*

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ABSTRACT : A total of 96 growing barrows (Duroc×Landrace×Yorkshire) at an average BW of 20.2 kg were used to investigate the effects of montmorillonite (MMT) or copper-bearing montmorillonite (Cu-MMT) on growth performance, intestinal microflora, digestive enzyme activities of pancreas and small intestinal contents, and the apparent nutrient digestion. The pigs were allocated to three groups with 32 pigs per treatment for 42 days and the average BW at the end of the experiment was 49.7 kg. The three dietary treatments were basal diet only (control group), basal diet +1.5 g/kg MMT, and basal diet +1.5 g/kg Cu-MMT. The results showed that supplementation with Cu-MMT significantly improved growth performance as compared to control and pigs fed with Cu-MMT had higher average daily gain than those fed with MMT. As compared to control, supplementation with Cu-MMT significantly reduced the total viable counts of *Escherichia coli* and *Clostridium* in the small intestine and proximal colon. Supplementation with MMT had no significant influence on intestinal microflora, although there was a tendency for *Escherichia coli* and *Clostridium* to be lower than the control. Pigs fed with Cu-MMT had lower viable counts of *Escherichia coli* in colonic contents than those fed with MMT. Although supplementation with MMT improved the activities of the digestive enzymes in the small intestinal contents, the tendency was not significant. Supplementation with Cu-MMT significantly improved the activities of total protease, amylase and lipase in the small intestinal contents. Supplementation with MMT or Cu-MMT improved the apparent nutrient digestion. (*Asian-Aust. J. Anim. Sci. 2004. Vol 17, No. 11 : 1575-1581*)

Key Words : Copper-bearing Montmorillonite, Growth Performance, Intestinal Microflora, Digestion, Pig

INTRODUCTION

Traditionally, clays have been incorporated in animal diets (10-20 g/kg) as a technological additive (lubricant or agglomerant) to improve feed manufacture (Angulo et al., 1995). However, recently a role as enhancer of the nutritive value of diets in ruminants and monogastric animals has been also proposed. It has been reported that the addition of clay to the feedstuffs improved the nutrient digestibility and the enzymatic activity of gastrointestinal secretions (Cabezas et al., 1991; Paolo et al., 1999; Ouhida, et al., 2000; Alzueta et al., 2002).

Montmorillonite (MMT), one of aluminosilicate clay, has a 2:1 layer structure. 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+}. Owing to these structural characteristics, MMT has specific physical-chemical properties such as high surface area, strong absorptive power, high structural stability, chemical inertia

and strong capacity to form stable suspensions at low concentrations (Borchardt, 1989). Animal feed containing MMT has been shown to promote weight gain and feed efficiency, to reduce bacterial contamination of the gut and to reduce the detrimental effects of mycotoxin-contaminated diets (Schell et al., 1993; Venglovsky et al., 1999; Tauqir et al., 2001).

An *in vitro* study of Hu et al. (2002) showed that MMT could adsorb *Escherichia coli* and *Staphylococcus aureus*, but showed no bacteriostatic or bactericidal effect. Nonmetallic minerals have been used as antimicrobial carriers for years (Hu et al., 2000; Wang et al., 2000). Ag^- carried on zeolite, montmorillonite and other clays has been reported as effective antibacterial materials (Rivera-Garza et al., 2000; Onodera et al., 2001). Recent experiments have revealed that Cu bearing montmorillonite (Cu-MMT), which is produced through Cu^{2-} exchange reaction, has antibacterial activity on *E. coli* K_{88}, *Clostridium* and *Salmonella* (Ye et al., 2003). Xia et al. (2003, unpublished data) studied the adsorption of *E. coli* K_{88} by MMT and Cu-MMT and found that the modified clays reduced bacterial numbers above 97% while the parent clays only produced reductions of about 20%. Up to now, there is no data on the effects of feeding diets containing Cu-MMT on pigs.

Therefore, an experiment was carried out to investigate the effects of dietary MMT or Cu-MMT on growth performance, intestinal microflora, digestive enzyme activities of pancreas and small intestinal contents, and the apparent nutrient digestion of growing pigs.

Table 1. Formula and chemical composition of the basal diet

Ingredients (%)	
Corn	61.5
Soybean meal	25.5
Wheat bran	7.5
Animal fat	2.5
Limestone	1.0
Dicalcium phosphate	1.2
Sodium chloride	0.3
L-lysine-HCl	0.2
Vitamin-mineral premix[1]	0.3
Analyzed chemical composition (% as feed)	
DE (MJ/kg) [2]	14.1
Crude protein	18.0
Ether extraction	5.3
Crude fiber	2.8
Lysine	1.0
Met.+Cys.	0.5
Calcium	0.8
Phosphorus	0.6
Copper (mg/kg)	9.2

[1] The vitamin/mineral premix provided (per kg feed): 2,000 IU vitamin A, 200 IU vitamin D_3, 20 mg vitamin E, 1 mg vitamin K, 1 mg thiamine, 3 mg riboflavin, 10 mg d-pantothenic acid, 0.5 mg folic acid, 1 mg pyridoxine, 20 mg niacin, 10 ug cobalamin, 500 mg choline chloride, 0.1 mg biotin, 0.2 mg Se, 0.2 mg I, 80 mg Fe, 5 mg Cu, 2 mg Mn and 80 mg Zn.

[2] DE was based on calculated values.

MATERIALS AND METHODS

Materials

MMT ore used in this work was a hydrothermal product of volcano sedimentary rocks from Chifeng, the Inner Mongolia Autonomous Region, China. Besides MMT, there were minor amounts of quartz and volcanic glass in the ore. To get rid of the impurities, the raw material was dried in an oven over night at 80°C and then milled to less than 300 mesh. The milled material was dispersed in water to form a 10% suspension that was churned up in a stirrer for about 10 min. Particles larger than 2 m were separated out by sedimentation while the suspension was centrifuged to get refined MMT. The refined MMT was dried at 80°C followed by another milling to less than 300 mesh for use. The formula of the purified MMT determined from chemical analysis is $[Na_{0.158}K_{0.082}Ca_{0.256}Mg_{0.063}]$ $[Mg_{0.376}Fe^{2-}_{0.014}Fe^{3+}_{0.136}Al_{1.474}][Si_{3.87} Al_{0.13}]O_{10}(OH)_2 \cdot nH_2O$. The cation exchange capacity (CEC) was 139.9 mmol/100 g, determined by leaching with 1 mol/L ammonium acetate at pH 7, washing with 90% ethanol, displacing the NH_4^+ with 1 mol/L NaCl and measuring the amount displaced with an autoanalyzer (Theng et al., 1997).

Cu bearing montmorillonite (Cu-MMT) was prepared by Cu^{2-} cation exchange reaction as follows. 5 g of the refined MMT was mixed with 100 ml of 0.1 mol/L $CuSO_4$ solution to form suspension by churning. The pH value of the suspension was adjusted to 5.0. The suspension was

placed at 60°C for about 6 h to accelerate the cation exchange. The product was centrifuged at a speed of 8,000 r/m for about 15 min. The clear liquid was pored out and was replaced by another 100 ml of solution. The product was washed with distilled water and centrifuged for 3 times. The product was dried at 80°C over night, and ground to a size less than 300 mesh. Cu content in the product is found to be 24.5 g/kg on the basis of atomic absorption spectrometry.

Animals and experimental diets

All procedures were approved by the University of Zhejing Institutional Animal Care and Use Committee. A total of 96 growing barrows (Duroc×Landrace×Yorkshire) at an average BW of 20.2 kg were allocated to 3 treatments for 42 days and the average BW at the end of the experiment was 49.7 kg, each of which was replicated four times with eight pigs per replicate. The pigs received the same basal diet and MMT or Cu-MMT was added to the basal diet at 1.5 g/kg respectively. Diets were formulated to meet or exceed nutrient requirements suggested by the NRC (1998) for 20 to 50 kg pigs. Antibiotic was excluded from all diets (Table 1). All pigs were given *ad libitum* access to feed and water. Average daily gain (ADG), average daily feed intake (ADFI), and feed/gain (F/G) were determined.

During the later period of feeding trials (on the 6th week of experiment), grab sampling as a technique for measuring apparent nutrient digestibility in group-housed animals was used (Kavanagh et al., 2001). Feeds including 0.25% chromic oxide as an indigestible marker were fed. A grab sample of feces was taken from eight pigs in each pen and pooled by the pen on the 5 th day after feeding the marked diets. Feces were dried in an air forced drying oven at 60°C for 72 h for chemical analysis. At the end of the feeding trial, eight pigs from each treatment (two pigs per pen) were slaughtered under general anaesthesia. The pigs were then immediately eviscerated for collection of GIT (gastrointestinal tract) digesta.

Chemical analyses

Feeds and feces were analyzed for organic matter (OM), crude protein (CP), ether extract (EE) according to the methods of AOAC (1990). Chromium was determined according to the procedure of Fenton and Fenton (1979).

Intestinal microbial populations

Samples of the contents from the small intestine (from the distal end of the duodenum to the ileo-caecal junction) and proximal colon were immediately collected into Qorpak glass containers under CO_2, sealed, and put on ice until they were transported to the lab for enumeration of microbial populations. Ten grams of mixed contents were blended under CO_2 in 90 mL of anaerobic dilution (ADS, Bryant

Table 2. Growth performance as affected by MMT or Cu-MMT in growing pigs[1]

	Control	MMT[2]	Cu-MMT	SEM[3]
ADG (g)	672 [b]	696 [b]	742 [a]	14
ADFI (g)	1,754	1,768	1,774	22
F/G	2.61 [a]	2.54 [ab]	2.39 [b]	0.05

[1] Values are presented as means: n=4 for ADG, ADFI and F/G per treatment. Means in a row with different letters differ significantly.
[2] MMT: montmorillonite, Cu-MMT: copper bearing montmorillonite, ADG: average daily gain, ADFI: Average daily feed intake. F/G: feed per gain.
[3] Standard error of the mean.

and Allison, 1961). Further serial dilutions were 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 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. Bacteria were enumerated on Wilkins Chalgren Agar (Oxoid; total anaerobes), MRS Agar (Oxoid; *Lactobacillus*). Reinforced Clostridial Agar plus supplements (Munoa and Pares, 1988; *Bifidobacterium*), Sulphite-Polymyxin Milk Agar (Mevissen-Cerhage et al., 1987; *Clostridium*), and MacConkey's No.2 (Oxoid; *Escherichia coli*). Single colonies were removed from selective media plates and grown in peptone yeast glucose (PYG) broth (Holdeman et al., 1977). 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).

Digestive enzyme activities in pancreatic tissue and small intestinal contents

Sampling procedure : The following procedures were according to the method described by Xu et al. (2002, 2003). The contents taken from the small intestine were digesta from the distal end of the duodenum to the ileocecal junction. A homogenous intestinal digesta sample was collected by massaging the tract from both ends. The digesta sample were stored immediately at -70°C until used. The small intestinal digesta samples were diluted 10×, based on the sample weight, with ice-cold PBS (pH 7.0), homogenized for 60 s, and sonicated for 1 min with three cycles at 30 s intervals. The sample was then centrifuged at 18,000×g for 20 min at 4°C. The supernatants were divided into small portions and stored at -70°C for enzyme assays.

The pancreas from slaughtered pigs was homogenized in ice-cold 0.2 M Tris-HCl buffer, pH 8.0 containing 0.05 M NaCl in the ratio 1:4 (w/v). The homogenate was centrifuged at 3,000×g for 15 min at 4°C and the supernatant was stored for enzyme assays.

Digestive enzyme assay : Assays for digestive enzymes

were carried out as described by Xu et al. (2003). Amylase (a-1, 4-glucan 4-glucanohydrolase. EC 3.2.1.1) activity was determined using the method of Somogyi (1960). Amylase activity unit (1 Somogyi Unit) was defined as the amount of amylase that will cause formation of reducing power equivalent to 1 mg of glucose in 30 min at 37°C per mg of intestinal digesta protein or pancreas. The substrate used in the analysis was cornstarch. All chemicals were purchased in a kit (No. 700) from Sigma Chemical Company (Sigma Chemical Co., St. Louis. MO 63178-9916). The analytical procedure was in accordance with Sigma instructions.

Lipase (triacylglycerol lipase. EC 3.1.1.3.) activity was assayed using the method described by Tietz and Fiereck (1966). Lipase activity unit (Sigma-Tietz Units) was equal to the volume (mL) of 0.05 M NaOH required to neutralize the fatty acid liberated during 6 h incubation with 3 mL of lipase substrate at 37°C per mg of intestinal digesta protein or pancreas. Olive oil was used as the substrate in this assay. All chemicals were purchased in a kit (No. 800) (Sigma Chemical Co., St. Louis, MO 63178-9916).

Protease activity was analyzed using the modified method of Lynn and Clevette-Radford (1984). The protease activity unit was defined as milligrams of azocasein degraded during 2 h incubation at 37°C per mg of intestinal digesta protein or pancreas. Azocasein was used as the substrate.

The intestinal digesta protein concentrations were determined by the method of Lowry et al. (1951). Ovine serum albumin was used as a standard. All chemicals were purchased in a kit (No. 690) (Sigma Chemical Co., St. Louis. MO 63178-9916).

Statistical analysis

One-way analysis of variance was performed using the general linear model procedure of 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

Growth performance of pigs is presented in Table 2. As compared to control, supplementation with 1.5 g/kg Cu-MMT significantly improved ADG and feed efficiency. While pigs fed with MMT had slightly greater ADG and feed efficiency than the control, the tendency was not significant. Pigs fed with Cu-MMT had higher ADG than those fed with MMT.

Intestinal microflora

As compared to control, supplementation with Cu-MMT significantly reduced the total viable counts of *Escherichia coli* and *Clostridium* in the small intestine and

Table 3. Effect of dietary MMT or Cu-MMT on intestinal microflora of growing pigs[1,2]

	Control	MMT[3]	Cu-MMT	SEM[4]
Small intestine				
Total anaerobes	9.71	9.62	9.87	0.29
Bifidobacterium	7.18	7.52	7.46	0.32
Lactobacillus	8.12	8.39	8.47	0.24
Clostridium	6.62[a]	6.24[ab]	5.72[b]	0.26
Escherichia coli	8.16[a]	7.80[ab]	7.15[b]	0.30
Proximal colon				
Total anaerobes	10.95	10.80	10.89	0.32
Bifidobacterium	8.40	8.52	8.58	0.26
Lactobacillus	9.73	9.95	9.84	0.25
Clostridium	7.52[a]	7.14[ab]	6.52[b]	0.30
Escherichia coli	8.43[a]	8.17[a]	7.46[b]	0.20

[1] Bacterial numbers are expressed as $\log_{10}$ cfu/g DM.
[2] Values are presented as means; n=8 per treatment. Means in a row with different letters differ significantly.
[3] MMT: montmorillonite, Cu-MMT: copper bearing montmorillonite.
[4] Standard error of the mean.

proximal colon of growing pigs (Table 3). Supplementation with MMT had no significant influence on intestinal microflora, although there was a tendency for *Escherichia coli* and *Clostridium* to be lower than the control. Pigs fed with Cu-MMT had lower viable counts of *Escherichia coli* in colonic contents than those fed with MMT.

There were no significant differences in the total anaerobes, *Lactobacilli* and *Bifidobacteria* in the small intestinal and colonic contents of pigs fed the basal diet only or with either MMT or Cu-MMT.

Digestive enzymes

The results of the effects of MMT or Cu-MMT on the digestive enzyme activities in the pancreas and the small intestinal contents of pigs are shown in Table 4. Although supplementation with MMT improved the activities of the digestive enzymes in the small intestinal contents, the tendency was not significant. Supplementation with Cu-MMT significantly improved the activities of total protease, amylase and lipase in the small intestinal contents. However,

Table 5. Effects of MMT or Cu-MMT on the apparent digestibilities of nutrients[1]

	Control	MMT[2]	Cu-MMT	SEM[3]
OM (%)	84.21[b]	86.52[a]	88.03[a]	0.66
CP (%)	79.64[b]	81.95[ab]	84.52[a]	1.36
EE (%)	65.86[b]	69.21[ab]	72.14[a]	1.63

[1] Values are presented as means; n=4 per treatment. Means in a row with different letters differ significantly.
[2] MMT: montmorillonite, Cu-MMT: copper bearing montmorillonite.
[3] Standard error of the mean.

the digestive enzymes in pancreas were not affected by dietary MMT or Cu-MMT.

Nutrient digestibility

The results of the effects of MMT or Cu-MMT on the apparent digestibilities of OM, CP and EE are shown in Table 5. Supplementation with MMT significantly improved the apparent digestibility of OM as compared to the control. Supplementation with Cu-MMT significantly improved the apparent digestibilities of OM, CP and EE as compared to the control.

DISCUSSION

Effects of dietary MMT or Cu-MMT on growth performance

Traditionally, clays have been incorporated in animal diets as a nonnutritive additive to improve growth and feed efficiency (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). The feeding value of clays is affected by the kind of clays, the producing area, the content, and the physical or chemical structural characteristics. Wu et al. (2000) studied the relationship between the content and the feeding value of natural clinoptilolite and showed that zeolite, whose content is below 40%, did not have significant feeding value. Animal feed containing MMT (10-30 g/kg) has been shown to promote weight gain and

Table 4. Effects of MMT or Cu-MMT on the digestive enzyme activities in the pancreas and the small intestinal contents of growing pigs[1,2]

Enzyme	Control	MMT[3]	Cu-MMT	SEM[4]
Pancreas				
Protease (unit)	135.26	137.11	128.73	9.61
Amylase (somogyi unit)	65.34	71.01	68.53	3.10
Lipase (sigma-tietz unit)	57.62	60.55	65.38	4.62
Small intestinal contents				
Protease (unit)	82.64[b]	94.82[ab]	103.17[a]	5.33
Amylase (somogyi unit)	15.75[b]	18.30[b]	24.26[a]	1.62
Lipase (sigma-tietz unit)	28.60[b]	33.65[ab]	39.82[a]	2.30

[1] Digestive enzyme activities in the pancreas and the small intestinal contents are expressed as U/mg pancreas and U/mg intestinal digesta protein, respectively.
[2] Values are presented as means; n=8 per treatment. Means in a row with different letters differ significantly.
[3] MMT: montmorillonite, Cu-MMT: copper bearing montmorillonite.
[4] Standard error of the mean.

feed efficiency of chickens and swine (Venglovsky et al., 1999; Tauqir et al., 2001). In the present study, supplementation with MMT had no effect on growth performance as compared with control. The reason of the lack of effects of MMT supplementation on pig growth performance in this study may be that the concentration of MMT (1.5 g/kg) was not adequate.

The present study found that supplementation with Cu-MMT significantly improved average daily gain and feed efficiency as compared to control. Pigs fed with Cu-MMT had higher average daily gain than those fed with MMT. These results indicate that MMT, which managed by ion exchange with Cu^{2+}, has an ability to improve the growth performance of growing pigs.

Effects of dietary MMT or Cu-MMT on intestinal microflora

It is well known that MMT can adhere to bacteria selectively (Girardeau, 1987). Adsorption was the main way between MMT and bacteria. The adsorption effect was related to layer charge density of MMT. In human medicine MMT has been applied as antidiarrheal remedies (Ahmed et al., 1993; Wang et al., 1995). Wu et al. (1999) used MMT as antidiarrheic to treat the diseases of colon bacillus and diarrheic syndrome in piglets and they found that the curative effects of MMT were higher in treating the diarrheic syndrome of early weaning piglets as compared with the antibiotics. Wang et al. (1995) reported that after the diarrheal children was treated with smectite for 5 days, the populations of *Bifidobacteria* increased 158 fold and those of *E. coli* decreased significantly.

Recent experiments have revealed that Cu bearing montmorillonite (Cu-MMT), which is produced through Cu^{2+} exchange reaction, has antibacterial activity on *E. coli* K_{88}. *Clostridium* and *Salmonella* (Ye et al., 2003). Hu et al. (2002) showed *in vitro* that MMT could adsorb *Escherichia coli* and *Staphylococcus aureus* while showed no bacteriostatic or bactericidal effect, but after exchange with Cu^{2+} then it showed these activities.

In the present study, supplementation with MMT had no significant influence on intestinal microflora, although there was a tendency for the *Escherichia coli* and *Clostridium* to be lower than the control. Supplementation with Cu-MMT significantly reduced the total viable counts of *Escherichia coli* and *Clostridium* in the small intestinal and colonic contents of growing pigs. These results indicated that Cu-MMT has higher antibacterial activity on *E. coli* and *Clostridium* than MMT.

Stadler and Schindler (1993) found that Cu^{2-} in aqueous solution with pH>4.5 tends to enter the interlayer position of MMT and forms $[Cu(AlO)_n(H_2O)_{4-n}]^{x+}$. When Na^- or Ca^{2-} was replaced by $[Cu(AlO)_n(H_2O)_{4-n}]^{x-}$, or Cu^{2+} entered the tetrahedron and octahedron, MMT would lose its

electrical balance. This made the mineral have surplus positive charge. On the other hand, bacteria cell wall is negatively charged due to functional groups such as carboxylates present in lipoproteins at the surface (Breen et al., 1995), so that Cu-MMT particles would attract bacteria, due to the opposite static charge. Xia et al. (2003, unpublished data) studied the adsorption of *E. coli* K_{88} by MMT and Cu-MMT and found that the modified clays reduced bacterial numbers above 97% while the parent clays only produced reductions of about 20%. A similar phenomenon had already been reported by Herrera et al. (2000). In their work, MMT was treated with cetylpyridinium. The product CP-MMT (cetylpyridinium-exchanged montmorillonite) was organic cation exchanged MMT, just like Cu-MMT, also with a surplus positive charge on surface. Under scanning electron microscopy they found that large amount of *Salmonella enteritidis* accumulated on CP-MMT surface, but untreated MMT was not attractive to *Salmonella enteritidis*. Surplus positive charge of Cu-MMT and CP-MMT was most probably an important factor for their antibacterial capability. Two possible mechanisms for the antibacterial effect of Cu-MMT can be assumed. One model involves the adsorption of the bacteria and immobilization on the surface of the Cu-MMT. Alternatively, Copper cation could dissociate from Cu-MMT and diffused into the solution. The active Cu^{2+} on the mineral surface was much higher than its concentration in the solution. The high concentration of Cu^{2-} will act directly on the attracted bacteria. Summary, electrostatic attraction and the antibacterial effect of Cu^{2+} ion on bacteria are two ways of the antimicrobial action of Cu-MMT.

Effects of dietary MMT or Cu-MMT on the digestive enzyme activities in the pancreas and the small intestinal contents

In the present experiment, supplementation with MMT improved the activities of the digestive enzymes in the small intestinal contents, although the tendency was not significant. It has been reported that the addition of clay to the feedstuffs improved the nutrient digestibility and the enzymatic activity of gastrointestinal secretions (Cabezas et al., 1991; Paolo et al., 1999; Ouhida et al., 2000; Alzueta et al., 2002). The following actions of clay on digestive enzymes have been reported. First, the ion-exchange properties of the zeolite could alter the pH and the ionic composition (including trace elements) of gastrointestinal fluids, thereby changing the enzymatic activity of gastrointestinal secretions (Martin-Kleiner et al., 2001). Secondly, Ouhida et al. (2000) reported that sepiolite increased significantly organic matter digestibility, in line with decreases in the water-relative viscosity of jejunal digesta in broiler chickens fed on three diets of different viscosities, such improving digestive enzymes. Thirdly,

some *in vitro* studies (Cabezas et al., 1991) have shown that pancreatic enzymes can be absorbed over the surface of sepiolite, forming complexes which are active over a range of different digestive pH. This is particularly relevant in the case of pancreatic amylase which is normally very intolerant to differing pH values. Although, the sepiolite-enzyme derivatives are less active than the native enzymes, the profile of the plot of enzymatic activity versus pH is complementary to that of native enzymes. Therefore, these enzyme-sepiolate derivatives are resistant to proteolysis and increase the amount of active digestive enzymes in the intestine.

We also found that the improvement of the small intestinal digestive enzyme activities supplemented with Cu-MMT was more than that supplemented with MMT. The intestinal microflora data showed that Cu-MMT significantly suppressed intestinal *Escherichia coli* and *Clostridium*. Such changes in microbial ecosystem in the presence of Cu-MMT might contribute to the observed effects on the small intestinal digestive enzyme activities. Gao (1998) reported that *Escherichia coli* and *Clostridium* may damage the villus and microvillus of intestinal mucosa and inhibit the secretion of digestive enzymes. Moreover, *Escherichia coli* could secrete a proteolytic enzyme, which may take the intestine digestive enzymes as selective nutrients, thus increasing the degradation of digestive enzyme (Conway, 1994).

Effects of Dietary MMT or Cu-MMT on nutrient digestibility

In the present experiment, the improvement of the nutrient digestibility with MMT supplementation was consistent with the previous studies of the clays. Different mechanisms have been proposed to explain the effect of clay on the digestive and productive results of the animals. First, the reported improvement of the digestive enzymatic activities with clay supplementation, also found in the present study, may be involved. Secondly, sepiolite is thought to promote increases on the digesta retention time in the gut of pigs and poultry (Tortuero et al., 1992; Ouhida et al., 2000). Sepiolite promoted significant increases in the times of retention of digesta in the gastrointestinal tract of birds fed on barley-wheat diets treated with enzymes (40 min longer, of which 20 min represented longer retention in the small intestine) (Ouhida et al., 2000). The slow down in rate of passage of digesta would allow the endogenous enzymes activity to be more effective in the digestion of fat, protein and carbohydrates (Tortuero et al., 1992). Thirdly, changes in the digesta viscosity could be involved. Ouhida et al. (2000) reported that sepiolite increased significantly organic matter digestibility, in line with decreases in the water-relative viscosity of jejunal digesta in broiler chickens fed on three diets of different viscosities.

We also found that the improvement of the nutrient digestibility supplemented with Cu-MMT was more than that supplemented with MMT. The more improvements in the intestinal microbial ecosystem and the digestive enzymatic activities supplemented with Cu-MMT than those with MMT may be contributed to this.

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