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# Effects of $Cu^{2+}$-exchanged montmorillonite on growth performance, microbial ecology and intestinal morphology of Nile tilapia (*Oreochromis niloticus*)

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**Abstract**

A total of 360 Nile tilapia at an average initial body weight of 3.9 g were randomly allocated to 4 treatments, each of which had three replicates of 30 fish/tank and used to investigate the effects of $Cu^{2+}$-exchanged montmorillonite (Cu-MMT) on growth performance, microbial ecology of the skin, gill and intestine, and intestinal morphology. The dietary treatments were: 1) basal diet, 2) basal diet +1.5 g/kg MMT, 3) basal diet +30 mg/kg copper as $CuSO_4$ (equivalent to the copper in the Cu-MMT treatment group), or 4) 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 fish fed with Cu-MMT had higher growth performance than those fed with MMT or $CuSO_4$. Supplementation with Cu-MMT reduced ($P<0.05$) the total intestinal aerobic bacterial counts and affected the composition of intestinal microflora with a tendency of *Aeromonas*, *Vibrio*, *Pseudomonas*, *Flavobacterium*, *Acinetobacter*, *Alcaligence*, *Enterobacteriaceae* decreasing as compared with control. Measurements of villus and microvillus heights at different sites of the intestinal mucosa indicated that dietary addition of MMT or Cu-MMT improved intestinal mucosal morphology. However, the microbial ecology of the skin and gill was not affected by the addition of $CuSO_4$, MMT or Cu-MMT. Supplementation with $CuSO_4$ had no ($P>0.05$) effect on the growth performance, microbial ecology and intestinal morphology. The results showed that Cu-MMT exhibited antibacterial activity *in vivo* and protected intestinal mucosa from invasion of pathogenic bacterium and toxins, resulting in a positive effect on the growth performance.
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*Keywords:* $Cu^{2+}$-exchanged montmorillonite; Growth performance; Microbial ecology; Intestinal morphology; Nile tilapia

## 1. Introduction

Bacterial disease is a major problem encountered in the rapidly developing fish farming industry. Although vaccines are being developed and marketed, they cannot be used alone as a universal disease control measure in aquaculture. Treatment with antibiotics continues to be important disease control measures in the aquaculture industry. However, abuse or overuse of antibiotics causes various side-effects and also results in the emergence and increase of bacteria resistant to antibiotics. Therefore, it is necessary to develop new types of antibacterial agents that can replace antibiotics.

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The metal-carrying inorganic antibacterial agents, being carried by non-metallic minerals, have attracted attention by durable antibacterial properties, non-resistance, and safety. Montmorillonite (MMT) is a sort of aluminum silicate with 2:1 layer structure of tetrahedral and octahedral layers. Between the structural sheets, there are exchangeable cations readily being replaced by other cations or compounds. Taking into account that 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, MMT may be suitable as an antimicrobial carrier. Our recent experiments revealed that MMT had no antibacterial activity, while $Cu^{2+}$-exchanged montmorillonite (Cu-MMT) has strong antibacterial ability on *Aeromonas hydrophila*, *Pseudomonas fluorescens*, *Vibrio parahaemolyticus* and *Escherichia coli* $K_{88}$ (Hu and Xia, 2005; Hu et al., 2006).

There are no data of the effects of Cu-MMT on fish *in vivo*. In the present study, the effects of Cu-MMT on growth performance, microbial ecology of the skin, gill and intestine, and intestinal morphology of Nile tilapia (*Oreochromis niloticus*) were investigated.

## 2. Materials and methods

### 2.1. Materials

MMT is a hydrothermal product of volcano sedimentary rocks from the Inner Mongolia Autonomous Region, China. Besides MMT, there were minor amounts of quartz and volcanic glass in the ore. The raw material was dried in 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 and kept for about 10 min with stirring. Particles larger than 1 μm 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 was $[Na_{0.158}K_{0.082}Ca_{0.256}Mg_{0.063}][Mg_{0.376}Fe^{2+}{}_{0.014}Fe^{3+}{}_{0.136}Al_{1.474}][Si_{3.87}Al_{0.13}]O_{10}(OH)_2{\cdot}nH_2O$.

Cu-MMT was prepared by $Cu^{2+}$-exchanged reaction. Ten grams of the refined MMT were mixed with 100 ml of 0.2 mol/L NaCl solution. The dispersion was kept for 5 h with stirring. The Na-MMT were then separated by centrifugation at a speed of 8000 g for about 15 min and washed with deionized water for three times. The washed Na-MMT was then dispersed in 200 ml of 0.05 mol/L $CuSO_4$ solution and pH value was adjusted to 5.0. The dispension was kept at 60 °C with stirring for 6 h. After centrifugation, the sediment was washed with deionized water for three times, dried at 80 °C over night, then ground to a size less than 300 mesh. The copper concentration in Cu-MMT was found to be 2.0% measured by atomic absorption spectroscopy.

### 2.2. Experimental design and fish rearing

Nile tilapia (*O. niloticus*) fingerlings were provided by WuYI fishery breeding field and acclimated to laboratory conditions for 2 weeks, fed with a conventional diet, after which time a total of 360 Nile tilapia at an average initial body weight of 3.9 g were randomly allocated to four dietary treatments for 56 days, each of which had three replicates of 30 fish/tank. The dietary treatments were: 1) basal diet, 2) basal diet +1.5 g/kg MMT, 3) basal diet +30 mg/kg copper as $CuSO_4$ (equivalent to the copper in the Cu-MMT treatment group), or 4) basal diet +1.5 g/kg Cu-MMT. Diets were formulated to meet nutrient requirements suggested by the NRC (1993) for Nile tilapia. No antibiotic was included in diets (Table 1).

The experimental system was a closed recirculation system consisting of 12 self-cleaning aquarium (50 cm wide × 100 cm long × 50 cm high), sedimentation tanks, a biological filter and a UV filter to prevent cross-contamination of micro-organisms between treatments. The system was installed in an environment-controlled laboratory maintained at 25 °C, with a photoperiod of 12 h light and 12 h darkness. The culture system was also provided with continuous aeration through an air compressor and heaters to keep water temperature at

Table 1
Ingredients and nutrient composition of diets

| Ingredients | % | Nutrition composition | % |
|---|---|---|---|
| Fish meal | 10 | Gross energy (kJ/g) | 18.1 |
| Soybean meal | 30 | Crude protein | 35.2 |
| Rapeseed meal | 25 | Crude lipid | 2.5 |
| Wheat middlings | 26 | Fibre | 6.8 |
| Corn oil | 2 | Ash | 11.1 |
| Vitamin premix [a] | 1 | | |
| Mineral premix [b] | 5 | | |
| Carboxymethyl cellulose | 1 | | |

[a] Vitamin premix (mg/ kg of diet): thiamine, 10; riboflavin, 20; pyridoxine, 10; cobalamin, 2; retinal, 4; cholecalciferol, 0.4; phylloquinone, 80; folic acid, 5; Calcium pantotheniate, 40; inositol, 400; niacin, 150; tocopherol, 60; choline, 6000; ascorbic acid, 500.

[b] Mineral premix (g/kg of diet): NaCl, 0.25; $MgSO_4$, 3.75; $KH_2PO_4$, 8; $Ca(H_2PO_4)_2$, 5; $FeSO_4$, 0.72; $(CH_2CHCOO)_2Ca.5H_20$, 0.88; $ZnSO_4.7H_2O$, 0.088; $MnSO_4.4H_2O$, 0.040; $CuSO_4.5H_2O$, 0.008; $CoCl_2.6H_2O$, 0.00025; $KIO_3.6H_2O$, 0.00075.

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