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Effect of montmorillonite on dietary lead (Pb) accumulation in tissues of tilapia (Oreochromis niloticus)

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ABSTRACT

The effect of montmorillonite (MMT) on dietary lead (Pb) accumulation in tissues of tilapia (Oreochromis niloticus) was investigated. Two hundred forty tilapia were randomly divided into four groups and fed with a basal diet, a basal diet supplemented with 0.5% MMT, Pb-contaminated basal diet (100 mg Pb/kg dry mass) and Pb-contaminated basal diet supplemented with 0.5% MMT, respectively. After 60 days, tilapia were sacrificed and sampled to determine Pb accumulation, zinc and iron concentration in the tissues of tilapia. MMT significantly reduced Pb accumulation in kidney, intestine, bone, stomach, gill, liver, spleen, muscle, spermary and brain of tilapia($P<0.05$). Concentration of zinc (Zn) and iron (Fe) in the sampled tissues (kidney, bone, liver, spleen and spermary) of tilapia were not affected by MMT ($P>0.05$). Addition of MMT in diet could protect tilapia from dietary Pb accumulation in tissues.

1. Introduction

Lead, as a non-essential metal, has become an important pollutant in aquatic ecosystem with the increasing use of this metal in industry, agriculture and anthropogenic activities. Fish are major aquatic ecosystem component and may be exposed to Pb contamination in food as well as in water. It was reported that body burden of Pb in contaminated benthic invertebrates, as one of the food sources of fish, was up to 792 μg/g dw (Woodward et al., 1994; Farag et al., 1999). Pb in food could be taken up by fish via gastrointestinal tract and accumulate in tissues, leading to reduced food safety of fish (Alves et al., 2006; Dai et al., 2009). Therefore, considerable efforts have been made to investigate economical and efficient methods to reduce dietary Pb accumulation in fish. Alves and Wood (2006) found addition of elevated calcium in diet alleviated dietary Pb uptake in gastrointestinal tract and might be an effective measure for the remedy of dietary Pb accumulation. Montmorillonite has high adsorption properties attributed to large specific surface area and high cation exchange capacity (Ramos and Hernández, 1996; He et al., 1999). The adsorption of Pb to MMT may offer an attractive and inexpensive option for the removal of bioavailable Pb in the diet (Liu et al., 2006; Sheng et al., 2009). MMT is used as a food additive to enhance growth in livestock (Venglovsky et al., 1999; Tauqir and Nawaz, 2001).

Pb can interfere with the absorption, transport and metabolism of many essential trace elements, such as Fe and Zn. Vivante et al. (2008) found Pb poisoning could induce Fe depletion and iron depletion could aggravate Pb poisoning in soldiers. Similar results were also observed in animals (Fick et al., 1976; Blanusa et al., 1989). Oral Pb exposure could disturb Zn metabolism in tissues of sheep, rat, dairy calves and bovine (Fick et al., 1976; Doyle and Younger, 1984; White et al., 1985; Blanusa et al., 1989).

In this study, montmorillonite was added in diet to investigate its effect on growth performance, Pb accumulation and concentration of Zn and Fe in tissues of tilapia (Oreochromis niloticus) exposed to 100 mg/kg dw dietary Pb.

2. Materials and methods

2.1. Materials

Male O. niloticus were purchased from an clean fishery farm (Hangzhou, China). MMT was obtained from the Inner Mongolia Autonomous Region, China and prepared according to the method of Kim et al. (2009).

2.2. Experimental design and fish rearing

Tilapia were acclimated to laboratory conditions in static aerated tanks at optimal temperature of 25 ± 1 °C for one month with of 12 h light:12 h dark photoperiod. Each tank ($120\times60\times70$ cm) contained

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350 L dechlorinated tap water from aeration and sedimentation tank (pH7.3 ± 0.2, dissolved oxygen 7.6 ± 0.2 mg/L, alkalinity as $CaCO_3$ 109 mg/L, hardness as $CaCO_3$ 118 mg/L). Fish were fed with commercial basal diet of tilapia (36% crude protein; 4.5% crude fat; 5.0% crude fiber ash) from Yueteng Feed Factory (Hangzhou, China) twice daily to satiation.

After acclimation, 240 fish weighing 32.25 ± 2.79 g were randomly divided into four groups with three replicate tanks (20 fish per replicate tank) and reared in the same conditions as acclimation period. Fish were fed with basal diet (control diet), basal diet added with 0.5% MMT, basal diet added with 100 mg Pb/kg dw and basal diet added with 0.5% MMT and 100 mg Pb/kg dw at 3–3.5% fresh body mass twice daily. Pb concentration in commercial basal diet was 1.09 ± 0.01 mg/kg dw. Pb-enriched diets were made according to Alves et al. (2006) by adding lead nitrate (Pb $(NO_3)_2$) into the same commercial tilapia basal diet. The commercial food was pulverized into a fine powder and mixed with Pb dissolved in water. The control diet and diet added with MMT only were prepared the same way except Pb was not added. To avoid waterborne Pb contamination, the unfed food and feces were removed every 2 h using a siphon tube, and with half (~175 L) of the water in the tank being renewed daily. Waterborne Pb was not detected throughout the experiment period.

2.3. Growth performance study

After exposed for 60 days, tilapia starved for 24 h were anesthetized with 200 mg/L MS222 (sigma) and weighted individually in each tank. Growth performance was analyzed in terms of daily mass gain (DWG), feed conversion ratio (FCR). The following formulae were used:

DWG (g) = (final body mass − initial body mass) / rearing period (days);
FCR (g / g) = food consumed / live mass gain.

2.4. Collection of tissue samples

At the end of the exposure period, fish were anesthetized and sacrificed. The intestine, kidney, bone, stomach, liver, gill, spleen, muscle, spermary and brain were dissected, rinsed with cold physiological saline, frozen in liquid nitrogen and stored at −80 °C for subsequent analysis.

2.5. Pb, Zn and Fe analysis

Tissue samples (0.5–1.0 g) were digested with 2 mL 68% nitric acid, 1 mL 30% hydrogen peroxide and 2 mL ultrapure water in a CEM Mars 5 microwave accelerated reaction system. Then, the resulting solution was made up to 25 mL with ultrapure water. Pb was determined by atomic absorption spectrophotometry with a graphite furnace and an acetylene-air flame (Solaar M6, Thermo Electron, USA). Zn and Fe concentration were determined by atomic absorption spectrophotometry with flame technique.

Table 1
Growth and food utilization of O. niloticus.

Parameters	0 mg Pb/kg		100 mg Pb/kg	
	0% MMT	0.5% MMT	0% MMT	0.5% MMT
Initial mass(g)	31.91 ± 0.33	32.33 ± 0.42	32.33 ± 0.37	32.41 ± 0.41
Final mass(g)	115.11 ± 9.62	116.37 ± 3.55	113.73 ± 5.60	116.86 ± 6.78
DWG (%)	1.39 ± 0.16	1.40 ± 0.06	1.36 ± 0.09	1.41 ± 0.12
FCR (g/g)	1.80 ± 0.10	1.79 ± 0.06	1.82 ± 0.06	1.81 ± 0.09
Survival rate (%)	100	100	100	100

Values are represented as mean ± SD derived from 3 samples each of 20 fish; values sharing no letters are not significantly different (P>0.05).

Table 2
Pb burden in sampled tissues of O. niloticus (μg/g tissue wet mass).

Tissue	0 mg Pb/kg		100 mg Pb/kg	
	0% MMT	0.5% MMT	0% MMT	0.5% MMT
Kidney	0.21 ± 0.04c	0.11 ± 0.02d	16.58 ± 2.76a	9.73 ± 1.30b
Intestine	0.02 ± 0.01c	0.02 ± 0.00c	6.76 ± 1.80a	4.64 ± 0.71b
Bone	0.21 ± 0.03c	0.14 ± 0.03d	4.81 ± 0.45a	3.03 ± 0.25b
Stomach	0.03 ± 0.01c	0.01 ± 0.01d	2.05 ± 0.46a	1.15 ± 0.12b
Gill	0.07 ± 0.02c	0.05 ± 0.02c	1.23 ± 0.18a	0.51 ± 0.06b
Liver	0.02 ± 0.00c	0.02 ± 0.01c	0.99 ± 0.11a	0.67 ± 0.12b
Spleen	0.05 ± 0.01c	0.02 ± 0.01d	0.39 ± 0.04a	0.21 ± 0.04b
Muscle	nd	nd	0.10 ± 0.02a	0.03 ± 0.01b
Spermary	nd	nd	0.04 ± 0.01a	0.02 ± 0.01b
Brain	nd	nd	0.02 ± 0.01	nd

Values are represented as mean ± SD derived from 6 samples each of 3 fish; values sharing the same letter are not significantly different (P>0.05), whereas those with different letters are significantly different (P<0.05); nd represents not detected.

2.6. Statistical analysis

The resulting data were expressed as mean ± SD and subjected to one-way analysis of variance (ANOVA, SPSS version 10.0) to determine significant differences among groups. Significant differences (P<0.05) were reanalyzed by least significant difference multiple-range test.

3. Results and discussion

3.1. Effect of dietary MMT on growth performance of tilapia

Montmorillonite has been used as food additive to improve feed manufacture and enhance the nutritive value of food (Hu et al., 2007). MMT(10–30 g/kg) added in diet could promote growth of chickens and swine (Venglovsky et al., 1999; Tauqir and Nawaz, 2001). There was no significant difference (P>0.05) in growth performance between MMT and control group in this study (Table 1). The reason might be attributed to low MMT concentration (0.5%) and short MMT exposure time (60 d). Xu et al. (2004) also found growing pigs fed with 0.5% MMT-added diet for 83 days exhibited no growth-promoting activity in growth. Dietary Pb concentration added in this study was selected to mimic environmentally relevant concentrations (0–792 μg/g dw) in terms of those found in benthic invertebrates at both contaminated and uncontaminated sites in the environment (Woodward et al., 1994; Farag et al., 1999). Consistent with the results of Alves et al. (2006), the addition of 100 mg/kg Pb in diet did not decrease the growth of tilapia compared to the control group(P>0.05), which might be resulted from strong tolerance of tilapia to Pb stress. Based on the above results, tilapia fed with 100 mg Pb/kg and MMT showed similar growth performance to fish fed with Pb only(P>0.05). In conclusion, There were no effects on growth performance for tilapia fed the MMT-only, Pb and MMT, and Pb-only diets when compared to the control diet.

Table 3
Zn concentration in sampled tissues of O. niloticus (μg/g tissue wet mass).

Tissue	0 mg Pb/kg		100 mg Pb/kg	
	0% MMT	0.5% MMT	0% MMT	0.5% MMT
Kidney	67.83 ± 8.73	67.17 ± 5.32	62.25 ± 6.12	64.22 ± 6.30
Bone	133.49 ± 12.45	135.68 ± 11.28	124.67 ± 16.21	133.28 ± 11.57
Liver	51.35 ± 5.67a	50.54 ± 5.99ab	44.58 ± 5.17b	48.56 ± 5.25ab
Spleen	35.37 ± 3.32	35.65 ± 3.63	33.58 ± 3.96	34.89 ± 3.39
Spermary	42.70 ± 4.74	42.56 ± 4.56	40.85 ± 4.06	42.71 ± 4.65

Values are represented as mean ± SD derived from 6 samples each of 3 fish; values sharing the same letter or no letter are not significantly different (P>0.05), whereas those with different letters are significantly different (P<0.05).

3.2. Effect of dietary MMT on Pb accumulation in tissues of tilapia

Exposure of tilapia to dietary Pb resulted in a significant Pb accumulation in the tissues of tilapia in the following order: kidney>intestine>bone>stomach>gill>liver>spleen>spermary>muscle>brain($P<0.05$). Pb accumulation in tissues of tilapia fed with basal diet were observed because Pb existed in the basal diet was bioavailable. There was a significant decrease in Pb burden in tissues of tilapia fed with MMT and Pb compared to fish fed with Pb only ($P<0.05$). It may be suggested that the MMT is binding Pb, thus reducing Pb bioavailability and uptake at the gastrointestinal tract (Ramos and Hernández, 1996; He et al., 1999; Xu et al., 2004).

3.3. Effect of dietary MMT on Zn and Fe concentration in tissues of tilapia

Compared with control group, reduced Zn concentration in sampled tissues of tilapia exposed to dietary Pb was observed (Table 2). Fe concentration also decreased in sampled tissues of tilapia exposed to dietary Pb compared to the tissues of tilapia in control group (Table 3). Similar results were observed in other studies (Blanusa et al., 1989; Vivante et al., 2008). Pb could compete with Fe and Zn in absorption in intestine (Cerklewski, 1984; Flora et al., 1989), however, the mechanisms involved in the competition between Pb and these two essential elements are still unknown. Iron and Zn concentrations in tissues of tilapia exposed to MMT and Pb increased compared to tilapia exposed to Pb only ($P>0.05$). This suggests that MMT in the diet most likely binds the dietary Pb in non-bioavailable form which in turn reduces Pb ability to interact or interfere with the transport and/or absorption of Fe and Zn at the gastrointestinal tract. Similar Fe and Zn concentrations were observed in tissues between MMT and control group (Tables 3 and 4). Overall MMT supplementation in diet did not affect Zn and Fe absorption at the gastrointestinal tract.

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