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Effect of Montmorillonite Superfine Composite on Growth Performance and Tissue Lead Level in Pigs

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Abstract A feeding trial was conducted to study the effect of montmorillonite superfine composite (MSC) on growth performance and tissue lead levels in pigs. Sixty barrows were randomly divided into two groups. They were fed the same basal diet supplemented with 0 or 0.5% MSC, respectively, for 100 days. Serum samples were collected and analyzed to study growth hormone secretion pattern. The mean lead concentration in selected tissues was analyzed. The results showed that average daily gain, average daily feed intake, and feed conversion ratio of pigs were improved by 8.97% ($p<0.05$), 3.90% ($p<0.05$), and 4.76% ($p<0.05$), respectively, with the supplementation of MSC compared to the control group. Serum sample analysis indicated that peak amplitude, base-line level, and mean level of growth hormone were increased by 117.14% ($p<0.01$), 42.78% ($p<0.01$), and 51.75% ($p<0.01$), respectively. Supplementation of MSC in the diet was found to significantly reduce lead concentration of tissues in blood, brain, liver, bone, kidney and hair.

Keywords Montmorillonite superfine composite · Lead · Growth performance · Tissue lead level · Pigs

Introduction

Lead (Pb), a non-biodegradable heavy metal, continues to pose health hazards in man and animal in China and elsewhere in the world. It affects each and every organ and system in the body [1]. Being a cumulative poison, Pb is accumulated in various organs, such as the liver, kidneys, bone, and hemopoietic system [2] and restrain badly the growth of animals [3, 4]. Several metal chelators have been used to manage Pb toxicity in the event of exposure, but none is suitable in reducing Pb burden in chronic Pb exposure [5]. Moreover, these chelators have toxic potential themselves [1] and often fail to remove Pb from all body tissues [6, 7].

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Montmorillonite is a layered silicate with the property of adsorbing 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 [8, 9]. Montmorillonite is commonly the main constituent of the clays known as bentonites, its chemical structure is $X_{0.3}Y_{2-3}Z_4O_{10}(OH)_2{\cdot}H_2O$, X=Ca^{2+}, Li^{2+}, Na^{2+}, Y=Al^{3+},Cr^{3+},Cu^{2+},Fe^{2+}, Z=Si, Al. It has been demonstrated that 2% of four different kinds of montmorillonite were able to adsorb aflatoxin [10, 11], fecal moisture [12], and bacterium [13]. However, its large supplementation (sometimes as high as 2%) has limited its use as a result of diluting nutrients. Recently, a special montmorillonite nanocomposite (MSC) has been developed by our research team, after specified physical and chemical modification, the material characterizes much higher adsorptive ability to certain substances than other regular montmorillonite [14, 15].

The main objective of this study was to evaluate the capacity of MSC to adsorb lead present in feeds and determine its effects on growth performance and tissue lead level in pigs.

Materials and Methods

Experimental Materials

MSC was provided by Feed Science Institute of Zhejiang University. It held 380 nm average particle diameter and 200–410 m^2/g specific surface area and 1.19 mmol/g cation exchange capacity.

Experimental Design

All procedures were approved by the University of Zhejiang Institutional Animal Care and Use Committee. Sixty barrows (Duroc×Landrace×Yorkshire) with an average body weight about 33.0 kg were selected from NingBo Zhenning breeding farm and were randomly assigned by weight to two groups, each of which was replicated three times with ten pigs. Half of the pigs were fed with corn–soybean basal diets containing 0.5% MSC, the other half acted as control groups, receiving no MSC. Feed was provided ad libitum and water was provided by automatic waterers. The feeding experiment lasted for 100 days after a 7-day adaptation period. Average daily gain (ADG), average daily feed intake (ADFI), and feed conversion ratio (FCR) were collected for all pigs throughout the experiment period. Experimental diets were formulated to meet or exceed the nutrient requirement for growing and finishing pigs recommended by the NRC [16]. The average content of Pb in the basal diets was 7.24 mg/kg. The composition of basal diets and their main contents are shown Table 1.

Sample Collections

Before the termination of the feeding experiment, blood samples were taken via the auriculares. At the end of the feeding trial, ten pigs from each group were chosen for slaughter. Pigs were stunned by electric shock and then killed by exsanguination. Portions of the brain, liver, kidney, long bone, hair, heart, spleen, pancreas, and longissimus muscle were collected in polyethylene bags without any preservative after sacrifice on the day. The samples were subsequently subjected to acid digestion for Pb estimation.

Table 1 Composition and Nutritive Value of Basal Diets

Ingredients (%)	Growers (30–60 kg)	Finishers (60 kg or over)
Corn	65.2	66.0
Soybean meal	20.0	18.0
Rapeseed meal	4.0	4.0
Wheat bran	5.0	7.5
Fish meal	2.0	1.0
Bone meal	1.5	1.2
Calcium carbonate	1.0	1.0
Salt	0.3	0.3
Premix[a]	1.0	1.0
Chemical composition (% as fed)[b]		
Digestible energy (MJl/kg)	13.63	13.46
Crude protein	17.56	16.55
Ether extract	2.26	2.31
Calcium	0.97	0.95
Phosphorus	0.59	0.63

[a] Contained per kg diet: Cu 25 mg; Zn 100 mg; Mn 40 mg; Se 0.1 mg; I 0.3 mg; V-A 6,660 IU; V-D_3 660 IU; V-E 88 IU; V-K 4.4 mg; V-B_2 8.8 mg; D-pantothenic acid 24.2 mg; niacin 33 mg; choline chloride 330 mg.

[b] All of the data are analytic values except digestible energy.

Laboratory Analysis

All samples were wet-digested (AOAC, 1984) using a 5:1 mixture of concentrated nitric acid and 70% perchloric acid in a heating block under low heat. The concentration of Pb in the acid digest was estimated by atomic absorption spectrophotometry (AA-6510, Japan) at 283 nm wavelength following the instrument's instruction manual. An air–acetylene mixture was used as the oxidant gas. The analytical quality was maintained by repeated analysis of the reference standards (Sigma Chemicals, St. Louis, MO). The results were expressed as microgram per gram of sample.

Porcine serum samples were thawed in room temperature; growth hormone (GH) levels were determined with the RIA kit (Northern Immune Technic Institute, Isotopes Company, China) in a beta-counter (Packard 8500, USA).

Table 2 Effects of MNC on the Growth Performance on Pigs

	MNC (%)		S.E.M
	0	0.5	
Initial weight (kg)	33.36	33.29	0.84
Final weight (kg)	102.17[a]	108.27[b]	1.66
ADG (g/d)	688.04[a]	749.77[b]	12.57
ADFI (kg pig^{-1} d^{-1})	2.31[a]	2.40[b]	0.03
FCR (g/g)	3.36[a]	3.20[b]	0.02

Values are presented as means; n=3 for ADG, ADFI and FCR per treatment. Means in a row with different letters differ significantly (p<0.05).

S.E.M Standard error of mean

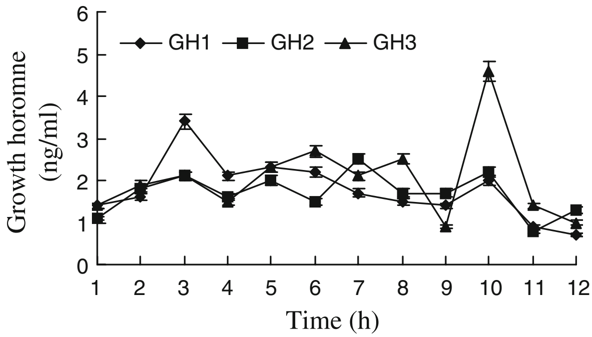

Fig. 1 *GH* secretion pattern of control group; *1*, *2*, and *3* are pig number. Pigs were bled at 15-min intervals for 3 h. Growth hormones in serum were determined with the RIA kit

Statistical Analysis

The data were analyzed statistically to determine whether there was any significant difference among difference treatment groups (ANOVA) using the general liner model procedures of PC SAS [17]. Data were considered by the model to be significantly different if $p<0.05$.

Results

Growth Performance

Growth performance of pigs fed MSC as compared to the control is presented in Table 2. Pigs treated with MSC had a significant improvement of 8.97% ($p<0.05$) in ADG, 3.90% ($p<0.05$) in ADFI, and 4.76% ($p<0.05$) in FCR as compared to the control group.

Growth Hormone Secretion

The results obtained showed that the serum GH peak amplitude, base-line levels, and mean levels were increased by 117.14% ($p<0.01$), 42.78% ($p<0.01$), and 51.75% ($p<0.01$), respectively, with the addition of MSC (Figs. 1, and 2, and Table 3).

Lead Content in Selected Tissues

Addition of MSC in the diet reflected in the appearance of various lead residues in selected tissues is shown in Table 4. The results indicated that MSC remarkably reduced lead

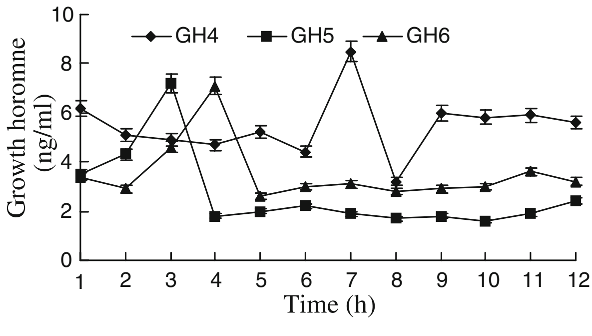

Fig. 2 *GH* secretion pattern of MNC treated group; *4*, *5*, and *6* are pig number. Pigs were bled at 15-min intervals for 3 h. Growth hormones in serum were determined with the RIA kit

Table 3 Effect of MNC and Lead on GH Level in Serum

	MNC (%)		S.E.M
	0	0.5	
Peak amplitude (ng/ml)	3.50[a]	7.60[b]	0.62
Base-line level (ng/ml)	1.73[a]	2.47[b]	0.12
Mean level (ng/ml)	2.86[a]	4.34[b]	0.18

Values are presented as means; $n=3$ per treatment. Means in a row with different letters differ significantly ($p<0.05$).

S.E.M Standard error of mean

concentration in such tissues as blood, brain, liver, bone, kidney, and hair by 59.72% ($p<0.01$), 46.84% ($p<0.01$), 48.16% ($p<0.01$), 34.75% ($p<0.01$), 35.02% ($p<0.01$), and 29.67% ($p<0.01$), respectively.

Discussion

The ADG, ADFI, and FCR of pigs were improved after feeding with MSC-containing diets. The results are similar to those described previously [4, 14]. It is evident that lead may be the important component causing performance inhibitory effects as shown in the study. Based on the former literature, Pb in the diet was found to inhibit the growth of animals in a dose-dependent manner. The inhibitory effect of Pb became statistically significant during the long time of exposure, especially when highest concentrations are considered. However, the addition of MSC in the feed held back obviously the effect of Pb toxicity on the growth performance in pigs.

The Pb levels in blood, brain, liver, kidney, long bone, hair, heart, spleen, pancreas, and longissimus muscle decreased significantly compared to the control. The results obtained showed that the concomitant use of nano-montmorillonite prevented the accumulation of Pb in these organs.

Table 4 Effect of MNC on Lead Concentration in Different Tissues of Pigs

	MNC (%)		S.E.M
	0	0.5	
Blood (μg/g)	1.44[a]	0.58[b]	0.22
Brain (μg/g)	0.79[a]	0.42[b]	0.13
Liver (μg/g)	2.72[a]	1.41[b]	0.25
Kidney (μg/g)	3.77[a]	2.46[b]	0.43
Long bone (μg/g)	3.74[a]	2.43[b]	0.41
Hair (μg/g)	4.55[a]	3.20[b]	0.52
Heart (μg/g)	0.43	0.39	0.12
Spleen (μg/g)	0.26	0.21	0.10
Pancreas (μg/g)	0.44	0.41	0.21
Longissimus muscle (μg/g)	0.47	0.43	0.26

Values are presented as means; $n=10$ per treatment. Means in a row with different letters differ significantly ($p<0.05$).

S.E.M Standard error of mean

The effect of Pb on hormone secretion was studied through blood sample taken via the auriculares. The results obtained showed that MSC can promote significantly the levels of serum GH. It is evident that Pb toxicity affected the secretion of GH and inhibited the growth of animals. When the feed was treated with MSC, the accumulation of Pb in the animals' body was prevented and its inhibitory effects were eliminated.

MSC has a larger specific surface area and more effective adsorption. These biologically active characteristics might have adsorbed Pb and enhanced its excretion from the body resulting in reduced Pb concentration in tissues and blood. Our unpublished results also showed that MSC increased the Pb concentration in the urine as well as in the feces of the animals. It can be suggested, therefore, that the ameliorative potential of MSC was perhaps due to combined effects both on metal absorption and on excretion from the body.

Our finding has also revealed that MSC has the ability to reduce residues of Pb in tissues (brain, liver, kidney, long bone, hair, heart, spleen, pancreas, *longissimus* muscle) as well as to increase the levels of serum GH in pigs as reported in other studies [18–20]. Therefore, it is concluded that MSC can be used for amelioration of Pb toxicity in animals and guarantee the safety of animal products. However, further researches are required to establish the dose and the molecular basis of the anti-toxic mechanism.

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