Effects of Montmorillonite Nanocomposite on Mercury Residues in Growing/Finishing Pigs

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ABSTRACT : The study was conducted to evaluate the effects of montmorillonite nanocomposite (MNC) on mercury residues in growing/finishing pigs. A total of 96 cross bred pigs (Duroc×Landrace×large white, 48 barrows and gilts respectively), with similar initial weight (27.87±1.15 kg), were used in this study. The animals were randomly assigned to two concentrations of mercury (0.1 and 0.3 ppm from $HgCl_2$) and two levels (0 and 0.3%) of MNC in a 2×2 factorial arrangement of treatments. Each group has 3 pens (replications), and each pen has 8 pigs (4 barrows and 4 gilts). The experiment lasted for 90 days. The results showed that pig growth performances were not affected significantly by inclusion of Hg and addition of MNC ($p \geq 0.05$). It indicated that the extent of intoxication in these pigs were not severe enough to impair growth performances. Both on the bases of 0.1 ppm and 0.3 ppm mercury supplementations, addition of 0.3% MNC markedly decreased mercury levels of blood, muscle, kidney and liver tissue ($p<0.05$). These results implied that the addition of non-nutritive sorptive material, MNC, could effectively reduce the gastrointestinal absorption of mercury via its specific adsorption, with a consequent reduction of mercury residues in body tissues. MNC had offered an encouraging solution to produce safe animal products with mercury contaminated feed. ***(Asian-Aust. J. Anim. Sci. 2004. Vol 17, No. 10 : 1434-1437)***

Key Words : Montmorillonite Nanocomposite, Mercury, Adsorption, Residue, Pig

INTRODUCTION

Mercury residues frequently occur in many environmental compartments because of increasing and widespread contamination from industrial and agricultural practices (Janicki et al., 1987; Nriagu et al., 1988). Furthermore, mercury is one of pollutants that could be enriched and amplified through biological food chains. It also can be accumulated in livestock and this sometimes causes toxic effects (Ammerman, 1977; Chang et al., 1977; Simpson et al., 1997). Nowadays concerns on food safety from consumers, society and governments soared to unprecedented heights. Safe and healthy food consumption has become a trend. Including mercury, heavy metals pollution in some feed ingredients and animal products has been fully reported and paid much attention by many scholars (Ammerman, 1977; Jeng et al., 1995; Nicholson et al., 1999). Many studies have been conducted on mercury contamination in fish and seafood, that are two of the main dietary routes of exposure for humans generally (Jorhem et al., 1991; Clarkson, 1992; Galal-Gorchev, 1993). However, the amount of data on mercury concentrations in terrestrial livestock and resultant meat products, is scant. In some certain regions or occasions, the Hg pollution in some feed ingredients has been higher to some extent (0.1 ppm is max. permission concentration for complete feed in China). Some ingredients including fishmeal, limestone and other trace element minerals, are much likely of higher mercury concentration (March et al., 1974; Nicholson et al., 1999). Therefore, farm animals and their products are at the risk of mercury pollution. Pork is the first staple item in meat consumption, especially in China. Furthermore, once mercury entrance to body tissues, it is difficult to remove out. No doubt, feed is the most important source of Hg residues in meat. So it'll be of great significance to reduce Hg residues in livestock by adsorption and reduction of absorption from the diet.

Montmorillonite is a type of better sorbent that can adsorb some toxins, heavy metals and bacteria in aqueous solution, and it has been applied to reduce those harmful contaminants in gastrointestinal tract as a non-nutritive additive (Wang et al., 2001). However, its large supplementation (sometime as high as 2%) has limited use as a result of diluting nutrients. Recently an especial montmorillonite nanocomposite (MNC) has been developed by our research team. Through specified physics and chemical modification, the material characterizes much higher adsorptive ability to certain materials than other regular montmorillonite. Data *in vitro* have showed that the kind of MNC took a noted role in mercury adsorption.

Therefore, the objective of present study was to investigate the effects of MNC addition on reduction of tissue mercury residues in growing/finishing pigs. Concerning the possible negative effect of higher Hg concentration on feed intake, two lower-intoxication-dose dietary Hg concentrations (0.1 and 0.3 ppm) were chose in the present experiment. Furthermore, the concentrations were similar to the natural Hg exposure. The level of MNC (0.3%) was decided in experiment, based on the data *in vitro*.

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Table 1. Composition of the basal diets for growing and finishing phases

Item	Growing	Finishing
Ingredient:		
Corn	50	50.6
Soybean meal	19	17.4
Rapeseed meal	4	5
Wheat meddling	18	17
Wheat bran	5.1	6
Limestone	1.3	1.3
Dicalcium phosphate	1.2	1.2
Salt	0.4	0.5
Premix [1]	1	1
Total	100	100
Calculated composition:		
DE, kcal/kg	3,087	3,070
CP, %	17.24	16.92
Lys, %	0.83	0.81
Met+cys, %	0.58	0.57
Calcium, %	0.86	0.86
Available P, %	0.35	0.35
Analyzed composition:		
Mercury, ppb	35.67	34.75

[1] Vitmins and minerals included to provide the following amounts per kilogram of diet: 180 mg of Zn; 150 mg of Fe; 150 mg of Cu; 50 mg of Mn; 0.3 mg of I; 0.3 mg of Se; 0.3 mg of Co; 6,500 IU of vitamin A; 750 IU of vitamin D_3; 20 IU of vitamin E; 3.5 mg of vitamin K_3; 2.8 mg of vitamin B_1; 6.2 mg of vitamin B_2; 33 mg of niacin; 18 mg of d-pantothenic acid; 3.5 mg of vitamin B_6; 0.85 mg of folic acid; 60 μg of biotin; 35 μg of vitamin B_{12}; and 600 mg choline chloride. Premix also provided 80 mg of chlortetracycline and 1,000 mg L-lysine HCl (purity, 98%) per kilogram of feed.

MATERIALS AND METHODS

Experimental design

A total of 96 cross bred pigs (Duroc×Landrace×Large White), 48 barrows and gilts respectively, with similar initial weight (27.87±1.15 kg), were used in this study. The animals were randomly allotted to two concentrations of mercury (0.1 and 0.3 ppm from $HgCl_2$) and two levels of MNC (0 and 0.3%) in a 2×2 factorial arrangement of treatments. Each group has 3 pens (replications), and each pen has 8 pigs (4 barrows and 4 gilts). The pigs were allowed *ad libitum* access to diets and water during the experiment, which last 90 days, 45 days in growing phase and 45 days in finishing phase. All pigs were cared for in accordance with the University Council on Animal care guidelines of animal welfare. The growing and finishing diets were showed in Table 1.

Body weights and feed intake were recorded to calculate average daily gain (ADG), average daily feed intake (ADFI) and feed/gain (F/G) for each pen on the 83rd day. Blood was collected in heparinized Eppendorf tubes via anterior vena cava venipuncture from two random selected pigs in each pen for a total of 24 pigs sampled (6 pigs per group) on d 30 (the 30th day), d 45, d 60, d 75 and d 90 respectively. Those blood samples were obtained at about 08:00 each time and kept in a -30°C freezer until analysis. Upon termination of the feeding trial, 6 randomly selected pigs (3 barrows and 3 gilts) from each treatment were slaughtered after a 24 h fast. Collected tissues samples included *longissimus dorsi* muscle, biceps femoris muscle, semimembranosus muscle, serratus muscle, renal cortex, renal medulla and liver tissues. And these tissue samples were also kept in a -30°C freezer until analysis.

Table 2. Effect of MNC on growth performance in growing/finishing pigs with 0.1 ppm and 0.3 ppm mercury administration

Item	Treatment				SEM
	0.1 ppm Hg		0.3 ppm Hg		
	0	MNC	0	MNC	
Initial weight, kg	27.48[a]	28.37	28.05	2`7.58	1.00
Final weight, kg	89.71	89.71	87.46	89.85	1.05
ADG, kg	0.73	0.74	0.72	0.75	0.02
ADFI, kg	2.18	2.17	2.19	2.21	0.05
F/G	3.01	2.94	3.05	2.95	0.06

[a] Means within a row with different superscripts are significantly different ($p<0.05$).

Mercury analysis

Instruments: Atomic absorption spectrophotometer (AA-6501, Shimadzuu, Japan); Mercury hollow-cathode lamp (Shimadzuu, Japan); Hydride vapor generator system (HVG-1, Shimadzuu, Japan).

Mercury of the samples were measured via hydride vapor generator coupled with atomic absorption spectrometer according to the method of Jackson (1995).

Statistical analysis

All the data were analyzed statistically by analysis of variances as a 2×2 factorial ANOVA procedure (SAS, 1989) and the treatment means were separated by Duncan's multiple range test. Statistical significance was at $p<0.05$ for all statistical tests.

RESULTS AND DISCUSSION

Growth performance

ADG, ADFI and F/G were not affected significantly by inclusion of Hg and addition of MNC ($p\geq0.05$), though there was a little difference on ADG and F/G between differed mercury levels (Table 2). It indicated that the extent of intoxication in these pigs were not enough severe to impair growth performance. And it seemed that 0.3% MNC addition would not impair pig growth performances. It suggested that animal normal growth performance might not decrease in those low-dose chronic intoxication cases after a short period. This was in agreement with results reported by March et al. (1974).

Table 3. Effect of MNC on blood mercury at varied exposure period

Item, ppb	Treatment				SEM
	0.1 ppm Hg		0.3 ppm Hg		
	0	MNC	0	MNC	
d 30	35.67[b]	27.58[c]	42.58[a]	38.17[b]	1.11
d 45	39.08[b]	31.83[c]	46.17[a]	40.58[b]	1.20
d 60	42.58[ac]	34.75[d]	46.58[a]	43.16[ab]	1.99
d 75	43.75[ac]	34.50[d]	45.75[a]	44.58[ab]	1.51
d 90	44.54[ac]	35.08[d]	46.00[a]	44.29[ab]	1.15

[a-d] Means within a row with different superscripts are significantly different ($p<0.05$).

Mercury residues

Table 3 showed that blood Hg levels were markedly higher in pigs with 0.3 ppm Hg supplementation than that in 0.1 ppm group on d 30 ($p<0.05$), d 45 ($p<0.05$), d 60 ($p<0.05$), d 75 ($p<0.05$) and d 90 ($p<0.05$) respectively. It was showed that Hg residues were much higher in 0.3 ppm groups than 0.1 ppm groups for each tissue sample in Table 4 ($p<0.05$). It suggested that the effect of dose-dependence was obvious. When mercury concentration increases in the diet, animal will have promoted mercury residues in blood and tissues. But, the interaction of mercury concentration ×MNC were not observed for each statistical item ($p\geq0.05$).

Compared with pigs not fed MNC, pigs fed MNC had significantly lower blood Hg levels on d 30 ($p<0.05$), d 45 ($p<0.05$), d 60 ($p<0.05$), d 75 ($p<0.05$) and d 90 ($p<0.05$) respectively (Table 3). As it was shown in Table 4, for pigs exposed to 0.1 ppm Hg, MNC addition lowered residues by about 47% in *longissmus dorsi* muscle ($p<0.05$), 53% in biceps femoris muscle ($p<0.05$), 40% in semimembranosus muscle ($p<0.05$), 50% in serratus muscle ($p<0.05$), 56% in renal cortex ($p<0.05$), 40% in renal medulla ($p\geq0.05$) and 68% in liver tissue ($p<0.05$). When pigs fed with 0.3 ppm Hg diet, MNC addition consistently lowered residues by about 32% in *longissmus dorsi* muscle ($p<0.05$), 34% in biceps femoris muscle ($p<0.05$), 31% in semimembranosus muscle ($p<0.05$), 34% in serratus muscle ($p<0.05$), 45% in renal cortex ($p<0.05$), 43% in renal medulla ($p<0.05$) and 40% in liver tissue ($p<0.05$). It was suggested that with supplementation of MNC, marked decreases of muscle, kidney and liver tissue mercury residues were got.

Following absorption, mercury could be transported and distributed into every tissue and organ of body via blood. However, the distribution and retention varied greatly for different tissues. In particular, there was a highest retention of mercury in the kidney tissue, second in liver tissue (Piper et al., 1971; Zalups et al., 1995; Zalups et al., 1997). This was quite consistent with results showed in Table 4. Mercury residues markedly declined in four different muscular tissues consistently. And the reduction occurred in renal cortex and medulla tissues. Blood and tissue Hg could reflect the extent of animal Hg intoxication, so reduction on Hg levels means the alleviation of intoxication. This suggested that addition of MNC in the diet could reduce the intoxication. Tissue mercury is from blood Hg, and blood Hg is mostly from diet. It was proposed that the decreases in blood and tissue Hg resulted from the reduced absorption of Hg in the gastrointestinal tract. Since no statistical difference on the ADFI was observed ($p>0.05$, Table 2), it was showed that the Hg residues differences were not caused by the differed feed intakes. We could conclude that the differences resulted from MNC adsorption to Hg. It was the adsorption that influenced dietary Hg to be absorbed and ingested in pig gastrointestinal tract. Furthermore, the MNC-mercury complex was stable and not affected by the different metabolizing enzymes in pigs' whole gastrointestinal tract. A large quantity of studies reported that certain clays could be used to adsorb Hg in aqueous solution (Undabeytia, 1993; Jin et al., 1999; Wang et al., 2001; Erickson, 2002). These materials have the property with porosity, huge surface area and strong ion exchange. Via proper and specific modification, MNC capacity of Hg adsorption is greatly enhanced. Therefore by addition of MNC to pig diet, Hg residues in meat and organs could be reduced to a considerably lower level. Now pork Hg maximum limit has been established in some countries, for example <50 ppb (in fresh weight) in China. With the supplementation of 0.3% MNC in pig growing/finishing diet, the produced pork is safe and of lower Hg residues, as

Table 4. Effect of MNC on mercury residues in muscle and organ tissues (in fresh weight)

Item, ppb	Treatment				SEM
	0.1 ppm Hg		0.3 ppm Hg		
	0	MNC	0	MNC	
Muscle tissue					
Longissimus dorsi	47.05[b]	24.85[c]	60.24[a]	40.89[b]	2.57
Biceps femoris	48.14[b]	22.47[c]	60.07[a]	39.42[b]	3.76
Semimembranosus	45.16[ab]	27.18[c]	53.96[a]	37.48[b]	3.30
Serratus	42.26[b]	21.16[c]	54.75[a]	36.25[b]	2.64
Organ tissue					
Renal cortex	414.16[b]	182.79[c]	956.82[a]	525.78[b]	72.46
Renal medulla	159.45[c]	97.39[c]	862.81[a]	495.46[b]	38.82
Liver	62.51[c]	20.28[d]	174.10[a]	104.05[b]	9.21

[a-c] Means within a row with different superscripts are significantly different ($p<0.05$).

well as no influence on growth performances.

The mercury concentrations in present study were not very high. As for a sorbent, it is much harder to adsorb those adsorbates in lower concentration than that in higher concentration. It was implied that MNC had a stronger affinity to mercury and would have satisfied effect in those higher contamination. Some researchers were afraid that higher concentration clays (0.5-1%) to animal diet would affect the availability of other nutrients (Ramos et al., 1997; Khajarern et al., 2003). More investigations should been done to testify whether clay or montmorillonite has the side effects. However, little changes on growth performances among four groups implied the adverse effects of MNC (0.3%) were slight and not obvious in present study.

No doubt it would be of great significance to apply MNC to solve the problem of Hg pollution in animal production industry. What's more, it could be used in a preventive manner. More studies should be done to explore MNC's role to higher mercury exposures and various form mercury pollutions. Use of MNC as a feed additive for mercury control requires much more studies of the possible long–term effects on the utilization of essential nutrients, such as vitamins, minerals etc. Though detailed mechanism of MNC's adsorption to dietary mercury has not been completely known at present, the possible hypothesis includes that MNC would bind mercury to form a considerably stable complex, which would not cross the luminal barrier of the intestine.

IMPLICATION

The present experiment suggests that the dietary supplementation of 0.3% MNC can effectively reduce tissue mercury residues in growing/finishing pigs fed contaminated feed. These testify that MNC can significantly decrease the gastrointestinal absorption of mercury via its specific adsorption. MNC have provided an encouraging solution to produce safe and low-residue animal products.

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