Effects of Lead and Particulate Montmorillonite on Growth Performance, Hormone and Organ Weight in Pigs*

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ABSTRACT : Seventy-two crossbred gilts of approximately 33 kg initial weight were used in this study. The gilts were randomly assigned into three groups. The three dietary treatments were basal diet only (control group), basal diet+10 mg/kg lead, and basal diet+10 mg/kg lead+0.5% particulate montmorillonite (PM). The results showed that the addition of lead to the diet decreased significantly the body weight and feed efficiency, but PM could restore body weight and feed efficiency of gilts compared to the Pb exposure group. There were no significant differences in weights of ovaries and uteri with addition of either lead or PM to the diet. Supplementing the lead in the diet of gilts also significantly increased the concentration of lead in blood, decreased circulating lutenizing hormone (LH) and estradiol (E_2) levels in serum, the addition of PM to the diet effectively adsorbed and lowered lead concentration in the blood. These data suggested that lead disrupts the signals between the hypothalamus and pituitary gland in gilts, and possibly suppressed the secretion of relative growth hormone and sex hormone. On the other hand, PM may ameliorate Pb toxicity in pigs. ***(Asian-Aust. J. Anim. Sci. 2005. Vol 18, No. 12 : 1775-1779)***

Key Words : Particulate Montmorillonite, Lead, Growth, Hormone, Pigs

INTRODUCTION

Lead (Pb), formally no element is ever bio-degradable heavy metal, continues to pose health hazards in man and animal worldwide. It affects each and every organ and system in the body (Goyer, 1990; Gilman et al., 1991). Reproductive defects associated with Pb poisoning have been noted since classical times (Bell and Thomas, 1980). Until recently, Pb was considered as a major environmental toxin, the animals may be exposed to low concentrations of Pb via contaminated feed, water, and feed additives (El et al., 2000). A lot of recent studies have demonstrated effects of Pb exposure on growth and development in humans and animals (Hamilton and O'Flaherty, 1995; Polák et al., 1995; Martin et al., 1996; Maboeta et al., 1999). Pb may also cause changes in the reproductive system of animals (Oldereid et al., 1993; Apostoli et al., 1999; Benoff et al., 2000; Crystel et al., 2001). In addition, suppression of postnatal growth rates and strong negative correlations between blood and Pb level in children and reduced growth stature have been reported (Badger et al., 1983; Angle and Kunzelman, 1989). The blood Pb levels at which these effects occur in humans are so low as to suggest that large segments of the population may be affected (Schwartz, 1992). In rat models, the results of reproductive toxicity have been reported following Pb exposure to animals. The effects described include changed circulating levels of testosterone, plasma and pituitary concentrations of luteinizing hormone (LH) and follicle-stimulating hormone (FSH) (Sokol et al., 1985; Sokol and Berman, 1991; Kempinas et al., 1994).

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 (Rodriguez et al., 1989; Dale et al., 1991). Montmorillonite are 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 (Masimango et al., 1978; Ramos and Hernandez, 1996), faecal moisture (Latif and Quisenberry, 1968) and bacterium (Hu et al., 2002). However, its large supplementation (sometime as high as 2%) has limited use as a result of diluting nutrients. Recently an especial particulate montmorillonite (PM) has been developed by our research team, after specified physics and chemical modification, the material characterizes much higher adsorptive ability to certain substances than other regular montmorillonite (Xu et al., 2003; Lin et al., 2004).

There are very few published researches on the effects of particulate montmorillonite and Pb in pigs. Therefore, The main objective of present study was designed to examine the effects of lead and particulate montmorillonite on growth performance, hormone and organ weight in pigs.

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Table 1. Composition and nutritive value of basal diets

Ingredients (%)	
Corn	62.4
Soybean meal	20.0
Rapeseed meal	4.0
Wheat bran	5.0
Rice bran	3.0
Fish meal	2.0
Bone meal	1.8
Calcium carbonate	0.5
Salt	0.3
Premix[1]	1.0
Chemical composition (% as fed)[2]	
Digestible energy (kcal/kg)[3]	3,175
Crude protein	17.50
Calcium	0.75
Phosphorus	0.50
Lysine	1.06
Methionine	0.45

[1] 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 33mg; choline chloride 330 mg.
[2] All of the data are analytic values except, digestible energy (DE) was calculated by De data of feed ingredient in Table of feed composition (NRC, 1998).
[3] The content of Pb in base diet was 7.24 mg/kg.

MATERIALS AND METHODS

Materials

Particulate montmorillonite (PM) was provided by Feed Science Institute of Zhejiang University. It held 380 nm average particle diameter, 200-410 m^2/g specific surface area and 1.19 mmol/g cation exchange capacity.

Animals experiments

All procedures were approved by the University of Zhejiang Institutional Animal Care and Use Committee. Seventy two gilts (Duroc×Labdrace×Yorkshine) with an average body weight about 33.0 kg were selected from NingBo Zhenning breeding farm and were randomly assigned by weight to three groups, each of which was replicated three times with eight pigs. Pigs in Group 1 were fed basal diet only (control). Group 2 was fed basal diet+10 mg/kg lead (the concentration of lead per kg of diet, lead nitrate, analytical grade, Shanghai Chemical reagent Com., Shanghai, China), and Group 3 was fed basal diet+10 mg/kg lead+0.5% particulate montmorillonite. Complete diets were formulated to meet all nutrients or above requirements (NRC, 1998). The composition of basal diets and their main contents are shown Table 1. Feed was provided *ad libitum* and water was provided by automatic waterers.

Sample collections

On 120th day of experiment (average final body weight about 103.0 kg), nine pigs from each group (three pigs per pen) were chosen randomly. They were fitted surgically with a polyvinyl cannulae into the jugular vein. Blood was collected at 4 h intervals over the 48 h and were placed in chilled heparinised tubes and centrifuged (10 min, 1.700 g, at 4°C). Serum was stored at -20°C until analysis of luteinizing hormone (LH), estradiol (E_2), prolactin (PRL) and progesterone (P_4). At the end of the sampling period gilts were euthanized. Ovaries and uteri were dissected and weighed.

Table 2. Effect of PM eliminating lead on the growth performance of gilts[1]

	Control	Lead	Lead+PM	SEM[2]
Initial weight (kg)	33.208	33.354	33.3632	0.90
Final weight (kg)	107.361^a	98.528^b	105.1674^a	1.66
ADG (g/d)	617.94^a	543.12^b	598.37^a	5.58
ADFI (kg/d)	2.3255^a	2.167^b	2.258^a	0.13
FCR	3.763	3.990	3.774	0.08

[1] Means with a row with different superscripts differ significantly ($p<0.05$).
[2] Standard error of the mean.

Blood lead analysis

The blood samples were subsequently subjected to acid digestion for Pb estimation by an atomic absorption spectrophotometer (AA-6501, Japan) at an absorption wavelength of 283.3 nm according to the method of Hsu et al. (1998).

Hormone analysis

GH, LH, E_2, PRL and P_4 levels in serum were determined by radioimmunoassay using kits purchased from Northern Immune Technic Institute, Isotopes Company, China. The intra- and inter- assay variation coefficients of the internal quality control pools for LH, E_2, PRL and P_4 were all within 10%.

Statistical analysis

One-way analysis of variance was performed using the General Linear Models Procedures of the SAS software (1989). Difference among means were tested using Duncan's multiple rang tests. A significant level of 0.05 was used.

RESULTS

Growth performance

Growth performance of pigs fed Pb or PM as compared to the control is presented in Table 2. The dietary addition of Pb significantly ($p<0.05$) decreased body weight, average day gain (ADG), average day feed intake (ADFI) in gilts, respectively, increased feed conversion ratio (FCR). However, similarly significant changes in body weight, ADG, ADFI and FCR were not found in gilts fed the diet treated with PM, but Pigs receiving PM had resumed body

Table 3. Effect of PM eliminating lead on blood lead concentration, ovarian and uterine weights of gilts[1]

	Control	Lead	Lead+PM	SEM[2]
Blood lead (μg/dl)	1.44[a]	2.08[b]	1.46[a]	0.27
Ovaries (g)	12.85[a]	12.76	12.81	0.54
Uteri (g)	511.0	508.94	514.38	4.21

[1] Means with a row with different superscripts differ significantly ($p<0.05$).
[2] Standard error of the mean.

weight. ADG, ADFI and FCR of gilts compared to the Pb-treated group.

Blood Pb levels and organ weights

Lead concentrations in whole blood sample of gilts from three groups are showed in Table 3. The blood lead level of Pb-treated gilts was significantly higher ($p<0.01$) than control animals. But the blood lead level of PM-treated animals declined significantly ($p<0.01$) after feeding PM compared to the Pb-treated group. The results also showed that neither Pb-treated gilts nor PM-treated gilts affected the weights of the ovaries or the uteri.

Serum hormone levels

The effects of lead exposure and PM supplementation on serum hormone levels are summarized in Table 4. Concentrations of GH, E_2 and LH in Pb- treated group were lower 27.13% ($p<0.01$), 12.48% ($p<0.05$) and 14.34% ($p<0.05$) than that of the control group, respectively. In comparison to Pb-treated group, the pigs fed the diet containing PM significantly increased the levels of GH, E_2 and LH in serum by 35.60% ($p<0.01$), 12.67% ($p<0.05$) and 20.57% ($p<0.01$), respectively. No significant effects were observed on contents of PRL and P_4 among three groups.

DISCUSSION

The growth performance of gilts fed lead diets are in agreement with the previously reported results for other species (Huseman et al., 1987; Hammond et al., 1990; Huseman et al., 1992; Hammond et al., 1993). It is evident that lead may be the important component causing growth 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. Huseman et al. (1987, 1992) have suggested that Pb inhibition of pituitary growth hormone (GH) release may play a role in growth deficits produced by Pb exposure. Comoratto et al. (1993) have demonstrated suppression of growth hormone-releasing hormone (GRF) stimulated GH release in rat following Pb exposure and GRF binding to the GRF receptor in isolated

Table 4. Effect of PM eliminating lead on serum hormone levels of gilts[1]

	Control	Lead	Lead+PM	SEM[2]
GH (ng/ml)	5.86[a]	4.27[b]	5.79[a]	0.30
E_2 (ng/ml)	116.24[a]	101.73[b]	114.62[a]	1.80
LH (ng/ml)	2.44[a]	2.09[b]	2.52[a]	0.32
PRL (ng/ml)	0.96	0.89	0.93	0.22
P_4 (ng/ml)	2.30	2.19	2.25	0.35

[1] Means with a row with different superscripts differ significantly ($p<0.05$).
[2] Standard error of the mean.

rat somatotroph membranes has been shown to be disrupted by Pb *in vitro* (Comoratto et al., 1993). However, the addition of PM in the feed held back obviously the effect of Pb toxicity on growth performance in gilts. It owe to the high specific surface area and strong heavy metal adsorption capacity of PM (Ramos and Hernandez, 1996; He et al., 1999; Hu et al., 2002).

The Pb level was increased significantly in blood of gilts receiving Pb alone. However, the results showed that the concomitant use of PM prevented the accumulation of Pb in blood and other organs efficiently, almost to the values recorded in healthy gilts. Dale et al. (1991) reported that montmorillonite could adsorb heavy metal cations either on its external surface or within its interlaminar spaces, by the interaction with or substitution of the exchange cations. These biologically active PM might have chelated Pb and enhanced its excretion from the body resulting in reduced Pb accumulation in blood.

In agreement with the previous studies in other mammals (Martin et al., 1996; Wiebe et al., 1988; El et al., 2000), the present experiment showed that the weight of ovaries and uterus in gilts fed Pb or PM diet did not differ from control animals. It seems that weight of reproductive organs in gilts is not affected by the diets containing low-dose Pb.

The endocrine and biochemical mechanisms underlying Pb-induced female reproductive toxicity have been little studied. These researches collectively suggest that Pb disrupted both hypothalamic and pituitary function. The results we have studied in gilts following Pb exposure is consistent with that of previous studies in female rats (Martin et al., 1996; Dearth et al., 2002; Dearth et al., 2004). Lead significantly decreased circulating LH and E_2 concentrations in serum. The present results supported previously reported data that Pb-treated animals manifested a hyperresponsiveness to GnRH stimulation as compared to control animals and are in agreement with a Pb-induced partial disruption of LH release from the pituitary gland. In addition, lead may interfere with calcium-based GnRH gonadotropin release via effects of its intoxication on calcium homeostasis and calcium-mediated cell function (Pounds, 1984; Krsmanovic et al., 1992; Verity, 1992; Skoczynska et al., 2001; Pillai et al., 2003), and then

circulating concentrations of LH and E_2 would be decreased. In contrast to serum LH and E_2 concentrations in gilts fed PM diet were significantly higher in comparison to Pb exposure gilts. It is indicated that PM may effectively adsorb and remove Pb in animal's body, ameliorate the effects of Pb toxicity on gilts, then resume the levels of serum LH and E_2 in gilts. In addition, the present study also showed that Pb or PM had no effects on the levels of serum PRL and P_4 in gilts. The reason is unclear.

In conclusion, we have studied a developmental gilt model in which the growth performance and reproductive toxicity effects observed in lead poisoning. On the other hand, we have explored the effects of PM on the growth, feed conversion, sexual organs weights and sex hormone in gilts following low-dose Pb exposure. The mechanisms underlying lead effects on reproductive growth performance and physiology appear to involve the dual sites of lead action at the level of the hypothalamic-pituitary unit and directly on gonadal steroidogenesis, possibly involving interactions with the calcium-dependent secondary messenger systems involved in regulation of relative growth hormone secretion and sex hormone biosynthesis. At the same time, we elicited that PM can effectively eliminate the effects of lead toxicity on reproductive incretion and growth performance in gilts. However, the molecular and biological mechanisms of lead toxicity, the dose and the molecular basis of the anti-toxic mechanism on PM should require further investigation.

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REFERENCES

Angle, C. R. and D. R. Kunzelman. 1989. Increased erythrocyte protoporphyrins and blood lead-a pilot study of childhood growth pattern. J. Toxicol. Environ. Health. 26:149-156.

Apostoli, P., S. Porru and L. Bisanti. 1999. Critical aspects of male fertility in the assessment of exposure to Pb. Scand. J. Work Environ. Health. 25:40-43.

Badger, T. M., J. S. Loughlin and P. G. Naddaff. 1983. The luteinizing hormone-releasing hormone (LHRH)-desensitized rat pituitary: Luteinizing hormone responsiveness to LHRH *in vitro*. Endocrinol. 112:793-799.

Bell, J. U. and J. A. Thomas. 1980. Effects of lead on mammalian reproduction. In Lead Toxicity (ED. R. L Singhal and J. A. Thomas). Urban & Schwartzberg, Baltimore/Zurich. pp. 167-187.

Benoff, S., A. Jacob and I. R. Hurley. 2000. Male infertility and environmental exposure to Pb and cadmium. Hum. Reprod. 6:107-121.

Comoratto, A. M., L. M. White, Y. S. Lau, G. O. Ware, W. D. Berry and C. M. Moriarty. 1993. Effect of exposure to low level lead on growth and growth hormone release in rats. Toxicol. 83:101-114.

Crystel, T., P. Joël, N. Francoise and L. Brigitte. 2001. Lead accumulation in the mouse ovary after treatment-induced follicular atresia. Reprod. Toxicol. 15:385-391.

Dale, J., M. Kowalska and D. L. Cocke. 1991. Interactions of montmorillonite with organic compounds adsorptive and catalytic properties. Chemosphere. 22:769-798.

Dearth, R. K., J. K. Hiney, V. Srivastava, S. B. Burdick, G. R. Bratton and D. W. Les. 2002. Effects of lead (Pb) exposure during gestation and lactation on female pubertal development in the rat. Reprod. Toxicol. 16:343-352.

Dearth, R. K., J. K. Hiney, V. Srivastava, D. W. Les and G. R. Bratton. 2004. Low level lead (Pb) exposure during gestation and lactation: assessment of effects on pubertal development in Fisher 344 and Sprague-Dawley female rats. Life Sci. 74:1139-1148.

El, F. A., F. Ghorbel, M. Smaoul, F. Makni-Ayadi and A. Kammoun. 2000. Effects du plomb d'origine automobile sur la croissance générale etl' activité sexuelle du rat. Gynécol Obstét Fertil. 1:51-59.

Gilman, A. G., T. W. Rall, A. S. Nies and P. Taylor. 1991. Goodman & Gilman's the pharmacological basis of therapeutics. Pergamon, New York, pp. 1592-1614.

Goyer, R. A. 1990. Lead toxicity: from overt to subclinical to subtle health effects. Environmental Health Perspectives. 86:177-181.

Hamilton, J. D. and E. J. O'Flaherty. 1995. Influence of lead on mineralization during bone growth. Fundamental and Applied Toxicology 26:265-271.

Hammond, P. B., D. J. Minnema and P. A. Succop. 1993. Reversibility of lead-induced depression of growth. Toxicol. Appl. Phdrmacol. 123:9-15.

Hammond, P. B., D. J. Minnema and R. Shukla. 1990. Lead lowers the set point for food consumption and growth in weanling rats. Toxicol. Appl. Pharmacol. 106:80-87.

He, H. P., J. G. Guo, X. D. Xie and J. L. Pen. 1999. Experimental studies on the selective adsorption of heavy metal on montmorillonite, illite and kaolinite and the influence of medium conditions. Acta. Mineralodica. Sinica. 19:231-235.

Hsu, P. C., M. Y. Liu, C. C. Hsu, L. Y. Chen and G. Y. Leon. 1998. Effects of vitamin E and/or C on reactive oxygen species-related lead toxicity in the rat sperm. Toxicol. 128(3):169-179.

Hu, X. R., G. L. Lu and L. S. Chen. 2002. Study on the mechanism of the interaction between and bacterium. Acta. Pharmaceutica. Sinica. 37:718-720.

Huseman, C. A., M. M. Varma and C. R. Angle. 1987. Childhood lead toxicity and impaired release of thyroid stimulating hormone. Environ. Res. 42:524-533.

Huseman, C. A., M. M. Varma and C. R. Angle. 1992. Neuroendocrine effects of toxic and low blood levels in children. Pediatrics. 90:186-189.

Kempinas, W. G., A. Farvaretto, V. Melo, T. L. Lamano Carvalho, S. O. Petenusci and R. M. Oliveira-Filho. 1994. Time-

dependent effects of lead on rat reproductive functions. J. Appl. Toxicol. 14:427-433.

Krsmanovic, L. Z., S. Stanko, M. Stojikovic and S. M. Dufour. 1992. Calcium signaling and episodic secretion of gonadotropin-releasing hormone in hypothalamic neurons. Proc. Natl. Acad. Sci. USA. 89:8462-8466.

Latif, M. A. and J. H. Quisenberry. 1968. Effects of dietary clays and sodium bicarbonate on the performance of commercial laying hens. Poult. Sci. 47:1688.

Lin, X. L., Z. R. Xu, X. T. Zou, F. Wang, X. H. Yan and J. F. Jiang. 2004. Effects of montmorillonite nanocomposite on mercury residues in growing/finishing pigs. Asian-Aust. J. Anim. Sci. 17(10):1434-1437.

Martin, J. J. R., M. B. Thomas, J. S. Sarah, K. R. Paula and S. Fatima. 1996. Reproductive toxicity and growth effects in rats exposed to lead at different periods during development. Toxicol. and appl. Pharmacol. 36:361-371.

Maboeta, M. S. M., A. J. M. Reinecke and S. A. Reinecke. 1999. Effects of low levels of lead on growth and reproduction of the asian earthworm perionyx excavatus (*Oligochaeta*). Ecotoxicology and Environmental Safety 44:236-240.

Masimango, M., J. Remacle and J. L. Ramault. 1978. The role of adsorption in the dlimination of aflatoxin B1 from contaminated media. Europ. J. Appl. Microbiol. 6:101-105.

National Research Council (NRC). 1998. Nutrient Requirements of Swine. 10th Ed. National Academy Press. Washington, DC.

Oldereid, N. B., Y. Thomassen, A. Attramada and P. K. Olaisen. 1993. Concentrations of Pb, cadmium and zinc in the tissues of reproductive organs of men. J. Reprod. Fertil. 99:421-425.

Pillai, A., L. Priya and S. Gupta. 2003. Effects of combined exposure to lead and cadmium on the hypothalamic–pituitary axis function in proestrous rats. Food Chem. Toxicol. 41:379-84.

Polák, J. and E. J. O'Flaherty. 1995. Physiologically based models of lead exposure in Children. Toxicology Letters 78:67.

Pounds, J. G. 1984. Effects of lead intoxication on calcium homeostasis and calcium-mediated cell function: a review. Neurotoxicol. 5:295-332.

Ramos, A. J. and E. Hernandez. 1996. *In vitro* aflatoxin adsorption by means of a montmorillonite silicate: A study of adsorption isotherms. Anim. Feed Technol. 62:263-269.

Rodriguez, J. M., A. J. Lopez and S. Bruque. 1989. Adsorption of phenamiphos by homoionic montmorillonites. Agrochimica. 33:312-21.

SAS Institute Inc. 1989. SAS/STAT User's Guide: Version 6, 4th edn. SAS Institute Inc., Cary, North Carolina.

Schwartz, J. 1992. Low level health effects of lead: Growth, developmental and neurological disturbances. In Human Lead Exposure (Ed. H. L. Needleman). CRC Press, Boca Raton, FL. pp. 233-242.

Skoczynska, A., J. Wrobel and R. Andrzejak. 2001. Lead-cadmium interaction effect on the responsiveness of rat mesenteric vessels to norepinephrine and angiotensin II. Toxicol. 162:157-170.

Sokol, R. Z., C. E. Madding and R. S. Swerdloff. 1985. Lead toxicity and the hypothalamic-pituitary-testicular axis. Biol. Reprod. 33:722-728.

Sokol, R. Z. and N. Berman. 1991. The effect of age of exposure on lead induced testicular toxicity. Toxicol. 69:269-278.

Verity, M. A. 1992. Ca^{2-}-dependent processes as mediators of neurotoxicity. Neurotoxicol. 13:139-48.

Wiebe, J. P., K. J. Barr, K. D. Buckingham. 1988. Effect of prenatal and neonatal exposure to Pb on gonadotropin receptors and stereoidogenesis in rat ovaries. J. Toxicol. Environ. Health. 24:461-476.

Xu, Z. R., Y. L. Ma, C. H. Hu, M. S. Xia, T. Guo and H. L. Jin. 2003. Effects of Cu-exchanged montmorillonite on growth performance, intestinal microflora, bacteria enzyme activities and morphology of broilers. Asian-Aust. J. Anim. Sci. 16(11):1673-1679.