Effects of Fluoride Levels on Lipid Peroxidation and Antioxidant Systems of Growing/Finishing Pigs

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ABSTRACT : Malondialdehyde (MDA) and total antioxidant capacity (T-AOC) levels, superoxide dismutase (SOD), glutathione peroxidase (GSH-Px), catalase (CAT), glutathione transferase (GST) and xanthine oxidase (XOD) activities were analyzed in serum, livers and kidneys of pigs treated with graded doses of fluoride (as NaF). Ninety-six Duroc-Landrace-Yorkshire crossbred growing pigs (48 barrows and 48 gilts, respectively), with similar initial weight 24.14±1.12kg, were randomly assigned to four different treatments. These treatments containing the following added F: basal control; 50 mg/kg F; 100 mg/kg F and 150 mg/kg F were randomly assigned to four pens (three barrows and three gilts) each in a completely randomized design. The results showed pigs treated with 150 mg/kg F significantly decreased average daily gain (ADG) ($p<0.05$) and increased feed/gain ratio (F/G) ($p<0.05$) compared to the controls. In the groups treated with fluoride, the contents of MDA increased, T-AOC levels and the activities of SOD, GSH-PX, CAT, GST and XOD decreased, and most of which altered significantly ($p<0.05$). The study therefore indicated the mechanism of excess fluoride on the impairment of soft tissues involved in lipid peroxidation and decreased the activities of some enzymes associated with free radical metabolism. ***(Asian-Aust. J. Anim. Sci. 2005. Vol 18, No. 4 : 552-556)***

Key Words : Growing/Finishing Pigs, Fluoride, Growth Performance, Lipid Peroxidation, Antioxidant Systems

INTRODUCTION

Fluorine (F), both essential and toxic, is a trace element for farm animals. Although the essentiality of trace amounts of F has been demonstrated (Messer et al., 1972), we have to face is excessive fluoride, but not absent. Fluorosis, caused by a long-term intake of high levels of fluoride, is an important public health problem all over the world, especially in developing countries (e.g. China and India). In China, except for the municipality of Shanghai, every province has areas that are afflicted with endemic fluorosis, nearly 70,000,000 patients with fluorosis (Liu et al., 2003). With the rapid development of feed industry and animal science, addition of raw rock phosphate and limestone as a calcium and phosphorus source, fluorosis has quickly increased in animal science. In China, fluorosis of domestic animals in fluorine polluting areas has been reported over 15 provinces and regions in recent years. Numerous studies showed that accumulation of excessive fluoride has toxic effects on many tissues and organs, resulting in serious damage and pathological changes (Whitford et al., 1979). There have been a number of studies on fluoride in laboratory animals, the mechanism of fluorosis has been reported by many scholars (Weber et al., 1969; Kragstrup et al., 1989; Maurer et al., 1990). It had been demonstrated that fluoride affects most, if not all, of the body's processes in one way or another. The mechanism has been probably clear that the effect of fluorosis on skeletal system, such as inhibition of bone mineralization and formation, delayed fracture healing and reductions in bone volume and collagen synthesis (Conrad, 2001); ameloblastic dysplasia, fracture and/or malformation and enamel hypoplasia of tooth (Maurer et al., 1990). However, the studies on the mechanism of non-skeletal fluorosis have only begun in recent years, and there exists some phenomena that couldn't be scientifically expatiated, so it is significant to enhance the studies related to non-skeletal fluorosis. Generation of free radicals, lipid peroxidation, and altered antioxidant defense systems are considered to play an important role in the toxic effects of fluoride on soft tissues (Patel and Chinoy, 1998; Han et al., 2004). Depending on this, in this study we mainly assess the effects of fluorosis on lipid peroxidation and antioxidant systems in serum, livers and kidneys of growing/finishing pigs, so as to further research the mechanism of fluorosis injuring soft tissues in animals and humans.

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MATERIALS AND METHODS

Animals and management

Ninety-six Duroc-Landrace-Yorkshire crossbred growing pigs (48 barrows and 48 gilts, respectively), with similar initial weight 24.14±1.12 kg, were randomly assigned to four different treatments. These treatments containing the following added F (as NaF): basal control; 50 mg/kg F; 100 mg/kg F and 150 mg/kg F were randomly assigned to four pens (three barrows and three gilts) each in a completely randomized design. Feed and water were supplied *ad libitum*. The experiment lasted for eighty-four

Table 1. Dietary ingredients and nutrient of basal diet

Item	Phases	
	Growing	Finishing
Ingredient (%)		
Yellow corn	64.32	67.25
Soybean meal	22.2	14.4
Wheat bran	10	15
Fish meal	0.25	
Monocalcium phosphate	0.8	0.8
Limestone	0.73	0.85
Salt (NaCl)	0.5	0.5
Lysine	0.2	0.2
Mineral/vitamin premix[a]	1	1
Calculated analysis[b]		
CP (%)	17.1	14.15
ME (Mcal/kg)	3.35	3.37
Ca (%)	0.56	0.53
Total P (%)	0.54	0.51

[a] Provided the following per kilogram of complete diet: vitamin A, 3,950 IU; vitamin D_3, 595 IU; vitamin E, 23 IU; vitamin B_2, 5.5 mg; vitamin B_{12}, 0.03 mg; biotin, 0.15 mg; nicotinic acid, 18 mg; Cu ($CuSO_4 \cdot 5H_2O$), 200 mg; Fe ($FeSO_4 \cdot 7H_2O$), 55 mg; Zn ($ZnSO_4 \cdot 7H_2O$), 120 mg; Mn ($MnSO_4 \cdot H_2O$), 60 mg; Se ($NaSe_2O_3$), 0.15 mg.

[b] Calculated analyses were based on nutrient contents of ingredients listed in NRC (1998).

days after seven days of adaptation period. Compositions of the basal diet and nutrient levels for growing and finishing phases are presented in Table 1. The content of fluorine was 37.39 mg/kg in the basal diet and 0.6 ppm in drinking water. Feed intake per pen was recorded for the experimental period, and each pig was weighed at the beginning and the end of experiment to determine average daily gain (ADG), average daily feed intake (ADFI) and feed/gain ratio (F/G).

Sample collection

At the end of 84 days feeding trial, two pigs (one barrow and one gilt) from each pen were randomly selected to slaughter after a 24 h fast. Blood samples were centrifuged at 3,000 rpm/min for 15 min, the serum separated by blood was packed in Eppendorf tubes, snap-frozen in liquid nitrogen, and stored at -70°C until analysis. The samples of livers and kidneys were collected from the left side of carcass within 15 min after exsanguinations, snap-frozen in liquid nitrogen, and stored at -70°C until they were analyzed.

Chemical analysis

The fluorine was determined with a fluoride ion-selective electrode.

Liver and kidney tissues were dissected, thoroughly washed with ice-cold normal saline, weighed, minced and homogenized (10%w/v) using 66 mmol/l phosphate buffer (pH 7.0). The homogenate that was centrifuged at 1,000 g for 20 min at 4°C was used for the estimation of lipid peroxidation. The supernatant obtained was further centrifuged at 12,000 g for 20 min at 4°C to obtain the postmitochondrial supernatant, which was used for the assays of T-AOC, SOD, GSH-Px, XOD, CAT and GST.

Lipid peroxidation was measured via the thiobarbituric acid color reaction for malondialdehyde (MDA) by the method of Wills (1966). The results were expressed as nmol MDA formed per milligram of protein (nmol/mgprot).

Total antioxidant capacity (T-AOC) was assayed by the method of Miller et al. (1993), the results were expressed as units per milligram of protein (U/mgprot). SOD was assayed by monitoring the rate of inhibition of reduction of nitroblue tetrazolium (NBT) by the enzyme (Asada et al., 1974). One unit of the SOD represents the amount of enzyme required to produce 50% inhibition of NBT reduction per minute. CAT activity was assayed by the method of Aebi (1984) based on the direct measurement of H_2O_2 decomposition at 25°C. One unit of CAT activity represents 1 μmol H_2O_2 decomposed per min. GSH-Px activity was determined according to the method of Flohe and Grunzler (1984). One unit of GSH-Px consumes 1 μmol NADPH/min. GST was assayed by the method of Habig et al. (1974), and the unit was defined as nanomole per liter of GSH used per minute. XOD was assayed using Xanthine as the substrate and by following the rate of reduction of nitroblue tetrazolium at 560 nm (Fried, 1974). One unit of XOD is defined as the amount of enzyme that produces 1 nmol of uric acid per minute.

All the enzyme activities were expressed as units per milligram of protein except for XOD (as units per gram of protein (U/gprot). The protein content was determined by the method of Bradford (1976) with crystalline bovine serum albumin as a standard.

MDA, T-AOC levels and SOD, GSH-Px, CAT, XOD and GST activities in serum were analyzed by the above methods, the results were expressed as nmol/ml (MDA), U/L (XOD) or units per millilitre of serum (U/ml).

Statistical analyses

The data were analyzed by analysis of variance for repeated measures using the general linear models procedure of SAS (Version 6.12). Significance was evaluated at the 0.05 level.

RESULT AND DISCUSSION

Growth performance

Compared to the control, ADG was decreased 5.29% ($p<0.05$) and 8.60% ($p<0.05$); F/G was increased 5.78% ($p<0.05$) and 8.50% when treated with 100/kg, 150 mg/kg F, respectively (Table 2). However, addition of fluorine in diets had no effect on ADFI of growing pigs. The results was similar to the findings of Burnell et al. (1986), they

Table 2. Growth performance of pigs

Item	Fluorine/ (mg kg^{-1})				SEM[1]
	0	50	100	150	
Initial wt (kg)	26.79^{a}	26.77^{a}	26.80^{a}	26.84^{a}	0.24
Final wt (kg)	89.18^{a}	87.17^{a}	85.89^{ab}	83.87^{b}	1.07
ADG (g)	742.81^{a}	719.18^{ab}	703.50^{bc}	678.91^{c}	12.36
ADFI (kg)	2.18^{a}	2.18^{a}	2.19^{a}	2.16^{a}	0.01
F/G	2.94^{a}	3.03^{ab}	3.11^{bc}	3.19^{c}	0.04

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

Table 3. Weights of livers and kidneys of pigs, expressed as a percentage of live body weight

Item	Fluorine/(mg kg^{-1})				SEM[1]
	0	50	100	150	
Liver (%)	1.54^{a}	1.52^{a}	1.76^{b}	1.84^{b}	0.07
Kidney (%)	0.35^{a}	0.34^{a}	0.33^{a}	0.30^{b}	0.01

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

Table 4. Fluorine concentration of serum, liver and spleen of pigs

Item	Fluorine/(mg kg^{-1})				SEM[1]
	0	50	100	150	
F (mg/L)	0.26^{a}	0.36^{b}	0.80^{c}	1.22^{d}	0.03
Liver (mg/kg)	1.21^{a}	1.40^{b}	1.85^{c}	2.03^{d}	0.06
Kidney (mg/kg)	1.14^{a}	1.48^{b}	1.74^{c}	2.06^{d}	0.05

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

reported ADG was reduced ($p<0.001$) for pigs consuming diets with F concentrations greater than 132 mg/kg. Studies on broilers (Huyghebaert et al., 1988), hens (Guenter and Hahn, 1986) and calves (Kapoor and Prasad, 1991) also showed that excessive fluorine in diets significantly lowered animal performances. Our results indicated that 37.39 mg/kg F in diets is enough to meet growing pigs, when the content of fluorine was higher, and then a decreased tendency of growth performance had been observed.

Relatives weight of livers and kidneys

As shown in Table 3, relative weights of livers were larger 19.48% ($p<0.05$) from pigs when 150 mg/kg F was added than those of the control. As a very active site of metabolism, the liver is especially susceptible to fluoride intoxication. In our experiment, abnormalities in the liver including degenerative and inflammatory changes and hepatic hyperplasia were observed when treated with 150 mg/kg F in diet. Compared to the control, relative weights of kidneys were smaller 14.29% ($p<0.05$) in 150 mg/kg F treatment, this decrease can be attributed to weight loss, degeneration of structure of kidneys. The results showed that accumulation of excessive fluoride induced abnormalities of livers and kidneys in pigs.

Table 5. MDA, T-AOC and antioxidants of serum

Item	Fluorine/(mg·kg^{-1})				SEM[1]
	0	50	100	150	
MDA (nmol/ml)	5.83^{a}	6.33^{a}	7.23^{b}	7.92^{b}	0.31
T-AOC (U/ml)	4.39^{a}	3.87^{ab}	3.56^{bc}	2.92^{c}	0.23
SOD (U/ml)	142.81^{a}	133.36^{ab}	129.42^{b}	116.10^{c}	3.31
GSH-Px (U/ml)	760.77^{a}	743.27^{a}	712.33^{ab}	684.07^{b}	17.55
XOD (U/L)	10.87^{a}	10.29^{ab}	9.62^{b}	9.25^{c}	0.30
CAT (U/ml)	39.10^{a}	31.32^{b}	30.18^{b}	25.42^{c}	0.83
GST (U/ml)	39.03^{a}	37.69^{ab}	36.54^{ab}	36.04^{b}	0.91

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

Table 6. MDA, T-AOC and antioxidants of livers

Item	Fluorine/(mg kg^{-1})				SEM[1]
	0	50	100	150	
MDA (nmol/mgprot)	1.63^{a}	1.77^{ab}	1.92^{ab}	2.09^{b}	0.12
T-AOC (U/mgprot)	24.05^{a}	19.84^{b}	18.45^{b}	14.79^{c}	1.06
SOD (U/mgprot)	72.04^{a}	67.91^{ab}	62.41^{bc}	61.48^{c}	1.98
GSH-Px (U/mgprot)	119.59^{a}	108.72^{ab}	105.94^{b}	91.97^{c}	4.08
XOD (U/gprot)	8.81^{a}	8.79^{a}	8.51^{ab}	7.18^{b}	0.54
CAT (U/mgprot)	61.37^{a}	58.83^{a}	51.82^{b}	48.28^{b}	1.96
GST (U/mgprot)	50.75^{a}	41.80^{b}	36.27^{c}	33.06^{c}	1.65

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

Tissue fluorine concentration

As shown in Table 4, compared with the control, serum, liver and kidney fluorine levels of three experimental groups were significantly elevated ($p<0.05$). The results confirmed that tissue concentrations of fluorine increased in response to an increase in dietary fluorine concentration.

Lipid peroxidation

It is well known that MDA is a terminal product of lipid peroxidation, so the content of MDA can be used to estimate the extent of lipid peroxidation. As shown in Table 5, 6 and 7, in the serum, livers and kidneys of pigs fed added 150 mg/kg F, MDA levels were significantly elevated 35.85, 28.22 and 39.27% compared to the controls ($p<0.01$). Shivarajashankara et al. (2001,2002) showed increased MDA levels in the red blood cells, liver and brain of rats. MDA contents were largely increased when treated with fluoride in different groups in chicks (Liu et al., 2003). Our study indicated that the action of lipid peroxidation was

Table 7. MDA, T-AOC and antioxidants of kidneys

Item	Fluorine/(mg kg^{-1})				SEM[1]
	0	50	100	150	
MDA (nmol/mgprot)	0.56^{a}	0.66^{ab}	0.74^{bc}	0.78^{c}	0.04
T-AOC (U/mgprot)	16.01^{a}	14.71^{ab}	13.06^{b}	9.05^{c}	0.64
SOD (U/mgprot)	73.03^{a}	68.06^{a}	67.34^{a}	60.75^{b}	2.04
GSH-Px (U/mgprot)	16.71^{a}	14.81^{ab}	13.17^{bc}	11.95^{c}	0.72
XOD (U/gprot)	7.95^{a}	7.79^{a}	7.42^{a}	7.09^{b}	0.21
CAT (U/mgprot)	58.87^{a}	56.33^{a}	49.82^{b}	46.91^{b}	1.83
GST (U/mgprot)	37.35^{a}	31.29^{b}	29.62^{bc}	26.82^{c}	1.26

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

increased by high fluoride.

Antioxidant systems

As shown in Table 5, 6 and 7, T-AOC levels, SOD, GSH-PX, CAT, XOD and GST activities of serum, livers and kidneys decreased with the increase of fluoride in diet. Especially, the concentrations of those significantly lowered when added 150 mg/kg F in diet ($p<0.05$) than those of the controls.

T-AOC, used to reflect the total capacity of antioxidant systems in the body in recent years, is an integrative index. No studies about the T-AOC records of pig fluorosis were reported by our searches, only several reports on other animal species. Their results indicated high fluoride significantly decreased the levels of T-AOC in chicks (Kang et al., 2001) and goats (Li et al., 2003). Our data showed T-AOC levels similarly decreased when treated with100 mg/kg F ($P<0.05$) and 150 mg/kg F ($p<0.001$) in serum, livers and kidneys of pigs.

Excess oxidants are captured by SOD, GSH-Px and CAT (Ko et al., 2004). And they are the mainly antioxidant enzymes in the body. These enzymes may scavenge unwanted $\cdot O_2^-$ and H_2O_2, ROOH produced by free radicals. For example, SOD catalyzes superoxide radical dismutation: $\cdot O_2^- + \cdot O_2^- + 2H^+ \rightarrow H_2O_2 + O_2$. The resulting hydrogen peroxide in turn is decomposed by the enzymes GSH-Px and CAT (Rzeuski et al., 1998). As shown the above expressed, activity decrease of each among them would induce increased free radicals, and so injuring the corresponding tissues. In this study, the results showed that all of SOD, GSH-Px and CAT activities were decreased in serum, livers and kidneys. Decreased SOD levels indicate the product of O_2^- radicals increased by lowered ability of the tissues that can scavenge free radicals, and similarly increased H_2O_2 in the tissues by decreased GSH-Px and CAT activities. In the same way, the conclusion had been confirmed by studies on mice, rat, goat and chicks. However, there were several different reports in which GSH-Px levels significantly elevated (Chow and Tappel, 1972; Shivarajashankara et al, 2001; 2002). Shivarajashankara et al. (2002) pointed out these differences in GSH-Px to the fluorosis in animals might be due to variations in dose, duration and route of fluoride administration, the stage of life at which fluoride was administered, animal species used and individual tissue response.

In agreement with findings reported by Dierickx and Beer (1983), they found fluoride inhibits GST activity in rat liver in a dose-dependent manner. Our studies also showed that GST activities in serum, livers and kidneys of pigs were lowered when fluoride was added in diet (Tables 5, 6 and 7). Similarly, GST activities in brain and gastrocnemius muscle of female mice (Vani and Reddy, 2000) were markedly decreased when treated with 20 mg/kg/body weight for 14 days. GST existed in various tissues, especially in the liver, it can remove free radicals and the levels can reflect the antioxidant capacity in the body.

Our data showed XOD activities in serum, livers and kidneys of pigs when treated with fluoride were decreased (Table 5, 6 and 7). XOD is also an enzyme related to the production of free radicals when oxygen was absent in the body. This further indicated that high fluoride increased free radical generation.

In present, studies about the effects of fluorosis on soft tissues in pigs are reported only several reports, especially papers about the mechanism of fluorosis are few. The results that had been researched on other animal species are inconsistent, even are inverse. The reason is various, including variations in dose, duration and route of fluoride administration, the stage of life at which fluoride was administered, animal species used and individual tissue response, and so on. So our studies are only an initial exploration, more studies need have been achieved to demonstrate the mechanism of fluorosis on soft tissues in the body.

IMPLICATION

The present study shows that high fluoride can markedly alter MDA, T-AOC levels and some enzymes activities associated with free radical metabolism in serum, livers and kidneys of growing/finishing pigs. The findings indicate lipid peroxidation and increased free radical production involved in the process of fluoride impairing the soft tissues in the body. Our data may provide some evidences for further studying the mechanism of excess fluoride accumulation on the impairment of soft tissues.

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