Turk J Vet Anim Sci
30 (2006) 65-70
© TÜBİTAK

Research Article

Effects of Dietary Fluoride Levels on Growth, Serum Indexes and Antioxidant Systems in Growing Pigs

Xin TAO[1,2,*], Zi Rong XU[2], Yi Zhen WANG[2]

[1]Institute of Animal Husbandry and Veterinary Science, Zhejiang Academy of Agricultural Sciences, Hangzhou 310021, P. R. China

[2]Key Laboratory for Molecular Animal Nutrition of Ministry of Education, Feed Science Institute, Zhejiang University, Hangzhou, 310029, P. R. China

Received: 18.11.2004

Abstract: Ninety-six growing pigs were used to evaluate the effects of fluoride levels on growth performance, serum indexes and antioxidant systems. Four dietary treatments were formulated by supplementing fluorine (as NaF) to provide the following added fluorine levels: 0, 50, 100 and 150 mg/kg. The results showed that pigs consuming diets with 100 and 150 mg/kg fluorine added had poor growth performance, and most serum biochemical indexes were significantly altered compared to the control ($P < 0.05$). On the other hand, serum and liver MDA concentration significantly increased due to the addition of 150 mg/kg fluorine ($P < 0.05$). T-AOC levels and the activities of SOD, GSH-PX, CAT and GST of serum and liver in fluoride added groups decreased, most of which altered significantly ($P < 0.05$). These results indicated that excessive fluoride ingestion had an adverse effect on animal health and performance.

Key Words: Fluoride, growing pigs, growth performance, serum indexes, antioxidant systems

Introduction

Fluorosis, caused by excessive fluoride ingestion, is an important public health problem all over the world. It has been reported that areas of endemic fluorosis occur in all inhabited continents (1). This condition is rampant in China. Except for the municipality of Shanghai, every province in China has areas that are afflicted with endemic fluorosis (2), with nearly 70,000,000 patients with fluorosis (3). In fluoride-contaminated areas, animals are as severely affected as humans are. In addition to endemic and industrial fluorosis, more mineral supplements added to diets with increasing development of the feed industry are direct important sources of excess fluorine intake by animals, which seriously affect their growth and production. The studies on the adverse effects of high fluoride ingestion on the health and performances of animals started early in the 1930s. However, most experiments had been conducted to study fluorosis in avian species, cattle, rabbits and sheep, little data were reported in pigs, especially since the 1980s. Yet fluorosis in pig groups in China was frequently reported in recent years. So it is necessary to determine the adverse effects of fluoride levels on pigs.

Depending on this, in this study it was mainly assessed the effects of excessive fluoride ingestion on growth performance, serum indexes and antioxidant systems in growing pigs.

Methods

Experimental design

A total of 96 crossbred growing pigs (68d) weighing 24.14±1.12 kg were randomly assigned to four treatments. These treatments containing the following added fluorine (as NaF) levels: 0, 50, 100 and 150 mg/kg fluorine were randomly assigned to four replications with 6 pigs (3 barrows and 3 gilts) per pen in a completely randomized design. Pigs were allowed ad libitum access to feed and water during the experiment. The experiment lasted 84 days after a 7-day adaptation period. All diets were formulated to meet or exceed the requirements described by the NRC (4). Fluorine content was 37.39

* E-mail: xindragon@sohu.com

mg/kg in the basal diet and 0.6 μg/ml in the drinking water. Feed intake per pen was recorded for the experimental period, and each pig was weighed at the beginning and the end of the experiment to determine average daily gain (ADG), average daily feed intake (ADFI) and feed gain ratio (F/G).

Sample collection

At the end of the 84-day feeding trial, 2 pigs (1 barrow and 1 gilt) from each pen were randomly selected for slaughter after a 24-h fast. Blood samples were centrifuged at 3000 rpm for 15 min, and the serum separated from the blood was packed in Eppendorf tubes, snap-frozen in liquid nitrogen, and stored at –70 ºC until analysis. The samples of liver were collected from the left side of the carcass within 15 min of exsanguinations, snap-frozen in liquid nitrogen, and stored at –70 ºC until they were analyzed.

Chemical analysis

Serum analysis for glutamic-pyruvic transaminase (GPT), glutamic-oxalacetic transaminase (GOT), alkaline phosphatase (ALP), lactic dehydrogenase (LDH) activities and for total protein, albumin (ALB), urea nitrogen, cholesterol, triglycerides and glucose were accomplished by described methods using a biochemical autoanalyzer (Beckman-CX9 Instruments, Inc.).

Serum Ca and Mg were determined by atomic absorption spectrophotometry (5), while serum phosphorus was analyzed using the Fiske and Subbarow (6) procedure.

Lipid peroxidation was measured via the thiobarbituric acid color reaction for malondialdehyde (MDA) by the method described by Wills (7). The results were expressed as nmol MDA formed per milligram of protein (nmol/mgprot).

Total antioxidant capacity (T-AOC) was assayed by the method reported by Miller et al. (8), and the results were expressed as units per milligram of protein (U/mgprot).

Superoxide peroxidation (SOD) was assayed by monitoring the rate of inhibition of reduction of nitroblue tetrazolium (NBT) by the enzyme (9). One unit of the SOD represents the amount of enzyme required to produce 50% inhibition of NBT reduction per minute.

Glutathione peroxidase (GSH-Px) activity was determined according to Flohe and Gunzler's method (10). One unit of GSH-Px consumes 1 μmol NADPH/min.

Catalase (CAT) activity was assayed by the method described by Aebi (11), based on the direct measurement of H_2O_2 decomposition at 25 ºC. One unit of CAT activity represents 1 μmol H_2O_2 decomposed per min.

Glutathione transferase (GST) was assayed as described Habig et al. (12), and the unit was defined as nanomole per liter of GSH used per minute.

All the enzyme activities were expressed as units per milligram of protein. The protein content was determined according to Bradford (13), with crystalline bovine serum albumin as a standard.

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.

Results

Growth performance

Compared to the control, ADG was decreased 5.29% and 8.60% ($P < 0.05$), F/G was increased 5.78% and 8.50% ($P < 0.05$) in the 100 and 150 mg/kg fluorine-treated groups, respectively (Table 1), and there was no significant difference between the 50 mg/kg fluorine group and the control group ($P > 0.05$). ADFI of growing pigs was not affected by the 3 fluorine levels treatments.

Serum biochemical values

As shown in Table 2, serum Ca and Mg concentrations of 150 mg/kg fluorine-added group were lower than those of the control group ($P < 0.05$). Serum P level was not affected by the addition of fluorine ($P > 0.05$).

The contents of serum albumin, cholesterol and triglycerides of the 150 mg/kg fluorine group tended to be lower ($P < 0.05$), while urea N and glucose levels of the 100 and 150 mg/kg fluorine-treated groups tended to be higher than those of the control group ($P < 0.05$).

The levels of serum GOT, GPT and LDH in pigs fed a diet supplemented with 150 mg/kg fluorine were significantly elevated compared to the control group ($P < 0.05$). Serum ALP levels of the 100 and 150 mg/kg fluorine groups increased 15.29% ($P < 0.05$) and 50.52% ($P < 0.001$) compared to the control group, respectively. All serum indexes in the 50 mg/kg fluorine group were similar to those in the control group.

Table 1. Growth performance of pigs.

Item	Fluorine (mg/kg)			
	0	50	100	150
Initial wt (kg)	26.79 ± 1.48	26.77 ± 1.29	26.80 ± 1.18	26.84 ± 1.29
Final wt (kg)	89.18 ± 5.96[a]	87.17 ± 4.41[a]	85.89 ± 4.95[ab]	83.87 ± 4.22[b]
ADG (g)	742.81 ± 61.09[a]	719.18 ± 63.92[ab]	703.50 ± 59.08[bc]	678.91 ± 53.86[c]
ADFI (kg)	2.18 ± 0.02	2.18 ± 0.02	2.19 ± 0.04	2.16 ± 0.05
F/G	2.94 ± 0.05[a]	3.03 ± 0.09[ab]	3.11 ± 0.10[bc]	3.19 ± 0.11[c]

[a-c] Means within a row with different superscripts differ significantly (P < 0.05).

Table 2. Serum biochemical values in pigs.

Item	Fluorine (mg/kg)			
	0	50	100	150
Ca, mmol/l	2.77 ± 0.18[a]	2.59 ± 0.23[ab]	2.57 ± 0.16[ab]	2.51 ± 0.13[b]
P, mmol/l	4.04 ± 0.58	4.10 ± 0.53	4.03 ± 0.51	3.64 ± 0.50
Mg, mmol/l	1.16 ± 0.11[a]	1.10 ± 0.10[a]	1.07 ± 0.09[a]	0.90 ± 0.09[b]
Total protein, g/l	88.59 ± 8.30	87.46 ± 8.56	86.78 ± 8.12	83.26 ± 10.19
Albumin, g/l	49.96 ± 3.96[a]	48.81 ± 4.27[ab]	47.47 ± 5.16[ab]	44.88 ± 3.15[b]
Urea N, mmol/l	6.08 ± 0.47[a]	6.38 ± 0.46[ab]	6.94 ± 0.63[b]	7.67 ± 0.76[c]
Cholesterol, mmol/l	3.58 ± 0.25[a]	3.54 ± 0.22[a]	3.49 ± 0.23[ab]	3.17 ± 0.29[b]
Triglycerides, mmol/l	0.85 ± 0.08[a]	0.82 ± 0.07[a]	0.77 ± 0.07[ab]	0.69 ± 0.05[b]
Glucose, mmol/l	6.86 ± 0.58[a]	6.89 ± 0.63[ab]	7.14 ± 0.46[b]	7.56 ± 0.67[b]
ALP, U/l	230.92 ± 26.65[a]	244.47 ± 32.58[ab]	266.23 ± 21.18[b]	347.59 ± 39.61[c]
GOT, IU/l	59.12 ± 5.02[a]	62.26 ± 5.79[a]	64.77 ± 6.37[ab]	70.81 ± 8.04[b]
GPT, IU/l	23.95 ± 2.01[a]	22.66 ± 2.01[a]	27.48 ± 2.18[a]	34.22 ± 2.98[b]
LDH, IU/l	519.63 ± 47.24[a]	513.67 ± 52.17[a]	544.23 ± 53.43 [a]	606.40 ± 58.63[b]

[a-c] Means within a row with different superscripts differ significantly (P < 0.05).

Lipid peroxidation

As shown in Tables 3 and 4, MDA levels in serum and liver were significantly elevated, 35.85% and 28.22%, due to 150 mg/kg fluorine addition compared to the controls, respectively ($P < 0.05$). Serum MDA level in 100 mg/kg fluorine additional group was also significantly higher than those of the control group ($P < 0.05$). Addition of 50 mg/kg fluorine had no effect on the MDA content of pigs ($P > 0.05$).

Antioxidant systems

As shown in Tables 3 and 4, T-AOC levels of serum and liver in the 100 and 150 mg/kg fluorine-treated groups significantly decreased compared to those in the controls ($P < 0.05$), and T-AOC concentrations in the liver of pigs fed the 50 mg/kg fluorine diet were significantly lower than those of the controls ($P < 0.05$). There was no significant effect of adding 50 mg/kg fluorine on serum T-AOC levels.

Table 3. MDA, T-AOC and antioxidants of serum.

Item	Fluorine (mg/kg)			
	0	50	100	150
MDA, nmol/ml	5.83 ± 0.64[a]	6.33 ± 0.42[a]	7.23 ± 0.61[b]	7.92 ± 0.70[b]
T-AOC, U/ml	4.39 ± 0.62[a]	3.87 ± 0.55[ab]	3.56 ± 0.51[bc]	2.92 ± 0.43[c]
SOD, U/ml	142.81 ± 11.45[a]	133.36 ± 11.39[ab]	129.42 ± 9.0[b]	116.10 ± 5.76[c]
GSH-Px, U/ml	760.77 ± 78.19[a]	743.27 ± 68.63[a]	712.33 ± 62.06[ab]	684.07 ± 51.82[b]
CAT, U/ml	39.10 ± 3.94[a]	31.32 ± 2.63[b]	30.18 ± 2.48[b]	25.42 ± 1.04[c]
GST, U/ml	39.03 ± 3.94[a]	37.69 ± 3.42[ab]	36.54 ± 3.88[ab]	36.04 ± 3.09[b]

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

Table 4. MDA, T-AOC and antioxidants of liver.

Item	Fluorine (mg/kg)			
	0	50	100	150
MDA, nmol/mgprot	1.63 ± 0.41[a]	1.77 ± 0.44[ab]	1.92 ± 0.48[ab]	2.09 ± 0.66[b]
T-AOC, U/mgprot	24.05 ± 2.36[a]	19.84 ± 2.32[b]	18.45 ± 1.75[b]	14.79 ± 1.02[c]
SOD, U/mgprot	72.04 ± 7.65[a]	67.91 ± 6.88[ab]	62.41 ± 6.21[bc]	61.48 ± 5.46[c]
GSH-Px, U/mgprot	119.59 ± 10.75[a]	108.72 ± 9.41[ab]	105.94 ± 9.57[b]	91.97 ± 8.86[c]
CAT, U/mgprot	61.37 ± 4.93[a]	58.83 ± 4.57[a]	51.82 ± 4.16[b]	48.28 ± 3.16[b]
GST, U/mgprot	50.75 ± 4.64[a]	41.80 ± 4.52[b]	36.27 ± 4.11[c]	33.06 ± 3.44[c]

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

The activities of serum SOD and CAT, liver SOD, GSH-Px and CAT of the 100 and 150 mg/kg fluorine-treated groups were markedly decreased compared to those of the control group ($P < 0.05$), and the activities of serum GSH-Px of the 150 mg/kg fluorine-treated group were significantly lower than those of the control group ($P < 0.05$). The addition of the 3 fluorine levels significantly decreased the serum CAT activities of pigs in comparison with the controls ($P < 0.05$).

GST activities of the liver in the groups given 3 concentrations of fluorine were significantly lower than those of the controls ($P < 0.05$), and the activities of serum GST of pigs fed the 150 mg/kg fluorine-added diet were decreased compared to the controls ($P < 0.05$). The data of GST activities in serum were similar among other 3 groups.

Discussion

The results in the present study were similar to the findings published by Burnell et al. (14), who reported reduced ADG ($P < 0.001$) for pigs consuming diets with 132 mg/kg fluorine concentration. Studies on broilers (15), hens (16) and calves (17) also indicated that excessive fluorine ingestion significantly decreased animal growth and production performances. It is conventionally considered that depressed appetite and consequently a feed consumption decrease resulted in the poor growth rate after feeding animals excessive fluorine levels. However, in the present study no significant difference was found in feed intake between fluorine–treated groups and the control group. On the other hand, the decreased F/G in the fluorine groups indicated that high fluorine intake resulted in poor growth performance of

animals by probably disturbing some nutrient utilization. Guenter and Hahn (18) pointed out that the toxic effects of fluorine on feed conversion of hens were not solely due to the reduced nutrient intake but also poor nutrient absorption and metabolic utilization also played an important role.

Fluorine interacts and alters the metabolism of calcium and magnesium (19) The decrease in serum calcium is related to a decrease of intestinal absorption of calcium by fluorine. A decrease of serum magnesium was considered due to an increase of magnesium fixation in tissues, mainly in bones and kidneys (20). However, serum phosphate content was not affected by fluorine in this study.

The liver, an organ of vital importance, was severely damaged by fluorine toxicity (21). Serum biochemical values and enzymatic activities of GPT, GOT, and LDH are sensitive serological indicators of liver toxicity. In our study these parameters were significantly altered by fluorine addition, suggesting that excessive fluorine might cause critical injury to the organ. These data indicated that the liver function of pigs was weakened by excessive ingestion of fluorine. The increased serum ALP following fluorine exposure may reflect a toxicity of fluorine for both osteoblasts (bone forming cells) and resorbing osteocytes (22).

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, the latter can indirectly reflect the status of the metabolism of free radicals, the degrees to which the tissue cells are attacked by free radicals and the lipid is peroxidated. In the present study, MDA concentrations in serum and liver of pigs in the fluorine groups largely increased, and correlated positively with the levels of fluorine. These results are consistent with the results reported by Shivarajashankara et al. (23), who demonstrated increased MDA levels in the red blood cells, liver and brain of rats. Similarly, MDA contents in serum of chicks were largely increased when treated with fluorine in different groups (3).

T-AOC, used to reflect the total capacity of antioxidant systems in the body in recent years, is an integrative index. Little is known about the effect of excessive fluorine intake on T-AOC in pigs; there are only reports on other animal species. Our data were similar to the results published by Kang et al. (24) and Li et al. (25), who reported that high fluorine intake significantly decreased serum T-AOC contents of chicks and its concentrations in the serum, liver, kidney, spleen and brain of goats.

SOD, GSH-Px and CAT are the main antioxidant enzymes in the body (26). These enzymes may scavenge unwanted $\cdot O_2^-$ and H_2O_2, and 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 (27). As shown above, decreased activity of each of them would induce increased free radicals, and so injure the corresponding tissues. In this study, the results showed that the activities of SOD, GSH-Px and CAT in the serum and liver of pigs in the fluorine-treated groups are lower than those of the controls to difference degrees, most of them significant ($P < 0.05$). These enzymes' activities showed a dose-effect correlation, and have significantly negative correlations with different levels of fluorine. 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. Our results were similar to the results from mice (28) and rats (29). However, there are several reports on elevated GSH-Px levels. We speculated that these differences in GSH-Px to the fluorosis in animals might be due to variations in dose, duration and route of fluorine administration, the stage of life at which fluorine was administered, the animal species used and individual tissue response.

GST existed in various tissues, especially in the liver. It can remove free radicals and its levels can reflect the antioxidant capacity of the body. This is in agreement with the findings reported by Dierickx and Beer (30); they found that fluorine inhibits GST activity in the rat liver in a dose-dependent manner. Our studies also showed that GST activities in the serum and liver of pigs were decreased when fluorine was added. Similarly, GST activities in the brain and gastrocnemius muscle of female mice (16) were markedly decreased when treated with 20 mg/kg/body weight for 14 days.

In conclusion, these results suggest that excessive ingestion of fluorine had a significant adverse effect on the growth performances and serum indexes of growing pigs. On the other hand, high fluorine intake can markedly alter MDA and T-AOC levels and some enzymes

activities associated with free radical metabolism in growing pigs. Our data may provide some evidence for further studying the mechanism of excess fluorine accumulation on the impairment of soft tissues.

References

1. Zhavoronkov, A.A., Strochkova, L.S.: Fluorosis: geographical pathology and some experimental findings. Fluoride, 1981; 14: 183-191.
2. Wei, Z.D., Wei, Y.: Fluoridation in China: a clouded future. Fluoride, 2002; 35: 1-4.
3. Liu, G.Y., Chai, C.Y., Cui, L.: Fluoride causing abnormally elevated serum nitric oxide levels in chicks. Environ. Toxicol. Pharmacol., 2003; 13: 199-204.
4. NRC. Nutrient requirements of swine [S]. (Tenth ed). Washington DC. USA: National Academic Press 1988.
5. Willis, J.B.: The determination of metals in blood serum by atomic absorption spectroscopy. I. Calcium. Spectrochim. Acta, 1960; 16: 259-278.
6. Fiske, C.H., Subbarow, Y.: The colorimetric determination of phosphorus. J. Biol. Chem., 1925; 66: 375-400.
7. Wills, E.D.: Mechanisms of lipid peroxide formation in animal tissues. Biochem. J., 1966; 99: 667-676.
8. Miller, N.J., Evans, C.R., Davies, M.J.: A novel method for measuring antioxidant capacity and its application to monitoring the antioxidant status in neonates. Clin. Sci., 1993; 84: 407-412.
9. Asada, K., Takahashi, M., Nagate, M.: Assay and inhibitors of spinach superoxide dismutase. Agric. Biol. Chem., 1974; 38: 471-473.
10. Flohe, L., Gunzler, W.A.: Assays of glutathione peroxidase. Methods Enzymol., 1984; 105: 114-121.
11. Aebi, H.: Catalase in vitro. Methods Enzymol., 1984; 105: 121-126.
12. Habig, W.H. Pabst, M.J., Jakoby, W.B.: Glutathione S-transferases: the first enzymatic step in mercapturic acid formation. J. Biol. Chem., 1974; 249: 7130-7139.
13. Bradford, M.M.: A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein dye binding. Anal. Biochem., 1976; 72: 248-254.
14. Burnell, T.W., Peo, E.R., Lewis, A.J., Crenshaw, J.D.: Effect of dietary fluorine on growth, blood and bone characteristics of growing-finishing pigs. J. Anim. Sci., 1986; 63: 2053-2067.
15. Huyghebaert, G., De Groote, G., Froyman, R., Derijcke, J.: Effect of dietary fluoride on performances and bone characteristics of broilers and the influence of drying and defatting on bone-breaking strength. Poult. Sci., 1988; 67: 950-955.
16. Vani, M.L., Reddy, K.P.: Effects of fluoride accumulation on some enzymes of brain and gastrocnemius muscle of mice. Fluoride, 2000; 33: 17-26.
17. Kapoor, V., Prasad, T.: Effect of dietary fluorine on growth, nutrient digestibility and mineral balances in calves. Indian J. Anim. Sci., 1991; 61: 1326-1329.
18. Guenter, W., Hahn, P.H.B.: Fluorine toxicity and laying hen performance. Poult. Sci., 1986; 65: 769-778.
19. Rich, C., Ensinck, J.: Effect of sodium fluoride on calcium metabolism in human beings. Nature, 1961; 191: 184-185.
20. Soldatovic, D., Nedeljkovic, M.: Change of calcium and magnesium levels in the organs of rabbits poisoned by fluoride. Acta Pharm. Jugoslav. 1974; 4: 101-105.
21. Kour, K., Koul, M.J., Koul, R.L.: Histological changes in liver following sodium fluoride ingestion. Fluoride, 1981; 14: 119-123.
22. Krook, L., Ronald R.M.: Fluoride and alkaline phosphatase. Fluoride, 1998; 31: 177-182.
23. Shivarajashankara, Y.M., Shibashamkara, A.R., Gopalakrishna, B.P., Hanumanth, R.S.: Brain lipid peroxidation and antioxidant systems of young rats in chronic fluoride intoxication. Fluoride 2002; 35: 197-203.
24. Kang, S.L., Guo, G.Q., Chai, C.Y.: Effects of fluoride on antioxidant contents in chickens. Chinese J. Vet. Med., 2001; 37: 15-16.
25. Li, S., Xu, S.W., Kang, S.L.: Studies on the antioxidant defense system of chronic fluorosis in goat. Chinese J. Vet. Sci. Technol., 2003; 33: 14-18.
26. Krajcovicova, K.M., Ursinyova, M., Blazicek, P., Spustova, V., Ginter, E., Hladikova, V., Klvanova, J.: Free radical disease prevention and nutrition. Bratisl. Lek. Listy., 2003; 104: 64-68.
27. Rzeuski, R., Chlubek, D., Machoy, Z.: Interactions between fluoride and biological free radical reactions. Fluoride, 1998; 31: 43-45.
28. Sharma, A., Chinoy, N.J.: Role of free radicals in fluoride-induced toxicity in liver and kidney of mice and its reversal. Fluoride, 1998; 31: S26.
29. Bian, J.C., Xian, S.M., Ye, P., Guan, S.F., Yuan, L., Ji, Q.X.: Changes of fluorine, anti-oxidantion enzymes and effect of selenium in rats with chronic fluorosis. Acta Nutrimenta Sinica, 1997; 19: 43-49.
30. Dierickx, P.J., Beer, D.J.O.: Interaction of fluoroacetamide with rat liver glutathione S-transferases: Evidence for detoxification roles by defluorination. Fluoride, 1983; 16: 145-151.