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Scientific Research

Effects of High Dietary Fluoride on Serum Biochemistry and Oxidative Stress Parameters in Broiler Chickens

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Abstract

The aim of this study was to investigate the effects of high dietary fluoride (F) on serum biochemistry and oxidative damage in broiler chickens. 280 one-day-old healthy avian broiler chickens were randomly allotted into four equal groups and fed with a corn-soybean basal diet containing 22.6 mg·F/kg (control group) or same basal diets supplemented with 400, 800, and 1200 mg·F/kg (high F groups I, II, and III) in the form of sodium fluoride for 42 days. At 42 days of age, the serum F content was markedly higher in the three high F groups than that in the control group. From 28 to 42 days of age, the contents of serum total protein (TP) and albumin (ALB) were significantly lower in the three high F groups. From 14 to 42 days of age, the activities of alkaline phosphatase (ALP), alanine aminotransferase (ALT) and the creatinine (Crea) contents in the serum showed a marked increase in the three high F groups; aspartate aminotransferase (AST) activity and uric acid (Ua) content were significantly increased, and a significant increase in the content of malondialdehyde (MDA) along with marked decreases in the activities of total superoxide dismutase (T-SOD), glutathione peroxidase (GSH-Px), catalase (CAT), the glutathione (GSH) content and the ability to inhibit hydroxyl radical were observed in the high F groups II and III. In conclusion, F has accumulated in the blood circulatory system and dietary F in the range of 800 - 1200 mg/kg could significantly induce abnormalities of bone, liver and kidney, inhibit the synthesis of protein, enhance lipid peroxidation and disturb the antioxidative system of broiler chickens.

Keywords

Biochemical Indexes, Broiler Chickens, High Dietary Fluoride, Oxidative Stress, Serum

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1. Introduction

Fluoride (F) is known to cause fluorosis in large doses and can even lead to death in excessive amounts [1]. In addition, F is a well-known inhibitor of numerous enzymes [2] [3]. Study has reported that changes on some biochemical parameters are possibly ascribed to increased oxidative stress in F toxication [4]. In recent decades, many investigations have focused on the relationship between F and free radical toxicity in humans, rats, mice, pigs, sheep or rabbits [5]-[10].

In our recent studies, we found that high dietary F induced oxidative stress in the cecal tonsil and the intestinal mucosa of broiler chickens [11] [12]. However, limited studies have focused on the effects of high dietary F on serum biochemistry and oxidative damage parameters in broiler chickens. Chen *et al*. [13] have reported that high dietary F decreases the superoxide dismutase (SOD), glutathione peroxidase (GSH-Px) activities and increases the malondialdehyde (MDA) content in the serum of broiler chickens. In addition, Liu *et al*. [14] have reported that F decreases the activities of SOD, catalase (CAT), GSH-Px and increases the MDA content in the serum of chicks, but the serum biochemical indexes, and the ability to inhibit hydroxyl radical and glutathione (GSH) content in the serum have not been reported in their experiments. Abdelhamid and Dorra [15] have studied that feed-borne F intoxication decreases the content of total protein (TP), albumin (ALB) and hepatic enzymatic activity in broiler chicks.

Thus, the objective of this study was to investigate the effects of high dietary F on serum biochemical parameters and oxidative stress indicators by detecting the activities of aspartate aminotransferase (AST), alanine aminotransferase (ALT), alkaline phosphatase (ALP), total superoxide dismutase (T-SOD), CAT, GSH-Px, the ability to inhibit hydroxyl radical and the contents of TP, ALB, creatinine (Crea), uric acid (Ua), GSH and MDA.

2. Materials and Methods

2.1. Broiler Chickens and Diets

280 one-day-old healthy avian broiler chickens (purchased from Wenjiang commercial poultry hatchery in Sichuan Province) were randomly divided into four equal groups with 70 broiler chickens in each and fed a corn-soybean diet as follows: a control group with 22.6 mg·F/kg, and three high F groups I, II, and III with 400, 800, and 1200 mg·F/kg diet, respectively. They were housed in corresponding cages with electrically heated units and had unrestricted access to water and the above-mentioned diets for 42 days.

All the dietary nutrition requirements were adequate according to the US National Research Council (NRC) (1994) [16].

Animal care and protocol of the experiment were approved by Sichuan Agricultural University Animal Care and Use Committee.

2.2. Serum Samples Collection

At 14, 28, and 42 days during the experiment, five broiler chickens in each group were phlebotomized from jugular vein to collect serum after a 12-h overnight fast. Non-anticoagulative blood samples were clotted for 15 min at room temperature and then centrifuged at 3000 rpm for 15 min. The serum samples were collected in eppendorf tubes and stored at −20˚C until analysis.

2.3. Determination of the Content of Serum F

Before determination, the serum samples were thawed. The content of serum F ions was determined with fluoride ion-selective electrode INESA PF-1-01 (China).

2.4. Determination of Serum Biochemical Parameters

The activities of T-SOD, CAT and GSH-Px and the contents of GSH, MDA, and the ability to inhibit hydroxy radicals in the serum were assayed by biochemical reagent kits (T-SOD, A001-1; CAT, A007-2; GSH-Px, A005; GSH, A006-1; MDA, A003-1; ability to inhibit hydroxyl radicals, A018). Meanwhile, the activities of AST, ALT and ALP, the contents of TP, ALB, Crea, Ua were measured using the corresponding reagent kits (AST, C0010-2; ALT, C009-2; ALP, A059-2; TP, A045-2; ALB, A028-1; Crea, C011-1; Ua, C012, respectively). All

the aforementioned commercial reagent kits were purchased from Nanjing Jiancheng Bioengineering Institute (China). All the experimental operating procedures followed the manufacturer's instructions. The absorbances of T-SOD, CAT, GSH-Px, GSH, MDA, ability to inhibit hydroxy radicals were measured at 550, 240, 412, 420, 532, 550 nm and the absorbances of AST, ALT, ALP, TP, ALB, Crea, Ua were detected at 510, 510, 520, 595, 628, 510 and 690 nm, respectively, with a microplate reader Bio-Rad 680 (USA).

2.5. Statistical Analysis

All the grouped data were statistically evaluated with SPSS software 17.0 for Windows and expressed as means ± standard deviation (SD). Comparison of means between four groups were conducted using one-way analysis of variance (ANOVA) followed by least significant difference (LSD) post hoc test and P-values of less than 0.05 ($p < 0.05$) were considered to indicate statistical significance.

3. Results

3.1. Changes in the Content of Serum F

As shown in **Table 1**, the serum F content was markedly higher in the three high F groups than that in the control group at the 42 days of age ($p < 0.01$).

3.2. Changes in the Serum Oxidative Stress Parameters

From 14 to 42 days of age, the activities of T-SOD, CAT, GSH-Px, the content of GSH and the ability to inhibit hydroxyl radicals were significantly lower ($p < 0.01$) in high F groups II and III than those in the control group, whereas the MDA content was significantly increased ($p < 0.01$) in the abovementioned high F groups in comparison with the control group (**Table 2**).

3.3. Changes in the Serum Biochemical Indexes

As shown in **Table 3**, the contents of serum TP and ALB were significantly lower in the three high F groups from 28 to 42 days of age ($p < 0.05$ or $p < 0.01$), the activities of ALP, ALT and the contents of Crea in the serum were markedly higher ($p < 0.05$ or $p < 0.01$) in the three high F groups; AST activity and Ua content were significantly higher in the high F groups II and III ($p < 0.05$ or $p < 0.01$) than those in the control group from 14 to 42 days of age.

4. Discussion

4.1. Effects of High Dietary F on the Content of Serum F in Broiler Chickens

In blood, about 75% of F remains free in plasma, therefore, determination of the content of serum F reflects the F content in blood to some extent. In our present study, the serum F content was markedly higher in the three high F groups than that in the control group at 42 days of age ($p < 0.01$). The increased content of serum F was reported by many anthors [17]-[19]. Likewise, the content of urine F significantly increases in rat [20], rabbit [18] and fluorotic human [19] has been reported. Kono *et al.* [21] reported that serum F concentrations in F-exposed workers with impaired renal function were strikingly higher than in other patients with chronic renal failure (CRF) and F-exposed workers. We may speculate that the increased content of serum F is due to renal dysfunction induced by F, which makes the ability to excrete F decreased and eventually leads to F accumulation in the blood circulatory system.

4.2. Effects of High Dietary F on Serum Oxidative Stress Parameters in Broiler Chickens

The generation and concentration of reactive oxygen species (ROS) in the body is controlled by the antioxidative system, which enables transformation of ROS into inactive and harmless compounds or molecules [22] [23]. Oxidative stress is a result of disturbed antioxidative/pro-oxidative balance and leads to biological damage, and is regarded as one of the causes of several pathologies that affect poultry growth [22] [24]. Lipid peroxidation is the fundamental parameter of oxidative stress and MDA is a terminal product of lipid peroxidation, and the content of MDA can be used to estimate the extent of lipid peroxidation [25] [26].

Table 1. Changes of the serum F content in broilers.

Days	Groups	F (μg/mL)
42 days	Control group	0.06 ± 0.01
	High F group I	0.22 ± 0.01**
	High F group II	0.75 ± 0.05**
	High F group III	1.79 ± 0.09**

Data are presented with the means ± standard deviation (n = 5). **p < 0.01, compared with the control group.

Table 2. Changes in the serum oxidative stress parameters of broilers.

Parameters	Groups	Days		
		14 days	28 days	42 days
T-SOD (U/ml)	Control group	127.74 ± 4.15	150.91 ± 2.10	162.34 ± 3.14
	High F group I	127.48 ± 2.52	150.65 ± 2.69	161.92 ± 2.37
	High F group II	102.25 ± 1.39**	122.65 ± 1.93**	145.77 ± 4.72**
	High F group III	101.56 ± 1.12**	119.20 ± 2.20**	140.91 ± 1.79**
GSH-Px (U/ml)	Control group	544.70 ± 6.94	497.70 ± 5.33	442.57 ± 14.17
	High F group I	527.63 ± 11.06*	483.51 ± 14.39	421.37 ± 11.12*
	High F group II	485.10 ± 4.95**	382.90 ± 16.02**	326.24 ± 10.19**
	High F group III	446.92 ± 13.87**	348.97 ± 14.16**	302.12 ± 6.55**
CAT (U/ml)	Control group	8.24 ± 0.58	12.99 ± 0.75	13.76 ± 0.55
	High F group I	7.80 ± 0.36	12.39 ± 0.76	13.12 ± 0.77
	High F group II	6.64 ± 0.52**	9.34 ± 0.33**	11.65 ± 0.51**
	High F group III	6.45 ± 0.50**	8.17 ± 0.38**	11.19 ± 0.57**
GSH (mgGSH/L)	Control group	13.88 ± 0.71	16.96 ± 1.06	17.41 ± 0.65
	High F group I	13.61 ± 0.86	16.05 ± 0.61	16.60 ± 1.04
	High F group II	11.57 ± 0.83**	12.92 ± 0.93**	13.78 ± 0.99**
	High F group III	11.02 ± 0.83**	10.61 ± 0.73**	11.83 ± 0.45**
MDA (nmol/ml)	Control group	3.44 ± 0.18	3.84 ± 0.76	5.96 ± 0.09
	High F group I	3.64 ± 0.05	4.66 ± 0.03	6.45 ± 0.33*
	High F group II	5.03 ± 0.96**	5.58 ± 0.85**	6.73 ± 0.17**
	High F group III	5.27 ± 0.33**	5.89 ± 0.46**	8.90 ± 0.40**
The ability to inhibit hydroxyl radicals (U/ml)	Control group	1357.86 ± 2.01	1361.47 ± 1.59	1362.05 ± 1.55
	High F group I	1356.67 ± 3.12	1357.80 ± 2.64	1360.33 ± 1.38
	High F group II	1348.80 ± 0.18**	1345.37 ± 3.59**	1355.48 ± 0.98**
	High F group III	1345.24 ± 1.56**	1344.64 ± 1.30**	1350.09 ± 3.30**

Data are presented with the means ± standard deviation (n = 5). *p < 0.05, compared with the control group. **p < 0.01, compared with the control group.

GSH-Px and CAT, as preventive antioxidants, and T-SOD, a chain-breaking antioxidant, play an important role in protecting against the deleterious effects of lipid peroxidation [27]. The limited activity of CAT causes accumulation of hydrogen peroxide, which in turn is the inhibitor of SOD [23]. GSH plays an important role in antioxidant defense against ROS through a nonenzymatic mechanism and regarded as an early biological marker of the oxidative stress [28] [29]. Meanwhile, GSH-Px metabolizes hydrogen peroxide to water by using GSH as a hydrogen donor [30]. The decreased GSH-Px activity may also be due to the decreased availability of GSH in the serum. Hydroxyl radical is one of the major oxygen radicals that can cause oxidative stress [12]. In our recent studies, we have found the abilities to inhibit hydroxyl radicals in the cecal tonsil [11] and intestinal mucosa

Table 3. Changes in the serum biochemical indexes of broilers.

Parameters	Groups	Days		
		14 days	28 days	42 days
TP (g/L)	Control group	27.47 ± 0.92	28.34 ± 1.13	30.85 ± 0.43
	High F group I	27.12 ± 1.00	26.77 ± 0.49*	25.73 ± 1.02**
	High F group II	24.33 ± 1.07**	23.57 ± 0.65**	25.94 ± 0.98**
	High F group III	18.09 ± 0.60**	18.72 ± 1.02**	22.49 ± 0.56**
ALB (g/L)	Control group	16.10 ± 0.77	19.90 ± 0.65	31.51 ± 1.13
	High F group I	17.60 ± 0.59**	17.30 ± 0.40**	28.93 ± 0.92**
	High F group II	19.80 ± 0.56**	17.10 ± 0.37**	27.27 ± 0.40**
	High F group III	18.70 ± 0.65**	15.19 ± 0.37**	23.70 ± 0.92**
ALP (U/100mL)	Control group	597.71 ± 8.82	781.70 ± 16.58	1260.39 ± 19.40
	High F group I	998.03 ± 12.11**	1884.29 ± 21.05**	2228.92 ± 21.15**
	High F group II	1133.82 ± 23.53**	2237.26 ± 14.23**	2407.08 ± 17.71**
	High F group III	1483.33 ± 15.56**	2566.02 ± 26.35**	2491.80 ± 22.19**
Crea (μmol/L)	Control group	204.28 ± 5.35	239.64 ± 2.48	269.11 ± 4.13
	High F group I	243.56 ± 4.36**	246.19 ± 2.96**	275.03 ± 5.27*
	High F group II	261.26 ± 4.59**	251.42 ± 2.71**	294.64 ± 1.39**
	High F group III	267.15 ± 3.34**	280.89 ± 1.61**	304.46 ± 3.08**
Ua (mg/L)	Control group	33.80 ± 0.75	36.62 ± 0.94	38.73 ± 0.92
	High F group I	34.68 ± 0.97	39.79 ± 0.84**	40.66 ± 0.67**
	High F group II	39.08 ± 1.30**	42.72 ± 0.80**	46.24 ± 0.92**
	High F group III	42.02 ± 0.84**	47.72 ± 1.31**	48.83 ± 0.97**
AST (U/L)	Control group	37.86 ± 2.85	40.05 ± 1.64	43.51 ± 1.96
	High F group I	40.94 ± 1.59	46.56 ± 1.90**	62.76 ± 1.29**
	High F group II	41.76 ± 1.37*	48.79 ± 1.70**	68.04 ± 1.91**
	High F group III	53.94 ± 2.49**	68.13 ± 2.87**	70.13 ± 2.09**
ALT (U/L)	Control group	2.50 ± 0.16	2.72 ± 0.09	6.21 ± 0.21
	High F group I	3.53 ± 0.14**	4.04 ± 0.11**	9.05 ± 0.74**
	High F group II	7.41 ± 0.42**	8.25 ± 0.66**	14.87 ± 0.56**
	High F group III	10.30 ± 0.34**	13.93 ± 0.99**	16.70 ± 0.92**

Data are presented with the means ± standard deviation (n = 5). *$p < 0.05$, compared with the control group. **$p < 0.01$, compared with the control group.

[12] of broiler chickens were decreased in the high F groups II and III. In the present study, a significant increase ($p < 0.01$) in the content of MDA along with the marked decreases ($p < 0.01$) in the activities of T-SOD, GSH-Px, CAT, the content of GSH and the ability to inhibit hydroxyl radical were observed in high F groups II and III from 14 to 42 days of age, suggesting that F inhibited the activities of serum antioxidants and increased the concentration of ROS in the blood circulatory system of broiler chickens. The results in this study are similar with the results from chicks [14], pigs [8]. Likewise, Li and Cao [31] have found a decrease in the activities of SOD and GSH-Px in people living in areas with endemic fluorosis. However, Chlubek *et al.* [32] found no changes in the activity of GSH-Px or concentrations of MDA in the pancreas of rats exposed to NaF in drinking water during four months. Reddy *et al.* [10] reported that there was no significant difference in the lipid peroxidation, GSH and vitamin C, as well as in the activities of SOD, GSH-Px, and CAT in the blood of fluorotic humans and rabbits. Shivarajashankara *et al.* [33] found the increased GSH level and GSH-Px activity in the erythrocytes, brain and liver of F-treated rats. Results obtained by different authors are contradictory, but most reports favor the oxidative stress theory of F.

4.3. Effects of High Dietary F on Serum Biochemical Parameters in Broiler Chickens

F toxicity in animals is multifarious [2]. F ions pass the intestinal barrier and are deposited in several organs and body through the blood system [34]. It has been reported that F toxication affects protein synthesis by primary causing destruction of polypeptide chains and weakness of amino-acid bindings in proteins [35] [36]. In our study, the serum TP and ALB contents were significantly decreased ($p < 0.05$ or $p < 0.01$) in the three high F groups I, II and III from 28 to 42 days of age, which demonstrated that excessive F caused a reduction of protein synthesis. Our results are agreed with other findings of the literature realized on mice [37], children [38], fish [39]. However, Cenesiz *et al*. [40] reported there was no significant change between the serum ALB values of control and fluorosed sheep.

As a very active site of metabolism, the liver is especially susceptible to F intoxication [37]. ALP, ALT and AST are important indicators of liver damage in clinic findings. These enzymes were secreted to blood in hepatocellular injury and their levels increase [41]. In the present study, a marked increase ($p < 0.05$ or $p < 0.01$) in the activities of serum AST, ALT and ALP was observed in the high F groups II and III or in the three high F groups from 14 to 42 days of age, which is similar with the observations of children [38], rats [42] and pigs [26]. These data indicated that the hepatic function of broiler was weakened by excessive ingestion of F. It remains controversial about whether or not excessive F intake induces damage to human liver functions. Xiong *et al*. [43] found no significant differences in the levels of serum AST and ALT in children with and without dental fluorosis. ALP also is the marker enzyme of F toxicosis and bone pathology [42]. The increased serum ALP activity following F exposure may reflect a toxicity of F for both osteoblasts and resorbing osteocytes [44]. In the present study, the increased serum ALP activity in the three high F groups may imply that dietary F induced bone disorder in broiler chickens. However, Pillai *et al*. [2] reported that a sublethal concentration of F induced a decline in the serum ALP activity.

The major route for the removal of F from the body is by the kidney [45]. High concentrations of F usually lead to kidney damage, including vacuolization and necrosis of tubules, hypertrophy and atrophy of glomeruli, interstitial edema, and interstitial nephritis [46]. Serum Crea levels are frequently used as a screening test for renal dysfunction [47]. Ua is the main end-product in nitrogen metabolism in birds [48]. Elevated serum Ua is a marker for decreased renal function [49]. The increased serum Ua and Crea in this study may be explained by the dysfunction of kidney due to the damage induced by F. Also Ua has a protective role in defence mechanisms of the body and is a lipid peroxidation inhibitor and a radical scavenger [50]. Decreased serum Ua was reported in children [51] and mice [7].

5. Conclusion

Based on the results observed in the present study and the aforementioned discussion, it is concluded that F has accumulated in the blood circulatory system and dietary F in the range of 800 - 1200 mg/kg could significantly induce abnormalities of bone, liver and kidney, inhibit the synthesis of protein, enhance lipid peroxidation and disturb the antioxidative system of broiler chickens. Our data provide some information for clinical diagnosis of fluorosis and for further studying the mechanism of excessive F accumulation on the damage of soft tissues in broilers.

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List of Abbreviations and Acronyms

GSH: Glutathione
T-SOD: Total superoxide dismutase
CAT: Catalase
GSH-Px: Glutathione peroxidase
MDA: Malondialdehyde
TP: Total protein
ALB: Albumin
ALT: Alanine aminotransferase
AST: Aspartate aminotransferase
Crea: Creatinine
Ua: Uric acid
ALP: Alkaline phosphatase

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