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Effects of Excess Dietary Fluoride on Serum Biochemical Indices, Egg Quality, and Concentrations of Fluoride in Soft Organs, Eggs, and Serum of Laying Hens

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Abstract This study was conducted to investigate the effects of excess dietary fluoride (F) on serum biochemical indices, egg quality, and concentrations of F in soft tissues, eggs, and serum of laying hens. Commercial laying hens (n = 576, 51 weeks of age) were randomly allotted to 6 treatments with 6 replicates of 16 birds. The basal diets contained fluorine inclusions at a level of 16 mg/kg, and graded sodium fluoride was added to the basal diet to achieve fluorine inclusions, respectively, at a level of 200, 400, 600, 800, and 1000 mg/kg in the experimental diets. Dietary F levels at 600, 800, and 1000 mg/kg decreased ($P < 0.05$) albumin height and yolk color, while eggshell strength and eggshell thickness significantly decreased at 800 and 1000 mg/kg, respectively, compared with the control group. Fluoride concentrations in eggshell, albumin, yolk, liver, kidney, ovary, and oviduct responded to dietary F levels positively, and F concentrations in eggshell were the highest. Fluorine concentrations in albumin and yolk increased with the feeding time at the same dietary F levels ($P < 0.05$). Dietary F level at 400 mg/kg increased serum calcium level and activity of glutamic oxalacetic transaminase ($P < 0.05$). In conclusion, dietary F levels at 600 mg/kg decreased albumin height and yolk color, while eggshell strength and eggshell thickness significantly decreased at 800 and 1000 mg/kg, respectively. F concentrations in soft tissues, albumin, yolk, and eggshell of layers had a positive correlation with dietary F levels. By disturbing Ca and phosphorus metabolism, dietary F levels affected the formation of eggshell, reducing eggshell strength and eggshell thickness.

Keywords Fluoride · Laying hen · Egg quality · Retention · Serum biochemical indices

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Introduction

It was believed that fluorine (F), naturally existing in soil, water, and food, is required in small quantities for the maintenance of human's and animals' health. A report of World Health Organization committee indicated that F is essential for life [1]. Fluoride is required for mineralization of bone and teeth; maintenance of fertility; and activation of certain enzymes, such as alkaline phosphatase [2]. However, excessive F intake for a long period of time may cause adverse impacts on skeleton; teeth [3]; and many other systems such as testicular tissues, liver, spleen, and kidney [4–9].

The rock phosphate, containing approximately 13–14% phosphorus and 3–4% fluoride, is the starting material for almost all chemically processed phosphates in mineral mixture and animal feed supplements. The defluoridation process of rock phosphate increases the cost of the feed supplements; hence, some manufacturers skip this step, leading to high fluoride concentrations in mineral mixtures and animal feed supplements [10]. There were several reports documenting mineral supplements as a major source of F toxicity in livestock, such as dairy cattle and buffalo [11–13]. Fluorosis in the layer industry have also appeared in different provinces of China in recent years. However, the information on the effect of excess dietary F on laying hens is unavailable. The aim of this study was to evaluate the effects of excess dietary F levels

on serum biochemical indices, egg quality, concentrations of F in eggs, soft tissues, and serum of laying hens.

Materials and Methods

The research was conducted with reference to the Chinese Guidelines for Animal Welfare and approved by the Animal Welfare Committee of the Animal Science College of Zhejiang University (Hangzhou, China).

Birds and Housing

Five-hundred and seventy-six 51-week-old Jinghong no. 1 commercial laying hens with similar performance, obtained from a commercial layer farm (Hangzhou, China), were randomly distributed to 6 treatments with 6 replicates of 16 hens. There were four hens in an individual cage (45 × 45 × 50 cm) equipped with two nipple drinkers and one feeder. Hens were kept in three-layer complete ladder cages and were fed ad libitum twice daily at 07:00 am and 15:00 pm, and water was available all the time. Cages were randomly located in a ventilated room with temperature between 20 and 25 °C and 16 h/day of illumination (10 to 20 lx).

Experimental Diets

In the control group, hens were fed a corn-soybean meal basal diet including 16-mg/kg F inclusion. Sodium fluoride (NaF; 99% purity, Hushi, Shanghai, China) was added to the basal diet to achieve concentrations of 200, 400, 600, 800, and 1000 mg F/kg of diet in experimental groups, respectively. The experiment lasted for 11 weeks, including a 2-week adaptation period and a 9-week experimental period. The diets were formulated in accordance with NY/T 33–2004 (Chicken Feeding Standard, Agricultural Industry Standard of the People's Republic of China), and ingredients as well as nutrient levels are presented in Table 1.

Table 1 Ingredient and nutrient composition of the basal diet (air-dry basis)

Items	Composition
Ingredients	Contents (%)
Corn	59
Soybean meal	23.9
Wheat bran	2
Limestone	8.45
Calcium hydrophosphate	1.65
Premix[a]	5
Total	100
Nutrient[b]	
Metabolizable energy (MJ/kg)	10.45
Crude protein (%)	15.45
Lysine (%)	0.79
Methionine (%)	0.36
Calcium (%)	3.43
Total phosphorus (%)	0.60

[a] The premix provided the following per kilogram of the diet: vitamin A, 7600 IU; vitamin D3, 2000 IU; vitamin E, 15 IU; vitamin K, 2 mg; thiamine, 1 mg; riboflavin, 8.5 mg; calcium pantothenate, 50 mg; niacin, 32.5 mg, pyridoxine, 8 mg; biotin, 2 mg; folic acid, 5 mg; vitamin B12, 5 mg; choline, 500 mg; Mn, 65 mg; I, 1 mg; Fe, 65 mg; Cu, 10 mg; Zn, 66 mg; Se, 0.12 mg

[b] Estimated from Chinese feed database provided with tables of feed composition and nutritive values in China (2015, 26th edition)

Sample Collection

At 61 weeks of age, 12 layers per treatment (2 layers per replicate) were sacrificed by bleeding of the jugular vein after 12-h fasting (water offered ad libitum) to collect liver, kidney, oviduct, and ovary samples. Samples were rinsed twice with ice-cold PBS and then dried with filter paper to avoid blood contamination. Blood samples collected during bleeding from jugular vein were centrifuged for 10 min (958×*g*) to separate out serum. In addition, 24 eggs (4 eggs from each replicate) for each treatment were randomly selected for egg quality at the end of the fourth week and ninth week and in the control, 400-, and 1000-mg/kg F groups; four eggs were collected separately to detect the F in albumin, yolk, and eggshells (eggshell membrane removed) at the end of the first, third, fifth, seventh, and ninth weeks. All samples were kept at −80 °C for analysis.

Experimental Parameters Measured

The contents of F in albumin, yolk, eggshell, serum, liver, kidney, oviduct, and ovary were measured according to a potentiometric method using an ion-selective electrode (Shanghai, Leici, PF-101) [14]. Eggs were weighed and cracked, and then albumin height, Haugh unit, yolk color, eggshell thickness, and eggshell strength were determined with a digital egg tester (DET-6000; Nabel Co. Ltd., Kyoto, Japan). Eggshell thickness (without the eggshell membrane) was measured using the middle part of the eggshell. The levels of serum total protein (TP), albumin (ALB), urea nitrogen (BUN), urea acid (UA), calcium (Ca), and phosphorus (P) and activities of glutamic oxalacetic transaminase (GOT) and glutamic pyruvic transaminase (GPT) were assayed and calculated followed by the protocols of commercial kits (Nanjing Jiancheng Bioengineering Institute, Nanjing, China).

Statistical Analyses

Procedures of one-way ANOVA from SPSS 20.0 software (SPSS Inc., Chicago, IL) were applied for egg quality,

Table 2 Effects of dietary fluoride (F) levels on egg quality

Items	Trail period (week)	F concentration in dietary (mg/kg)						SEM	*P* value
		16	200	400	600	800	1000		
Albumin height (mm)	4th	8.79 a	8.73 ab	8.78 a	8.17 bc	8.22 abc	7.92 c	0.19	0.000
	9th	7.23 a	7.11 ab	7.09 ab	6.96 b	6.87 b	6.92 b	0.086	0.003
Yolk color	4th	7.59 a	7.46 ab	7.42 ab	7.33 ab	6.99 bc	6.75 c	0.18	0.000
	9th	7.25 a	7.08 ab	6.89 bc	6.81 bc	6.71 c	6.33 d	0.1	0.000
Haugh unit	4th	90.98	90.32	90.70	89.38	88.50	88.19	1.07	0.06
	9th	82.76 a	83.01 a	81.84 ab	81.41 ab	80.90 ab	79.25 b	1.14	0.03
Eggshell strength (kgf/m^2)	4th	3.74 a	3.38 ab	3.22 ab	3.14 bc	2.90 bc	2.62 c	0.18	0.000
	9th	3.27 a	3.07 abc	3.16 ab	3.04 abc	2.82 c	2.88 bc	0.11	0.003
Eggshell thickness (mm)	4th	0.37 a	0.36 ab	0.35 ab	0.34 bc	0.33 bc	0.32 c	0.01	0.000
	9th	0.36 a	0.35 a	0.35 a	0.34 ab	0.32 bc	0.32 c	0.01	0.000

Results are the mean and SEM of six replicates, with four eggs per replicate. Means within a row with no common lowercase letters differ significantly ($P < 0.05$)

biochemical indices, and F concentration-level data analysis. When significant differences were found ($P < 0.05$), Tukey post hoc tests were performed.

Results

Effect of Dietary F Levels on Egg Quality

The data of albumin height, yolk color, Haugh unit, eggshell strength, and eggshell thickness are presented in Table 2. Adding F to the basal diet significantly decreased ($P < 0.05$) the levels of albumin height, yolk color, eggshell strength, and eggshell thickness, both at the end of the fourth week and ninth week, compared to the control groups, respectively. At the end of ninth week, dietary F level at 1000 mg/kg significantly decreased Haugh unit ($P < 0.05$), compared to the control group.

Effects of Dietary F Levels on Contents of F in Partial Organs and Eggs of Laying Hens

Table 3 shows that the F residues in eggshell, liver, kidney, ovary, and oviduct of laying hens increased significantly with the increase of dietary F supplemental levels ($P < 0.05$). Dietary F levels from 200 to 1000 mg/kg accelerated the F deposition in eggshell, liver, kidney, ovary, and oviduct significantly ($P < 0.05$).

Contents of F in albumin and yolk are illustrated in Fig. 1. At the end of the first week, the contents of F in albumin and yolk did not differ in 400-mg/kg F group, while those had a significant increase in 1000-mg/kg F group ($P < 0.05$), compared to the controls. At the end of the third, fifth, seventh, and ninth weeks, the contents of F in albumin and yolk had a significant increase in 400- and 1000-mg/kg F groups ($P < 0.05$), as compared to those in the controls, and a significant difference was found between 400- and 1000-mg/kg F groups ($P < 0.05$). The contents of F in albumin and yolk in

Table 3 Effects of dietary F level on contents of fluoride (F) in egg shell, serum, oviduct, ovary, liver, and kidney

Item	F concentration in dietary (mg/kg)						SEM	*P* value
	16	200	400	600	800	1000		
Eggshell	6.58 f	19.15 e	52.86 d	79.15 c	164.71 b	203.26 a	1.93	0.000
Ovary	3.38 e	3.56 de	4.97 cd	5.97 bc	6.99 b	9.16 ba	0.46	0.000
Oviduct	3.54 d	3.75 d	3.69 d	4.88 c	6.11 b	6.63 a	0.10	0.000
Liver	3.97 d	4.32 cd	5.26 bcd	5.88 bc	6.75 b	8.56 a	0.59	0.000
Kidney	2.74 c	4.37 c	6.20 b	6.76 b	9.47 a	10.46 a	0.52	0.000
Serum	11.26	11.31	11.94	13.04	12.10	11.28	1.39	0.77

Each value represents the mean of six replicates of two birds each. Means within a row with no common lowercase letters differ significantly ($P < 0.05$)

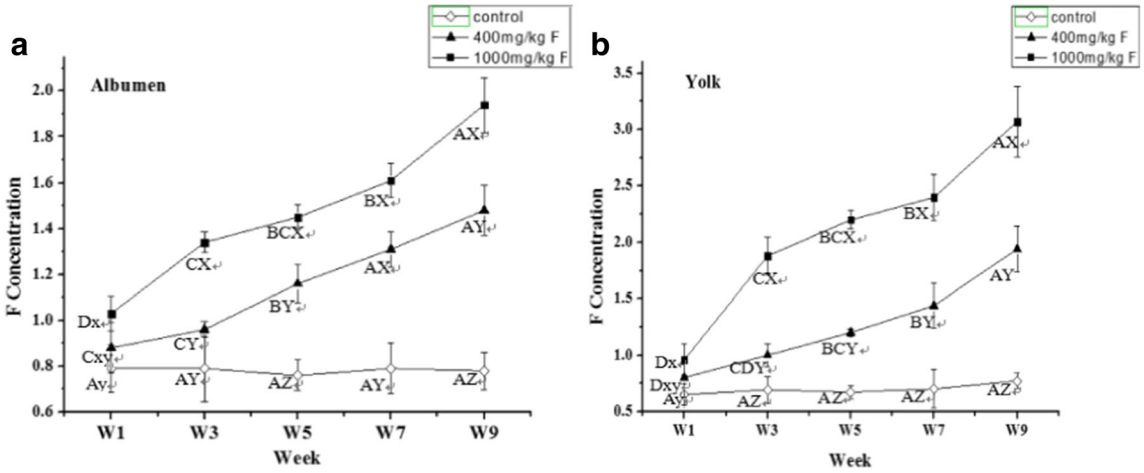

Fig. 1 Effect of dietary F levels on F concentration in albumin and yolk response to dietary F levels. Laying hens were treated with basal diet containing concentrations of 16 (control), 400, and 1000 mg/kg F, respectively. **a** F concentration in albumin response to dietary F levels. **b** F concentration in yolk response to dietary F levels. *x-z* means that F concentration in albumin and yolk differed ($P < 0.05$) at the same time point, *X-Z* means that F concentration in albumin and yolk differed ($P < 0.05$) at the same point, and *A-D* means that F concentration in albumin and yolk differed ($P < 0.05$) at the same dietary F levels. Data are expressed as means ± SD, $n = 6$

400- and 1000-mg/kg F groups increased ($P < 0.05$) at the end of the end of third, fifth, seventh, and ninth weeks compared with those at the end of the first week, respectively. Fluorine concentrations in eggshell were higher than those in albumin, yolk, liver, kidney, ovary, and oviduct.

Effects of Dietary F Levels on Biochemical Indices

As shown in Table 4, serum Ca, P, and UA levels and GOT and GPT activities increased significantly in response to dietary F concentration ($P < 0.05$), compared to the control groups, respectively. However, there were no significant changes in serum TP, ALB, and BUN levels.

Discussion

Fluoride is an essential trace element for humans and animals, while long-time or excessive exposure to F can result in body damage and diseases [15, 16]. From the previous work in our lab, we found that excess F in layer diet markedly decreased the laying rate (data are now in the publication process; see Table 5). In the feeding experiment, we also found that excess fluoride made the eggs more fragile, so in the following experiment, we examined the effect of fluoride on egg quality. Haugh unit and albumin height are important characteristics concerning internal egg quality. This study showed that dietary F levels at 1000 mg/kg significantly decreased Haugh unit and F levels at 600 mg/kg or higher levels significantly

Table 4 Effects of dietary fluoride (F) level on biochemical index

Item	F concentration in dietary (mg/kg)						SEM	*P* value
	16	200	400	600	800	1000		
TP (g/L)	35.46	35.85	37.57	35.08	35.64	36.66	1.76	0.75
ALB (g/L)	16.70	16.70	18.18	16.60	19.40	16.67	1.43	0.32
BUN (mmol/L)	2.22	2.39	2.62	2.61	2.78	2.48	0.21	0.17
UA (mg/L)	180.43 b	186.07 b	208.81 ab	229.83 a	233.94 a	248.99 a	12.25	0.001
Ca (mmol/L)	2.40 c	2.74 bc	2.80 b	2.81 ab	3.05 ab	3.17 a	0.12	0.000
P (mmol/L)	0.95 b	0.83 b	1.12 b	0.97 b	1.00 b	1.96 a	0.18	0.000
GPT (IU/L)	40.07 d	42.32 cd	43.82 bcd	45.55 bc	46.52 b	51.47 a	1.38	0.000
GOT (IU/L)	5.85 e	7.14 e	10.39 d	13.03 c	15.67 b	19.11 a	0.76	0.000

Results are the mean and SEM of six replicates, with two hens per replicate. Means within a row with no common lowercase letters differ significantly ($P < 0.05$)

TP total protein, *ALB* albumin, *BUN* urea nitrogen, *UA* urea acid, *Ca* calcium, *P* phosphorus, *GPT* glutamic pyruvic transaminase, *GOT* glutamic oxalacetic transaminase

Table 5 Effects of dietary fluoride (F) levels on laying performance

F concentration (mg/kg)	Laying performance			
	Laying rate (%)	Egg weight (g)	ADFI (g)	FCR
Control (16)	87.50 a	64.65 a	105.97 a	1.89 b
200	86.85 a	64.53 a	105.68 a	1.87 b
400	85.38 a	64.50 a	105.50 a	1.95 b
600	83.43 a	63.74 a	102.79 b	1.96 b
800	76.25 b	62.73 ab	98.88 c	2.12 a
1000	67.78 c	60.83 b	92.11 d	2.24 a
SEM	1.53	0.684	0.610	0.039
P value				
Linear	0.000	0.000	0.000	0.001
Quadratic	0.000	0.014	0.000	0.001

Results are the mean and SEM of 6 replicates, with 16 hens per replicate. Means within a column with different lowercase letters differ significantly ($P < 0.05$)

ADFI average daily feed intake, *FCR* feed conversion ratio

decreased albumin height of laying hens, respectively. Eggshell strength and eggshell thickness are two important indicators for reflecting eggshell quality. Eggshell strength ultimately affects the soundness of the shell, and weaker shelled eggs are more likely to have cracks and breakages followed by subsequent microbial contamination [17]. In the current research, dietary 800 and 1000 mg/kg of F significantly decreased eggshell strength and eggshell thickness ($P < 0.05$), both in the fourth week and ninth week. However, previous studies reported that eggshell thickness was unaffected under F exposure [18].

Results in Table 3 showed that F concentrations were generally high in eggshell, kidney, and ovary and the highest in eggshell; lower in liver, oviduct, and yolk; and the lowest in albumin (Fig. 1), which agreed with previous studies reporting that F is mainly deposited in the eggshell and fluoride levels in albumin and yolk are much lower than those in eggshell [19, 20]. Hence, eggshell F concentration is more sensitive to F exposure than other soft organs [21].

Fluoride is absorbed from the gastrointestinal tract, respiratory tract, or skin and mucous membrane, then reaching different organs and body tissues via blood circulation. It was demonstrated that the clearance of F from plasma mainly depends on the skeleton, which acts as a natural sink for F [10, 22]; hence, its accumulation in soft tissues was very low. In our study, we found that kidney F contents were higher than other soft tissues. This phenomenon may be due to the osmotic function of kidney, being capable to elaborate urine hypertonic to blood and to excrete the urine out of the body [23], accompanied by the exclusion of F; hence, kidney contained higher F levels than any other soft tissues [24].

The metabolism of calcium and phosphorus is closely related to the formation of eggshell, and if one is deficient, the other would be interfered with proper utilization [25]. Dietary calcium is absorbed into the blood stream, and then, it is either stored in the bones until needed for shell formation or transported directly to the shell gland to be used in the synthesis of calcium carbonate in eggshell [26]. The current experiment showed that F concentration in eggshell was the highest, which was in agreement with previous observations [10, 20, 24], and serum calcium levels increased with the increase of dietary F supplemental levels. The formation of eggshell accompanied with shell gland secreting calcium (Ca^{2+}) and HCO_3^-, combining to sparingly soluble calcium carbonate, which accounted for 94.03% of eggshell components [26, 27]. However, calcium ion is more likely to combine with F to form insoluble calcium fluoride, with the increase of F concentration, which disturbs the process of eggshell calcification and diminishes absorption of calcium [28, 29]. In another hand, eggshell formation is the most lengthy process in the whole egg formation, taking roughly 18–20 h to create the membranes and shell [26]. Therefore, the excess ingested F was almost removed by eggshells, causing much lower accumulation of F in album and yolk [20].

It was reported that serum TP and ALB levels were two critical indexes for reflecting liver protein anabolism, and serum BUN and UA were two important indexes for reflecting liver protein catabolism [30]. Results of this study showed that F supplementation did not affect serum TP, ALB, and BUN levels, so it indicated that birds fed with excess dietary F (F content 16 to 1000 mg/kg) for 9 weeks would not cause the disorder of liver protein metabolism. However, previous studies indicated that serum TP and ALB levels decreased in fluorotic cattle [31] and goats [32]. The inconsistent outcomes triggered by dietary F addition might contribute to dose, duration of exposure, and animal species [33].

Aminotransferases, such as GOT and GPT, are always used as biomarker of hepatic cellular impairment in blood stream

[34, 35]. This experiment showed that serum GOT and GPT activities increased with the increase of dietary F supplemental levels, which disagreed with previous investigations indicating that excessive F inhibited the activities of serum transaminases [31, 32]. Hence, it was speculated that the reason for the increase of serum GPT and GOT activities might be the compensatory regeneration of liver mild damage, and if the level of dietary F exceeded 1000 mg/kg or the time of F exposure prolonged over 9 weeks, the liver would be seriously damaged. However, mechanism of this phenomenon is not well understood.

In summary, our results indicated that F concentrations in soft tissues and eggs had a positive correlation with dietary F levels and dietary F might affect the formation of eggshell by affecting the levels of serum Ca and P. When dietary F levels reached 600 mg/kg, it will significantly reduce the albumin height and yolk color of laying hens and with the increase of dietary F levels, eggshell strength and eggshell thickness will also be significantly decreased.

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Compliance with Ethical Standards All animal procedures followed the guidelines of the National Institutes of Health Animal Care and Use Guidelines, Department of Environment, Forests, and Climate Change.

Conflict of Interest The authors declare that they have no conflict of interest.

References

1. WHO (1973) Trace elements in human nutrition: a report of a WHO expert group. Technical series no 532. World Health Organization, Geneva
2. Kirck KL (1991) Biochemistry of the elemental halogens and inorganic halides. Plenum, New York, pp 19–68
3. Everett E (2011) Fluoride's effects on the formation of teeth and bones, and the influence of genetics. J Dent Res 90:552–560
4. Perumal E, Paul V, Govindarajan V, Panneerselvam L (2013) A brief review on experimental fluorosis. Toxicol Lett 223:236–251
5. Sun Z, Niu R, Wang B, Jiao Z, Wang J, Zhang J, Wang S, Wang J (2011) Fluoride-induced apoptosis and gene expression profiling in mice sperm in vivo. Arch Toxicol 85:1441–1452
6. Ghosh D, Das Sarkar S, Maiti R, Jana D, Das UB (2002) Testicular toxicity in sodium fluoride treated rats: association with oxidative stress. Reprod Toxicol 16:385–390
7. Mukhopadhyay D, Chattopadhyay A (2014) Induction of oxidative stress and related transcriptional effects of sodium fluoride in female zebrafish liver. Bull Environ Contam Toxicol 93:64–70
8. Podder S, Chattopadhyay A et al (2010) Histopathology and cell cycle alteration in the spleen of mice from low and high doses of sodium fluoride. Fluoride 43(4):237–245
9. Sharma A, Chinoy NJ (1998) Role of free radicals in fluoride-induced toxicity in liver and kidney of mice and its reversal. Fluoride 31:S26
10. Ranjan R, Ranjan A (2015) Fluoride toxicity in animals. Springer International Publishing, New York City
11. Griffith-Jones W (1977) Fluorosis in dairy cattle. Vet Rec 100:84–89
12. Hillman D, Bolenbaugh DL, Convey EM (1979) Hypothyroidism and anaemia related to fluoride in dairy cattle. J Dairy Sci 62:416–423
13. Singh JL, Swarup D (1995) Clinical observations and diagnosis of fluorosis in dairy cows and buffaloes: case report. Agri Practice 16: 25–30
14. Del Razo LM, Corona JC, Garcia-Vargas G, Albores A, Cebrian ME (1993) Fluoride levels in well-water from a chronic arsenicism area of northern Mexico. Environ Pollut 80:91–94
15. Bouaziz H, Croute F, Boudawara T, Soleilhavoup JP, Zeghal N (2007) Oxidative stress induced by fluoride in adult mice and their suckling pups. Exp Toxicol Pathol 58(5):339–349
16. Monsour PA, Kruger BJ (1985) Effect of fluoride on soft tissues in vertebrates. Fluoride 18:53–61
17. Yuan C, Song HH, Zhang XY, Jiang YJ, Zhang AT, Azzam MM, Zou XT (2014) Effect of expanded cottonseed meal on laying performance, egg quality, concentrations of free gossypol in tissue, serum and egg of laying hens. Anim Sci J 85(5):549–554
18. Pattee OH, Wiemeyer SN, Swineford DM (1988) Effects of dietary fluoride on reproduction in eastern screech-owls. Arch Environ Contam Toxicol 17(2):213–218
19. Guenter W, Hahn PH (1979) Fluorine toxicity and laying hen performance. Poult Sci 65:769–778
20. Górecki H, Chojnacka K, Dobrzański Z, Kołacz R, Górecka H, Trziszka T (2006) The effect of phosphogypsum as the mineral feed additive on fluorine content in eggs and tissues of laying hens. Anim Feed Sci Technol 128(1):84–95
21. Carriere D, Bird DM, Stamm JW (1987) Influence of a diet of fluoride-fed cockerels on reproductive performance of captive American kestrels. Environ Pollut 46:151–159
22. Whitford GM (1994) Intake and metabolism of fluoride. Adv Dent Res 8:5–14
23. Korr IM (1939) The osmotic function of the chicken kidney. J Cell Comp Physiol 13(2):175–193
24. Shupe JL (1980) Clinicopathologic features of fluoride toxicosis in cattle. J Anim Sci 51:746–758
25. Kebreab E, France J, Kwakkel RP, Leeson S, Darmani H, Dijkstra J (2009) Development and evaluation of a dynamic model of calcium and phosphorus flows in layers. Poult Sci 88:680–689
26. Hunton P (2005) Research on eggshell structure and quality: an historical overview. Revista Brasileira de Ciência Avícola 7(2):67–71
27. Abdel-jabbar N, Al-asheh S (2009) Factorial design for the analysis of packed-bed sorption of copper using eggshell as a biosorbent. J Environ Protec Sci 3133–139
28. Pasternak K, Truchlinski J (1999) Fluorine and its influence on animal bodies. Med Weter 55(10):643–645
29. Ensminger AH (1995) The concise encyclopedia "food and nutrition". CRC, California
30. Robin JP, Cherel Y, Girard H, Geloen A, Le Maho Y (1987) Uric acid and urea in relation to protein catabolism in long-term fasting geese. J Comp Physiol B 157:491–499
31. Arya HO, Wittwer F, Villa A, Ducom C (1990) Bovine fluorosis following volcanic activity in the southern Andes. Vet Rec 126:641–642
32. Tsunoda H, Makaya S, Sakurai S, Itai K, Yazaki K, Tatsumi M (1985) Studies on the effect of environmental fluoride on goats. In: Susheela AK (ed) Fluoride toxicity. International Society for Fluoride Research, New Delhi
33. Yuan C, Song HH, Jiang YJ, Azzam MMM, Zhu S, Zou XT (2013) Effects of lead contamination in feed on laying performance, lead

retention of organs and eggs, protein metabolism, and hormone levels of laying hens. The Journal of Applied Poultry Research 22(4):878–884

34. Ramaswamy M, Thangavel P, Panneer Selvam N (1999) Glutamic oxaloacetic transaminase (GOT) and glutamic pyruvic transaminase (GPT) enzyme activities in different tissues of *Sarotherodon mossambicus* (Peters) exposed to a carbamate pesticide, carbaryl. Pestic Sci 55:1217–1221
35. Gaim K, Gebru G, Abba S (2015) The effect of arsenic on liver tissue of experimental animals (fishes and mice)—a review article. International Journal of Scientific and Research Publications 5(5): 1–9