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IMMUNOTOXIC EFFECTS OF SODIUM FLUORIDE IN CHICKENS

(With 8 Tables and 9 Figures)

By

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التأثيرات السمية المناعية لفلوريد الصوديوم فى الدجاج

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تم دراسة تأثير الفلورين على البدارى وعلى الأمهات البياضة، واستمرت التجربة فى القسم الأول لمدة ثلاثة أشهر تم فيها تقسيم ٩٠من البدارى الى ثلاث مجموعات أعطيت للأولى والثانية منها ١٠٠٠، ١٥٠٠جزء فى المليون من الفلورين فى صورة فلوريد الصوديوم على التوالى مضافا الى العليقة المقدمة أما المجموعة الثالثة فقد استخدمت كضابط للتجربة. بينما اشتمل القسم الثانى مجموعتان إحداهما أضيف الى علائقها الفلورين بمقدار ١٥٠٠جزء فى المليون واستخدمت الأخرى كضابط للتجربة لمدة شهرين. وقد تم قياس معدلات النمو والزيادة فى الوزن وقياس نسبة الفلورين فى الدم والبروتين الكلى والألبيومين والجلوبيولين والنسبة بين الألبيومين والجلوبيولين بالاضافة الى الفحص الهستوباثولوجى للغدة الدرقية والطحال وغدة فابريشيش. وقد دلت النتائج على ارتفاع معنوى شديد للفلورين فى الدم لازمه انخفاض شديد فى معدلات البروتين الكلى والألبيومين والجلوبيولين وخاصة الجاما منه أما نتيجة التحليل الكهربائى للأمصال فقد أوضحت انخفاضا شديدا فى معدلات الألبيومين. كما انخفضت النسبة المئوية للخلايا الأكولة مقارنة بضوابط التجربة علاوه على انخفاض معدلات الاستهلاك ومعدل النمو فى قسمى التجربة. كما دلت نتائج الفحص الهستوباثولوجى عن وجود تغيرات مرضية بالأعضاء المفحوصة والتى تؤكد خطورة هذه الملوثات البيئية على الجهاز المناعى للجسم وكمية انتاج اللحوم والبيض مما يدعونا الى ضرورة التربية بعيدا عن هذه الأماكن الصناعية وتقليل وصول الملوثات الى أجسام الحيوانات. ومن هذا يخلص البحثون الى أنه على الرغم من أن كميات قليلة من الفلورين هامة جدا لعظام وأسنان قوية، فإن كميات كبيرة منه قد تؤدى الى حدوث تغيرات مناعية وهستوباثولوجية ضارة تؤثر تأثيرا مباشرا على الثروة الداجنة حيث أنها تصبح أكثر عرضة للعدوى بمسببات الأمراض المختلفة.

SUMMARY

The major goal of this investigation is to determine the immunotoxic effects of sodium fluoride (NaF) in chickens. Two experiments were conducted to achieve this goal. In the first experiment, two groups of baladv chickens were exposed to two doses (1000 and 1500 ppm) of NaF and third group served as control for three months. In the second experiment, two groups of white high line laying hens were also used, The first group was treated with 1500 ppm NaF and the second was kept as control for two months. Relative weight of lymphoid organs as well as phagocytosis percentage were

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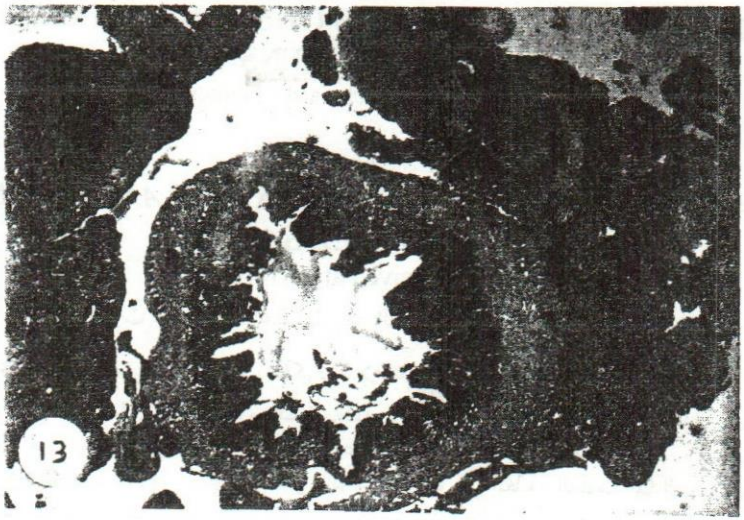
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measured 30, 60 and 90 days after exposure of broiler chicken to NaF and 30 and 60 days in case of laying hens. Body weight and body weight gain were also taken into consideration in both experiments. Significant reduction in the relative weight of both thymus and bursa of fabricius in all chickens exposed to NaF was observed. Phagocytosis percentage was decreased parallely to the weight reduction of thymus and bursa of fabricius. Body weight and body weight gain were significantly decreased allover the course of the experiment in the two doses as well as the two experiments. Total serum protein level, albumin, globulin and A1/g1 ratio were also reduced with the administration of NaF. The reduction of serum albumin and globulin was positively correlated with the dose and time. Electrophoretic pattern of the tested chickens sera revealed a significant decrease in serum albumin percentrage in the group of chicken's treated with 1000 ppm NaF. Significant disturbances ($P< 0.01$) were observed in serum globulin fractions IgM, IgA and IgG in our results. Fluoride levels in the serum of tested chickens increased with the increase of the dose and time. Histopathological description of lymphoid organs in treated chickens revealed severe to moderate pathological changes especially with the large dose of NaF after long time of exposure. Most disturbances caused by the fluoride exposure were dose and time related.

Keywords: *Immunotoxicity-sodium fluoride-chickens-electrophoresis*

INTRODUCTION

Some experimental studies on certain animals, and a few studies concerned with the sensitivity of some humans to fluorides have reported that fluorides, at normal and elevated drinking water concentrations could cause significant physiological stress as well as pathological conditions (LU *et al., 1965; COOK, 1973; GODBER, 1973; JOLLY et al., 1973 and SINCLAIR, 1973).*

Fluorine, the most reactive halogen, is ubiquitous mainly in the form of inorganic fluoride compounds. The essentiality of trace amounts of fluorine in animal nutrition has been demonstrated *(MESSER et al., 1972 and SCHWARZ and MILNE, 1972).* Interest in the role of fluoride in poultry nutrition arose not from deficiency symptoms but rather from toxicity problems induced by feeding raw rock phosphate as a calcium and phosphorus source *(GUENTER and HAHN, 1986).*

In the chicken, antibody-mediated immunity is dependent on the normal development of the bursa of fabricius *(GLICK, 1970).* Chemical interference with the bursa during embryonic development *(GLICK and SADLER, 1961; MUELLER et al., 1960 and WARNER et al., 1962)* or neonatally *(GLICK, 1967, 1970,1971; LERMAN and WEIDANZ, 1970; and TOIVANEN et al., 1972)* will be reflected in an elimination or reduction in immunoglobulin G (1gG) and antibody production and maturation of plasma cells. Although thousands of different chemicals are currently used in the course of industrial processes as well as medical purposes, investigations dealing with the influence of these chemicals on

the immune response have so far been very few.

It is the objective of this paper to report the toxic effects of sodium fluoride administration on the immune system as well as some other parameters in chickens.

Histopathological changes in the lymphoid organs as well as phagocytosis percentage were taken into consideration.

MATERIALS and METHODS

Sixty Balaby broilers and fourty laying hens have been used in this study in two experiments.

Experiment I:

In this study we have used 90 clinically healthy Balady broilers, vaccinated, free from internal or external parasites, weighing 400-550 g, and aging two months. These chickens were classified into three groups, the first two groups fed on a ration contaning 1000 and 1500 ppm of sodium fluoride for 3 months and the third group was kept as control, each group contained 6 males and 24 females.

Experiment II:

Fourty apparently healthy, white high line laying hens of six months, weighing from 1.00 to 1.500 kg were used. Birds were classified into two groups, each of them contained 20 hens (4males and 16 females). The first group fed on a ration containing 1500 ppm of NaF for two months while the second was used as a control.

Both experiments were conducted in an environmentally controlled daylighted laboratory. Tape water (0.02 ppm fluoride) was provided *ad libitum*. Commertial ration was used in the feeding of birds containing 3.60 ± 0.26 ppm NaF.

Chemicals:

Sodium fluoride (99% purity) purchased from Aldrich chemical company Ltd.

Sampling:

Serum samples were taken 1,2 and 3 months after exposure of broilers of the 1st experiment to fluoride in ration for estimation of serum fluoride, total protein, albumin and electrophoretic pattern. Also, 1 and 2 months for laying hens previosuly exposed to 1500 ppm of fluoride in ration (Experiment II) were administered to the same parameters. Birds were sacrificed monthly from the two experiments to examine the histopathological lesions of lymphoid organs.

Methods:

Fluoride level, total protein and albumin in the sera were determined according to *FRY and TAVES (1970), WEICHSELBAUM (1946) and DRUPT (1974)* methods respectively. Protein fractions horizontal zone electrophoresis were carried out *(SCHALM, 1979)*.

Macrophages were collected by peritoneal washing with Hank's balanced salt solution (HBSS), which was chilled and contained 5 Uml^{-1} heparin. The viable counts of macrophages were assessed using the trypan blue dye exclusion method in a Neubauer haemocytometer, and total counts were assessed with Turk's solution as described by *SINGH et al. (1984)*.

The phagocytic activity of peritoneal macrophages was evaluated using sheep red blood cells (SRBC) according to the method of *KOLLER et al. (1980)*, with the modification of *RAISUDDIN et al. (1990)*. One million cells in a fixed volume were spread over a glass microcoverslip (22 mm^2) kept in a plastic petridish of 4.0 Cm diameter. The cells were allowed to adhere at 37°C for one hour. The non-adherent cells were washed with phosphate-buffered saline(PBS; PH 7.2) at 37°C. Washed SRBC were coated with antisheep haemolysin 1: 500 dilution so as to opsonize them at 37°C for one hour. Opsonized cells were washed with PBS, and a 1% suspension was prepared in RPMI-1640 medium. One mililitre of 1% SRBC was spread over the coverslip on which the macrophages were adhered. These were incubated at 37°C for one hour, then washed with PBS. For microscopic examination, cells were stained with Giemsa-Wright stain and examin-ed in oil immersion under a light microscope. A macrophage was considered positive for phagocytosis if two or more SRBC were seen engulfed (Fig. 10). The percent phagocytosis was calculated according to the following formula:

$$\text{Phagocytosis} = \frac{\text{Macrophages ingulfing more than 2 SRBC}}{\text{Total number of macrophages}} \times 100$$

Statistical analysis of the obtained data was performed according to the method of *KALTON (1967)*.

RESULTS

The toxic effects of sodium fluoride on the body weight and body weight gain in chickens are summarized in Table 1. Relative weight of thymus and bursa of fabricius as well as phagocytosis percentage are summa-rized in Table 2. Total serum protein, albumin, globulin and albumin/ globulin ratio in adult hens exposed to 1500 ppm NaF in ration for 3 months are presented in Table 3, Table 4 shows the electophoretic pattern of hens serum after the exposure to 1500 ppm NaF for 3 months. Fluoride levels in serum of hens exposed to 1500 ppm sodium fluoride for 3 months are presented in Table 5. Table 6 summarized total serum protein, albumin, globulin levels and albumin/ globulin ratio in broiler chickens exposed to NaF in ration in a dose of 1000 and 1500 ppm. Electrophoresis picture in the serum of brolier chickens exposed to NaF in a dose of 1000 and 1500 ppm for two months is presented in Table 7. Table 8 summarized the fluoride levels in the serum of broiler hens exposed to 1000 and 1500 ppm NaF for two months.

Electrophoretic patterns of serum proteins in control, 1000 and 1500 ppm sodium fluoride are presented in Fig. 1, 2 and 3 respectively.

The results of histopathological examination are presented in Fig. 4-9. Thymus gland, spleen and bursa fibrica of chicken given 1000 ppm of fluorine did not show any detectable histopathological changes, only a

moderate to servere degree of lymphoid exhaustion is observed in the thymus gland from chicken killed after three months. Histopathological changes of moderate degree were observed in the thymus gland from chicken exposed to a dose of 1500 ppm of fluorine and sloughtered after one month. These changes consisted of wide spread small focal areas of hyalinization of the thymic tissue and mild degree of exhaustion of lymphoid tissue. Hemoglobin pigments were observed with the macrophage cells in the area of hyalinization. Spleen and bursa from this group of chicken showed no detectable microscopic changes. Thymus gland from chicken slaughtered after two months of administration of 1000 and 1500 ppm showed a relatively large area of hyalinization along with a prominent depletion of lymphoid population (Fig. 4 and 5). However, the spleen from this group of chicken showed no detectable histopathological changes. Bursa fibrica from these chicken showed depletion of lymphoid population and the epithelial cells were necrosed or degenerated and hyalinized (Fig. 6). Thymus gland from chicken killed after 3 months of adiministration of 1500 ppm of fluorine showed a wide spread destruction of lymphoid element along with hyalinization of the thymic tissue (Fig. 7). Spleen from this group of birds revealed mild depletion of lymphoid cells population along with proliferation of reticuloendothelial system cells (Fig. 9). Bursa fibrica from such birds was completely fibrosed and contained no lymphoid elements (Fig. 8).

DISCUSSION

In recent reports, several environmental contaminants were shown to be synergistic to infections agents. Many suggestions have been made in the literature to explain the relationship of high fluoride diets to retarded growth of chickens. Our results presented in Table 1 reported a highly significant reduction in body weight and body weight gain in chickens administered 1000 and 1500 ppm sodium fluoride. This reduction seems to be dose and time dependent. *MICHEL et al. (1984)* reported that fluoride caused a growth inhibition by the restriction of feed consumption which is systemic in nature and independent of any action in the digestive tract. Relative weight of thymus and bursa of fabricius as well as phagocytosis percentage are summarized in Table 2. The present study showed that sodium fluoride has an obvious effect on thymus body weight ratio with both 1000 and 1500 ppm doses allover the time of the experiment, meanwhile bursal relative weight was affected after 30 days only with 1500 ppm sodium fluoride and after 45 days with the two used doses. Phagocytosis percentage decreased in a dose and time dependent manner. According to *DESCOTES (1986)*, toxicological manifestations in the immune system following chemical exposure may appear as changes in lymphoid organ weights and/or histology. He added that it is essential in any immunotoxicity evaluation to measure the weight of thymus, spleen and peripheral lymph nodes. The decrease in relative weight of thymus

and bursa in treated groups of chicken could be attributed to the effect of sodium fluoride administration.

Our results presented in table 3 and 6 revealed a highly significant decrease in total protein in treated hens both with 1000 and 1500 ppm NaF, allover the experiment. This decrease in total protein could be attributed to the inhibitory effect of fluoride on protein synthesis which have been proved before by *VESCO and COLAMBO (1970)*. Fluoride inhibits protein synthesis *(HOERZ and McCARTY, 1971; GODCHAUX and ATWOOD, 1976)* and the incorporation of radioactive amino acids into protein is reduced in the presence of low concentrations of fluoride *(HELGELAND, 1976; LIN et al., 1976; HOLLAND, 1979)*. It has been indicated that this reduced incorporation of precursors may be due to reduced uptake into the cells *(HELGELAND, 1977)*, and fluoride induced reduction in uptake of other substances has been reported by *DOST et al. (1977)*. It has also been reported that fluoride inhibits Na^+, K^+- activated ATPase, an enzyme essential for the uptake of amino acids since it is involved in the transport of Na^+ and K^+, which is necessary for active uptake of amino acids *(HOLLAND and HONGSLO, 1979)*. *ABDEL-HAMID and DORRA (1993)* proved that feeding of broiler chickens on graded levels of fluorine as sodium fluoride resulted in poor growth and feed conversion efficiency, high mortality, disorders of bone formation, decreased relative weights of pituitary, adrenals, heart, liver, spleen, lungs, kidneys and gizzard and changes in intestinal measurements. Their biochemical tests revealed changes in blood composition in the form of low total protein as well as albumin and globulin fractions.

Our results revealed severe reduction in serum albumin levels in treated chickens in comparsion to control group of chickens. Serum albumin/globulin ratio seems to behave in the same manner as it decreases in the treated group to half of control chickens. Albumin/ globulin ratio has been used to aid in interpretation of total protein values *(AHLAM et al., 1994)*. This ratio will remain normal if both protein fractions are uniformly altered and be abnormal if this alteration was in one fraction *(ROBERT and KEITH, 1988)*. As shown in tables 4 and 7 at 1000 and 1500 ppm NaF, the serum IgG level was reduced and IgM level was increased compared to controls. The increase in serum IgM and the decrease in IgG are interesting finings in our result as IgG antiboy synthesis is thought to be more thymus depenent than IgM synthesis. Hence, our results could suggest an impairment in the function of T helper cells. IgA an IgD were also affected in variable manner during this experiments, meanwhile there was no effect on IgB except in the first month samples in the group of chicken treated with 1500 ppm NaF.

Fluoride levels in the serum of chickens were presented in Tables 5

and 8. There was a highly significant increase in fluoride levels in treated hens in comparison to controls. This increase in serum fluoride concentration found to be time and dose dependent in our experiments. There is an accumulation of fluoride in the serum of all animals and *GROTH (1975 a, b)* has suggested that this is one of the most notable characteristics of fluoride as a pollutant. Thus, serious adverse effects are possible even at low levels of exposure if this persists for lengthy periods of time. In addition, fluorides are known to cause chromosome damage and mutation in animals *(ROSE and MARIER, 1977).*

In the assessment of immunotoxic action in experimental animals, histopathology of lymphoid organs is a cornerstone *(VOS, 1977; KRAJNC-FRANKEN et al., 1990; KUPER et al., 1973 and SCHUURMAN et al., 1991). DEN and VOS (1986) and SCHUURMAN et al. (1994)* stated that routine histopathology of lymphoid organs is useful in assessing the immunotoxicity of a chemical. Our results of histopathological examination of thymus, spleen, and bursa of fabricius are presented in figs. 4-9. Histopathological changes shown in our experiments were correlated to the other immune responses as well as fluoride concentrations in the serum of affected hens.

It could be finally concluded that the administration of NaF for 2 to 3 months could alter the immune response and reduce host resistance to infection leading to economic losses in poultry industry.

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Table (1): Effect of sodium fluoride administration on body weight gain in chickens

Time of exposure (day)	Dose (ppm)	Body Weight Mean ± S.E. (Kg)	Feed consumption (g/day)	Body weight gain (g)
0.0	1000	0.462±0.017		
	1500	0.453±0.011		
	Control	0.458±0.010		
15	1000	0.549±0.013**	50.62±5.42*	0.087±0.002**
	1500	0.526±0.007**	46.62±5.33**	0.073±0.004**
	Control	0.584±0.012	60.00±5.80	0.126±0.002
30	1000	0.662±0.013**	70.68±6.55	0.113±0.003
	1500	0.624±0.019**	67.37±6.20*	0.098±0.002*
	Control	0.696±0.012	73.50±8.21	0.112±0.003
45	1000	0.759±0.024**	66.66±6.35*	0.097±0.005**
	1500	0.709±0.020**	72.30±6.51	0.085±0.006**
	Control	0.856±0.027	74.10±6.42	0.160±0.002
60	1000	0.850±0.040**	71.12±6.45	0.091±0.006**
	1500	0.794±0.030**	71.52±5.88	0.085±0.005**
	Control	0.958±0.032	72.40±7.01	0.102±0.002
75	1000	0.946±0.040**	70.55±6.68	0.096±0.004**
	1500	0.930±0.030**	72.90±5.95	0.136±0.002**
	Control	1.160±0.023	71.65±6.65	0.204±0.007

* Significant at P≤ 0.05 ** Significatn at P≤ 0.01

Table (2): Relative weight of thymus and bursa of fabricius and phagocytosis % of broiler exposed to NaF

Time of esposure (day)	Dose (ppm)	Thymus	Bursa	Phagocytosis %
30	1500	2.125±0.233**	1.380±0.125**	60.00±1.50**
	1000	2.025±0.250**	2.222±0.250	40.00±5.20
	Control	4.250±0.305	2.455±0.236	50.00±1.50
60	1500	1.910±0.289**	0.690±0.115**	47.50±3.55**
	1000	1.760±0.295**	1.540±0.103**	44.20±2.66**
	Control	4.740±0.267	2.150±0.155	55.50±2.55
90	1500	2.135±0.098**	0.855±0.095**	46.500±4.44**
	1000	2.230±0.115**	1.650±0.085**	43.666±5.05**
	Control	3.655±0.115	2.370±0.210	53.085±4.42

* Significant at P≤ 0.05 ** Significant at P≤ 0.01

Table (3): Total serum proteins, Albumin, globulin and Albumin/globulin ratio in adult hens exposed to 1500 ppm sodium fluoride in ration for three months.

Times of exposure (month)	Dose (ppm)	Total protein (g/dl)	Albumin (g/dl)	Globulin (g/dl)	Globulin %			Alb./Glob. ratio
					α	β	ν	
One	1500	4.804± 0.291**	1.437± 0.100**	3.357± 0.202	12.50± 0.62	8.85± 0.57**	48.56± 3.20	0.726± 0.009*
	Control	7.258± 0.678	3.268± 0.088	3.990± 0.440	12.30± 1.00	4.40± 0.60	48.38± 1.40	0.831± 0.03
Two	1500	4.036± 0.270**	1.245± 0.106**	2.791± 0.205**	14.40± 0.80	5.10± 0.60	48.10± 2.46	0.450± 0.03**
	Control	6.650± 0.147	2.996± 0.84	3.654± 0.120	12.90± 1.02	4.76± 1.26	46.92± 2.01	0.819± 0.024
Three	1500	4.576± 0.080**	1.460± 0.025**	3.116± 0.060**	15.05± 1.12	12.30± 0.60**	41.0± 0.68**	0.468± 0.007**
	Control	7.376± 0.094	3.099± 0.055	4.277± 0.099	13.82± 1.06	5.00± 0.56	48.75± 0.33	0.736± 0.016

* Significant at P≤ 0.05

** Significant at P≤ 0.01

Table (4): Electrophoretic pattern in Balady laying hens serum exposed to 1500 ppm of sodium fluoride in ration for 3 months.

Time (month)	Dose (ppm)	Pre-Albumin (%)	Albumin (%)	Total Albumin (%)	Globulin %								
					α1	α2	β	Globulin				
								IgM	IgA	IgG	IgT	IgB
One	1500	5.75± 1.12	24.46± 0.23**	30.21± 0.52**	4.95± 0.42	7.55± 1.57	8.85± 0.57**	8.90± 0.80**	6.35± 0.075	18.40± 1.30*	9.46± 1.77**	4.00± 0.25**
	Control	9.0± 1.4	36.40± 2.40	45.40± 2.36	6.10± 1.0	6.20± 0.80	4.40± 0.60	5.40± 0.62	4.40± 1.20	26.06± 2.4	1.02± 0.75	1.50± 0.75
Two	1500	5.83± 1.33**	26.45± 2.00	32.28± 2.40**	8.05± 0.57*	6.35± 0.22	5.10± 0.15	7.40± 0.22*	9.15± 0.475**	17.36± 0.08**	10.25± 2.37**	3.95± 1.47
	Control	16.53± 1.28	28.76± 2.39	45.30± 0.80	5.16± 0.8	7.64± 1.13	4.76± 1.26	6.13± 0.49	5.03± 0.55	21.4± 0.67	2.066± 0.36	2.33± 0.57
Three	1500	9.50± 0.15	22.15± 0.225**	31.65± 1.650**	9.80± 1.4	5.25± 0.323	12.30± 0.60**	5.10± 0.75	18.85± 0.275**	8.00± 0.45**	5.30± 0.35**	3.00± 1.12
	Control	12.61± 1.20	29.8± 1.8	42.41± 0.60	6.3± 0.6	7.52± 1.10	5.0± 0.66	5.90± 0.20	5.12± 0.60	24.3± 1.04	1.99± 0.20	1.44± 0.31

Significant at P≤ 0.05 ** Significant at P≤ 0.01

Table (5): Fluoride levels in serum of hens after exposure

Time (month)	Dose	Mean ± S.E.
One	1500 ppm	0.415 ± 0.012**
	Control	0.066 ± 0.0033
Two	1500 ppm	0.940 ± 0.073**
	Control	0.062 ± 0.0065
Three	1500 ppm	1.06 ± 0.021**
	Control	0.079 ± 0.0055

Significant at P≤ 0.05 ** Significant at P≤ 0.01

Table (6): Total serum proteins, Albumin, Globulin and Albumin/Globulin ratio in broiler chickens exposed to sodium fluoride in ration (1000 and 1500 ppm)

Times (month)	Dose	Total protein (g/dl)	Albumin (g/dl)	Globulin (g/dl)	Globulin % α	Globulin % β	Globulin % ν	A/G
One	1000	5.084± 0.203*	1.502± 0.045**	3.582± 0.197	12.66± 0.70	8.46± 1.62	53.88± 1.00**	0.419± 0.016**
	1500	4.043± 0.156**	1.190± 0.044**	2.953± 0.122**	11.55± 0.20	2.70± 0.55**	58.20± 1.50**	0.403± 0.02**
	Control	6.002± 0.282	2.100± 0.040	3.902± 0.193	11.39± 2.10	5.73± 0.475	49.56± 0.56	0.540± 0.02
Two	1000	4.026± 0.236**	1.232± 0.052**	2.757± 0.211**	16.90± 0.80	11.95± 0.475**	38.00± 0.80**	0.396± 0.023**
Three	1500	4.076± 0.235**	1.038± 0.068**	3.138± 0.153*	18.78± 1.22	10.50 ±1.9	45.75± 0.78*	0.346± 0.015**
	Control	5.551± 0.108	1.934± 0.0192	3.617± 0.121	14.15± 1.20	7.250± 0.37	42.15± 1.02	0.536± 0.010

* Significant at P<0.05
** Significant at P<0.01

Table (7): Electrophoretic pattern in broilers chicken serum after exposure to sodium fluoride in ration (1000, 1500 ppm) for 2 months.

Time (month)	Dose (ppm)	Pre Albumin (%)	Albumin (%)	Globulin (%)								
				α1	α2	ß	Globulin					
							IgM	IgA	IgG	IgGT	IgGB	IgGE
One	1000	7.366± 1.49	17.43± 2.31**	7.966± 1.53	4.200± 0.59	8.460± 1.62	7.200± 0.39	5.33± 0.43	32.23± 5.36	6.433± 2.34	2.690± 0.42*	0.00± 0.00
	1500	5.350± 1.52	23.30± 2.35	6.300± 0.15	5.250± 0.275	2.700± 0.55**	11.100± 0.85**	8.100± 0.65**	18.30± 6.15	18.100± 5.05**	1.70± 0.15*	1.00± 0.50
	Control	8.73± 1.60	26.46± 1.64	6.933± 2.36	4.466± 1.70	5.733± 0.59	7.233± 0.93	5.266± 0.42	26.166± 3.12	4.566± 0.85	4.70± 0.67	1.63± 0.92
Two	1000	6.700± 0.25**	24.45± 0.025**	10.500± 0.250**	6.40± 0.45	11.950± 0.475**	4.350± 1.123	8.60± 1.20*	20.75± 0.125**	1.750± 0.375	2.55± 0.475	0.00± 0.00
	1500	9.300± 0.45**	15.050± 3.37	6.33± 0.625	12.45± 1.025**	10.50± 2.90	8.200± 0.83*	9.950± 0.975**	18.80± 2.1*	3.750± 1.275	2.250± 0.675	2.80± 0.45
	Control	18.65± 0.325	16.75± 0.325	6.800± 0.25	7.350± 0.125	7.250± 0.375	6.100± 0.300	5.400± 0.05	24.5± 0.75	2.300± 0.000	2.80± 0.25	1.05± 0.52

* Significant at P< 0.05 ** Significant at P< 0.01

Table (8): Fluoride levels (mean ± S.E.) in serum of broilers after exposure to sodium fluoride in ration (1000, 1500 ppm)

Time of exposure (month)	Dose (ppm)	Fluoride (ppm)
One	1000	0.220 ± 0.015**
	1500	0.426 ± 0.035**
	Control	0.056 ± 0.0022
Two	1000	0.242 ± 0.023**
	1500	0.456 ± 0.320**
	Control	0.048 ± 0.003

* Significant at P< 0.05 ** Significant at P< 0.01

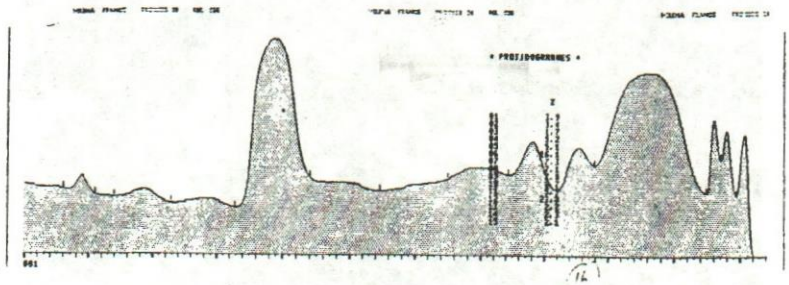

Fig. 1: Electrophoretic pattern of control serum

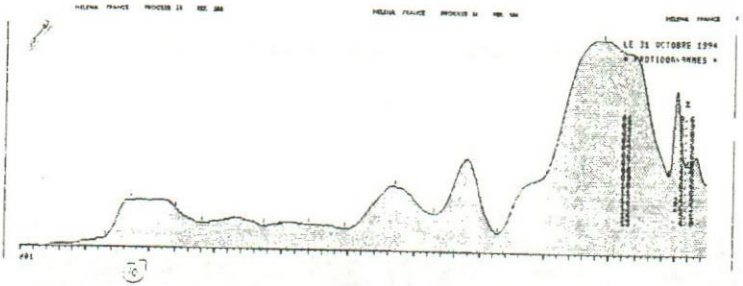

Fig. 2: Electrophoretic pattern of serum after 1000 ppm NaF

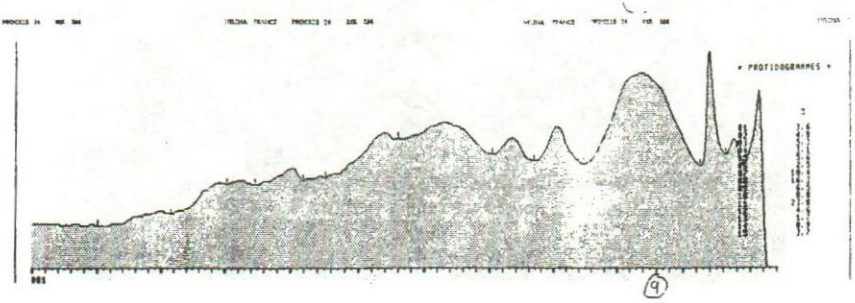

Fig. 3: Electrophoretic pattern of serum after 1500 ppm NaF

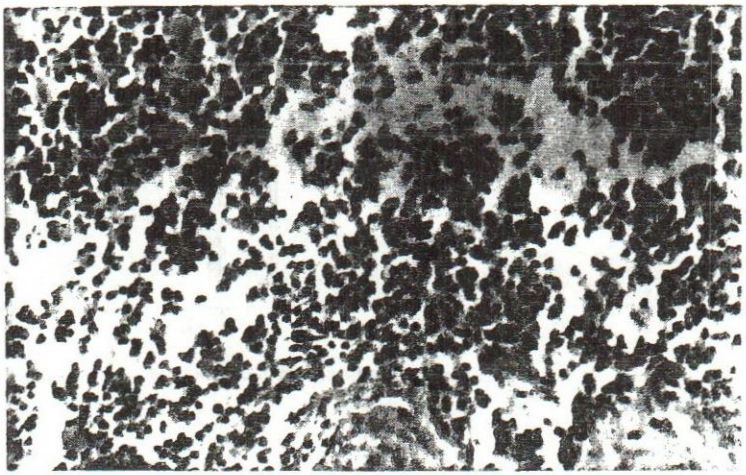

Fig. 4: 1000 ppm NaF after 3 months: depletion of lymphoid population of thymus gland. H & E stain (25X)

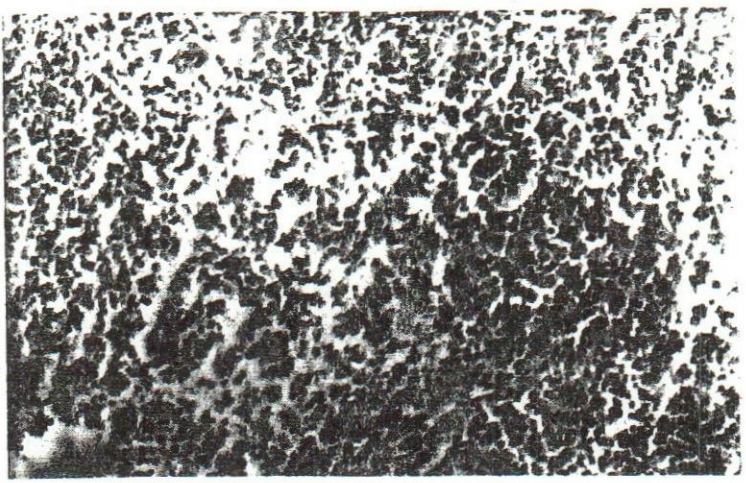

Fig. 5: 1500 ppm NaF after 2 months: severe degree of depletion of lymphoid population. H & E stain (10X)

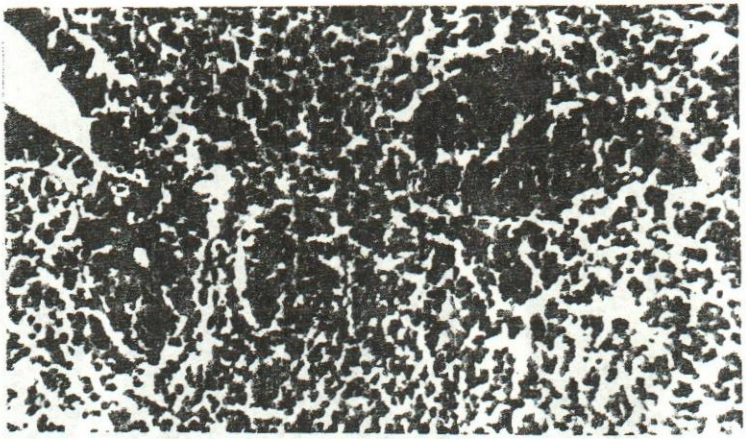

Fig. 6: 1500 ppm NaF after 2 months: depletion of lymphoid tissue in the bursa with necrosis and hyalinization of the epithelium H & E stain (10X)

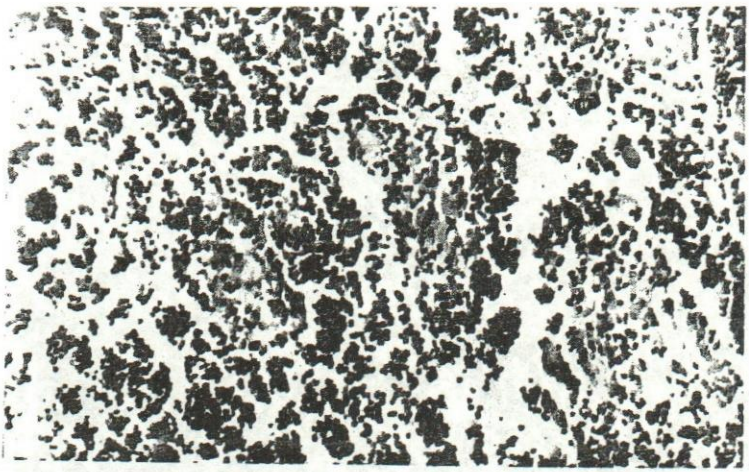

Fig. 7: 1500 ppm NaF after 3 months: severe exhaustion of lymphoid population and hyalinization of thymic tissue. H&E stain (10X)

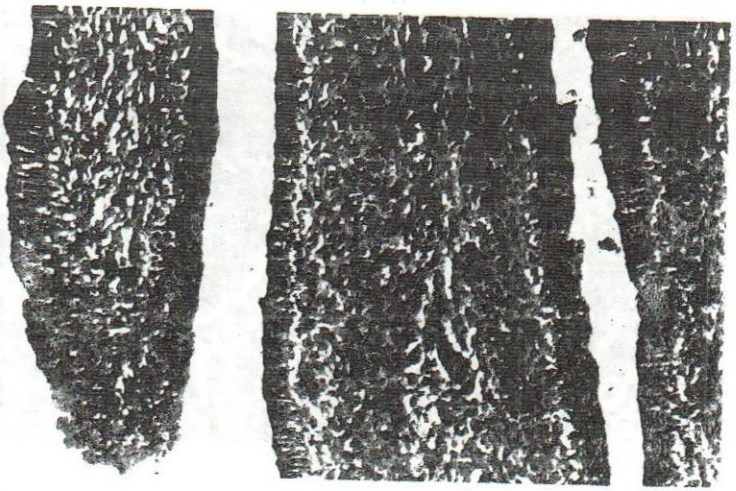

Fig. 8: 1500 ppm NaF for 3 months showed fibrosis of the bursa fibrica alongwith loss of the most of the lymphocytic population H&E stain (X10)

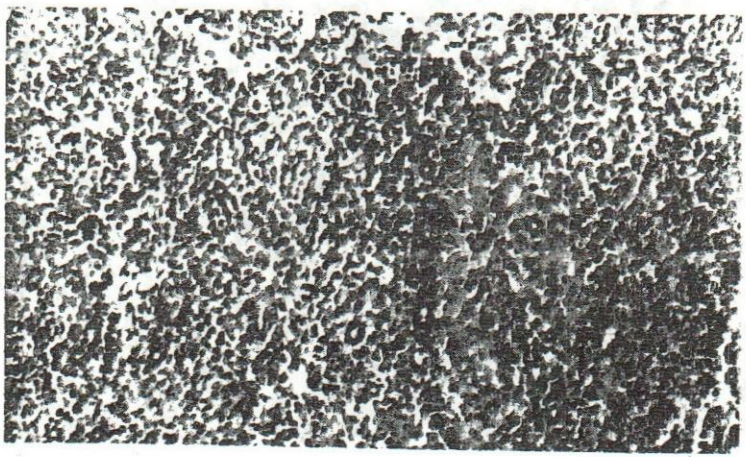

Fig. 9: 1500 ppm NaF for 3 months: spleen showed mild deplation of lymphoid cells population along with proliferation of the reticuloendothelial cells H&E stain (10X)