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HEMATOLOGICAL AND BIOCHEMICAL STUDIES OF FLUORINE POISONING IN CHICKENS, A TRIAL FOR TREATMENT

(With 10 Tables)

By

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دراسات دموية وبيوكيميائية للتسمم بالفلورين فى الدجاج مع محاولة للعلاج

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تم أجراء هذا البحث لدراسه الاثار السميه لفلوريد الصوديوم على الدجاج وقد تم أستعمال مائة وسبعون من بدارى الدجاج البلدى وستون من الدجاج البياض تم تقسيمها الى ثلا ث مجموعات رئيسية أستمرت الأولى والثانية لمدة ثلاثة أشهر متصلة وقد أحتوت علائقها على الفلورين بجرعات ١٠٠٠، ١٥٠٠ جزء فى المليون للتجربة الأولى (بدارى) وعلى ١٥٠٠ جزء فى المليون للتجربة الثانية (دجاج بياض) بينما خصصت المجموعة الثالثة (بدارى) لدراسة مدى أمكانية علاج حالات التسمم المزمن بالفلورين فى الدجاج بأستعمال أكسيد الالومنيوم مع أستمرار التعرض للفلورين. وقد تم دراسة صورة الدم وعمل قياسات تحليلية لعناصر الفلورين والكالسيوم والفسفور والماغنسيوم واليود وأنشطة خمائر الفوسفاتاز ومستويات تى ٣ و تى ٤ وكذلك الترای جلیسرید والتأثير على أوزان الدجاج وأنتاج ووزن البيض فى الدجاج البياض. وقد وضح جليا مدى التأثيرات الضارة لفلوريد الصوديوم والدور الايجابى للعلاج بأكسيد الألمونيوم فى تخفيض نسبة الفلورين من الجسم وعودة الغدة الدرقية للعمل طبيعيا الا أن مخاطر جسام قد ظهرت أيضا عند أستعمال أكسيد الألمونيوم فى العلاج لزيادة أنشطة خمائر الفوسفاتاز القلوى والترای جلسرید وأنخفاض نسبه الكالسيوم والماغنسيوم وما ترتب عليها من ضرر بالجسم ولذا فلابد من أستمرار المحاولات العلاجيه حتى يمكن تعميمها دون ترك أى أثار ضاره أخري.

SUMMARY

This study was carried out to investigate the toxic and hazardous effects of sodium fluoride (NaF) on chickens. One hundred and seventy Balady broiler and sixty laying hens have been used in this study. Three experiments were conducted to achieve this goal. In the first experiment, two groups of balady chickens were fed on 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 used The first group was treated with 1500 ppm NaF while the second was kept for three months as control. In the third experiment, balady broiler chicken previously intoxicated by sodium fluoride (1000, 1500 ppm) for three months were used and treated with alluminum oxide. Hematological picture and biochemical analysis of Fluorine, Calcium, Phosphorous, Magnesium, Iodine, Triiodothyronine (T3), Thyroxine (T4), Alkaline Phosphates activity, and Triglycride revealed highly significant variation than normal. Also, mean egg weight, feed consumption, and body weight gain were decreased. The use of aluminum oxide has some beneficial effects for treatment of florine toxicity in broiler chicken.

Key words: *Fluorine poisoning - Chickens - Treatment.*

INTRODUCTION

Fluorides are salts of hydrogen fluoride. The element fluorine is the most electronegative of all elements. Its affinity to a number of elements is very considerable. Due to the increase uses of fluorides in the treatment of certain metabolic bone diseases, particularly Osteoporosis in one hand and its application as feed additives as calcium source and as a result of ecosystem contamination from industrial pollution specially by super-phosphate factory on another hand, the present experiments were conducted to determine the exerted toxic effect of fluorine on laying hens with special interest to their performance.Abdel Nasser et al (1995) reported a highly significant reduction in body weight and body weight gain in fluorine treated chickens They concluded in their work that NaF alter the immune response and reduce host resistance to infection. Hasanien et al. (1995) recorded chromosomal aberrations in chickens treated with NaF. As fluorine has tendency to combine with other elements, the experiment included the picture of most essential elements that related strongly to fluorine in addition to the thyroid hormones and iodine. The application of aluminum compounds as an alleviating elements for fluorosis had a special interest. So an experiment was done to detect the efficiency of aluminum (AI) on the exerted toxic effects of fluorine toxicosis either on the hematological picture, thyroid hormones,or on related elements as Ca, P, Mg and Iodine. The aim of this study was to put a clear line on the application of fluoride in one hand and the alleviation of its effect by using Aluminum.oxide.

MATERIALS and METHODS

One handred and seventy Balady broilers and sixty laying hens have been used in this study in three experiments.

Experiment I:

In this experiment, 90 clinically healthy Balady broilers were used, vaccinated against Newcastle, 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 a ration containing 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:

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

Experiment III:

Eighty Balady broilers of five months age were used in this experiment. These chickens were classified into five groups, the first two groups fed a ration containing 1000 and 1500 ppm of sodium fluoride for 4 weeks, the third and fourth groups fed a ration containing 1000 ppm sodium fluoride + 8000 ppm aluminum oxide and 1500 ppm sodium fluoride + 12000 ppm aluminum oxide for 4 weeks while the fifth group was kept as control, each group contained 4 males and 16 females.

The three experiments were performed in an environmentally controlled daylighted laboratory. Tape water (0.02 ppm fluoride) was provided *ad libitum.* Commercial ration was used in the feeding of birds containing 3.60 ± 0.26 ppm fluoride.

Chemicals:

Sodium fluoride (99% purity), Aluminum oxide (99% purity) were purchased from Aldrich chemical company Ltd.

Sampling:

Blood samples were obtained from wing veins for hematological examination and serum samples were taken 1, 2 and 3 months after exposure of broilers to fluoride in the 1^{st} experiment for estimation of serum contents of fluoride, calcium, phosphorous, magnesium, triglycerides, iodine, triiodothyroxine, thyroxin, and alkaline phosphatase activity; 1, 2 and 3 months for laying hens previously exposed to 1500 ppm of fluoride in experiment II and 1, 2, 3 and 4 weeks in the third expirment were administered to the same parameters.

Methods:

Red blood cells (RBCs), White blood cells (WBCs), Hemoglobin concentration (HB), packed cell volume (PCV), Mean corpuscular volume (MCV), Mean corpuscular hemoglobin (MCH), and Mean corpuscular hemoglobin concentration (MCHC) were determined according to methods cited by Coles (1986),

Fluorine, calcium, inorganic phosphorous, magnesium and iodine serum levels were determined according to Fry and Taves (1970), Bett and Fraser (1959), Mornil and Prox (1973),Grinder and heth (1971) and Morin et al. (1975) respectively.

Serum triglyceride concentration, and alkaline phosphatase activity were determined according to the methods optained by Fletcher (1968) and Balfiedld and Goldberge (1971) .Quantitative determination of T3, and T4 in serum were obtained after Zoasoo (1975). Statistical analysis of data was performed after Kalton (1967).

RESULTS and DISCUSSION

Fluorine element (Tables 6, 7, 8) was increased to reach the toxicologic level which (over 0.5 ppm) indicates recent fluorosis (Greenwood et al., 1964) at the beginning of the second month of the second experiment (Table 8). Fluorine level (0.988 ppm) increased to reach the hazardous level at the 1^{st} week in the 1^{st} experiment (Table 7) at 1500 ppm dose level, meanwhile in the group treated with 1000 ppm fluorine, the level were increased above toxic level at the end of the 4^{th} week (0.53 ppm). Elevated levels of fluorine had been detected in hens exposed to industrial fluorosis at Manqubad area (Ahlam et al., 1994).

The effect of aluminum oxide as a line of treatment of fluorosis in chicken was observed in the form of decrease in fluorine levels during the 4 weeks in aluminum treated group than the levels in fluorosed control hens. This fact of decreased plasma fluorine had been described by Spencer et al.

(1980). This decrease in plasma fluorine level could be attributed to the inhibition of intestinal fluorine absorption (Spencer and Lender, 1979). Aluminum compounds are the most frequently used alleviators of fluorosis (Becker et al., 1950).The effect of Al on Phosphorus appeared to inhibit its absorption from intestine. Phosphorus levels in treated group exposed to 1000 ppm was decreased while birds exposed to 1500 ppm showed increased levels in comparison to non treated fluorosed one. It was postulated that Al combines with phosphates in the intestine and induce phosphorus depletion which associated with increased bone resorption (Spencer and Lender, 1979; Brudevold et al., 1972 and Lotz et al., 1968).

Iodine levels increased in all hens exposed to fluorine either in the level of 1000 or 1500 ppm whatever, young or adult, treated or non treated. This increase in iodine levels is cotradictionary to the finding of Ahlam et al., (1994) in endemic fluorosed hens present in the area of superphosphate producing plant. Thyroxin and Triiodothyronine (T4 & T3) elevated levels indicates a stimulant effect of fluorine on the thyroid gland. This increase in both hormones in addition to the increased level of iodine in serum needs more work for the clarification of this stimulating effect of fluorine.

Triglycerides indicated a highly significant increase in the serum of exposed hens whatever treated or not. The effect of fluorine on triglycerides was detected in Guinea pigs after inhalation of HF where the triglycrides synthesis was accelerated in the liver and the extrahepatic lipoprotein lipase activity was also decreased in the fluoride group; this could be related to the decrease in apo protein C (Douset et al., 1984). The previous findings could explain the plasma triglycerides accumulation observed after exposure to NaF in all exposed hens.

The analysis of magnesium in serum of fluorine exposed hens revealed an increase in all treated and non treated groups. This result is in accordance with Seddek (1988) who found an increase in Mg in serum of goats showing endemic fluorosis. This increase was positively correlated to the increase in bone Ca and Mg.

The analysis of Ca in blood serum revealed an increase in Ca levels in the 1st week in the 1000 and 1500 ppm level while the use of aluminum led to a significant decrease in Ca levels. Calcium levels were decreased after one month of exposure. The decrease of Ca level in aluminum treated groups were evident during the 4th weeks of exposure. Although Ca is particularly ameliorative, and no other cation can substitute for required Ca, it seems unlikely that aluminum involves simple displacement of Ca from Ca-requiring binding sites (Deleers, 1985). In the other hand Ca increased during 1st month of adult exposed, the values decreased in the 2^{nd} and 3^{rd} month of exposure. This could be related to the inhibition of Ca absorbance from the intestine due to CaF combination. Hatfield et al. (1942) found a considerable decrease in blood Ca with increased fluorine intake.

Alkaline phosphatase showed increased activity in the birds of the three experiments where the increase appeared in the 1^{st} week, this finding ensure that fluorine act on periostium which is the main source of AP enzyme, and the hyperactivity of the periostium is considered the main cause of increased AP activity (Rosenberger et al., 1979). This finding is in agreement with that of increased AP in experimentally exposed cows to fluorosis by Potmann (1979) who found an increase in AP activity in cows exposed to fluorine in rations over one year exposure to CaF.

The hematological picture of hens in the 1^{st} experiment showed a highly significant decrease in RBCs number. The decrease was evident along the 3 months period. The dose 1500 ppm showed more effect on inducing anemia. PCV values were non significantly decreased in the 1^{st} month while the values were unchanged in the 2^{nd} and 3^{rd} month. Hemoglobin values were decreased which inversely correlated to the exposure dose. The mean corpuscular volume show a highly significant increase in all exposed birds indicating that anemia in this type of exposure of a macrocytic hypochromic one. The results of mean corpuscular hemoglobin concentration ensure this finding of hypochromacia which appeared clearly in the higher doses. In the second experiment the changes behave the same way in RBCs number, PCV, and hemoglobin concentration. In the 3^{rd} experiment, the changes in total number of RBCs appeared in the 2^{nd} week, hemoglobin showed no significant change, but MCHC values decreased at 3^{rd}, 4^{th} week. The PCV values showed a decrease correlated with the dose allover the test period. The AL treatment appeared to have no therapeutic effect on blood constituents in this part of experiment. The white blood cells showed no significant changes allover the exposure time in all the three experiment. It is apparent that there is no effect of fluorine on the WBCs. The change in RBCs had been recorded in cattle in India where the most significant results of fluorosis was the continuous fall in the hemoglobin and red cell contents of blood (Majumdar and Ray, 1946) Slagvold (1934) in sheep. Shupe et al. (1963) showed that fluorine had no effect on blood or haemobiotic centers. Potmann (1979) found no significant changes in blood constituents in cows fed CaF in ration for one year. The exerted effect in our result could be referred to the high dose used in ration(1000, 1500 ppm).

The effect of fluorides on body weight and productive activity have been shown before (Schmidt et al. 1954) who found no significant differences in the milk or butter fat production of the controls or the animals fed various amounts of sodium fluoride. In the same time fluorine exerted no detrimental effect upon weight during the 1st thirty six months of experiment. No differences could be detected in milk cow's yield and productivity with respect to weight gain and lactation in cows fed one year CaF in ration (Potmann, 1979). Our results showed a significant effect of fluoride as NaF in ration on body weight gain at a dose of 1500 ppm from the 1st week and in the 2nd week for 1000 ppm ration. This result could be attributed to less feed consumption which also decreased. Egg production and weight were decreased in fluorosed birds than control. In the same time application of aluminum as an alleviating element has a significant effect on correction of Fluoride , Iodine , T3 ,and T4 levels beside egg production in fluorosed hens.On other hand application of aluminum oxide in the treatment has undesirable effect on the calcium, and triglysride levels as well as on alkaline phosphatase activity. Body weight gain (Table 10) appeared to be not affected with aluminum addition to the diet during the 4 weeks of experiment.

In conclusion the previous findings explain the toxic effects of exposure to 1000 and 1500 ppm fluorine. Aluminum as an alleviating ion to fluoride had diminished its level in serum but had no effect on its exerted toxic effect of decreasing Ca, Mg, p in serum. Aluminum had also no effect on the treatment of the hematological changes. The changes in egg weight, egg production, body weight gain were also not significantly affected by the addition of AI to ration. The ill response of the aforementioned exerted toxic effects of fluorides to aluminum compound could clarify that the use of aluminum must be in rations containing 1000 ppm as a maximum level. Addition of Ca, P, Mg to the ration is recommended to equilibrate theire level changes during absorption from the intestine or by their exhaustion from bones due to their decrease in plasma of fluorosed birds. Fluorine exerted its toxic effect not only on bone and teeth but also on the elemental status and hormones such as thyroxin and triiodothyronine which needs more investigations on the human beings exposed to industrial fluorosis either in aluminum or superphosphate industry.

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Table (1): Hematological picture in Broiler chicken after exposure to sodium fluoride in ration (1000, 1500 ppm) for three months.

Time (month)	Dose (ppm)	RBCs $(10)^6$ µl	Hb g/dl	PCV %	MCV cu/u	MCH µ/ug	MCHC %	WBCs $(10)^3$
1	1000	1.960±0.210**	11.140±0.560*	25.900±1.795**	150.180±4.22**	58.860±4.250	17.090±1.010**	4.625±0.279
	1500	1.930±0.229*	12.840±0.220	27.620±1.660**	148.570±3.22**	66.520±3.450**	15.030±0.980**	7.500±1.300
	Control	2.988±0.220	13.020±0.503	34.000±1.200	112.850±4.11	46.570±4.55	23.940±1.11	5.625±0.620
2	1000	2.656±0.141	11.010±0.216**	32.460±0.980	130.200±6.250**	46.450±3.510	24.120±2.400	6.800±0.770
	1500	2.012±0.135**	11.120±0.194**	34.255±0.940	170.250±9.650**	58.260±4.760**	18.090±0.860**	5.428±0.626
	Control	2.948±0.106	12.675±0.284	31.580±0.094	108.130±4.500	40.070±2.500	24.650±1.660	6.125±0.675
3	1000	2.672±0.220	10.200±0.740**	29.200±2.450	119.220±5.600	62.620±4.500*	28.840±1.250	8.750±1.640
	1500	2.120±0.220**	10.660±1.122**	34.750±2.720	161.320±6.600**	48.480±2.600	19.880±1.330*	4.370±0.210
	Control	2.895±0.100	13.000±1.130	33.000±1.130	115.200±7.550	45.050±3.500	23.980±0.980	5.950±0.689

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

Table (2): Hematological picture in laying hens after exposure to sodium fluoride in ration (1500 ppm) for 3 months.

Time (month)	Dose (ppm)	RBCs $(10)^6$ µl	Hb g/dl	PCV %	MCV Cu/u	MCH U/µg	MCHC %	WBCs $(10)^3$
1 M	1500	2.160±0.138*	10.675±0.790**	25.000±1.060**	115.760±8.980	50.020±2.340	20.430±2.010	6.375±0.660
	Control	3.167±0.154	14.800±0.588	35.440±1.220	111.900±11.020	47.840±3.420	21.690±1.980	6.000±0.250
2 M	1500	2.310±0.090**	9.950±0.820**	28.000±0.220*	122.210±5.240	43.070±1.220	23.810±2.110	6.440±0.660
	Control	2.802±0.125	13.900±0.440	31.050±1.010	108.800±4.640	48.010±1.550	20.750±1.650	7.200±0.840
3 M	1500	2.381±0.080*	27.500±0.320**	27.500±0.320**	115.490±10.620	42.350±1.020	23.710±1.750	7.880±0.620
	Control	2.740±0.090	13.650±0.330	30.980±0.400	113.060±12.400	50.820±1.110	20.380±1.850	7.540±0.788

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

Table (3): Mean of egg weight / week in fluoretic and treated hens.

Time in week	Control	1000 ppm		1500 ppm	
		F.	F. + AL	F.	F. + AL
1	37.060±0.085	33.545±0.230**	35.330±0.33*D	33.666±0.650**	37.500±0.960 D
2	38.118±0.554	32.428±0.286**	35.230±0.110**D	37.800±0.186	40.200±0.677
3	39.684±0.071	33.875±0.208**	37.180±0.660*D	35.214±0.267**	38.250±0.780 D
4	38.810±0.026	32.600±0.630**	37.526±0.687 D	38.250±0.280	38.312±0.117

Table (4): Mean of week egg production / hen in fluoretic and treated hens.

Time in Week	Control	1000 ppm		1500 ppm	
		F.	F. + AL	F.	F. + AL
1	2.428±0.182	1.857±0.311	1.200±0.163**	0.600±0.157**	0.400±0.097**
2	2.225±0.331	1.125±0.195*	1.950±0.137 D	1.400±0.185*	1.050±0.086**
3	2.500±0.346	1.125±0.066**	2.550±0.210 D	1.400±0.173**	1.400±0.141**
4	4.000±0. 21	0.800±0.133**	2.966±0.298* D	1.400±0.133**	2.975±0.15**D

** Significant at $P < 0.01$
* Significant at $P < 0.05$
D Significant in comparison with treated hen at $P < 0.01$

Table (5): Hematological picture in fluoretic broiler chicken after treatment by aluminum oxide for one months

Time	1st week					2nd week					3rd week					4th week				
Parameter	C.	1000 F	1000 F+A	1500 F	1500 F+A	C.	1000 F	1000 F+A	1500 F	1500 F+A	C.	1000 F	1000 F+A	1500 F	1500 F+A	C.	1000F	1000 F+A	1500 F	1500 F+A
RBCs $(10)^6$	3.01 ± 0.12	2.88 ± 0.25	2.98 ± 0.26	2.65 ± 0.12	2.84 ± 0.21	2.45 ± 0.11	1.88 ± 0.05**	2.44 ± 0.01 D	2.05 ± 0.14*	2.72 ± 0.11 D	3.21 ± 0.20	2.38 ± 0.25**	2.66 ± 0.16**	2.01 ± 0.10**	2.80 ± 0.20 D	3.19 ± 0.07	2.85 ± 0.02**	1.80 ± 0.02**D	2.35 ± 0.12**	2.13 ± 0.16**
Hb g/dl	13.4 ± 0.71	12.0 ± 0.26	12.2 ± 0.60	10.6 ± 0.20**	11.6 ± 0.30	12.2 ± 0.45	10.1 ± 0.56	13.6 ± 0.34 D	12.4 ± 0.33	12.0 ± 0.01**	13.6 ± 0.96	11.0 ± 0.87	13.0 ± 1.02	13.0 ± 1.02	13.2 ± 0.88	13.1 ± 1.01	9.45 ± 0.44**	12.0 ± 0.54 D	11.8 ± 1.02	11.6 ± 0.88
PCV %	36.4 ± 1.11	30.4 ± 1.56	30.21 ± 2.02*	28.1 ± 1.22**	29.2 ± 2.15	29.1 ± 0.75	32.7 ± 1.66	34.0 ± 2.41	38.5 ± 2.14**	31.0 ± 1.25 D	33.6 ± 2.02	29.1 ± 2.21	28.5 ± 1.36*	30.6 ± 2.50	31.5 ± 2.11	36.0 ± 2.52	39.0 ± 0.47	40.5 ± 2.54	45.2 ± 2.35*	41.7 ± 1.44
MCV Cu/u	120. ± 4.26	105. ± 5.56	101. ± 4.44**	106. ± 5.65	102. ± 4.56**	116. ± 5.02	174. ± 5.42**	139.0 ± 12.4M	187.0 ± 12.4**	112.0 ± 10.6 D	110.0 ± 11.2	127.0 ± 9.84	107.0 ± 10.7	152.0 ± 12.2*	110.0 ± 10.2M	112.0 ± 8.66	136.0 ± 9.88	223.0 ± 20.1**D	192.0 ± 19.2*	193.0 ± 19.2*
MCH U/µg	44.7 ± 2.44	41.7 ± 3.55	40.9 ± 2.88	40.2 ± 3.66	41.0 ± 2.68	48.4 ± 1.50	53.7 ± 5.66	55.8 ± 1.66**	60.6 ± 2.44**	43.4 ± 4.22 D	42.4 ± 2.44	46.3 ± 3.55	49.0 ± 3.25	46.6 ± 5.33**	46.1 ± 3.66M	41.1 ± 2.40	33.1 ± 3.30	66.4 ± 5.32**D	50.6 ± 4.33	54.1 ± 3.50**
MCHC %	22.3 ± 2.40	23.9 ± 1.86	24.4 ± 2.61	24.8 ± 2.66	24.3 ± 3.54	20.4 ± 1.56	18.6 ± 1.40	17.8 ± 1.65	16.4 ± 1.13	23.0 ± 2.12M	23.5 ± 1.23	21.5 ± 2.10	20.3 ± 1.88	15.4 ± 1.20**	21.6 ± 2.40	24.3 ± 1.80	30.1 ± 3.20	15.0 ± 1.30**D	19.7 ± 1.20*	18.4 ± 1.66**
WBCs $(10)^3$	6.75 ± 0.32	7.65 ± 0.34	6.74 ± 0.26	6.66 ± 0.28	7.76 ± 0.55	6.50 ± 0.65	5.50 ± 0.44	6.20 ± 0.58	7.01 ± 0.50	6.23 ± 0.42	7.20 ± 0.66	6.65 ± 0.26	5.85 ± 0.36	7.01 ± 0.26	6.86 ± 0.37	6.38 ± 0.13	5.00 ± 0.20	5.50 ± 0.22	4.50 ± 0.20	3.50 ± 0.18

C : Control F : Fluoride A : Aluminum oxide
S.E. : Standard Error * : Significant at $P < 0.05$ ** : Significant at $P < 0.01$
M : Significant at $P < 0.05$ in comparison with treated hen D : Significant at $P < 0.01$ in comparison with treated hen

Table (6): Biochemical analysis of broiler chickens serum after exposure to sodium fluoride in ration (1000, 1500 ppm) for three months.

Time	1st week					2nd week					3rd week					4th week				
Parameter	1000 F	1000 F+A	1500 F	1500 F+A	C.	1000 F	1000 F+A	1500 F	1500 F+A	C.	1000 F	1000 F+A	1500 F •	1500 F+A	C.	1000 F	1000 F+A	1500 F	1500 F+A	C.
Fluorine (ppm)	0.273 ± 0.014 **	0.266 ± 0.006 **	0.988 ± 0.035 **	0.790 ± 0.009 **D	0.0407 ± 0.004	0.290 ± 0.022 **	0.185 ± 0.008 **D	10.024 ± 0.065 **	0.644 ± 0.045 **D	0.042 ± 0.003	0.385 ± 0.021 **	0.195 = 0.009 **D	1.132 ± 0.068 **	0.388 ± 0.028 **D	0.034 ± 0.005	0.536 ± 0.049 **	0.201 ± 0.008 **D	1.090 ± 0.086 **	0.483 ± 0.038 **D	0.033 ± 0.006
Calcium (mg%)	8.465 ± 0.361	4.455 ± 0.286 **D	8.257 ± 0.440	5.340 ± 0.320 **D	7.428 ± 0.520	7.308 ± 0.640	4.329 ± 0.320 **D	4.590 ± 0.265 **	3.109 ± 0.310 **	7.020 ± 0.440	7.622 ± 0.522	4.010 ± 0.325 **D	4.66 ± 0.285 **	3.030 ± 0.320 **	7.220 ± 0.466	6.680 ± 0.713	3.890 ± 0.199 **D	4.120 ± 0.232 **	3.170 ± 0.217 **	8.010 ± 0.433
Phosphorus (mg%)	4.838 ± 0.375	4.322 ± 0.259	5.035 ± 0.820	4.640 ± 0.422	4.110 ± 0.307	5.035 ± 0.325	3.557 ± 0.31 D	4.220 ± 0.322	5.230 ± 0.250	4.420 ± 0.470	5.120 ± 0.52	4.990 ± 0.66	4.68 ± 0.38	4.880 ± 0.42	5.040 ± 0.32	5.200 ± 0.24	4.880 ± 0.28	4.760 ± 0.31	5.220 ± 0.29	4.880 ± 0.38
Magnesium (mg%)	2.41 ± 0.226	2.16 ± 0.250	1.68 ± 0.178	1.93 ± 0.144	2.36 ± 0.320	2.91 ± 0.110	2.01 ± 0.170	2.705 ± 0.304	2.80 ± 0.230	2.58 ± 0.248	2.84 ± 0.110	2.86 ± 0.120	2.54 ± 0.165	2.54 ± 0.133	2.65 ± 0.122	2.65 ± 0.110	2.722 ± 0.20	2.200 ± 0.210	2.80 ± 0.233	2.45 ± 0.244
Iodine (ppm)	0.142 ± 0.002 **	0.128 ± 0.005 **M	0.155 ± 0.012 **	0.145 ± 0.008 **	0.090 ± 0.004	0.145 ± 0.005 **	0.130 ± 0.004 **M	0.165 ± 0.009 **	0.140 ± 0.002 **M	0.09 ± 0.005	0.156 ± 0.005 **	0.110 ± 0.007 **D	0.168 ± 0.005 **	0.14 ± 0.006 **M	0.088 ± 0.006	0.164 ± 0.010 **	0.126 ± 0.002 **D	0.175 ± 0.008 **	0.143 ± 0.00 **D	0.086 ± 0.003
T_3 (µ/L)	2.334 ± 0.012	0.863 ± 0.099	1.048 ± 0.073	0.992 ± 0.136	1.220 ± 0.026	1.440 ± 0.039 **	1.228 ± 0.007 **	1.286 ± 0.014 **	1.210 ± 0.010 **	1.144 ± 0.01	1.323 ± 0.012 **	1.198 ± 0.008 D	1.402 ± 0.010 **	1.245 ± 0.01 D	1.210 ± 0.018	1.350 ± 0.009 **	1.200 ± 0.017 **D	1.420 ± 0.006 **	1.270 ± 0.012 **D	1.080 ± 0.002
T_4 (µ/L)	8.831 ± 0.857 **	5.541 ± 0.158 **D	6.126 ± 0.139 **	4.336 ± 0.080 **D	2.580 ± 0.295	7.825 ± 0.695 **	3.750 ± 0.146 *D	5.168 ± 0.239 **	3.462 ± 0.057	8.74 ± 0.299	7.666 ± 0.321 **	2.960 ± 0.323 D	6.866 ± 0.084 **	4.22 ± 0.065 **D	2.497 ± 0.226	9.010 ± 0.342 **	2.455 ± 0.233 D	5.662 ± 0.224 **	2.954 ± 0.346 D	2.550 ± 0.276
Alkaline Phosphats (IU/L)	131.0 ± 5.593	235.0 ± 21.37 **D	289.17 ± 20.42 **	342.10 ± 22.42 **	108.85 ± 10.83	270.0 ± 24.32 **	431.06 ± 20.21 **D	294.57 ± 16.42 **	429.56 ± 31.17 **D	135.4 ± 7.073	165.2 ± 10.45	448.00 ± 12.390 **D	284.26 ± 5.660 **	460.8 ± 24.24 **D	217.73 ± 16.520	220.4 ± 8.550	384.10 ± 28.550 **D	205.42 ± 0.959	41.60 ± 22.220 **D	202.17 ± 16.05
Triglycride (mg/dl)	266.4 ± 5.82 **	285.5 ± 6.22 **	273.82 ± 8.44 **	210.20 ± 6.22 D	207.33 ± 4.25	288.6 ± 10.20 **	286.44 ± 9.12**	244.44 ± 8.77**	206.66 ± 8.88 D	212.2 ± 8.66	290.5 ± 12.66 **	310.54 ± 12.33 **	287.32 ± 7.68**	215.6 ± 10.2 D	198.54 ± 11.65	466.5 ± 23.55 **	335.66 ± 10.85 **D	429.66 ± 7.81 **	315.58 ± 10.66 **D	204.66 ± 8.780

C: Control **F:** Fluoride **A:** Aluminum oxide ***:** Significant at P < 0.05 **** :** Significant at P < 0.01

S.E.: Standard Error **D:** Significant at P < 0.01 in comparison with treated hen **M:** Significant at P < 0.05 in comparison with treated hen

Table (7): Biochemical analysis of broiler chicken serum after exposure to sodium fluoride in ration (1000, 1500 ppm) for 3 months.

time	1 month			2 months			3 months		
Parameter	**C**	**1000**	**1500**	**C**	**1000**	**1500**	**C**	**1000**	**1500**
Fluorine (ppm)	0.056 ± 0.002	0.220 ± 0.015**	0.428 ± 0.035**	0.048 ± 0.003	0.242 ± 0.023**	0.456 ± 0.032**	0.038 ± 0.002	0.268 ± 0.016**	0.868 ± 0.048**
Iodine (ppm)	0.083 ± 0.0003	0.125 ± 0.002**	0.083 ± 0.001	0.062 ± 0.001	0.121 ± 0.015**	0.131 ± 0.006**	0.077 ± 0.002	0.126 ± 0.0002**	0.158 ± 0.0003**
Calcium (mg %)	7.171 ± 0.298	8.281 ± 0.504	4.469 ± 0.401**	7.860 ± 0.542	7.620 ± 0.465	4.330 ± 0.560**	8.850 ± 0.524	5.157 ± 0.505	6.011 ± 0.420**
Phosphorus (mg %)	5.220 ± 0.050	5.290 ± 0.219	3.170 ± 0.195**	5.020 ± 0.220	4.005 ± 0.146**	3.780 ± 0.271**	5.330 ± 0.24	3.090 ± 0.250**	3.650 ± 0.310**
Magnesium (mg %)	3.422 ± 0.310	3.401 ± 0.220	2.950 ± 0.250	3.860 ± 0.260	4.010 ± 0.360	3.990 ± 0.280	3.540 ± 0.400	4.186 ± 0.180	3.880 ± 0.300
T_3 (µ/L)	1.236 ± 0.079	1.743 ± 0.002**	1.678 ± 0.330	0.402 ± 0.015	1.655 ± 0.044**	1.359 ± 0.018**	0.791 ± 0.016	1.558 ± 0.054**	1.293 ± 0.042**
T_4 (µ/L)	2.607 ± 0.078	5.802 ± 0.650**	4.060 ± 0.220**	2.013 ± 0.122	4.540 ± 0.402**	6.085 ± 0.546**	2.112 ± 0.080	4.565 ± 0.212**	5.707 ± 0.651
Alkaline Ph. (IU/L)	207.617 ± 16.28	609.325 ± 24.515**	955.37 ± 350.19**	182.66 ± 16.88	566.93 ± 73.07**	493.80 ± 14.188**	232.57 ± 16.99	470.00 ± 50.07**	491.87 ± 17.86**
Triglyceride (mg/dl)	154.73 ± 8.10	186.37 ± 5.48**	197.696 ± 9.17**	162.44 ± 12.52	419.67 ± 64.69**	265.08 ± 9.09**	155.122 ± 4.75	266.45 ± 11.63**	283.95 ± 13.93**

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

Table (8): Biochemical analysis of laying hens serum after exposure to sodium fluoride in ration (1000, 1500 ppm) for three months.

Time	1 month		2 months		3 months	
Parameter	C	1500	C	1500	C	1500
Fluorine (ppm)	0.066 ± 0.0033	0.415 ± 0.012**	0.062 ± 0.0065	0.940 ± 0.073**	0.079 ± 0.0055	1.060 ± 0.021**
Iodine (ppm)	0.104 ± 0.0006	0.132 ± 0.002**	0.099 ± 0.001	0.142 ± 0.0006**	0.098 ± 0.0003	0.198 ± 0.014**
Calcium (mg %)	10.172 ± 0.720	8.010 ± 0.210*	9.633 ± 0.699	7.510 ± 0.330*	10.240 ± 0.580	7.864 ± 0.680*
Phosphorus (mg %)	4.470 ± 0.030	3.880 ± 0.080	4.820 ± 0.320	2.412 ± 0.38**	5.51 ± 0.44	3.66 ± 0.21**
Magnesium (mg %)	4.050 ± 0.250	3.820 ± 0.410	3.960 ± 0.320	3.850 ± 0.250	4.120 ± 0.320	3.665 ± 0.130
T_3 (µ/L)	0.664 ± 0.024	1.317 ± 0.011**	0.686 ± 0.090	1.804 ± 0.028**	0.586 ± 0.020	1.908 ± 0.033**
T_4 (µ/L)	2.052 ± 0.279	4.919 ± 0.422**	2.078 ± 0.366	5.602 ± 0.665**	1.875 ± 0.052	6.033 ± 0.422**
Alkaline Ph. (IU/L)	120.86 ± 3.94	596.33 ± 21.075**	108.75 ± 10.25	472.24 ± 74.42**	93.97 ± 6.55**	387.91 ± 19.05
Triglycride (mg/dl)	216.63 ± 18.89	485.42 ± 22.13**	218.25 ± 28.228	448.40 ± 13.56**	201.42 ± 21.06	534.025 ± 31.46**

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

Table (9): 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)	Excess of body weight gain (g)
1 - 7	1000	0.946 ± 0.040**	70.68 ± 6.55	0.113 ± 0.003
	1500	0.930 ± 0.030**	67.37 ± 6.20*	0.098 ± 0.002*
	Control	1.160 ± 0.023	73.50 ± 8.21	0.112 ± 0.003
8 - 15	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
16 - 23	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
24 - 30	1000	0.956 ± 0.031**	70.55 ± 6.68	0.096 ± 0.004**
	1500	0.864 ± 0.025**	72.90 ± 5.95	0.136 ± 0.002**
	Control	1.062 ± 0.022	71.65 ± 6.65	0.204 ± 0.007

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

Table (10): Effect of Aluminum oxide administration on body weight gain of fluoretic chickens.

Time	1st week					2nd week				
Parameter	C.	1000 F	1000 F+A	1500 F	1500 F+A	C.	1000 F	1000 F+A	1500 F	1500 F+A
Body weight Mean±S.E. (k.g.)	1.02 ± 0.03	1.15 ± 0.02	1.08 ± 0.04	1.01 ± 0.02	1.05 ± 0.03	1.03 ± 0.04	1.15 ± 0.04	1.20 ± 0.04	1.02 ± 0.03	1.08 ± 0.03
Feed consumption (g./day)	73.5 ± 6.11	71.9 ± 5.95	72.6 ± 6.65	70.9 ± 4.76	73.5 ± 6.11	74.9 ± 5.95	72.6 ± 6.65	72.9 ± 4.76	71.9 ± 4.76	73.5 ± 6.11
Excess of body weight gain (g.)	36.4 ± 1.11	30.4 ± 1.56 *	30.2 ± 2.02 *	28.1 ± 1.22 **	29.2 ± 2.15	29.1 ± 0.75	32.7 ± 1.66	34.0 ± 2.41	38.5 ± 2.14	31.0 ± 1.25 D

Time	3rd week					4th week				
Parameter	C.	1000 F	1000 F+A	1500 F	1500 F+A	C.	1000 F	1000 F+A	1500 F	1500 F+A
Body weight Mean±S.E. (k.g.)	1.09 ± 0.03	1.60 ± 0.04	1.26 ± 0.03	1.01 ± 0.02	1.09 ± 0.03	1.15 ± 0.04	1.80 ± 0.02	1.32 ± 0.02	1.02 ± 0.02	1.15 ± 0.01
Feed consumption (g./day)	75.4 ± 6.44	71.7 ± 6.22	73.1 ± 4.74	72.1 ± 4.76	74.3 ± 6.11	76.1 ± 6.24	71.9 ± 5.23	73.8 ± 5.74	72.8 ± 4.44	73.9 ± 5.22
Excess of body weight gain (g.)	33.6 ± 2.02	29.1 ± 2.21	28.5 ± 1.36 *	30.6 ± 2.50	31.5 ± 2.11	36.0 ± 2.52	39.0 ± 0.47	40.5 ± 2.54	45.2 ± 2.35 *	41.7 ± 1.44

C : Control F: Fluoride A: Aluminum oxide *: Significant at $P < 0.05$ **: Significant at $P < 0.01$
S.E. : Standard Error D: Significant at $P < 0.01$ in comparison with treated hens M: Significant at $P < 0.05$ in comparison with treated hen