

Effect of Fluorine on Growth, Calcification and Parathyroids in the Chicken*

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IN THE rather extensive literature relating to the effects of fluorine on the animal body, there is no reference, so far as we know, to the effect of feeding fluorides to chickens. However, certain experiments with the use of rock phosphate containing fluorides as a mineral supplement for chickens have a bearing on this problem, and will be referred to in connection with our experiments.

EXPERIMENTAL

Three experiments were carried out with growing chicks and one with pullets. In the first, Barred Plymouth Rock baby chicks were kept on the Wisconsin ration plus 2 percent cod liver oil, for nine days, after which various amounts of sodium fluoride were incorporated in this ration, except in the case of those animals kept as controls. The Wisconsin ration was made up of 80 pounds ground yellow corn, 20 pounds wheat middlings, 3.8 pounds steamed bone meal, 5 pounds calcium carbonate and 1 pound sodium chloride, plus 2 percent of cod liver oil.†

In the case of the two higher levels of sodium fluoride which were used, the fluoride was incorporated by direct admixture with a generous portion of ball milled corn. For the lower levels, a 4 percent solution of

sodium fluoride was evaporated on the ball milled corn at approximately 60°C. in the drying room. The cod liver oil was mixed with the rest of the ration ingredients from time to time, in order to prevent rancidity and oxidative changes. At first, skim milk was fed *ad libitum*, but records of food and milk consumption in the first two weeks of the experiment showed that the groups receiving the higher levels of NaF were consuming much more milk in proportion to dry mash than the other lots. To avoid this difficulty, 25 percent of dried skim milk was incorporated into the dry mash, and the chicks were given water to drink. On the basis of the total food intake, the proportion of sodium fluoride in the rations was as follows; none, 0.015, 0.037, 0.075, 0.15, 0.3, 0.6, and 1.2 percent. Each group consisted of eleven or twelve chicks. Two groups were kept as controls. The ration was fed *ad libitum* and the chicks were weighed weekly. They were kept indoors on shavings at first, but were later transferred to screens when cases of coccidiosis appeared among them.

In the second experiment, 21 Bar. Ply. Rock chicks nine weeks old were used. The chicks were divided into three groups, one being kept as control, and the others receiving 0.6 and 1.2 percent of sodium fluoride, incorporated in the Wisconsin chick ration.

Because of the accidental occurrence of coccidiosis in the baby chicks, the third experiment was designed essentially to repeat the first. Day-old White Leghorn chicks

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† Furnished by E. R. Squibb and Sons, New York.

were used, and these were kept on screens from the beginning. All were given the basal ration for three weeks, at which time 40 chicks which had made satisfactory gains were selected. They were divided into four groups, two of which were given the basal ration plus 0.15 and 0.3 percent of sodium fluoride respectively. The remaining groups were kept as controls. Each control group was offered the amount of food that had been consumed by the corresponding fluoride-fed group on the preceding day. Contrary to our expectations, it proved difficult to equalize the food consumption of the chicks receiving 0.15 percent sodium fluoride and their controls, inasmuch as the controls regularly ate a little less than the others. Therefore, after 2 weeks, these groups were subdivided, after which the food consumption was successfully equalized for two groups of five. The remaining chicks from these lots were allowed food *ad libitum*.

We supplemented our study of the effect of fluorine on the growing chick by an experiment on six young White Leghorn pullets. They had been receiving a ration composed of 96 percent white corn, 3 percent dried pork liver and 1 percent irradiated yeast[‡], fed together with skimmilk and oyster shells *ad libitum*. Two of the pullets were kept on this ration as controls, and four were given the ration plus 1.6 percent sodium fluoride, which was added to the dry mash. As in the case of the growing chicks, feed and milk consumption records kept at the beginning of the experiment showed that the pullets receiving sodium fluoride ate less of the dry mash and more milk than controls. Subsequently, dried skimmilk was added in the proportion of 40 percent of the total food ingested, which was approximately what the control pullets consumed when skimmilk was fed *ad libi-*

tum. Sodium fluoride then constituted 0.96 percent of the ration.

GROSS EFFECTS ON BODY WEIGHT

For the first experiment, the approximate average feed consumption per chick per day for seven weeks together with the total average gain for the same period, is shown in Table 1. While these group consumption records are only approximately correct, it appears that the gain per gram of feed intake did not vary greatly for the several groups, although group VI ate much less and gained less than the others. Deaths occurred so soon in Groups VII and VIII that feed consumption records were not obtained for them very long after the dried skimmilk was added to the mash.

Figure 1 shows the average weight curves for some of the chicks in the experiments. It will be noted that sodium fluoride at levels of 0.6 and 1.2 percent of the ration proved very detrimental to the young chicks. After three and four weeks' feeding, one-third of the chicks in each of the lots were dead. The remainder were killed to obtain blood samples for analysis, and tissues for examination. Of the remaining chicks, those receiving .015-.15 percent NaF, grew approximately as well as the controls. However, in the group receiving 0.3 percent sodium fluoride growth was distinctly inferior to that of the controls. It is noteworthy that the higher levels of sodium fluoride were less toxic to the older chicks used in Experiment II, than to the baby chicks. With one exception the two-months-old chicks which received 0.6 percent sodium fluoride, made fair gains during the four weeks of the experiment. One lost 20 grams in the fourth week, and appeared quite ill. Of the seven chicks which received 1.2 percent sodium fluoride, four lost weight, one gained slightly, our two made excellent gains of over 400 grams in four weeks.

[‡] Obtained from Standard Brands, New York City, and irradiated in our laboratory.

The effect of sodium fluoride on weight gains in relation to food consumption for the chicks in the third experiment is shown in Table 2. No depression of appetite was noticed in the groups fed 0.15 percent sodium fluoride. In fact, it will be noted that in the lots fed *ad libitum*, the weight gain was somewhat better for those given 0.15 percent sodium fluoride than for the corresponding controls.

was depressed aside from the effect on feed intake. Inasmuch as this level of sodium fluoride proved distinctly less toxic to the twenty-one-day old chicks than to the nine-day-old chicks, it was decided to increase the level of sodium fluoride to 0.6 and 1.2 percent respectively for two groups of six chickens each. In contrast to the marked toxic effect of the ration containing 0.6 percent sodium fluoride on the young chicks

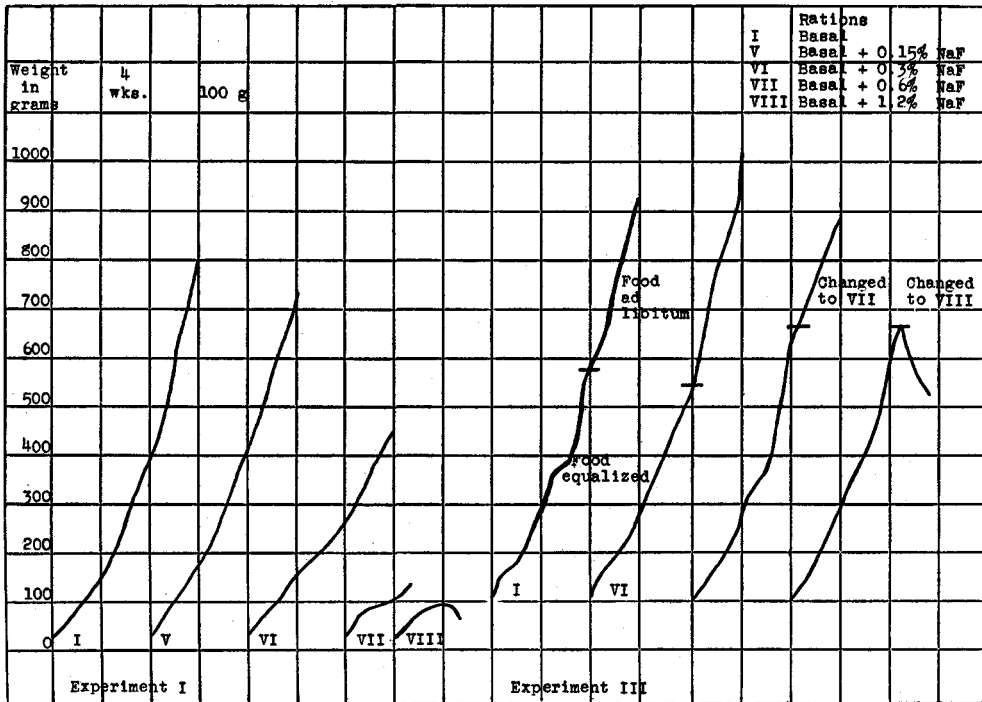


FIG. 1. Weight curves of chicks receiving sodium fluoride.

When the feed intake was equalized, the weight gain for the chicks receiving 0.15 percent NaF was practically identical with the controls. When 0.3 percent sodium fluoride was fed, the intake of feed and the increase in weight were decreased, but somewhat less than in the previous experiment, in which the chicks were started on the ration at an earlier age. Although the consumption records were necessarily approximate, there is some indication that growth

the three-months-old chickens continued to grow at a satisfactory rate on this ration. However, 1.2 percent of sodium fluoride in the ration proved distinctly toxic to the older chickens, as indicated by an immediate and rapid loss of weight, with the occurrence of two deaths in the third week.

It should be emphasized that the lot receiving 0.15 percent sodium fluoride appeared as thrifty in every way as the controls. The combs of cockerels which re-

ceived 0.3 percent sodium fluoride were somewhat pale, but were erect. In other respects these chickens appeared to be in good condition. However, the combs of the cockerels which received 0.6 percent sodium fluoride were pale and droopy, while those

those of Buckner, Martin, and Insko (1929) who found that rock phosphate, which is well known to contain fluorine, caused poor growth and early mortality of baby chicks. The survivors were given bone meal and limestone for four weeks, during which they

TABLE 1. Influence of sodium fluoride on feed consumption and gain to seven weeks

Lot number	NaF in ration	Average feed consumption per chick per day	Average gain in weight for period
	per cent	grams	grams
I	0.00	44	531
II	0.015	39	466
III	0.037	46	485
IV	0.075	39	493
V	0.15	46	479
VI	0.30	24	266
VII	0.60	died early	—
VIII	1.20	died early	—

which had received 1.2 percent sodium fluoride lacked tone altogether.

Thus it is seen that 0.3 percent sodium fluoride, which markedly inhibited the growth of baby chicks, had only a slight effect where the chicks were allowed stock ration for 3 weeks before the sodium fluoride was added. Furthermore, 0.6 percent

recovered. When the chicks were returned to rock phosphate as a mineral supplement, they grew well for the remainder of 12 weeks. Halpin and Lamb (1932) found in three growth experiments that no harm was produced by replacing 1 percent of steamed bone meal in the Wisconsin chick ration with rock phosphate, but that some depres-

TABLE 2. Effect of sodium fluoride on gains

Supplement	Feed intake	Distribution of sexes	Initial average weight	Range of weight at 11 weeks	Average weight at 11 weeks
None 0.3% NaF	Equalized	5♂, 3♀	grams 109	grams 475-820	grams 591
		5♂, 3♀	108	450-675	537
None 0.15% NaF	Equalized	3♂, 2♀	116	562-690	630
		4♂, 1♀	110	515-770	627
None 0.15% NaF	<i>Ad libitum</i>	2♂, 3♀	100	515-690	619
		2♂, 3♀	106	545-845	687

sodium fluoride, which caused death of young chicks in approximately four weeks, permitted fairly good growth in chickens 2 and 3 months of age. However, 1.2 percent of sodium fluoride in the ration proved very toxic to all groups.

Our observations are in harmony with

sion of growth occurred when 2 percent was used, and that 3 percent produced serious harm to growth.

The weights of the pullets varied considerably from week to week, but there was a tendency for the controls to maintain their weight while the fluorine-fed pullets lost. At

the termination of the experiment, the controls weighed on the average 73 grams less than at the beginning, while the fluorine fed pullets had lost an average of 346 grams. The animals were kept on the experiment from 21-25 weeks. One death occurred after 24 weeks of fluorine feeding.

Egg production

Previous to the experiment, five of the six pullets had been laying on an average of 20 eggs per month. Of the laying hens,

small chicks in Experiment I, the blood was pooled for analysis, but individual determinations were made on the blood of the larger chickens. Determinations were made in duplicate by the method used in our experiments with rats (Hauck, Steenbock, and Parsons, 1933).

The results of serum calcium determinations on chicks receiving sodium fluoride are summarized in Table 3. It is seen that in the young chicks which had received 0.6 and 1.2 percent sodium fluoride, the serum

TABLE 3. Serum calcium of chicks receiving sodium fluoride

Age of chick weeks	Duration of experiment weeks	Average serum calcium—mg. per 100 cc.				
		No NaF	0.15% NaF	0.3% NaF	0.6% NaF	1.2% NaF
Experiment I						
4	3	16.6 (3)*				7.6 (8)
5	4	16.1 (1)			10.5 (8)	
16	15	16.8 (7)		15.2 (8)		
Experiment II						
13	4	17.0 (7)			11.5 (6)	12.6 (6)
Experiment III						
8	5	11.3 (2)		12.2 (2)		
15	12	12.8 (2)	9.9 (1)	12.9 (1)		
15†	3½				13.8 (2)	11.7 (2)
16	13	10.5 (2)				
16†	4½					12.3 (2)
20	17	14.9 (4)	12.9 (4)	12.8 (2)		
20†	9				13.6 (3)	

* Figures in parentheses indicate the number of chicks.

† These are tabulated separately because the higher levels of fluorine were introduced late in this experiment.

the controls continued to lay averaging 19 eggs per month, whereas the fluorine fed hens laid only occasionally, averaging 4 eggs per hen per month. This is in agreement with the work of Buckner, Martin, and Peter (1923), who fed rock phosphate *ad libitum* and of Halpin and Lamb (*loc. cit.*) who found that 3 percent rock phosphate markedly decreased egg production.

Serum calcium and inorganic phosphorus

The chicks whose growth has been described were killed by bleeding from the jugular vein and blood samples were analyzed for serum calcium. In the case of the

calcium was distinctly low. A slight depression of serum calcium also occurred in the chicks that were given these levels of sodium fluoride at two months of age, but no effect of sodium fluoride on serum calcium was observed in older chicks.

The average serum calcium for the fluorine-fed hens which were not laying at the termination of the experiment was 10.8 mg., that for the fluorine-fed hens which were laying at the termination of the experiment was 16.0 mg., and that for the controls was 23.0 mg. per 100 cc. Although the serum calcium for the laying, fluorine-fed hens was less than for the controls, our data are too

limited to permit us to draw conclusions.

In Experiment III, the inorganic phosphorus of the serum was determined using the method of Fiske and Subbarow (1925). These data are presented in Table 4. All of the determinations were within the normal range for chicks. No effect of sodium fluoride on the inorganic phosphorus was observed.

Bone ash

For data on bone, tibiae from representative chicks were dissected from the fresh tissues, and were ashed as usual after thorough extraction with hot alcohol. The data on bone ash are presented in Table 5. It

Kidney phosphatase

In view of the fact that sodium fluoride has been shown to affect the *in vitro* activity of various enzymes, including phosphatase, it seemed desirable to determine whether phosphatase activity was affected by sodium fluoride feeding. Kidney phosphatase was extracted, and its activity determined essentially according to the technique of Kay (1926, 1928). The data obtained are summarized in Table 6. No significant differences in kidney phosphatase were observed. These findings are noteworthy inasmuch as Phillips (1932) has reported that plasma phosphatase is an index

TABLE 4. Serum phosphorus of chicks receiving sodium fluoride

Age of chick weeks	Duration of experiment* weeks	Average inorganic phosphorus—mg. per 100 cc.				
		No NaF	0.15% NaF	0.3% NaF	0.6% NaF	1.2% NaF
Experiment III						
15	12	7.1 (2)	8.2 (1)	6.0 (1)		
15*	3½				6.4 (2)	5.9 (2)
16	13	7.2 (2)				
16*	4½					6.0 (1)
20	17	8.4 (4)	6.5 (4)	6.8 (2)		
20*	9				6.6 (3)	

* These are tabulated separately because the higher levels of fluorine were introduced late in this experiment.

will be noted that in the young chicks which received 0.6 and 1.2 percent of sodium fluoride, the percentage of ash was somewhat higher than for the controls. However, growth in these groups was greatly retarded. It is possible that the increase in percentage ash was related to stunting. This seems the more probable since, in the older chickens, whose growth was not so depressed by fluorine feeding, no consistent difference in bone ash was observed. In this connection it is of interest to note that Halpin and Lamb (*loc. cit.*) found no measurable effect of rock phosphate feeding on the ash of tibiae of birds at 17 and 20 weeks of age, although there was considerable incidence of crooked breast bone at the 2 and 3 percent levels.

of the degree of fluorine poisoning in cattle. It is possible that this difference may be caused by difference in species or else that kidney phosphatase is less variable in fluorine poisoning than plasma phosphatase. On this we have no data.

Parathyroids and other organs

We became interested in the parathyroid relations because of the similarity in changes found by Erdheim (1911a, b, c, d) and Toyofuko (1911) in the incisors of the rat, produced by parathyroidectomy and by fluorine feeding (McCoulum *et al.*, 1925). We anticipated some difficulty in making observations of the parathyroid on so small an animal as the rat and therefore decided to study these glands in the chick as well.

TABLE 5. Bone ash of chicks receiving sodium fluoride

Supplement	Number of chicks	Age—weeks	Duration of experiment—weeks	Range in percentage ash	Average percentage ash
Experiment I					
None	3	4	3	44.0-48.6	46.1
1.2% NaF	8	4	3	47.7-55.4	49.6
0.6% NaF	8	5	4	44.2-54.5	49.9
None	2	16	15	46.9-48.4	47.7
0.3% NaF	2	16	15	43.0-47.8	45.4
Experiment III					
None	3	16	13	52.8-57.8	55.1
1.2% NaF	2	16	5	55.2-55.9	55.6
None	1	15	12		52.5
1.2% NaF	2	15	4	56.2-56.6	56.4
0.3% NaF	1	15	12		52.0
None	2	20	17	55.1-57.7	56.4
0.6% NaF	3	20	9	56.5-62.5	59.6
0.3% NaF	2	20	17	54.9-55.7	55.3

The main lobes of the parathyroid in the chick are easily observed with the naked eye, and can readily be dissected from the surrounding tissue.

Accordingly, when the experiments were terminated the parathyroids were dissected out and examined carefully for gross evidence of variation from the normal in size. Parathyroids from 33 chicks of various ages were weighed while fresh, and the glands from 31 chicks and 6 hens were examined histologically as well. No consistent differ-

ence in size of parathyroids of fluorine-fed and control animals was observed by these methods. Certainly no such marked differences occurred as have been observed in rachitic chicks by Nonidez and Goodale (1926) and Higgins and Sheard (1928). This is in harmony with our observations (1933) on rats, in which fluorine was not found to affect the size of the parathyroid. Histological examination proved that the glands of fluorine-fed chicks killed at 4 and 5 weeks of age were normal in appearance.

TABLE 6. Kidney phosphatase of chickens receiving sodium fluoride

Number of animals	Percent of NaF in ration	Range in kidney phosphatase units per gram	Average kidney phosphatase units per gram
<i>Chicks</i>			
Experiment I			
5	None	2.9-4.0	3.4
5	0.075	2.6-3.8	3.2
5	0.3	1.6-4.8	3.0
Experiment II			
5	None	2.3-3.7	3.0
5	0.6	1.2-5.4	2.8
5	1.2	1.3-3.8	2.6
<i>Hens</i>			
2	None	2.7-3.6	3.2
3	0.96	1.4-4.1	3.1

In the chicks which were three months or more of age, all of the variations in histological appearance which were noted by Higgins *et al* (1928, 1930) were apparent in the parathyroids of our chicks, both fluorine-fed and controls. We therefore have no evidence that fluorine feeding affects either the size or the histological character of the parathyroids.

The only gross change noted at autopsy was the occurrence of small local hemorrhages varying from the size of a pin point to approximately one-fourth inch in diameter, in the duodenal loop of some of the fluorine fed chickens at all levels. This was at first assumed to be a manifestation of coccidial infection. However, it was not observed in controls which were known to have coccidiosis, but did occur in some fluorine-fed chicks in Experiment III, none of which gave any evidence of this infection. These lesions were less severe, and did not occur as regularly as the hemorrhages observed in the pyloric mucosa of our fluorine-fed rats.

SUMMARY

Chicks of various ages were fed sodium fluoride in amounts ranging from 0.015 to 1.2 percent of the ration.

No unfavorable effect on the appearance or weight of the chicks was noted with levels up to 0.15 percent of sodium fluoride.

The 0.3 percent level of sodium fluoride depressed appetite and the weight of young chicks, but had little effect on chicks two and three months old. Sodium fluoride at a 1.2 percent level proved markedly toxic to chicks of all ages.

The ingestion of 0.6 percent and 1.2 percent of sodium fluoride resulted in an apparent lowering of serum calcium in the young chicks. There was no evidence of any effect of sodium fluoride on the serum cal-

cium or inorganic phosphorus of older chicks.

No consistent effect of sodium fluoride on bone ash was observed within the limits of these experiments.

Sodium fluoride ingestion did not have any significant effect on the kidney phosphatase of chicks.

No evidence was obtained that fluorine feeding influences either the size or the structure of parathyroids.

Small hemorrhages were observed in the duodenal loop of some of the fluorine fed chickens at all levels of fluorine intake.

The ingestion of 0.96 percent of sodium fluoride caused depression of weight and marked decrease in egg production of hens.

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