

## The Effect of Dietary Sodium Fluoride on Internal Organs, Breast Muscle, and Bones in Captive American Kestrels (*Falco sparverius*)

D. M. Bird, D. Carrière, and D. Lacombe<sup>1</sup>

Raptor Research Centre of McGill University, 21,111 Lakeshore Road, Ste. Anne de Bellevue, Quebec, H9X 1CO, Canada

**Abstract.** In 1982, 29 7-day-old American kestrel (*Falco sparverius*) chicks from captive stock were randomly assigned to one of three dietary regimens: (1) 10 birds were fed daily with cockerel mash (0 ppm of F<sup>-</sup>: control birds); (2) 10 birds were fed daily with cockerel mash containing 1,120 ppm of F<sup>-</sup>; (3) 9 birds were fed daily with cockerel mash containing 2,240 ppm of F<sup>-</sup>. Growth of the kestrels was not significantly affected by NaF in their diet. No significant differences were found among the 3 groups for length of duodenum, jejunum and ileum. Rectum was longer as more fluoride was added to the diet. Weights of adrenals, brain, gizzard, spleen, heart, kidneys, liver, pancreas, and pectoral muscle were not significantly affected by treatment, although kidneys, spleen and adrenals tended to become lighter. Percent bone ash was significantly ( $P < 0.05$ ) increased, while bone breaking strength was significantly ( $P < 0.05$ ) decreased by treatment.

The effect of fluoride on growth of poultry species has been extensively studied (Halpin and Lamb 1932; Anderson *et al.* 1955; Chan *et al.* 1973; Nahorniak *et al.* 1983), and it has been used to increase bone-breaking strength (Merkley 1981). Conversely, very little is known of the effect of dietary fluoride to wild bird species (van Toledo 1978; Seel 1983; Seel and Thomson 1984; Seel *et al.* 1986; Pattee *et al.* 1988; Henny and Burke 1990). Previous studies have shown that fluoride accumulated in bones and eggs of American kestrel (*Falco sparverius*) fed day-old cockerels dipped in sodium fluoride (Bird and Massari 1983) or containing sodium fluoride in their femur (Carrière *et al.* 1987). Kestrel chicks with fluorotic bones did not suffer from growth depression (Carrière 1985), which is known to be the first visible sign of fluoride poisoning (Halpin and Lamb 1932; Nahorniak *et al.* 1983). However, serious physiological and biochemical changes may occur where dietary fluoride causes no inhibition of growth. Changes in the function (Suketa and Terui 1980) and a redistribution of reducing substances were observed in tissues (Phillips and Chang 1934) and this effect is associated with changes in the biochemistry and growth of internal organs such as increases in the weights of gizzard,

heart, kidneys, liver (Yu and Driver 1982), and adrenal glands (Rao and Sushella 1979). Calcium deposits occurred in liver and kidneys, which would likely increase the weight of these organs (Marier 1981). In addition, histological changes were reported in the liver of guinea pigs, varying from extensive degenerative changes to complete necrosis (Kour *et al.* 1981). Concentrations of cyclic AMP was significantly higher in the heart of fluoride-treated rats (Stroková and Zhavoronkof 1983). Using captive American kestrels, we wanted to determine the first signs of fluoride poisoning in growing kestrel chicks and to examine the effect of fluoride on various internal organs, including muscles and bones.

### Methods

Kestrel chicks used for this experiment were obtained from the captive colony of American kestrels maintained at McGill University (Bird 1982). Artificial incubation practices for kestrel eggs were described by Bird and Laguë (1982). Twelve hours after hatching, chicks were fed mashed 1-day-old cockerels with down, beaks and legs removed. A partial analysis of the nutrient content of day-old cockerels was evaluated by Bird and Ho (1976). No vitamin or mineral supplements were added to the diet. Both control and treatment diets were prepared in advance and frozen in small blocks of about 5 g. One hour prior to the first daily meal, appropriate food quantities were left to thaw to room temperature. Treatment started at day 7 and at that early age, it was not possible to determine sex of kestrels. Available kestrels were randomly assigned to the following groups:

- (A) 0 ppm of F<sup>-</sup> (control group): 4 males and 6 females were hand-fed 5 g of mashed cockerels containing 0.0497 g of NaHCO<sub>3</sub> prior to normal feed rations.
- (B) 1,120 ppm of F<sup>-</sup>: Prior to their normal rations 5 males and 5 females were fed daily 5 g of cockerel mash containing 0.0056 g of F<sup>-</sup> (0.0123 g of sodium fluoride) and in addition 0.0248 g of NaHCO<sub>3</sub> in order to balance the level of sodium with the other two regimens.
- (C) 2,240 ppm of F<sup>-</sup>: 6 males and 3 females were fed daily 5 g of cockerel mash containing 0.0112 g of F<sup>-</sup> (0.0246 g of sodium fluoride) prior to their normal rations.

The treatment was administered until day 27. All birds were hand-fed to satiation four times daily. Birds were weighed daily prior to their first meal. Bill depth, tarsus length, third toe length, antebra-

<sup>1</sup> Address correspondence to D. Lacombe.

**Table 1.** Mean fluoride content (ppm/dry weight and ppm/ash weight) and percent bone ash in the diaphyses of femuræ, and tibiae breaking strength (maximum load: LOAD, maximum stress: STRESS, and modulus of rupture: MOR) of growing hand-reared American kestrels

Treatment	Fluoride (ppm/d wt <sup>b</sup> ) ( $\bar{X} \pm S.D.$ )	% Bone Ash ( $\bar{X} \pm S.D.$ )	Fluoride (ppm/a wt <sup>c</sup> ) ( $\bar{X} \pm S.D.$ )	Tibia breaking strength ( $\bar{X} \pm S.D.$ ) <sup>a</sup>		
				LOAD (kg)	STRESS (kg/cm <sup>2</sup> )	MOR <sup>f</sup> (MN/m <sup>2</sup> )
0 ppm	247.0 ±103.1	51.71 ±3.39	477.66 ±199.38	2.99 ±0.40	1,328.06 ±278.62	130.3 ±27.3
1,120 ppm	6,137.9** <sup>d</sup> ±823.8	56.23** ±2.96	10,897.92** ±1,465.06	2.75 N.S. <sup>e</sup> ±0.52	1,215.97 ±300.66	119.3 N.S. ±29.5
2,240 ppm	10,272.6** ±865.8	57.21** ±3.51	17,955.95** ±1,513.37	2.75 N.S. ±0.44	878.42** ±220.17	86.2** ±21.6

<sup>a</sup>  $\bar{X} \pm S.D.$ : mean  $\pm$  standard deviation

<sup>b</sup> d wt: dry weight; ppm =  $\mu\text{g/g}$

<sup>c</sup> a wt: ash weight; ppm =  $\mu\text{g/g}$

<sup>d</sup>\*\* : Significant difference between means of fluoride-fed and control birds within a column ( $P < 0.01$ ). Statistics: One-way ANOVA and Scheffé's test

<sup>e</sup> N.S.: no significant difference between means of fluoride-treated and control birds within a column ( $P > 0.05$ )

<sup>f</sup> MOR = Modulus of rupture

chium and manus were measured following procedures outlined by Bird and Laguë (1982). Every 3 days from day 0 to day 27, linear measurements were taken on the left side of the body using a Vernier caliper accurate to 0.1 mm.

At day 28, birds were sacrificed and dissected. The fresh weights of the brain, gizzard, heart, kidneys, spleen, adrenals, liver, pancreas and pectoralis muscle were recorded using a Sartorius balance. The weights for paired organs refer to the total weight of right and left organs. Total gut length, as well as length of different sections of intestinal tract (duodenum, ileum, jejunum, and rectum) were measured immediately after the carcasses were opened. To account for size differences due to body weights, organ weight and gut length were divided by body weight (Moss 1972).

Bone-breaking strength was measured with an Instron Universal Testing Machine, model IIC (Instron Engineering Corp., Canton, MA, USA). The fresh tibia was mounted on two pivots and pressed in the center until breakage (van Toledo 1981). The tibia was chosen because it is a long straight cylindrical bone with no flat surface. Hence, it was easier to calculate the maximum stress it can resist without being permanently folded.

Bone-breaking strength is calculated as follows:

$$S = MC/I$$

where S stands for STRESS, M for MOMENT, C for the DISTANCE FROM NEUTRAL FIBER and I for the FIRST MOMENT OF AREA. In detail, the formula is written as:

$$S = \frac{F(L/2)(D_o/2)}{(D_o^4 - D_i^4) \pi/64}$$

where

- L = distance between the two pivots
- $D_o$  = external diameter of the tibia
- $D_i$  = internal diameter of the tibia
- F = force applied

This index of bone strength is independent of its diameter, length or mass. To allow comparison of our study with others, STRESS (kg/cm<sup>2</sup>) can also be expressed as the MODULUS OF RUPTURE (MOR) which is the maximum bending stress expressed in Newtons per square meter (N/m<sup>2</sup>) (Wainwright *et al.* 1976; Gere and Timoshenko 1984).

Fluoride assays on bones were performed with a fluoride-specific ion electrode (Orion Research, Boston, MA, USA) (Singer and

Armstrong 1968). Bones were defleshed, split and cleaned of all marrow. They were then defatted through two 12-h treatments of alcohol-ether (Kuo and Stamm 1974). After being dried and weighed, bones were placed in a covered porcelain crucible and heated at 600°C for 4 h. Once cooled, the samples were weighed once more in order to determine the percentage of ash in the weight of the dry fat-free bone (Kuo and Stamm 1974). Ash was separated so as to make 3 replicates for each bone. Replicates were weighed and the ash was diluted in 2 ml of perchloric acid (HClO<sub>4</sub> 1.13 N). Fixed amounts of TISAB solution and NaOH 1 N were added, pH adjusted to 5.2 and total volume brought up to 20 ml. Results of fluoride concentration in bones are given on a dry fat-free basis.

Bone fluoride values were log-transformed and averaged by group. Treatment means were compared by one-way analysis of variance (ANOVA) since no sexual dimorphism was observed for bone lengths in this experiment (Carrière 1985). However, sexual dimorphism in body weight, with females being heavier than males is a normal phenomenon in raptorial birds and this could influence the weight of the internal organs. Thus, the effect of treatment on the intestinal tract and the ratio of organ weight/body weight were determined using a 2-way ANOVA where treatment and sex were considered (SAS Institute Inc 1982). In all tests where significant effects were detected, Sheffé's test was used to locate differences among means. The Shapiro-Wilk test was used to check the normality of the distribution of the data and verify the appropriateness of the use of parametric statistics.

## Results and Discussion

Contrary to what was observed in domestic bird species (Halpin and Lamb 1932; Nahorniak *et al.* 1983), none of the standard anatomical measurements, *i.e.*, body weight, skull width, bill depth, tarsus length and diameter, antebrachium and manus length, was significantly affected by the NaF treatment compared to the control group ( $P > 0.05$ : 1,120 ppm and 2,240 ppm of F-). These results indicate that kestrels apparently grew normally.

The amount of fluoride deposited in the bones of growing kestrels increased in a dose-dependent manner with dietary fluoride levels (Table 1). Treated birds had fluoride levels significantly ( $P < 0.01$ ) different from the control group.

Similarly, fluoride did accumulate in bones with increasing dietary fluoride and duration of exposure in eastern screech-owls (*Otus asio*) (Pattee *et al.* 1988) and in turkeys (*Meleagris gallavo*) (Anderson *et al.* 1955). The efficiency with which food is assimilated during growth of semi-altricial birds such as the American kestrel is higher than in precocial birds (Ricklefs 1973). This might explain why kestrels accumulated fluoride in their bones at concentrations higher than those found in studies using adult and growing chickens. Low levels of  $\text{Ca}^{++}$  in the diet also facilitated the ingestion of fluoride. In this study, calcium supplement was not added to the diet of growing kestrels. Bone fluoride concentrations for treated kestrels were much higher than those found in a preliminary study using calcium supplement (Carrière 1985), almost reaching the saturation point of 15,000 to 20,000 ppm and being indicative of poisoning as suggested for domestic animals (Phillips and Suttie 1960; Shupe 1980). Bone fluoride (ppm/ash weight) increased with age in black-crowned night-herons (*Nycticorax nycticorax*) (Henny and Burke 1990), but no attempt was done to show the same effect in kestrels. Thus, bone fluoride in growing kestrels fed dietary fluoride was 10 to 16 times greater than that found in hatch year male black-crowned night-herons, but only two to three times higher than that found in adult herons. Percent bone ash, which represents the proportion of inorganic component of the bone, also increased significantly with dietary fluoride in the treated groups compared to that of the control group ( $P < 0.01$ ) (Table 1). In other bird species, increases in bone ash were observed, as well as decreases in bone-breaking strength (Rogler and Parker 1972; Chan *et al.* 1973; Nahorniak *et al.* 1983). In virtually all studies, the fluoride content of different bones (sternum and femur) followed similar patterns (Merkley 1981; Bird and Massari 1983; Mehdi *et al.* 1983).

Bone quality seemed to be affected since significant decreases in bone-breaking strength and significant increases in bone mineralization were observed in fluoride-treated kestrels. When the breaking strength (LOAD) was expressed as the maximum load the bone can carry, no significant differences were detected among groups (Table 1). However, when these figures are used to calculate the maximum stress (STRESS and MOR) the bone can resist, bone quality clearly decreased as more fluoride was added to the diet of growing kestrels. The calculated maximum stress for the 0 ppm of F<sup>-</sup> (control group) and the group fed with 1,120 ppm of F<sup>-</sup> was significantly higher ( $P < 0.01$ ) than the value determined for the group fed with 2,240 ppm of dietary fluoride (Table 1). Bone-breaking strength (119.3 and 86.2 MN/m<sup>2</sup>) measured in growing kestrels fed high fluoride levels in their diet was lower but in the range of that measured in hatch year black-crowned night-herons (175.8 MN/m<sup>2</sup>) (Henny and Burke 1990). However, second year, third year and adult herons had higher bone-breaking strength (240.8 to 262.0 MN/m<sup>2</sup>) than young kestrels fed fluoride. Since bone quality of both black-crowned night-herons (Henny and Burke 1990) and white leghorn pullets (Merkley 1981) exposed to fluoride increased during growth, we might expect that adult kestrels fed fluoride would have bones more resistant to breakage than growing kestrels exposed to fluoride. No study has yet been done to demonstrate such an effect. The decreased bone-breaking strength caused by fluoride ingestion may result from a decreased bonding strength between the crystal

material and collagen matrix of the bone (Wolinsky *et al.* 1972). Fluoride might induce a decrease in the bone lipids acting as a chemical link between the organic and inorganic phases of the bone. This would in turn produce decreased elasticity and increased brittleness. Moreover, collagen synthesized and laid down during fluoride exposure is under hydroxylated and inadequately crosslinked (Sushella and Sharma 1982; Monsour and Kruger 1985). As a consequence, this collagen is rapidly catabolized and collagen content of the bone decreased. Also, a decrease in the regularity of collagen fibrils, as well as degradation of fibrils is observed, part of complex disturbances of the fluorotic bone (Bély *et al.* 1988). Changes in collagen structure are followed by damage to the matrix (proteoglycan aggregate) (Bély *et al.* 1988).

None of the ratios of organ weight/body weight was significantly affected by treatment ( $P > 0.05$ ) nor by sex ( $P > 0.05$ ). However, spleen and adrenal weights, although not significant but very close to the 0.05 level ( $P = 0.0893$  and  $P = 0.0518$  respectively), showed a trend to become lighter with the increase of fluoride in the diet (Table 2) and sex had no effect on these parameters ( $P > 0.05$ ).

Ingestion of dietary fluoride in growing kestrels was not associated with any significant change in total gut length ( $P > 0.05$ ) and sex has no effect on this parameter ( $P > 0.05$ ). The rectum was the only section of the intestinal tract to be significantly affected by treatment (1,120 ppm of F<sup>-</sup>:  $P < 0.05$ ; 2,240 ppm of F<sup>-</sup>:  $P < 0.01$ ) but not by sex ( $P > 0.05$ ), becoming longer as more sodium fluoride was added to the diet (Table 2). This result is difficult to explain since various factors are known to affect gut size (for a review see Moss, 1972; Bailey 1981). Parasite loads, gut microbes, rhythm of feedings, as well as the amount of food eaten and diet composition were considered in kestrels, but none of these factors can adequately account for a difference at only the terminal section of the gut. Gut microbes are likely to affect the weight of the small intestine, not the rectum length and no obvious parasites were found during dissection. In this experiment the rhythm of feeding was fixed at four times daily and fluoride was the only variable. A change in rectum length could be due either to a discrete physiological disorder (such as a change in hormonal levels provoked by fluoride intake) or more likely to simple irritation of the rectum due to the presence of salt (NaF) in the excreta. Excretion of fluoride does occur (Sushella *et al.* 1982) and heavy grey mucous covering the membrane of the duodenum has been observed in birds fed with fluoride (Anderson *et al.* 1955). In our dissections of kestrels we did not split open the intestinal tracts to determine if irritation was present in birds with longer rectums. If such an effect is confirmed, the method of experimental administration of fluoride may have to be modified.

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**Table 2.** Organ and muscle weights (g of tissue/100 g of body weight), and lengths of total gut and 4 sections of digestive tract (cm/100 g of body weight) in captive American kestrels fed dietary fluoride (F-)

Organ	0 ppm of F- ( $\bar{X} \pm S.D.$ ) <sup>a</sup>	1,120 ppm of F- ( $\bar{X} \pm S.D.$ )	2,240 ppm of F- ( $\bar{X} \pm S.D.$ )	
Gizzard	2.17 ± 0.94 (4) <sup>c</sup>	1.87 ± 0.28 (7)	2.11 ± 0.49 (7)	N.S. <sup>b</sup>
Heart	0.76 ± 0.12 (5)	0.79 ± 0.09 (7)	0.79 ± 0.07 (8)	N.S.
Pectoralis	10.15 ± 0.31 (4)	10.35 ± 0.75 (7)	9.75 ± 0.80 (7)	N.S.
Adrenals	0.0148 ± 0.0035 (5)	0.0119 ± 0.0023 (6)	0.0110 ± 0.0010 (7)	N.S.
Kidneys	0.84 ± 0.16 (5)	0.74 ± 0.06 (8)	0.78 ± 0.09 (8)	N.S.
Liver	3.20 ± 0.43 (4)	3.18 ± 0.47 (7)	2.99 ± 0.19 (8)	N.S.
Pancreas	0.31 ± 0.04 (5)	0.29 ± 0.06 (7)	0.33 ± 0.05 (8)	N.S.
Brain	1.96 ± 0.14 (5)	1.93 ± 0.15 (7)	1.99 ± 0.07 (8)	N.S.
Spleen	0.0780 ± 0.0246 (5)	0.0677 ± 0.0104 (7)	0.0576 ± 0.0112 (8)	N.S.
Total gut	40.83 ± 2.62 (5)	38.27 ± 3.32 (7)	39.41 ± 3.53 (8)	N.S.
Duodenum	10.19 ± 0.32 (5)	10.29 ± 0.01 (7)	9.76 ± 1.67 (8)	N.S.
Jejunum	16.40 ± 1.19 (5)	15.19 ± 2.61 (7)	16.35 ± 1.93 (8)	N.S.
Ileum	13.89 ± 3.78 (5)	11.64 ± 2.28 (7)	12.02 ± 2.21 (8)	N.S.
Rectum	1.01 ± 0.03 (5)	1.26 ± 0.24 <sup>*d</sup> (7)	1.38 ± 0.24 (8)	**

<sup>a</sup>  $\bar{X} \pm S.D.$ : mean ± standard deviation

<sup>b</sup> N.S.: no significant difference between means of fluoride-treated and control birds ( $P > 0.05$ ) within a row

<sup>c</sup> (N): number of birds of each treatment used to collect the internal organs

<sup>d</sup> Significant difference between means of fluoride-fed and control birds within a row. \* =  $P < 0.05$ ; \*\* =  $P < 0.01$ . Statistics: Two-way ANOVA and Scheffé's test; sex had no significant effect on all these parameters ( $P > 0.05$ )

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