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Effects of Dietary Sodium Fluoride on Bone Fluoride Levels and Reproductive Performance of Captive American Kestrels*

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ABSTRACT

Fifteen breeding pairs of captive American kestrels Falco sparverius *were randomly assigned to one of four daily dietary regimes: 4 pairs received 10 ppm NaF, 5 pairs 50 ppm NaF, 1 pair 500 ppm NaF, and 5 pairs served as controls. Administration of dietary NaF via their diet of day-old cockerels had no effect on clutch size, percent hatchability and percent fledging success, but was associated with a higher percent fertility than controls. Eggs laid by kestrels fed 50 ppm NaF had significantly thicker eggshells than the 10 ppm and control groups. The pair receiving 500 ppm NaF died after 6 days of treatment. Fluoride content in eggshells was significantly higher in the 10 and 50 ppm groups, the latter having the highest values. The last egg laid in the clutch had the highest fluoride content. Fluoride tended to bioaccumulate in bone, and more in females than males.*

INTRODUCTION

Industrial fluoride emissions have become an environmental concern, especially when dealing with chronic fluorosis in domestic farm animals (Krook & Maylin, 1978) and humans (Czerwinski & Lankosz, 1978). While fluoride levels have been monitored in various indigenous plants and animals (Kay *et al.*, 1975), little is known of the movement of fluoride through a predator–prey system. Rose & Marier (1977) reported that bones of carnivorous species contained more fluoride than those of

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herbivorous species, but felt that 'data are insufficient to permit firm conclusions regarding food-chain build-up of fluoride'.

Studies of fluoride effects on avian reproduction have been limited mainly to domestic fowl (Rogler & Parker, 1972; Chan *et al.*, 1976; Yu & Driver, 1978). Balazova & Hluchan (1968) and Rippel (1971) have shown that fluoride emissions can affect fluoride content in eggs as well as bones. Van Toledo's (1978) finding of high levels of fluoride in wild bird eggs, particularly owls, and a lame barn owl *Tyto alba* containing 1000 ppm in its bones, suggests that fluoride has the potential to biomagnify itself at the top of food chains.

American kestrels *Falco sparverius* are easily bred in captivity and have been used in many laboratory studies (Bird & Rehder, 1981). A captive colony of over 200 kestrels maintained at McGill University facilitated an assessment of the effect of dietary sodium fluoride (NaF) on bone fluoride content and reproductive performance of these birds.

Our specific objectives were as follows:

(i) to examine the effects of dietary NaF on clutch size, eggshell thickness, fertility, hatchability and fledging success in captive kestrels;
(ii) to determine whether dietary NaF would accumulate in eggshells produced by captive kestrels;
(iii) to determine the effect of dietary NaF on fluoride content in the diaphyses of femurs and humeri of male and female kestrels.

MATERIALS AND METHODS

Fifteen male and 15 female captive kestrels of up to 2 years old were paired and, avoiding related matings, randomly assigned to each of 15 exterior polyethylene breeding cages described by Bird & Laguë (1982). Each pair was randomly assigned to one of four daily dietary regimes: 4 pairs received 10 ppm NaF, 5 pairs 50 ppm NaF, 1 pair 500 ppm NaF, and 5 pairs served as controls. The NaF was mixed with flour and smudged on to the exposed abdomens of frozen–thawed day-old cockerels. The control pairs received only flour on their food. Each pair received a total of 3 cockerels daily. Raptors rarely drink water (Cooper, 1978), so this was not available.

Breeding pairs were checked daily during egg-laying and each egg was individually marked. Four days after the laying of the 1st clutch, usually

4–5 eggs, the entire clutch was removed and placed into artificial incubators, as described by Bird *et al.* (1976). The day following clutch removal, the experimental diet was administered and monitored for 10 days. Each pair recycled to produce a 2nd clutch, generally 11–14 days after removal of the 1st clutch (Bird & Laguë, 1982). Four days after the completion of the 2nd clutch the pair was sacrificed and prepared for fluoride assays, and the 2nd clutches removed for artificial incubation. Eggshells from hatched and infertile eggs were frozen for later analyses.

Assays on the diaphyses of both the femur and humeri of each bird were conducted according to procedures outlined by Singer & Armstrong (1968) and Kuo & Stamm (1974). The defatting procedure was omitted and results are presented on an ash weight basis.

The outer membranes of the eggshells were removed with a trypsin digest. The eggshells were rinsed in deionised distilled water, air-dried for several minutes, and dried in an oven at 100 °C. The eggshells were pulverised with an agate mortar and pestle into a fine powder. Up to five 200 mg powdered shell samples of each egg were placed in a plastic beaker. 1 ml of deionised distilled water and 5 ml of 1 N HCl were mixed in. The beaker covered by parafilm stood overnight. 3 ml of Orian TISAB solution were added, adjusted with 1 N NaOH to a pH of 5·0 and brought to a total volume of 10 ml. Readings were conducted with a combination fluoride electrode and an Orian digital pH-meter. The data were not corrected for recovery. All eggshell values were log-transformed and based on a 200 mg sample, not the entire egg.

Thicknesses of eggshells with membrane were measured by randomly selecting and measuring three spots on the egg's equator with a Starret ratchet-stop micrometer. Percent fertility was defined as the percentage of fertile eggs of all eggs laid; percent hatchability was defined as the percentage of fertile eggs that hatched; and percent fledging success refers to the percentage of fledged young from hatched eggs.

Analyses of variance and Duncan's multiple-range tests were conducted according to Steel & Torrie (1960) to determine any significant differences between sexes, clutches, treatments, and laying sequence of eggs.

RESULTS

The pair of kestrels receiving 500 ppm NaF died only 6 days after commencement of the experimental diet. The actual cause was unknown,

but definitely resulted from fluorosis. All other pairs survived in apparent good health and recycled to lay 2nd clutches.

Sample sizes did not permit adequate statistical analysis of the reproductive data, but certain trends were obvious (Table 1). Second clutches were smaller than 1st clutches in all treatment groups. No notable differences appeared in percent hatchability and percent fledging success. In fact, young fledging from fluoride-treated pairs behaved and

TABLE 1
Effects of Dietary Administration of Sodium Fluoride on Clutch Size, Fertility, Hatchability and Fledging Success in Captive Kestrels

		Mean clutch size	% *fertility*	% *hatchability*	% *fledged*
Control	1st clutch	4·6 (5)[a]	41 (9/22)[b]	88 (7/8)[c]	57 (4/7)[d]
	2nd clutch	4·0 (5)	35 (7/20)	83 (5/6)	100 (3/3)
10 ppm	1st clutch	4·8 (4)	84 (16/19)	94 (15/16)	73 (11/15)
	2nd clutch	4·0 (4)	81 (13/16)	85 (11/13)	60 (6/10)
50 ppm	1st clutch	4·8 (5)	65 (13/20)	92 (12/13)	80 (8/10)
	2nd clutch	4·2 (5)	77 (13/17)	85 (11/13)	70 (7/10)

[a] Mean size of 4·6 eggs in 5 clutches.
[b] 9 fertile of 22 eggs laid (1 unknown).
[c] 7 of 8 fertile eggs hatched.
[d] 4 young fledged from 7 hatched eggs.

appeared no different from young not involved in the experiment. There were no major differences in clutch size and percent hatchability between 2nd clutches in each treatment group. Percent fertility in 2nd clutches in the 10 and 50 ppm groups was more than twice that of the control group. Fledging success of 2nd clutches was somewhat higher in the control group than in both fluoride groups, but may be an artefact of a small sample size in the former.

There were no significant ($p > 0{\cdot}05$) differences between eggshell thickness of eggs in 1st and 2nd clutches (Table 2). However, eggs laid by kestrels in the 50 ppm NaF group had significantly ($p < 0{\cdot}000\,1$) thicker eggshells than those laid by birds in the control and 10 ppm groups. There was a distinct tendency for eggs to have thicker shells as more NaF was added to the diet (Table 2).

With respect to fluoride content in eggshells (Table 3), there were significant differences ($p < 0{\cdot}05$) between 1st and 2nd clutches, fluoride treatment and clutch–treatment interaction. For clarification, all 1st-clutch eggs in each treatment group served as controls because treatments were administered after the laying of the 1st clutch. There was no significant ($p > 0{\cdot}05$) difference between eggshell fluoride content in the 1st and 2nd clutches of the control group. However, in this regard, the 2nd

TABLE 2
Eggshell Thickness (Plus Membrane) of Eggs Laid by Captive Kestrels Administered Two Dietary Levels of Sodium Fluoride

Clutch[a]	*Treatment*[b]	*N* (*eggs*)	*Shell thickness* (*mm*)	*Range*
1st	Control	18	$0{\cdot}157 \pm 0{\cdot}015$	0·130–0·188
1st	10 ppm	15	$0{\cdot}162 \pm 0{\cdot}012$	0·147–0·189
1st	50 ppm	22	$0{\cdot}173 \pm 0{\cdot}015$	0·150–0·212
2nd	Control	14	$0{\cdot}162 \pm 0{\cdot}008$	0·144–0·172
2nd	10 ppm	10	$0{\cdot}168 \pm 0{\cdot}016$	0·146–0·199
2nd	50 ppm	18	$0{\cdot}177 \pm 0{\cdot}015$	0·149–0·207

[a] No significant ($p > 0{\cdot}05$, *F*-test) differences between 1st and 2nd clutches.
[b] 50 ppm treatment was significantly ($p < 0{\cdot}000\,1$, *F*-test, Duncan's multiple-range test) different from control and 10 ppm treatments.

clutches of the 10 ppm group were significantly ($p < 0{\cdot}05$) higher in fluoride than the 1st clutches of the 10 ppm and both 1st and 2nd control clutches. Similarly, the 2nd clutches of the 50 ppm group were significantly ($p < 0{\cdot}05$) higher than all other clutches in the 10 ppm and control groups.

Fluoride content of the eggs also varied relative to where they were laid in the laying sequence in the clutch. The last egg laid in the clutch was the most heavily contaminated (Table 3).

According to Table 4, there were no significant ($p > 0{\cdot}05$) differences between fluoride contents in diaphyses of femurs and humeri with respect to sex or treatments. However, there was a definite tendency for females to accumulate more fluoride in these bones than males, and this is especially evident in the male and female administered 500 ppm NaF for only 6 days. There was also a slight increase in fluoride content in bones of kestrels fed dietary NaF, particularly when dietary levels reached

TABLE 3

Mean Fluoride Content (Dry Weight) in 200 mg Samples of Eggshells Produced by Captive Kestrels Fed Two Levels of Dietary Sodium Fluoride

Clutch	*Treatment*	*Sequence of laying of eggs*				
		1	*2*	*3*	*4*	*5*
1st	Control	0·09 ± 0·13 (2)[a]	0·21 ± 0·23 (4)	0·31 ± 0·28 (3)	0·41 ± 0·35 (4)	1·29 ± 1·10 (2)
1st	10 ppm	0·23 ± 0·21 (3)	0·23 ± 0·22 (3)	0·20 ± 0·31 (4)	0·39 ± 0·46 (4)	1·05 ± 1·40 (3)
1st	50 ppm	0·28 ± 0·47 (4)	0·25 ± 0·27 (4)	0·03 ± 0·04 (3)	0·04 ± 0·08 (3)	2·23 ± 2·70 (4)
2nd	Control	0·93 ± 0·87 (3)	0·13 ± 0·16 (4)	0·25 ± 0·39 (4)	0·62 ± 0·13 (2)	2·60 ± 2·80 (2)
2nd	10 ppm	3·93 ± 1·22 (3)	4·53 ± 2·12 (4)	3·45 ± 0·26 (2)	10·99 ± 11·07 (3)	17·09 — (1)
2nd	50 ppm	39·78 ± 15·19 (4)	18·34 ± 14·40 (4)	25·18 ± 15·62 (4)	53·61 ± 20·08 (4)	—

[a] Three 200 mg samples of each of 2 eggs contained a mean ± SD of 0·09 ± 0·13 ppm dry wt.

TABLE 4

Fluoride Content (ppm F^-) in the Diaphyses of Femurs and Humeri of Captive Kestrels Administered Three Levels of Dietary Sodium Fluoride

Treatment	*Femur*		*Humerus*	
	Female	*Male*	*Female*	*Male*
Control	524 ± 211[a] (5/15)[b]	515 ± 134 (4/12)	302 ± 117 (5/15)	430 ± 120 (5/14)
10 ppm F^-	839 ± 534 (4/11)	549 ± 222 (4/11)	—	—
50 ppm F^-	820 ± 360 (5/15)	646 ± 263 (5/14)	562 ± 255 (5/15)	421 ± 237 (5/15)
500 ppm F^-	2 127 ± 5 (1/3)	896 ± 22 (1/3)	1 494 ± 29 (1/3)	842 ± 9 (1/3)

[a] Mean value ± SD in ash weight of bone diaphyses.

[b] 5 birds examined for a total of 15 samples, usually 3 samples per bird.

500 ppm. The femurs and humeri of the 500 ppm female contained more than double the fluoride content of any other group.

DISCUSSION

Second clutches smaller in size than 1st clutches are typical of captive kestrels forced to renest (Bird & Laguë, 1982), and are probably not related to the dietary fluoride treatments. The higher percent fertility in 2nd clutches of the pairs fed NaF compared with control pairs is inexplicable at this time.

The thicker eggshells with higher fluoride intake are also difficult to account for. Merkley (1981) found no reduction in egg quality or production in poultry fed up to 300 ppm NaF in their drinking water. It is plausible that the added NaF stimulated bone resorption via the parathyroid gland (Franke, 1979) to make more calcium available for eggshell production.

The significantly higher accumulation of fluoride in eggshells after treatment with NaF, as well as significantly higher accumulation in clutches of the 50 ppm group, provides conclusive evidence that dietary NaF can find its way into at least the eggshells of American kestrels. Whether the fluoride levels also rise in other parts of the kestrel egg, e.g. yolk, albumen, must be determined by further experimentation. Van Toledo (1978) analysed eggs from poultry and two wild bird species selected from nests in areas contaminated to various degrees by fluoride emissions. Fluoride content in eggs laid in contaminated areas was close to three times higher than in those laid in non-contaminated regions. It is difficult to relate our findings to van Toledo's simply because our birds were fed a certain amount of NaF over a short period of time, 10 days, and admittedly we could not accurately control exactly how much NaF was ingested by each bird. Our results showed an average fluoride content of 8·0 ppm and 34·2 ppm in 200 mg samples of eggshells of kestrels fed 10 ppm and 50 ppm, respectively. Van Toledo reported values for fluoride content in eggshells of *Parus major* ($N = 78$) and *Strix aluco* ($N = 3$) nesting in contaminated areas as being 110 ppm and 240 ppm, respectively. None of the latter species' eggs hatched.

If eggs are to be removed from wild nests for fluoride analysis, the order in which the eggs have been laid in the clutch seems important. Eggshells of the last eggs laid in the clutch had the highest fluoride contents. One might

suppose that fluoride stored originally in the medullary bone is entering the bloodstream as a result of medullary bone breakdown for egg production. Whether such a relationship would be observed in wild birds on a chronic diet of fluoride from contaminated prey bears further investigation.

Kestrels fed 10 ppm and 50 ppm NaF for 10 days did not exhibit significantly higher fluoride content in femurs and humeri. Our technique of administration of NaF did not facilitate careful monitoring of exact intakes by the kestrels. The pair fed 500 ppm definitely had extremely elevated values, quite comparable with those reported for the lame barn owl by van Toledo (1978). The high levels of fluoride reported in potential prey items of birds of prey by Kay *et al.* (1975) and the ability of birds of prey to consume and digest osseous material (Duke *et al.*, 1975) make a strong case for more study in this area, as well as monitoring of wild-living birds of prey and their eggs.

Generally, females accumulated more fluoride in their bones than males, but not significantly so. This could be a function of the continual build-up and breakdown of medullary bones in females undergoing egg production.

Finally, the role of magnesium intake may be important (Marier, 1981) as grasshoppers heavily consumed by kestrels in summer may contain large amounts of this mineral (Bird *et al.*, in press). Rogler & Parker (1972) showed that magnesium and fluoride can work synergistically to depress growth in chicks.

CONCLUSIONS

(i) Administration of dietary NaF at 10 ppm and 50 ppm for 10 days had no effect on clutch size, percent hatchability and percent fledging success, but was associated with higher percent fertility than controls. Eggs laid by kestrels fed 50 ppm NaF had significantly thicker eggshells than those of the 10 ppm and control groups.

(ii) Fluoride content in eggshells was significantly higher in kestrels fed 10 ppm and 50 ppm NaF, the latter having the highest values. First and 4th eggs in the order of laying in the clutch had significantly higher fluoride content than 2nd, 3rd and 5th eggs.

(iii) Although neither was significant, there were definite tendencies for

fluoride to bioaccumulate in bone and for females to contain higher accumulations of bone fluoride than males.

(iv) There is a definite need for further research to quantify dose–response relationships under even more controlled conditions to discover the role of diet and determine the mechanisms of bioaccumulation and excretion of fluoride in avian species and, most important, to determine the impact, if any, on the reproductive performance and health of wild-living birds of prey.

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