AFRICA ENVIRONMENTAL REVIEW Journal

ISSN: 1727-8341

AER Journal Volume 4, Issue 2, pp. 73-81, Aug, 2021

RESEARCH ARTICLE

Available Online at *http://www.aer-journal.info*

Fluoride in Chicken (*Gallus domesticus*) Feathers from Nakuru, Kenya

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Abstract

Fluoride (F) toxicity in avian species has elicited global attention. To study the fluoride risks in chicken domesticated in Nakuru County of Kenya, maize and water samples were considered as potential sources of fluoride excessive intake while feathers gave an indication of fluoride body burden. The fluoride concentrations were measured and correlated to the levels in the poultry water, chicken feathers and in maize samples obtained from farms where the poultry was reared. The resulting data indicated a fluoride concentrations range of 10.4 ± 3.5 to 20.2 ± 3.5 mg/kg in the maize sampless, whereas in chicken feathers the fluoride levels ranged from 6.3 ± 2.4 to 16.6 ± 8.4 mg/kg. The F concentration in poultry water (0.4 ± 0.5 to 2.8 ± 2.2mg/L), was correlated to those in chicken feathers and the results showed that poultry water was major contributing factor to overall F exposure of farm birds. It was observed that labile F from high altitude areas percolated and polluted water and biological systems down-slope at distances controlled by the slope of land. For this reason, the areas at the rift valley floor were at greatest risk of fluoride toxicity.

Keywords: Fluoride, Chicken, Nakuru, Altitude, Slope

INTRODUCTION

Recent reports on occurrence of high fluoride (F) in farm products have heightened concerns over the fate of F in environments (Alonso & Camargo, 2011; Amenu *et al.*, 2013). Overexposure to F leads to fluorosis and it has been linked to hypothyroidism, depression, low concentration spans, chronic memory lapses, impaired hearing, and low IQ scores in children (Prystupa, 2011). F contamination also impairs the quality of agricultural products, and compromise safety of consumers of polluted products. For that reason, high F has become a major impediment to marketability of farm produce from affected areas and the assessment of F exposure in farm animals is therefore of essence but current methods for F assessment in livestock are tedious and expensive (Choubisa *et al.*, 2011). Luckily, environmental residues picked by poultry are known to accumulate proportionately in the birds' feathers in relation to the bird's total body burden (Burger & Gochfeld, 2015). Consequently, birds' feathers play vital roles in storing and eliminating undesirable residues from birds' bodies and assessment of pollutants in birds' feathers provides convenient and inexpensive ways for bio-monitoring distribution of environmental pollutants in the ecosystems (Dauwe *et al.*, 2020).

The present work investigated F contamination in chicken feathers from high-F areas of Nakuru County of Kenya. in order to determine the extent of F pollution and its impact in agricultural systems. Chicken feathers were collected from poultry farms and analyzed for F content using ion specific electrodes. The water that is normally provided to the chickens for drinking and maize (*Zea mays*) samples from the farms were also collected and analyzed. It was anticipated that understanding F distribution in chicken (*Gallus domesticus*) feathers could avail additional data that could inform future mitigation efforts to enhance food safety and safeguard public health of communities in the F affected areas.

MATERIALS AND METHODS

Figure 1 shows the map of study area (A) in relation to the map of Kenya (B) and the map of Africa (C). The area, which comprises Nakuru County of Kenya, covers 2,300 km^2 and is bound by latitudes 00^o 00'N and 01 ^{o}00'S and longitudes 35^o 30' E and 36^o 30'E. Sampling was conducted between 28th and 31st August in 2017 between long (March-July) and short (September-November) rain seasons.

Poultry farmers were selected randomly from the entire study area. A total of 167 chickens were picked from the flocks and three feathers plucked from each (Wambu *et al.,* 2014). Poultry water samples and samples of maize grain from the same farms were also collected. The feathers and the maize samples were packed into separate *khaki* envelopes and the water samples into 500-mL plastic bottles, respectively. The metadata including farmer's name, farm GPS coordinates, administrative location and primary source of poultry water were recorded. The water samples were fixed on-site by addition of 1.0 mL of 6 N HNO_3 and transported to the laboratory in a cool box.

At the laboratory, the feathers were washed in acetone, rinsed in a detergent solution, and then in 2 N sulphuric (IV) acid and in excess de-ionized water. Excess water was allowed to drip off and samples dried in an oven (UN 55 plus –en, Memmert, Germany) at 105^{o}C for 12 h before they were ground to fine powder using a grinder (model TLG-200L4, Zhejiang xilling, Zhejiang, China). They were then preserved in air-tight plastic sample vials pending analyses.

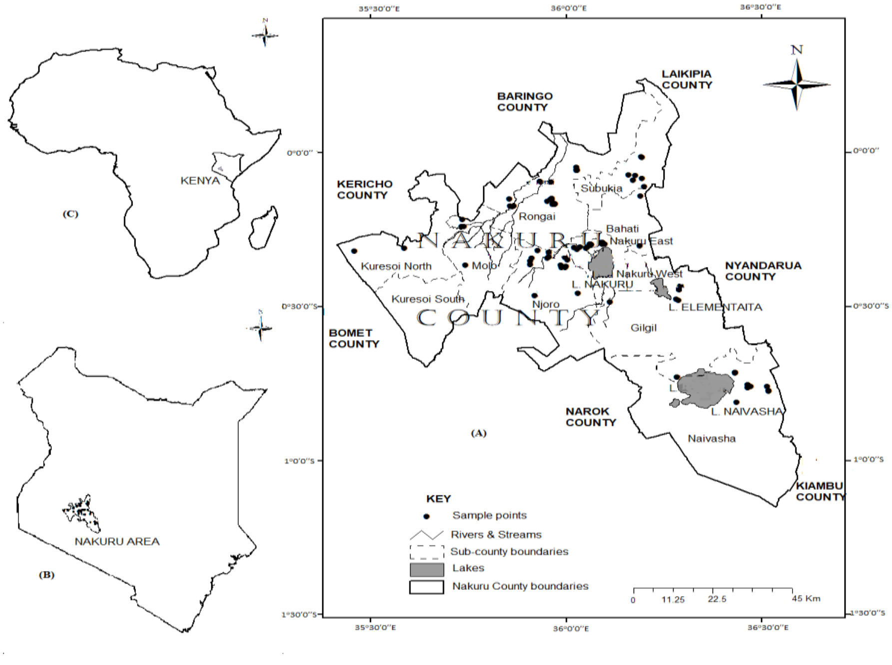


Figure 1: Location Map of Nakuru Study Area (A) in Relation to Map of Kenya (B) and Map of Africa (C).

The maize samples, on the other hand, were washed in excess de-ionized water, dried, ground, and preserved in the same way.

Exactly 1.25 g portions of each of ground feathers and maize samples were treated with 10 mL of 6 M sodium hydroxide in separate digesting tubes and heated over a water bath until they dissolved in the base.

The resulting solutions were allowed to cool to room temperature and neutralized by addition of 8 M sulphuric (VI) acid. The mixtures were transferred into 50-mL volumetric flasks and the solutions made up to 50 mL with de-ionized water. The F activity of the water samples and of the feathers and the maize samples extracts in milivolts (mV) was measured using a Jenway® fluoride ion specific electrode (ISE) with a Jenway® fluoride meter (Jenway Ltd., Essex, UK) [10]. The F concentration of water samples was calculated using a calibration curve constructed from F standards of 0.1–100 mg/L

The original F content (mg/kg) in maize and chicken feathers was calculated from Eq. 1 as follows:

$$C_s = C_l \times \frac{V}{m} \quad \text{…….eq 1}$$

where, C_s is F concentration (mg/kg) in solid feathers samples, C_l is F concentration mg/L) in solution, v is volume (L) of digested sample solution used, *m* is mass (kg) of the portion of sample used. Trends in variation of F content with altitude of sample points were analyzed by plotting trend curves based on 10-interval moving averages of F distribution versus sample point elevations. All experiments were conducted in triplicate and data analyzed in *Microsoft Excel* by calculating the mean, standard deviation and range on replicate experimental values. Causative effects were determined by calculating the correlation coefficients (R^2).

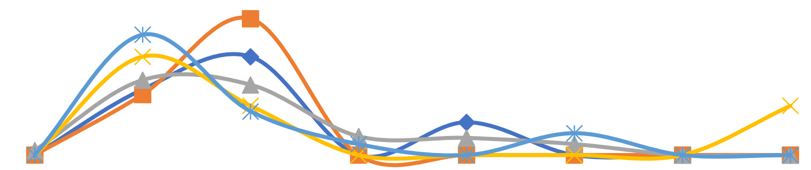
RESULTS

Distribution of chicken breeds by sampling sub-regions is presented in Table 1. Majority of chickens studied were Kenya's indigenous (*Kienyeji*) chicken (75.4%) followed by layers (10.7%), Kenya Agricultural Research Institute (KARI)-improved *Kienyeji* chicken (10.3%), exotic broilers (4.8%), and Kuroiler chicken (3.2%), which were introduced in Kenya from India through Uganda in the year 2010. The population of *Kienyeji* chicken was highest in rural areas in Njoro (27.8%), Rongai (21.4%), Naivasha (15.1%), and Subukia (94.4% %) whereas the other commercial chicken breeds were concentrated in urban areas in Nakuru and Naivasha sub-counties.

Figure 2 shows that mean F levels in feathers followed the order: Kuroiler chicken (16.9±14.20 mg/kg)> broilers (12.4±5.38 mg/kg) > *Kienyeji* chicken (12.1±5.62 mg/kg) > layer (11.0±5.67 mg/kg) > improved KARI (10.0±2.21 mg/kg) breeds. However, greater part of feathers samples (79.0%) had F levels of 6.0–15.0 mg/kg but the data was skewed towards greater values of F and 85.7% of the outliers lay in the upper quartiles.

Table 1: Distribution of Chicken Breed by Sub-Counties of Nakuru County

Sub-counties	**NUMBER OF CHICKEN BY BREEDS (n, %)**					**Overall**
	Broilers	**Improved KARI**	***Kienyeji***	**Kuroilers**	**Layers**	
Gilgil	0 (0.0%)	1 (7.7%)	3 (2.4%)	1 (25.0%)	0 (0.0%)	5 (3.0%)
Molo	0 (0.0%)	0 (0.0%)	5 (4.0%)	0 (0.0%)	0 (0.0%)	5 (3.0%)
Naivasha	1 (16.7%)	3 (23.1%)	19 (15.1%)	0 (0.0%)	2 (11.1%)	25 (15.0%)
Nakuru east	2 (33.3%)	0 (0.0%)	9 (7.1%)	1 (25.0%)	3 (16.7%)	15 (9.0%)
Nakuru west	1 (16.7%)	0 (0.0%)	11 (8.7%)	1 (25.0%)	11 (61.1%)	24 (14.4%)
Njoro	0 (0.0%)	4 (30.3%)	35 (27.8%)	1 (25.0%)	0 (0.0%)	40 (24.0%)
Rongai	1 (16.7%)	5 (38.5%)	27 (21.4%)	0 (0.0%)	2 (11.1%)	35 (21.0%)
Subukia	1 (16.7%)	0 (0.0%)	17 (13.5%)	0 (0.0%)	0 (0.0%)	18 (10.8%)
Overall	**6 (3.6%)**	**13 (7.8%)**	**126 (75.4%)**	**4(3.4%)**	**18 (10.8%)**	**167 (100.0%)**



	0-4	5-9	10-14	15-19	20-24	25-29	30-34	35-39
% Broilers	0.0	33.3	50.0	0.0	16.7	0.0	0	0
% Improved KARI	0.0	30.8	69.2	0.0	0.0	0.0	0	0
% Indigeneous	2.4	38.1	35.7	9.5	8.7	5.6	0	0
% Kuroilers	0.0	50.0	25.0	0.0	0.0	0.0	0	25
% Layers	0.0	61.1	22.2	5.6	0.0	11.1	0	0

Figure 2: Variation in F Levels of the Feathers by Chicken Breeds.

Highest incidences of high poultry water F (Table 2) occurred in areas around Lakes Nakuru and Elmentaita at the rift valley floor and a large percentage of poultry water from Nakuru East (73.4%), Nakuru West (76%), and Naivasha (85.8%) sub-counties, which are in close proximity, contained F levels above World Health Organization (WHO) allowable maximum concentration for human consumption of 1.5 mg/L [11]. Nevertheless, poultry water F levels decreased with increasing distance from the rift valley floor and lowest F levels occurred in high altitudes in Molo (0.5±0.6 mg/L), Subukia (0.5±0.4 mg/L), and Rongai (0.4±0.5 mg/L) where 80% of poultry water had F levels below 0.7 mg/L recommended by EPA [12]. Between the two extremes, there were intermediate F levels in Njoro (1.8±2.1 mg/L) and in Gilgil (1.2±2.1 mg/L), which lie within transitional altitude areas.

The trends in water F were reflected in F levels in chicken feathers even though the mean F levels in the latter samples were an order of magnitude higher.

Table 2: Spatial Distribution of F in the Chicken Feathers by Regions

SUB-COUNTIES	**AV. ALTITUDE ((x±SD) m)**	**AV. F CONCENTRATION (x±SD)**		
		WATER (mg/L)	FEATHERS (mg/kg)	MAIZE (mg/kg)
Molo	2434±19	0.5±0.6	6.3±2.4	20.1±3.3
Subukia	2310±96	0.5±0.4	11.9±4.4	11.4±7.7
Naivasha	2042±107	2.8±1.8	10.4±3.0	10.4±3.5
Njoro	2033±199	1.8±2.1	12.2±6.0	12.9±5.9
Gilgil	1900±24	1.2±2.1	9.4±0.9	20.2±3.5
Nakuru East	1813±50	2.8±2.2	16.6±8.4	14.5±6.2
Nakuru West	1806±13	2.8±2.2	11.3±4.7	15.8±6.6
Rongai	1767±90	0.4±0.5	12.2±6.4	13.7±3.3

Therefore, Figure 3 revealed matched phases in the graph of F levels in chicken feathers and that for poultry water F content. However, sections in water F curve occurred at altitudes that were10–80 m lower than those for corresponding segments in the feathers' F curve. The differences in altitude of similar sections for the two curves were greater in high altitudes and steeper slopes than in lowlands at the rift valley floor.

No compelling similarities could however be observed between the maize F levels and the water and the feathers F content. Instead, Figure 3 showed divergent F trends for the maize samples relative to those observed in the poultry water and feathers. Also, the maize F content reached limiting values of 13–15 mg/kg in areas of particularly high water and poultry feathers F.

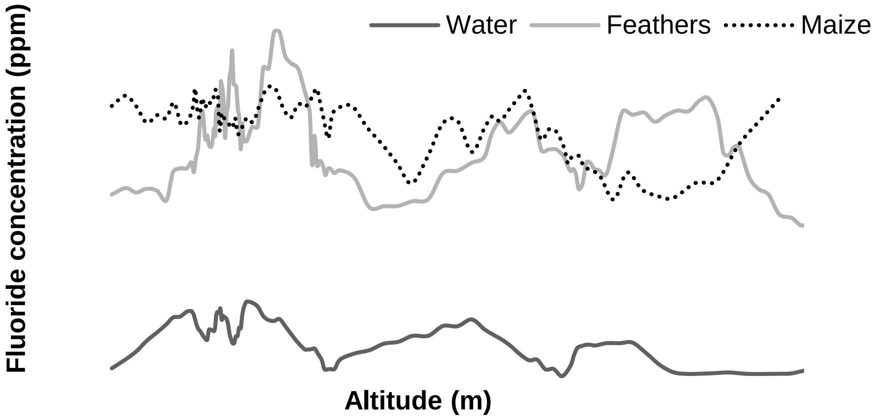


Figure 3: Trends in Variations in F Concentration in Water Sources, Chicken Feathers and Maize Samples with Altitude.

DISCUSSION

Incidences of high F in poultry are linked to birds consuming excessive F through diets (Komsa *et al.,* 2016). High F in environmental water and soil samples is associated with areas of little rainfall, high temperatures, low slopes, and expansive drainage basins (Kahama *et al.*, 1997). In the current study, areas of high F in chicken feathers as well as in the poultry water were those linked to the hot and dry zones at the rift valley floor and lowest F in water (≈ 0.5 mg/kg) occurred among highland areas in Molo, Subukia, and Rongai, which was consistent with results of previous studies that had placed water F levels in Molo Gikunju *et al*. (1995) and in Njoro Moturi (2004) at 0.188-0.443 mg/kg and 0.69-7.75 mg/kg, respectively. Occurrence of high water F in the lowland areas results from hydrogeological translocation and deposition of labile F released from F-rich rocks in the highlands and from higher rates of evaporation due to elevated temperatures, which tend to raise the F levels in environmental samples among the lowlands. Also, chickens in hot and drier areas consume larger volumes of water Frame (2008); Jacob (2018) which exacerbates their exposure to F content from the environment.

The trends in the F levels of chicken feathers related to the distribution of poultry water F and areas of high poultry water F were also high in F content of chicken feathers and vice versa (Figure 3). Poultry drinking water was, therefore, the key factor that controlled the birds' exposure to excessive F from environment (Jacob, 2018). This is because dispersion of F in the natural environment follows the prevailing drainage patterns (Wambu *et al.*, 2014). Besides, water is the single nutrient that is consumed by birds in the highest proportions and chicken, in particular, consume twice as much water as the amounts of feeds they eat (Frame, 2008). Chicken exposure to F among low-altitude areas is further aggravated by the fact that birds tend to consume increasingly more of water (and progressively less amounts of feeds) under conditions of increasing temperature Lyons (1997) which means that the lower the altitude of the drainage basin, the higher temperatures and amounts of water consumption exposing the birds to greater proportions of pollutant.

Comparison of water F and feathers F, which is depicted in Figure 3, further showed that phases in water F appeared at altitudes that were lower than those for corresponding phases of F in chicken feathers. Differences in altitudes of water F and feathers F were, however, greater in areas of steeper slopes than in lowlands of at the rift valley floor. It showed that soluble F from enriched rocks in the highlands percolated to enrich and impact environmental samples and chicken flocks in the lower altitudes of the current study areas. It also showed, as expected, that translocation of labile F was greater and faster among the steeper highland areas than in the lowlands of gentler slopes at the rift valley floor.

Even so, birds intake of water and other dietary rations depends on birds' breed, the mode of rearing, and the type of feeds among other factors (Trautner & Einwag, 1989). Egg-laying chicken breeds, for instance, require diets that are enriched in calcium and phosphorus, which are needed for egg shell formation Jacob *et al*. (2011), whereas meat-type chicken need protein-enriched diets for body development (Jacob, 2018). It can be assumed that the egg-producing chicken could, therefore, be at greater risk of F exposure through feeds than the meat-type chicken breeds. This is because calcium and phosphorus sources have generally been linked to greater incidences of elevated chicken exposure to F than protein sources (Ammerman *et al.,* 1977). Nevertheless, nutrition requirements of birds and the potential of birds' exposure to environmental residues is also a function of their levels of productivity [16] and hens that are laying eggs have greater likelihood of exposure to environmental residues than those that are not in production as the former require more feeds than the latter (Jacob & Pescatore, 2012).

The rate of maturation and the resulting age-dependent nutritional needs of chicken vary according to the birds' body sizes and play a major role in determining the degree of exposure of birds to environmental residues. Larger chicken breeds grow faster and have greater nutritional requirements than smaller birds of same age. The larger birds, therefore, consume greater amounts of feeds and water and are exposed to greater amounts of dietary residues compared to smaller chicken (Jacob *et al*., 2011). Thus, the meat-type chicken, which are typically larger-bodied, also mature faster Jacob (2015) consume greater amounts of feeds and water, and were, therefore, expected to exhibit greater F in their feathers than the smaller chicken breeds. As a result, the Kuroiler Farmer's Trend and broiler Bizna Digital birds, which are large breeds of chicken, exhibited higher F levels in their feathers compared to other breeds. Also, the *Kienyeji* chicken, which is generally small chicken breeds Lwelamira & Kifaro (2010), showed low F levels in their feathers even though majority of *Kienyeji* chicken were pasture-fed and were expected to exhibit to greater F content in their feathers.

Then, broiler chicken is typically slaughtered at six to eight weeks of age Owings (1995) whereas layers remain in flocks for 3-5 years (Jacob & Pescatore, 2012). It was expected, therefore, that the egg-producing chicken flocks would exhibit greater F levels in their feathers due to their greater longevity in the farms than meat-type chicken. Nonetheless, the meat-type chicken breeds in the present study showed greater F levels in their feathers indicating that most of the birds' feathers F burden was accumulated during the early years of the birds' growth when calcified body tissues were most rapidly developing. It also showed that the capacity of chicken to retain F in the feathers diminished greatly with maturity of the birds. The current data, therefore, further showed that broiler chickens, due to their greater nutrition requirements during the growing stages, consumed greater amounts of feeds and water, which exposed them to greater F content than the other chicken breeds of same age.

Similarly, browsing chicken were expected to be exhibit greater risk of F exposure than birds reared under more confined systems of management (Jacob & Pescatore, 2012; Deng *et al.,* 2007). This is because pasture-fed chickens are typically more active, have greater energy requirements, consume greater amounts of dietary rations, and are more exposed to uncontrolled feedstuffs from the environment than chicken flocks reared under intensive management systems. The bulk of *Kienyeji* chickens in rural areas are kept under free-range systems of managements [21] and they were expected to exhibit greater F levels in their feathers. Nonetheless, greater prevalence of F was found in chicken feathers of exotic broilers and layers breeds, which are typically reared under intensive systems of management. This could be ascribed in part to the fact that, in the current study, commercial chicken farming rather than *Kienyeji* chicken rearing were more concentrated in urban areas around Nakuru and Naivasha towns, which are also well known endemic high F areas in the world (Jirsa *et al.,* 2013).

CONCLUSION

Excess F was reported in poultry water, chicken feathers, and maize samples from all parts of the study area. F levels in chicken feathers correlated to those in poultry water showing that water was the primary pathway by which poultry got exposed to excessive environmental F. Excessive F released from highlands translocated downstream and impacted water and poultry farms at distances determined by land gradient. Incidences of high F in poultry water and poultry feathers were, therefore, highest in lowlands with long gentle slopes, low average rainfall and high climatic temperatures. On the other hand, meat-type chicken breeds were most susceptible to F accumulation in their feathers and high F susceptibility of the chicken breeds was linked to their high productivity and fast rates of maturation.

Acknowledgement

This project received funding from European Union's Horizon 2020 Research and Innovation Programme under Grant Agreement No. 690378.

Conflict of Interest

The authors declared that there is no conflict of interest.

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