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## Some Morphometric and Radiological Features of the African Bush Fowl (*Francolinus bicalcaratus*): Insights for Domestication and Conservation Strategies

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Running title: Morphometry and Radiology of the Gastrointestinal Tract of African Bush Fowl

### Abstract

The Double-Spurred Francolin (*Francolinus bicalcaratus*), or the African Bush Fowl, is a keystone savannah species. The bird is edible but faces threats from habitat loss and hunting pressure despite being economically valuable to several populations. This calls for concern about research and methods in supporting conservation and population viability measures. The significance of environmental requirements and population monitoring in ensuring conservation measures is brought out by earlier research work conducted on the other francolin species.

The gastrointestinal ecology and health of free-living birds depend on gastrointestinal transit time (GIT) and radiology examinations. While GIT transit time affects a bird's diet, foraging habits, and nutrient absorption, radiology is used to detect gastrointestinal disease and illustrate food transit. When used together, these tests give a complete picture of the digestive system of free-living birds, which can be used to guide conservation and wildlife management strategies. This information can be utilized to conserve wild bird species by informing habitat, diet, and population management.

This study explores the anatomical and radiological diameter of the GI tract of the African Bush Fowl on radiographs. Prior to radiography, in which ten adult African Bush Fowls (five males and five females) were immobilized and anesthetized, prior to being humanely put down, there was plain radiography for GIT measurement in size and GI transit times analysis on contrast-enhanced images. Morphometric measurement was used to measure and quantify the digestive organs after euthanasia.

The presence of grit required for processing food was demonstrated via the mineral composition of the crop and gizzard. This might also be attributed to ingestion of grain by birds. In comparison to photographs, the transit time of GI was on average forty minutes. A correlation between carcass weight and organosomatic marker or GI tract linear measurement did not exist. This is in agreement with some research in fish and mammals indicating that any-sized animals are able to adapt the morphophysiology of the digestive system. Such independence may be initiated by the environment, adaptive evolutionary mechanisms, or large-scale differences in individual variation. To gain complete appreciation of such mechanisms and how they impact domestication and conservation plans, more studies are required.

## Introduction

Second to the guinea fowl in economic value in the biological belt of the African savannah is the African Bush Fowl, or double-spurred francolin (*Francolinus bicalcaratus*) (Owolabi and Akinpelu, 2020; Nigel et al., 2021). One of the most significant game animals and food sources, the species is highly valued as a culinary delicacy by much socioeconomic classes and is mostly obtained through hunting (Aro et al., 2020). However, as a consequence of intense hunting pressure, habitat degradation may be enhanced by climate change and the endangered status of the species, francolins and African bush fowl have declined in numbers, and this has concerned stakeholders like hunters, conservation biologists, and wildlife enthusiasts (Aro et al., 2020).

The double-spurred Francolin is endangered in Morocco. Population density monitoring and reintroduction of captive-bred birds to the wild have been highlighted by conservation (Dörgeloh, 2005; Saâd and Abdeljebbar, 2014). Conservation design should be effective, and it is necessary to know breeding practices, habitat needs, and threats (Robertson et al., 1997).

In order to optimize feeding efficiency and feed conversion ratio, the avian stomach must be known in order to design adaptation mechanisms for domestication. Experiments conducted on various birds, including utilized as raptors, cassowaries, and chickens, revealed that histological features

of the digestive tract change with regional diet, influencing the ability of birds to digest nutrients (Dini et al., 2022). Moreover, poultry studies have aimed to describe the transcriptome of various parts of the gastrointestinal tract for the purpose of finding genes implicated in digestion activities, highlighting digestive efficiency as being critical in feedstuff utilization over a wider array of feedstuffs (Amélie et al., 2018). The worth of knowing the gut to give optimum nutrient usage and disease resistance in poultry is again highlighted through studies on morphometrics and microstructure of the gastrointestinal tract in free-ranging birds, which have shown correlations between ranging profiles, intake during foraging, and gut development (Joanna et al., 2021).

The location, volume, and shape of the GI organs can all be determined using contrast radiography. Transit times can be assessed when given in series (Morescoo and Goncalves, 2022). Because it has a short transit time and causes fewer adverse effects than some other contrast media like barium sulphate, iohexol is a safe and appropriate low-osmolality nonionic iodinated contrast medium for use in birds (Moresco & Goncalves, 2022). In avian medicine, it is preferable to have a rapid transit time because this allows speedy diagnosis with little stress handling. The purpose of this study is to evaluate the African Bush Fowl's gastrointestinal tract anatomical sizes from gross and radiographic perspectives.

## Materials and Methods

### *Animals*

Ten adult double-spurred Francolins (*Pternistis bicalcaratus*) five males, and five females of weight 167 to 350 grams were purchased from a bad market in Badagry, Lagos, Nigeria. Before sample collection began, the birds were allowed to acclimatize to natural light/darkness for two weeks in the Experimental Animal House, Department of Veterinary Anatomy, Faculty of Veterinary Medicine, University of Ibadan, Ibadan, Nigeria. They were fed moist growers' marsh in ventilated cardboard boxes to prevent damage due to confinement. Ten birds of both sexes were purchased, and 0.02 mg/kg body weight of low-dose ketamine was used to sedate them (Elsayed et al., 2019). Two view radiographs were obtained before administering positive contrast media to assess transit time to examine the gastrointestinal tract.

### **Ethical approval**

Ethics approval for the research was provided by the University of Ibadan Animal Care and Use Research Ethics Committee (UI-ACUREC), with certificate number UI-ACUREC/18/0114.

### **Radiography**

Intramuscular ketamine hydrochloride (0.02 mg/kg) was used for sedating the birds. Plain radiography was taken for comparing the arrangement of bones in birds with their digestive tracts. Radiography was taken on the Allengers 525 Fluo machine. Two standard radiographic views, dorsoventral and lateral views, were taken to acquire the full view of the digestive tract. General

morphology of the digestive organs and any structural abnormalities were evaluated in the radiographs.

### **Transit Time**

Bolus transit time was measured by giving the oral suspension of barium sulfate (1.5 mL/kg body weight) to the birds and taking serial radiographs until barium reached the cloaca at five-minute intervals. Transit time was the time elapsed between the administration and arrival of the barium in the cloaca.

### **Morphometric Analysis**

#### **Weight and Linear Measurements**

A precision electronic balance were used to measure the birds once they had been killed through cervical dislocation and then having their respective digestive organs excised post-mortem. The linear (length) measurements of the digestive organs were taken with a digital caliper. These included the esophagus, crop, proventriculus, gizzard, duodenum, jejunum/ileum, ceca, colorectum, and the cloaca.

#### **Analysis of numerical Data**

GraphPad Prism software version 8 (GraphPad Software, San Diego, CA, USA) was employed to analyze the data. The numerals of all produced data were reported by means  $\pm$  standard error of means. Pearson correlation coefficient was applied to determine whether the relationship exists between carcass weight and gastrointestinal tract organosomatic indices; a p-value  $<0.05$  was the statistical significance.

## Results

In the cranial chest, the plain radiographs showed the crop and the trachea. Most of the plain radiographs showed the air sacs, and the crop and gizzard appeared to contain mineral opacity, which is consistent with the fact that the gizzard contains stones, which is used to grind food particles.



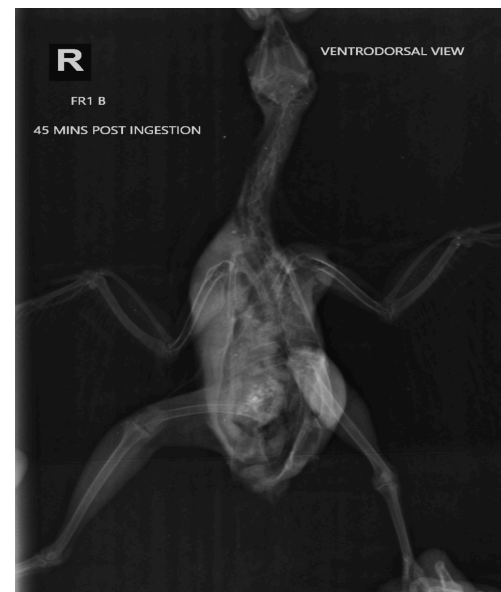
**Figure 1:** Plain radiograph of male the African Bush Fowl in lateral view showing the trachea, grit within the crop (red arrowhead), stones within the true stomach (blue arrowhead) and intestinal loops are visible (white arrowhead).



**Figure 2:** Plain radiograph of female African Bush Fowl in dorsoventral view.



**Figure 3:** 5 mins post contrast view of African bush fowl. The contrast agent was at the crop (arrowheads) at the time of the radiograph.



**Figure 4:** 45 mins post contrast view of African Bush Fowl within the large intestine (arrows).

**Table 1: Mean ±SEM (range) Organosomatic indices of the Gastrointestinal tract of African Bush Fowl**

Carcass Weight	Esophagus	Duodenum	Jejunum /Ileum	Colo/ rectum	Caecum	Proventriculus	Gizzard	Liver
185.1 ± 12.05 (167.3- 208.1)	0.5991 ± 0.03770 (0.5286- 0.6575)	0.881 ± 0.08612 (0.7173- 1.009)	604.1 ± 326.2 (2.639- 1124)	0.9881 ±0.1233 (0.7689- 1.195)	1.167 ± 0.1768 (0.8368- 1.442)	0.4065 ± 0.01105 (0.3844- 0.4184)	3.153 ± 0.08252 (2.989- 3.25)	1.606 ± 0.1202 (1.375- 1.778)

**Table 2: Correlation of Organosomatic indices of the Gastrointestinal tract of African Bush Fowl**

	Carcass weight vs. Duodenum	Carcass weight vs. Jejunum /Ileum	Carcass weight vs. Colorectum	Carcass weight vs. Caecum	Carcass weight vs. Proventriculus	Carcass weight vs. Gizzard	Carcass weight vs. Liver
Pearson r							
R	0.9771	0.9771	-0.9771	0.9771	0.9771	0.9771	0.9771
95% confidence interval							
R squared	0.9547	0.9547	0.9547	0.9547	0.9547	0.9547	0.9547
P value							
P (two-tailed)	0.1366	0.1366	0.1366	0.1366	0.1366	0.1366	0.1366
P value summary	ns	ns	ns	ns	ns	ns	ns
Significant? (alpha = 0.05)	no	no	no	no	no	no	no

**Table 3: Mean ± SEM Linear measurements (cm) of the Gastrointestinal tract of African Bush Fowl**

	Carcass Weight	Esophagus	Duodenum	Intestine	Colorectum	Caecum	Proventriculus
Mean	234.7	8.650	8.650	38.00	6.450	7.650	3.300
Std. Error of Mean	± 20.42	± 1.530	± 1.068	± 2.598	± 0.1443	± 1.068	± 0.4041

**Table 4: Carcass Weight/ Linear Correlation of the Gastrointestinal tract of African Bush Fowl**

	Carcass Weight Vs. Esophagus Length (Cm)	Carcass Weight Vs. Duodenum Length (Cm)	Carcass Weight Vs. Intestine Length (Cm)	Carcass Weight Vs. Colorectum Length (Cm)	Carcass Weight Vs. Caecum Length (Cm)	Carcass Weight Vs. Proventriculus Length (Cm)
Pearson r						
R	-0.9771	0.9771	0.9771	-0.9771	0.9771	0.9771
95% confidence interval						
R squared	0.9547	0.9547	0.9547	0.9547	0.9547	0.9547
P value						
P (two-tailed)	0.1366	0.1366	0.1366	0.1366	0.1366	0.1366
P value summary	ns	ns	ns	ns	ns	ns
Significant? (alpha = 0.05)	no	no	no	no	no	no

## Discussion and Conclusion

The radiographic results validated the existence of mineral opacity in ventriculus/gizzard and crop and air opacity within the trachea (Figure 1), which indicated the use of grit or gravel for grinding food because of muscular activity (Al-Samawy et al., 2021). Contrast agent appears to have passed through the entire gastrointestinal system in 40 minutes, from visibility in the evidence by contrast radiographs showing trace of contrast in the crop after 5 minutes (Figure 3) to that contained in the large intestine after 45 minutes (Figure 4). Although a mean transit time of 40 minutes is seen in this species, it may be necessary to increase the contrast dose to adequately mark various gastrointestinal tract regions.

This study did not find any relationship of carcass weight with linear measurements and organosomatic ratios of the digestive tube in *Francolinus bicalcaratus*. Whereas, in this case, the research failed to find any correlation between organosomatic ratios and linear measurements of the digestive tube and carcass weight in *Francolinus bicalcaratus*. This result was consistent with those reported from *Pirapitinga* fish (Fagner et al., 2019) and certain mammals (María et al., 2021), which also demonstrated that there was no relationship between weight of the carcass and measurements of the digestive system, while other birds, such as vultures (Anika et al., 2023) and pigeons (Dariusz et al., 2020), exhibited significant correlations between body weight and parameters of the digestive system. This disparity suggests that, in some animals, morphophysiology of the digestive system may be separate from body size, and

it requires investigation of the cause and consequence of such separation. Highly significant relationships between body weight and characteristics of the digestive system have been reported in studies of other birds, such as vultures (Anika et al., 2023) and pigeons (Dariusz et al., 2020).

In this study, the presence of mineral opacity in the crop and gizzard conforms to the key function of grit in the gastrointestinal digestion of *Francolinus bicalcaratus*. The contrast radiography showed a rapid transit time of the contrast medium (approximately 40 minutes), although increased dosage of contrast will most likely enhance visualization of different gut parts. Besides, the lack of correlation between carcass weight and GIT measurement signifies independent digestive system operation concerning this species' weight. This outcome contrasts with what is observed in other avians but parallels what occurs in certain fishes and mammals. This supports the significance of further work on specie specific digestive adaptations

## Clinical Recommendation

The rapid transit time observed justifies clinicians to consider altering the contrast doses in studying gastrointestinal motility in *Francolinus bicalcaratus* to achieve optimal viewing of all sections. Dietary assessment should also recognize the contribution grit makes in francolin digestion. Future studies should be formulated along environmental, diet and physiology interaction and their contribution towards adapting this species

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