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Full length Research Article

Morphologic and Histologic Studies on the Tongue of the Juvenile Cattle Egret (*Bubulcus ibis*)

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Summary: Cattle egret (*Bubulcus ibis*) is a cosmopolitan heron species, with least concern conservation status. There are limited literatures on the anatomy of this bird, especially in relation to its sensory organs, hence this study described the morphologic and histologic features of its tongue. The tongues of twelve juvenile cattle egrets were examined in situ for morphological features and morphometric measurements were determined ex situ. Histological assessment was conducted on the tongue section with parameters such as epithelial and lamina propia heights, lingual muscle and entoglossal cartilage heights evaluated. Grossly, the tongue was divided into three parts namely; apex, body and the root. It was arrow shaped, conforming to the shape of the beak, with a laryngeal mound bounded caudally by the pharyngeal papillae at its root. A massive entoglossal cartilage formed the core of the cranial apex, ventral body portion, and caudal aspect of the root. Histologically, the lingual mucosa possessed keratinized squamous epithelium in all its divisions, with spinous conical papillae being characteristic of the cranial apical mucosa. The body lingual mucosa possessed foliate papillae on the dorsal aspects, while filiform papillae were prominent in the ventral portions. The lingual root uniquely possessed numerous glandular ducts in its lamina propia as well as localized adipocytes. Overall, the regression analysis showed that the bird's body weight can be conveniently predicted from tongue parameters. This study, thus provided additional knowledge on the anatomy of the birds and, the generated data could be useful in comparative regional avian anatomy.

Keywords*: cattle egret, entoglossal cartilage, histomorphometry, lingual papillae, morphometry*

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INTRODUCTION

The bird, Cattle Egret (*Bubulcus ibis*) is a species of heron found all over the world, with least concern conservation status (Telfair and Raymond, 2006). The bird belongs to the order Pelecaniformes, family Ardeidae and, are native to Africa; mostly found in dry grassy habitats, in association with wildlife and livestock (Hancock, 1984). They are associated with both small and large flocks, feeding in loose aggregates. The birds feed on insects, earthworms, spider, frogs and most especially ticks present on cattle (Siegfried, 1971). Other prey of the birds include grasshoppers, crickets, and moths (McKilligan, 2005). Generally, cattle egrets are colonial in nature and whitish in colour. Both sexes of this bird are similar, and the male is slightly bigger and possess marginally longer breeding plumes than the female. The juvenile age group lacks coloured plumes and have a black beak (Mckilligan, 2005), with the adult possessing greyish-yellow feet and sharply pointed yellow short bill (Seedikkoya *et al*., 2007).

Studies on the gross morphometry and histomorphomtery of the cattle egret are scarce. The descriptive anatomy on its appendicular (Rezk 2015a) and axial skeletons (Rezk, 2015b), gross morphometrics of the fore- and hind-limb skeleton (Ekeolu *et al*., 2016), the gross and morphometrical studies on the humerus (Sasan *et al*., 2019), and more recently, the craniofacial and ocular morphometrics (Azeez *et al*., 2022a) of the cattle egret have been published. With the exception of the report of Al-Zahaby (2016) on gross and histological description of tongue of the adult egret, there is a dearth of information on the gross and histo-morphometric parameters of the tongue of the juvenile cattle egret and this study objective was to fill part of that knowledge gap. Aging-associated morphological and morphometric indices are very important for comparative, developmental and evolutionary assessments. Hence, the description of the lingual macro-anatomical structure and histology of cattle egret juvenile age category can provide supplementary data to the basic information on the adult egret tongue, as studies across age groups are of great value to set standards of comparison for research. Furthermore, considering the vital sentinelling role played by cattle egrets in the assessment of agro-ecosystem pollution and its importance in serving as excellent animal model in foraging study as well as biological insect pest control agent in the agro-ecosystem, there is need to comprehensively study its tongue morphology, a suitable indicator of its feeding habit, more particularly in a juvenile cattle egret which is yet to be

investigated.The tongue is a very muscular organ, constituting a larger part of the oral cavity. Structurally, it possesses a free apex, body and an attached root. It is a very sensitive organ owing to it's very rich innervation, possesses a huge mass of skeletal muscles that rest on the floor of the oral cavity (Abayomi *et al*., 2009; Igado *et al*., 2015). The lingual gross and histological features such as its' lateral boundary and median sulcus conformation, papillae types and predominance, degree of keratinization of lingual epithelia and the placement of the lingual glands are crucial considerations that determine the level of tongues' mobility and its ability to capture preys as well as manipulate and swallow food items (Jackowiak and Godynicki, 2005; Emura *et al*., 2008).

This study described the morphologic and histologic features of the tongue of juvenile *Bubulcus ibis* (cattle egret) found in Nigeria.

MATERIALS AND METHODS

Animals: The animals, cattle egret, used were captive and of the juvenile age group. They were aged according to the detailed morphologic description by Mckilligan (2005) and Seedikkoya *et al*., (2007), while the juveniles possess black feet and beaks, and lack coloured plumes, the adults in contrast possess yellow feet and sharply-pointed short yellow bill. Of note, cattle egret is a medium sized bird, possessing short-legs and thick necks, in comparison to other egrets (Ivory, 2000). Twelve apparently healthy juvenile cattle egrets (mixed sexes) were used in this study. The birds were captured alive in a single batch from their habitat at Yelwa Fulani Community, Naraguta B Ward in Jos North Local Government Area of Plateau State, Nigeria (GPS coordinates: 9°59'21.5"N; 8°52'49.7"E). The birds were then transported to the Gross Anatomy Laboratory of the Department of Veterinary Anatomy, Faculty of Veterinary Medicine, University of Jos, Nigeria. Live body weight was measured and recorded using the mechanical laboratory weighing scale sensitive to 0.1 gm (Camry Emperor Table Scale J1712420834, China). Care was taken to ensure the bird did not experience undue pain or discomfort. The ethical clearance (Reference number: UJ/FPS/F17-00379) for the use of this bird was obtained from the Animal Care and Use Committee of the University of Jos, Nigeria. The experimental protocols used were also in conformity with the National Institute of Health Guide for the Care and Use of Animals (NIH Publications No. 80–23) and the European Communities Council Directive of November 24, 1986 (86/609/EEC).

Sedation and Organ Excision: The birds were euthanized by the administration of ketamine (40 mg/kg) and xylazine (10 mg/kg) through the intravenous route (Lierz and Korbel, 2012). Following deep anaesthesia, the birds were exsanguinated by cardiac puncture. The head was carefully dissected and the upper beak reflected to expose the tongue in situ.

Morphological Studies: Gross observations were made on the tongue in both in situ and ex situ positions. Gross features of the dorsal and ventral surfaces of the tongue were captured using a Sony® Digital Camera (DSC-H300 20.1 megapixels with 35x Optical Zoom). Tongue weight was obtained using the analytical weighing balance (Ohaus GmbH, Nänikon, Switzerland), sensitive to 0.01 gram, following its careful dissection from the lower jaw (ex situ). Linear morphometric parameters (defined in subsequent section) were measured with the aid of graded ruler (in centimeters). The nomenclature adopted in this study for the anatomical descriptions was as described in the sixth edition of the Nomina Anatomica Avium (2017).

Morphometric Parameters: The parameters measured are highlighted below, with weight recorded in grams (g) and linear measurements in centimeters (cm):
a). Weight of the animal (WOA) - measured with the aid of a mechanical laboratory weighing scale. b). Weight of the tongue (TOW) - measured using the analytical weighing balance.
c). Apex width (AW): The widest distance between the lateral margins of the apex of the tongue.
d). Body width (BW): The widest distance between the lateral margins of the body of the tongue.
e). Root width (RW): The widest distance between the lateral margins of the root of the tongue
f). Tongue length (TL): The longitudinal distance between the tip of the apex and the caudal-most part of the tongue (i.e the cranial margin of the laryngeal mound).
g). Apex length (AL): The longitudinal distance between the tip and the terminal end of the apex of the tongue.
h). Body length (BL): The longitudinal distance between the caudal end of the apex and the terminal end of the body of the tongue.
i) Root length (RL): The distance between the caudal end of the body and the terminal end of the root of the tongue (i.e. the cranial margin of the laryngeal mound).

Histological Studies: Immediately after the birds were euthanized, the tongues were dissected out and fixed in 4% phosphate-buffered paraformaldehyde (Loba Chemie PVT Ltd, India). The tissue samples were processed using the routine histological technique for light microscopic examination, as described by Azeez *et al*., (2022b). Surgifield SM-202A rotatory microtome was used for the sectioning at 7 µm. Longitudinal sections were obtained and stained using Haematoxylin (CDH Lab Reagent, New Delhi, India) and Eosin (Kem Light Lab Reagent, Mumbai, India). Histological examination was carried out on the processed tissue samples and photomicrographs taken at ×40, 100 and 400 magnifications through an Olympus Microscope equipped with industrial digital camera using an imaging software, MII ImageView version 3.7.9229 (YSC Technologies, Fremont, CA, USA). Photomicrographs of the sections of the apex, body and root were evaluated for regional variations.

Histomorphometry: Lingual histomorphometry was carried out using Motic Image Plus (MIP) 2.0 software by following the modified approach of Ahmed (2018). The parameters evaluated based on the tongue's division (as illustrated in Figure 2) of the cattle egret include:
(a) At the cranial (ACC I) and caudal (ACC II) aspects of the lingual apex, the following measurements were done:
- lingual epithelial height (EH) – the distance between the epithelial mucosal apex and its base border with laminal propria,

- lamina propria height (LP) – the distance between the base of the epithelial mucosa and the cranial border of the dorsal lingual muscle,
- dorsal lingual muscle length (DM) – the distance between the caudal most part of the lamina propria and the most cranial part of the endochondrial sheath of the entoglossal cartilage,
- entoglossal cartilage width/height (EC) – The distance between the dorsal and ventral endochodrial sheath summits.
- ventral lingual muscle- (VM) – The distance between the ventral border of endochodrial sheath and the outermost layer of the ventral lingual muscle.

(b). At the dorso-ventral (BDV) divisions (I, II and III) of the lingual body, the morphometric parameters (EH, LP, DM, EC and VM) assessed for the apex were recorded, except for the absence of the dorsal lingual muscle, entoglossal cartilage in DV I, and also, the same ventral lingual muscle shared by DV I and DV II.

(c). For the dorso-ventral (DV) divisions (1, II, III and IV) of the lingual root, the complete lingual parameters described for the apex (i.e., EH, LP, DM, EC and VM) were determined.

Data analysis: The numeric data generated were analyzed and expressed as mean ± SD, and the Scatter plot and the Pearson correlation coefficient (R two-tailed test) were used to measure the direction and strength of relationship between the variables. The regression analysis was done to forecast the imapact of the lingual parameters on the body weight prediction were also done. The John's Macintosh Project Statistical Analysis Software (JMP SAS/STATR) software version 10.0 (SAS Institute Inc., Carry, NC, USA) was used for statistical analyses.

RESULTS

Gross Characteristics: The tongue of the juvenile cattle egret revealed a long, protrusible structure, bounded; on the dorsal surface by the palate, laterally by the paired lower beak, and ventrally by visibly folded oral mucosa with patterned longitudinal fold (Plate. 1). It conforms to the shape of the lower beak which is V-shaped in outline (Plates. 1A and B). Grossly, it is divided into three parts; apex, body and the root. The apex of the tongue of the *Bubulcus ibis* evaluated in this study and their surrounding oral mucosa were reddish in colour in situ. The body and root of the tongue had a similar coloration as the apex. The apical aspect of the tongue is pointed assuming a spear-like shape and wider at its base. On its dorsal surface, it is characterized by a longitudinal furrow known as median groove (Figs. 1A and C). At the point of transition into the body of the tongue, a dorsally located transverse rows of caudally pointing papillae were situated (Plates 1A and C). The significant core of the apex is constituted by entoglossal cartilage. Similarly, the body of the tongue is supported by entoglossal cartilage. The ventral surface of the tongue of egret more specifically at the body of the tongue is anchored to the oral floor by mucosal fold referred to as frenulum (Plate. 1B). The root has the widest outline compared to other divisions of the tongue. Its caudal relation is formed by the laryngeal mound which is characterized by a median slit (glottis), an entrance into the laryngeal cavity and raised caudally pointing projections (pharyngeal papillae) on its dorsal summit (Plates 1A and C).

Morphometrics and analysis: Body and organ weights and, the morphometric parameters results are presented in Table 1. The scatter plot matrix and the Pearson's correlation co-efficient values of the variables from Table 1 are presented in Fig. 1. The regression analysis for the body weight prediction from lingual parameters is shown in Table 2. The apex had the longest length relative to the body and root. Conversely, the root had the longest width, in comparison with the apex and the body. Correlation of the body weight of the birds with tongue parameters using Pearson's correlation showed highest values for tongue weight (r=0.9351).

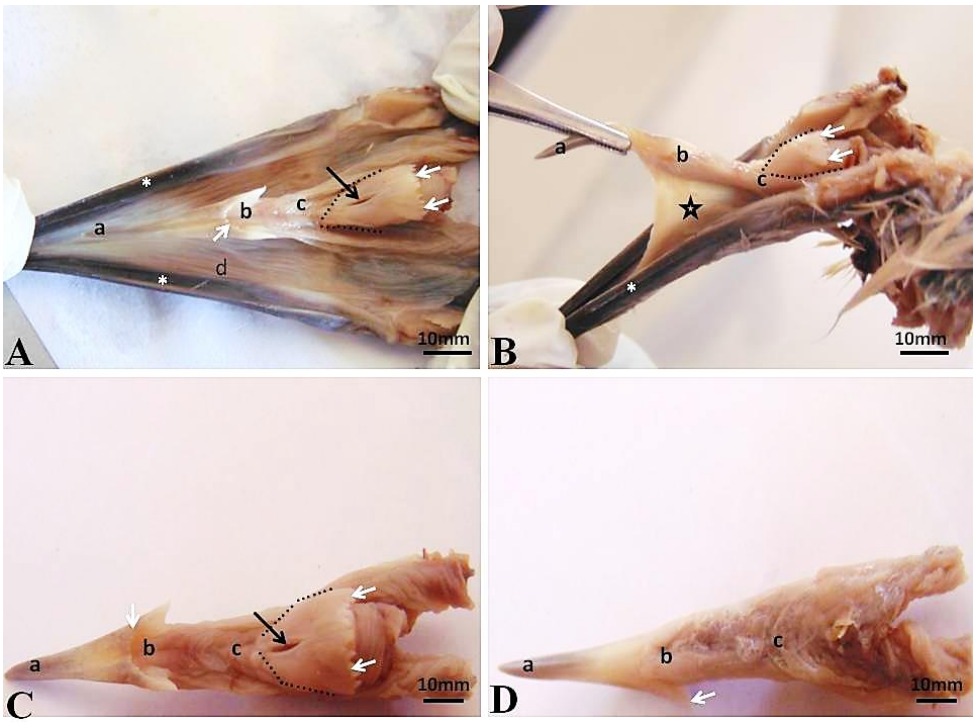

Plate 1.
Photographs of the tongue of juvenile cattle egret (*Bubulcus ibis*) following post-fixation. **A. Dorsal view** (*in situ*) **B. Dorso-lateral view** (*in situ*) **C. Dorsal view** (*ex situ*) **D. Ventral view** (*ex situ*). Note the divisions of the tongue (a- apex, b- body c- root) and these lingual and paralingual features; White arrow – pharyngeal papillae, Black star – frenulum linguae, Black arrow – glottis (laryngeal cleft), Dashed lines – laryngeal mounds, d – oral mucosa (folded and numerous), white asterisk – (lower beak).

Juvenile cattle egret tongue morphology

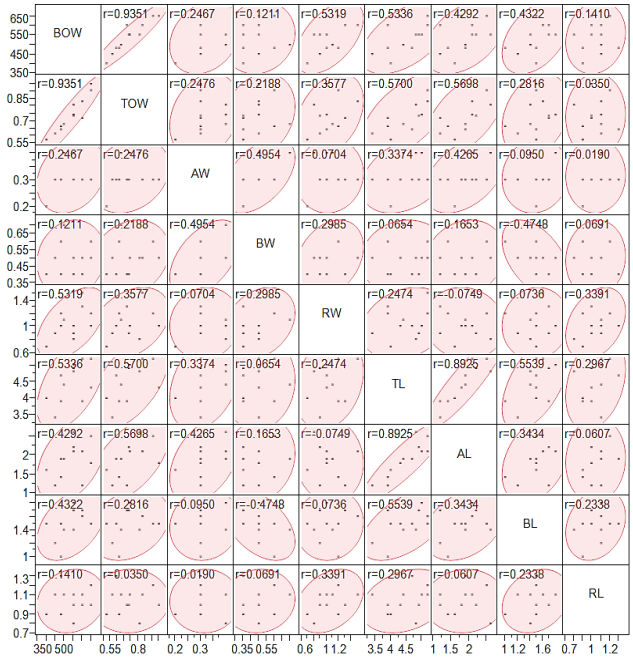

Figure 1:
Correlation analysis and scatter plot matrix of the measured parameters Correlation coefficients (r) were captured within the scatter plots. The scatterplot showed the relationships of the different variables as well as the density ellipses in the shaded portions.

Table 1.
Morphometric characteristics of the tongue in the juvenile cattle egret

Variables (n=12)	mean±SD
Weight of the animal (BOW) (g)	538.33±78.72
Weight of the tongue (TOW) (g)	0.74±0.12
Apex width (AW) (cm)	0.32±0.06
Body width (BW) (cm)	0.50±0.10
Root width (RW) (cm)	1.05±0.22
Tongue length (TL) (cm)	4.48±0.58
Apex length (AL) (cm)	1.88±0.46
Body length (BL) (cm)	1.48±0.22
Root length (RL) (cm)	1.04±0.13

Regression analysis was done to predict the weight of the bird animal from tongue morphometric data. As shown in Table 2 below, the equation for the predictor of the weight of the body is Y= a+bx, where Y= predictor value for the body weight, a=intercept, b=regression coefficient and x=explanatory variable. Hence, Y= -18.14+582.08TOW+85.54RW-22.62TL+93.43BL, where R2 = 0.9665 and adjusted R2 = 0.9473.

Histological Examination: For easy identification at histological examination, a schematic illustration of the tongue was made from the gross appearance shown in Fig. 1. As shown in Fig. 2 the apex was divided into cranial (illustrated as ACC I) and caudal (illustrated as ACC II) portions.The body was divided into 3; dorsal and ventral (BDV): I, II and III. The root was divided into 4; dorsal and ventral (RDV): I, II, III and IV.

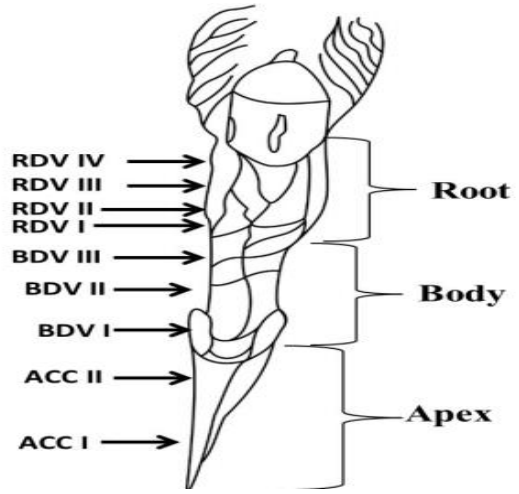

Figure 2.
Schematic diagram of the lingual (tongue) divisions of the juvenile cattle egret (*Bubulcus ibis*). Note the three main divisions of the tongue; Apex, body and root. Drawing was done using the modified approach of Erdogan and Iwasaki, (2014).

Table 2.
Regression analysis for the prediction of body weight from tongue parameters.
Current Estimates

Lock	Entered	Parameter	Estimate	nDF	SS	"F Ratio"	"Prob>F"
X	X	Intercept	-18.141929	1	0	0.000	1
	X	TOW	582.079385	1	32210.37	98.681	2.24e-5
		AW	0	1	164.8324	0.467	0.52009
		BW	0	1	5.006322	0.013	0.91236
	X	RW	85.5405486	1	3482.197	10.668	0.01375
	X	TL	-22.619614	1	964.149	2.954	0.12937
		AL	0	1	202.1073	0.582	0.47436
	X	BL	93.4361198	1	3224.848	9.880	0.01631
		RL	0	1	24.38251	0.065	0.80768

RSquare	RSquare Adj
0.9665	0.9473

The analysis shows the stepwise fit for body weight (BOW) in relation to other measured parameters. The BOW value can successfully be predicted from the intercept, explanatory variable and regression coefficient values generated. TOW – tongue weight, RW – root width, TL – tongue length, AW – apex width, BW- body width, RW – root width, AL – apex length and BL – body length

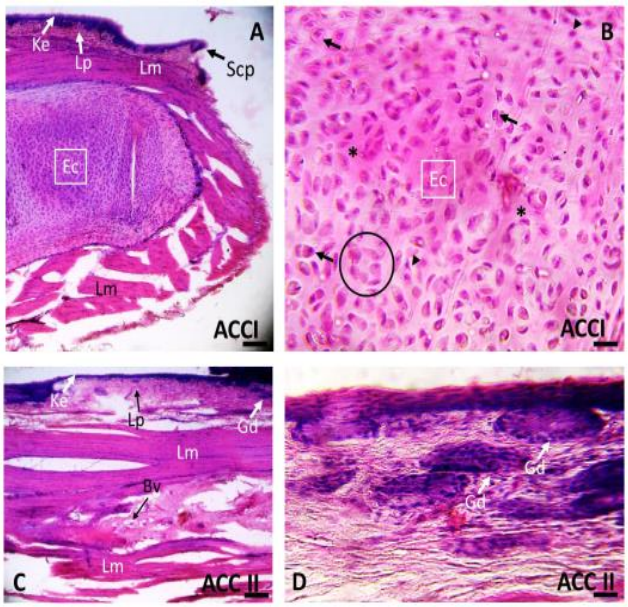

Plate 2.
Photomicrographs of the longitudinal section of the apical aspect of the tongue of the juvenile cattle egret (*Bubulcus ibis*). A. Cranial apical part. B. High power micrograph of the entoglossal cartilage (Ec) in A. C. Caudal apical part. D. High power micrograph of the glandular duct (Gd) in C. Lp – Lamina propria, Bv – Blood vessel, Ke - keratinized squamous epithelium, Lm - lingual muscles, Scp - spinous conical papillae, ACC I and ACC II are the cranial and caudal apical portions, respectively. Stain: H&E. Scale bar: A-D = 100 µm

The Apex: The histological profile of the longitudinal section of the cranial apex (the early and later part) of juvenile egret tongue is characterized by lingual mucosa containing keratinized squamous epithelium with exclusive presence of spinous conical papillae (Scp) in the mucosa of the early part of the apex. The bulk of the cranial part is typified by the presence of a centrally located entoglossal cartilage (Ec) bounded dorsally and ventrally by lingual muscles (Lm) (Plate 2A and B). However, although the histological appearances of the caudal apex of the tongue also featured keratinized squamous epithelium (Ke), lingual muscles predominate its architecture, with few glandular ducts (Gd) (Plate 2C and D).

The Body: The histology of the body aspect (early dorsal part) of the egret tongue is characterized by the presence of keratinized squamous epithelium and appearance of foliate papillae on the lingual mucosa (Plate 3A). The later dorsal part of the tongue's body bears both keratinized and non-keratinized epithelium most especially on the ventral aspect. It also has on its dorsal most part foliate and on the ventral part filiform papillae (Flp) as well as a prominent entoglossal cartilage (Plate 3D). The final dorsal part (i.e. caudal-most part) of the tongue's body (Plate 4A) showed keratinized squamous epithelium and foliate papillae on the lingual mucosa. Moreover, there was a presence of relatively thin lingual muscle layer dorsal to centrally located massive entoglossal cartilage layer (Plate 4A). Conversely, the final ventral part of the tongue's body is characterized by the presence of entoglossal cartilage layer and a thin layer of lingual muscle (Plate 4B and C).

The Root: The histo-architecture of the root (cranio-dorsal part) of the tongue is typified by the presence of keratinized squamous epithelium, multiple glandular ducts within the lamina propria and uneven distribution of lingual muscles (Plate 5A). The later dorsal part (mid-portion) of the tongue's root was observed to be lined by both keratinized and non-keratinized epithelia most especially on the cranio-ventral aspect. It also bears on the dorsal most part foliate papillae and filiform on the cranio-ventral part coupled with the presence of substantial entoglossal cartilage and few lingual muscles (Plate 5B).

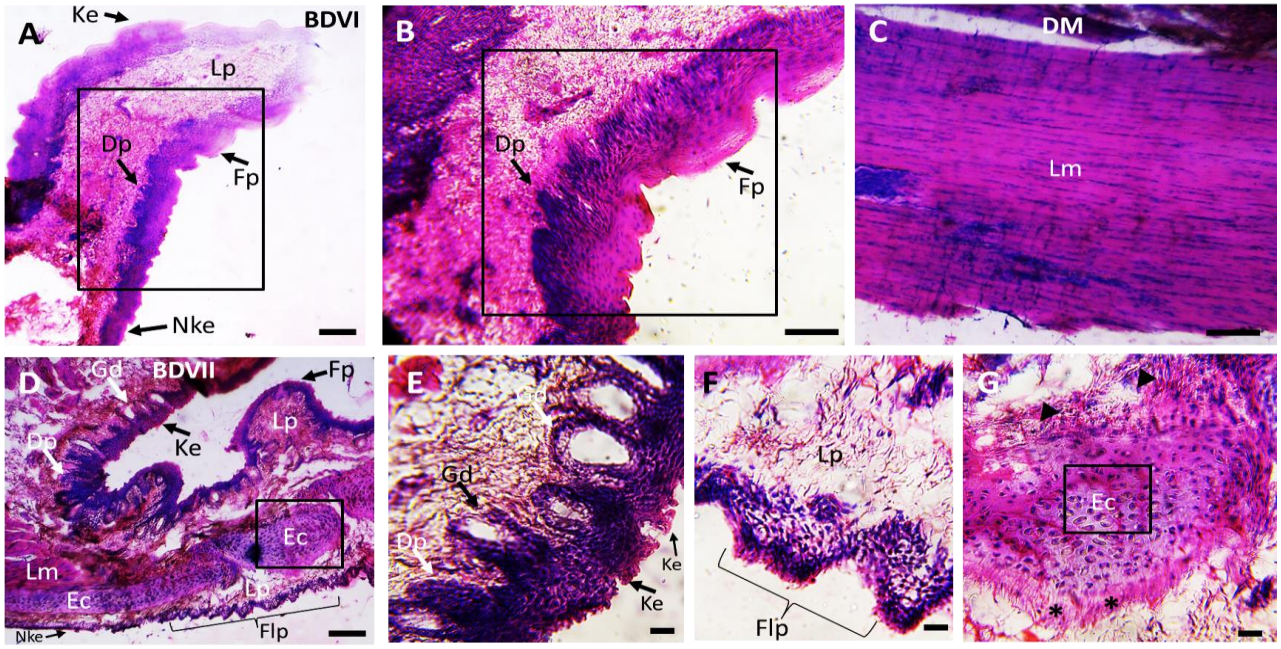

Plate 3.
Photomicrographs of the longitudinal section of the body aspect of the tongue of the juvenile cattle egret (*Bubulcus ibis*). **A.** Early dorsal part of the tongue's body **B.** Higher power micrograph of the boxed area in A. **C.** Early ventral part of the tongue's body **D.** Mid-dorsal part of the tongue's body. **E-G:** These are high power micrographs of keratinized squamous epithelium (Ke), filiform papillae (Flp) and entoglossal cartilage (Ec) (boxed area) of D. Stain: H&E. Scale bar: A-G = 100 µm

For the more later dorsal part of the tongue's root (Plate 5C), the following features were recognized; Keratinized squamous epithelium, localized adipocytes, glandular ducts within lamina propria and lingual muscles. On the other hand, there was presence of substantial entoglossal cartilage and more lingual muscles in the most caudal dorsal part (Plate 5D). With respect to the ventral aspect of the tongue's root, its' bulk is constituted by lingual muscles.

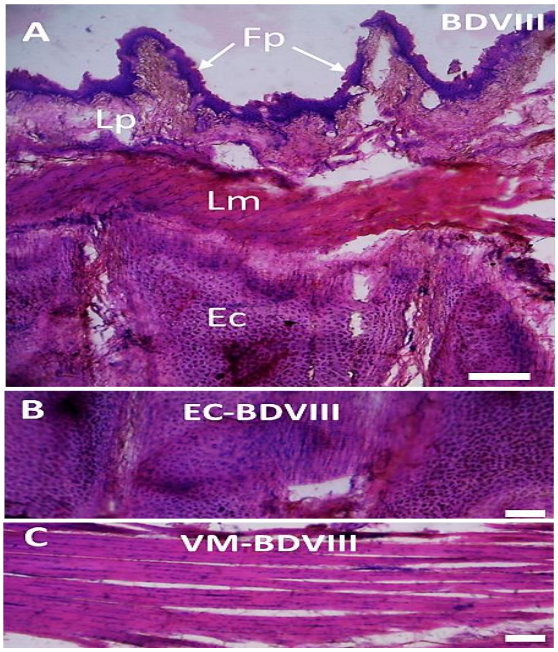

Plate 4.
Photomicrographs of the longitudinal section of the body aspect of the tongue of the juvenile cattle egret (*Bubulcus ibis*). A. Dorso-caudal extremity of the tongue's body. B-C. Dorso-ventral extremity of the tongue's body. Fp – foliate papillae, Lp – lamina propia, and entoglossal cartilage (Ec). Scale bar: A-C = 100 µm

Histomorphometry: The epithelial height (EH) was found to be highest in the cranial aspect of the body of the tongue compared to the apex and root. For the lamina propia height (LP), the highest value was recorded in the cranial aspect of the dorsoventral segment of the root of the tongue compared to the apex and body (Table 3). Similar values were recorded for the dorsal lingual muscles of the body (middle portion) and the root (the most caudal part) (352.88±15.60 µm and 310.31±22.54 µm, respectively) compared to the lesser value recorded for the apex (188.26±17.83 µm). In contrast, the ventral lingual muscle (VM) was most dominant in the caudal portion of the body in comparison with the apex and root. With regards to the entoglossal cartilage (EC), it was massive in the caudal portions of the root and in the body. Of note, the lamina propia height (LP) values of the body and the root decreased cranio-caudally (Table 3).

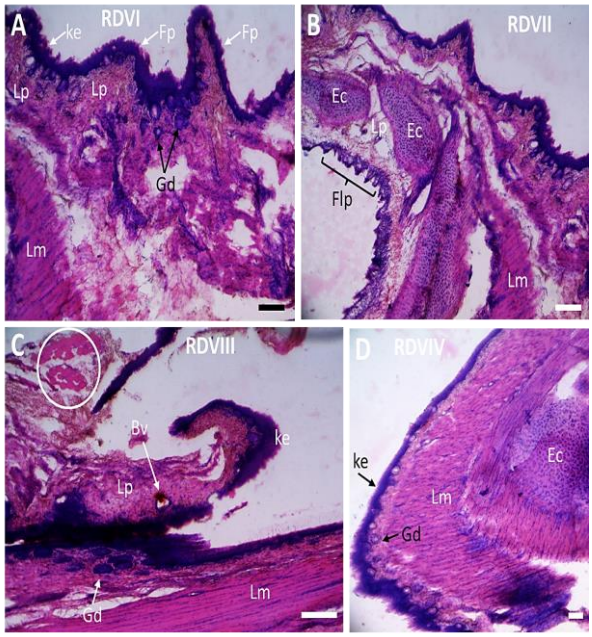

Plate 5.
Photomicrographs of the longitudinal section of the root aspect of the tongue of the juvenile cattle egret (*Bubulcus ibis*). **A-B.** Cranial dorsal portions of the tongue's root. **C-D.** Caudal dorsal portions of the tongue's root. Ke - keratinized squamous epithelium, Fp - filiform papillae, Ec - entoglossal cartilage, Lp – lamina propia, Lm - lingual muscles, Flp - filiform papillae and Gd – glandular ducts Stain: H&E. Scale bar: A-D = 100 µm.

Table 3.
Histomorphometric measurements of the tongue in the juvenile cattle egret

Apex Parameters (µm)	**EH-ACCI**	**LP-ACCI**	**DM-ACCI**	**EC-ACCI**	**VM-ACCI**	**EH-ACCII**	**LP-ACCII**	**DM-ACCII**	**EC-ACCII**	**VM-ACCII**
	33.91 ±3.97	29.35 ±4.63	159.61 ±10.76	454.96 ±11.44	254.39 ±16.62	24.62 ±2.09	50.56 ±4.65	188.26 ±17.83	335.18 ±18.41	231.66 ±6.99
Body Parameters	EH-BDV1	LP-BDV1	EH-BDVII	LP-BDVII	EC-BDVII	DM-BDVII	VM-BDVII	EH-BDVIII	LP-BDVIII	EC-BDVIII
	54.69 ±6.6	139.02 ±10.74	48.8 ±4.54	112.73 ±12.47	166.67 ±15.07	352.88 ±15.6	398.8 ±22.59	53.72 ±6.41	101.01 ±11.71	830.13 ±100.9
	DM-BDVIII	VM-BDVIII	EH-RDV1	LP-RDV1	EC-RDV1	VM-RDVI	EH-RDVII	LP-RDVII	EC-RDVII	VM-RDVII
	256.5 ±13.97	637.74 ±42.15	43.78 ±10.15	164.43 ±16.87	775.53 ±30.06	458.02 ±24.24	49.99 ±6.27	82.05 ±6.55	177.41 ±13.49	454.83 ±18.08
Root Parameters	EH-RDVIII	LP-RDVIII	EC-RDVIII	DM-RDVIII	VM-RDVIII	EH-RDVIV	LP-RDVIV	EC-RDVIV	DM-RDVIV	VM-RDVIV
	36.73 ±6.79	27.62 ±5.88	1173.52 ±157.26	254.3 ±21.15	526.13 ±14.7	32.7 ±4.49	35.21 ±5.22	503.65 ±18.24	310.31 ±22.54	525.61 ±28.29

The parameters were expressed as mean±SD. Measurements were based on the schematic illustration in Fig. 2. Legends: EH, LP, DM, EH, VM, ACC, BDV and RDV were as earlier state

DISCUSSION

The tongue constitutes a major part of the lingual apparatus and it is situated on the lower beak (mandible) floor. Lingual apparatus components include: the tongues' hyoid apparatus (a cartilaginous and bony structure), blood vessels, connective tissues and innervations (Homberger, 1989). The tongue is endowed with the intrinsic and extrinsic muscles; while the extrinsic muscles in conjunction with the extrinsic connective tissues link the lingual apparatus with the skull, the intrinsic tongue muscles join the hyoid apparatus bones, thereby enhancing their relative movements and changing lingual conformation (Crole and soley, 2009). Meanwhile, in contrast to most mammalian tongues, only the extrinsic muscle is consistently present in almost all birds (Huang *et al*., 1999), except the Parrot (Homberger, 1989).

Variations exist in the morphological and structural adaptations of the avian tongue to the various ecological conditions, especially their feeding patterns and behaviours, with correlations documented between the food intake type and lingual morphology (Iwasaki 2002; Sabry, 2015). The disparity in lingual features such as the lateral boundary and median sulcus conformations, papillae types and predominance, degree of keratinization of lingual epithelia and the placement of the lingual glands determine the level of tongues' mobility and its ability to capture preys as well as manipulate and swallow food items (Jackowiak and Godynicki, 2005; Emura *et al*., 2008).

The avian tongue is a triangular shaped organ, assuming the shape of the oral cavity, and it is non-protrusible. Generally, birds possess a poorly developed sense of taste (Dyce *et al*., 2016). In the present study, the tongue of the juvenile cattle egret (*Bubulcus ibis*) was observed to be divided into three portions namely; the apex, body and root. The observed divisions of egret tongue were consistent with the reports of Erdogan and Iwasaki (2014) on the avian tongue. The juvenile cattle egret has a V-shaped outline, with its apical part assuming a spear-like shape cranially, dorsally possessing a median groove. The arrow shaped tongue of the juvenile egret and its observed conformation to the shape of the beak could largely be associated with their kind of diet and the mechanism of food intake. Seedikkoya *et al*., (2007) proposed that the dagger-like apex of the egrets' tongue provides suitable eating prospects for searching small food items, especially insects in waste dumps and ticks on cattle's body. This morpho-functional attribute with respect to the shape of egret tongue concur with our observation and the previous report by Harrison, (1964).

The laryngeal mound of birds is caudal to the base of the tongue and the glottis is found on it, appearing as a median slit. Interestingly, the glottis of birds is not guarded by the epiglottis (Dyce *et al*., 2016). In this study, there was a laryngeal mound that is bounded caudally by the pharyngeal papillae at the root end of the tongue. These pharyngeal papillae are believed to perform functions connected with the retaining of food in the oral cavity and directing food into the oesophagus. The observations on the pharyngeal papillae gross appearance in the egret further validate the findings of McLelland (1979), Kobayashi *et al*. (1998), and Erdogan and Iwasaki (2014).

The average body weight of the juvenile cattle egret in this study was 538.33±78.72 g. This is in line with the weight range (270 - 512 g) of the cattle egret as documented by Telfair and Raymond, (2006). The whole length of the juvenile cattle egret tongue was recorded as 4.48 mm, this is less than the value recorded for the length of the adult cattle egret tongue (6.00 mm) by Al-Zahaby (2016). Conversely, in this study,a slightly higher value (4.40 mm) for the whole tongue width was recorded, relatively to the reported value (4.00 mm) by Al-Zahaby (2016). Overall, the regression analysis data indicates that the weight of the bird can be excellently predicted from weight of the tongue, width of the root, length of the tongue and the breadth of the body. With respect to the earlier investigations on the gross morphometry of the tongue of other avian species, Bello *et al*., (2015) observation on the tongue of Muscovy duck revealed that the duck's tongue was triangular in shape with a mean length of 55 mm, the apex and root mean widths of 10mm and 16mm, respectively and the root length of 7.5 mm. Also, Pourlis (2014) observed that the tongue of the quail was triangular in shape with a slightly rounded apex and an average length of 1.2 cm. More recently, İlgün *et al*., (2020) equally reported in Guinea fowl tongue, features like flattened, pointed and triangular shaped tongue with the mean length of 18.21 mm.

The presence of frenulum, a membrane fold attaching the lingual ventral aspect to the floor of oral cavity and the free nature of the tongue at the apex seen in this study seemed to be morphological features that assist in the anchorage of the tongue within the oral cavity. These gross findings on the tongue of the juvenile egret have structural resemblance with the typical morphological appearance of the tongue documented by McLelland (1979), Homberger and Meyers, (1989) in chicken.

Histologically, previous reports indicated that the dorsal surface of the apex of avian tongue was lined by slightly keratinized stratified squamous epithelium comprised of six to eight layers of polyhedral cells with coverings by a few-layered keratinized epithelia (Farouk and Hassan, 2015), and the ventral surface possessed thinner and highly keratinized epithelium with a slightly wavy basement membrane (El-Bakary, 2011). The adult cattle egret was documented by Al-Zahaby *et al*., (2016) to possess keratinized lingual epithelia, with its' largely parakeratinized epithelia leading to the slough-off and desquamation of its superficial cells to varying degrees, except for epithelia of the tongues' root which was smooth and less exfoliated.

The epithelial keratinization type (stratified squamous) and the papillae (conical and filiform) distribution observed on the lingual mucosa of the different divisions of the juvenile cattle egret tongue seemed to vary with the regions, with the exclusive presence of numerous glandular ducts and localized adipocytes in the lamina propia of the apex and body. The present study also showed that the lamina propia height of the tongue was higher in the body and root of the tongue compared to the apex. This could be due to the constant external assaults on the apex of the tongue since it is the first line of contact with food. The relatively larger proportions of the lingual body muscles could be attributed to the tapered nature of the tongue. Qureshi *et al*., (2017) reported that the mean thickness of the lingual epithelium, submucosa and muscularis and lamina propia of the immature Duck (Anas platyrhynchos) were 96.13±12.04, 150.87±0.002, 232.67±24.78 and 118.93±7.03 µm,

respectively, although the authors did not state from which region(s) of the tongue the measurements were made. The authors also reported that there was an increase in thickness from the immature stages to the adult stages after which it remained fixed till old age.

The observed variations in the nature of the epithelial stratification and keratinization as well as the progressive cranio-caudal decrease in the epithelial thickness of the tongue from the apex to the root in the cattle egret here studied could be attributed to the functional resistance of the lingual epithelium to external assaults. The lingual epithelial nature described in this study wholly concurs with reports of Iwasaki *et al*. (1997) and Skieresz-Szewczyk *et al*. (2014) in the tongue of Middendorf's bean goose and the tongue of the domestic duck, respectively. It however, contrasted the lingual epithelial nature described by Crole and Soley (2009) in Emu tongue.

The dense irregular connective tissue, adipose cells, strong layer of striated muscle fibres and numerous blood vessels observed as components of the lamina propria in the egret tongue could be responsible for the firmness of the tongue of this species. The lamina propria profile seen in this work is similar to Erdogan and Iwasaki (2014) report on function-related morphological characteristics and specialized structures of the avian tongue. This presence of tubulo-alveolar glands surrounded by connective tissue beneath the dorsal lingual epithelium in the egret tongue could be suggestive of higher demands for lubricating fluid for ingested food particles. McLelland, (1990) mentioned that the structure of lingual glands is more developed and complex in birds that feed on dry food compared to those consuming naturally well-lubricated food. Therefore, their presence validates the above statement.

The histological features of the entoglossal cartilage of the juvenile cattle egret were typical of hyaline cartilage. Importantly, the massive nature of the entoglossal cartilage of the juvenile cattle egret tongue and its envelop formed by a definite perichondrium consisting of a fibrous connective tissue and striated muscle fibres could provide an important morphological support to the tongue and could also be suggested to be of importance in the movement of the tongue out of the oral cavity. The morpho-physiological details of entoglossal cartilage of egret assessed are similar to those reported by Erdogan and Alan, (2012) in Chukar partridges' tongue, Igwebuike and Anagor (2013) in Muscovy duck, Pasand *et al*. (2010) in male ostrich, and Parchami and Dehkordi (2011) in domestic pigeon.

This study revealed that the tongue of the juvenile cattle egret is divided into three portions of the apex, body and the root, grossly. It demonstrated that the shape of the tongue of the juvenile cattle egret is in conformation with the shape of the beak and this is largely associated with their kind of diet and mechanism of feed intake. Altogether, the above datasets could come handy in the clinical and regional anatomical studies of this bird. Further studies are required especially on the histochemical and immunohistochemical demonstration of the tissue components in the tongue of the juvenile cattle egret as this will further elucidate its morphology, and as well studies could be carried out on the variation of the tongue morphology across different age groups of the cattle egret.

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