ORIGINAL COMMUNICATION

Anatomy Journal of Africa. 2021. Vol 10 (1): 1908-1917

ANATOMICAL INVESTIGATION ON THE CRANIOFACIAL AND OCULAR MORPHOMETRICS OF THE MALE WESTERN CATTLE EGRET (*Bubulcus ibis*)

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ABSTRACT

The heron species, Cattle egret (*Bubulcus ibis*) can be found all over the world. In the South-Western region of Nigeria, they are colonial, white in colour, and are seen in open lands and around water bodies. Morphometric data are important parameter in the assessment of functional morphology, hence we here present for the first time, data on the craniofacial indices of cattle egret. The cranium, nasal, orbital and beak morphometric indices and correlation data of the cattle egret were investigated on the fresh specimen and macerated skull of the male cattle egrets, using the GraphPad Prism. The height of the cranium was 33.92±0.970 cm, while the cranium length and breadth were 52.92±1.800 and 34.48±1.714, respectively. The right eye socket length, 36.17±5.636 and breadth, 31.67±2.160 were slightly greater than the left eye socket length, 35.92±4.128 and breadth, 31.00±2.757. There was a strong positive correlation between the right eye socket breadth and the left eye socket breadth (r=0.940), and also, a very strong positive correlation was found between the right and the left eye socket length (r=0.981). Results showed the foramen magnum length and breadth to be 19.17±0.753 and 18.83±0.753, respectively, however, a low positive correlation was recorded between the foramen magnum length and breadth (r=0.059). A strong positive correlation was observed between the upper beak length and the lower beak length (r=0.901), while similar values were recorded for the nasal breadth and length, respectively. Data generated from this study will prove useful in comparative, regional and clinical anatomy and could also help in identifying archaeological remains of the cattle egrets.

Keywords: cattle egret, cranium, orbit, beak, nasal, morphometrics, craniofacial indices

INTRODUCTION

The cattle egret was first described by Carl Linnaeus in his *Systema naturae* (Linnaeus 1758). Cattle egret (*Bubulcus ibis*) is a cosmopolitan species of heron and is present in worldwide (Rezk 2015). Geographically, the cattle egret has two races which are classified as, the western cattle egret, *B. ibis*, and eastern cattle egret, *B. coromandus,* and these two forms were described by McAllan et al. (1988). In the South Western part of Nigeria, cattle egrets are colonial, white in colour, and are found in open lands and also, around water bodies. The cattle egret has widely been described based on the color of their plumage, habitat, and feeding habits (Hasan 2015). This bird is of huge economic importance in the

Submitted 11th June 2019. Published 1st November 2020. To cite: Azeez Idris Ayodeji, Ekeolu Oyetunde Kazeem, Usende Ifukibot Levi, Olumuyiwa Abiola Adejumob, Adetogun Adefunke. Anatomical investigation on the craniofacial and ocular morphometrics of the male western cattle egret (*Bubulcus ibis*)·· Anatomy Journal of Africa. 2021. Vol 10 (1): 1908-1917.

control of ectoparasites of cattle as well as land pests (Hussein and Rezk n.d.). While detailed account of the fowl skeleton (Getty 1975) and wings and pelvic bones anatomy of emu (Kumar and Singh 2014), buzzard (Atalar et al. 2007) and kit (Sharma and Dubal 2018; Tiwari et al. 2011) have been described, studies of the gross morphometry of the skeleton of the cattle egret is scarce and the scarcity applied to evolutionary study of the bird morphology (Ekeolu et al. 2016). Rezk (2015) published the descriptive anatomy on its appendicular skeleton and Ekeolu et al., (2016) reported data the on comparative gross morphometrics of the forelimb and hind limb skeleton. More recently, Sasan et al. (2019) published data on the gross and morphometrical studies on humerus of cattle egret. Morphometrical studies of the skull not only reflect the genetic and eco-phenotypic variation of individuals and animals but also provide foundations for clinical and surgical stereotaxic practices (Wehausen and Ramey 2000), hence, we here provide for the first time, baseline information on gross morphometric data on the cranium, nasal and orbital bones and the beak of *B. ibis*, thereby adding to the limited body of knowledge on its anatomy.

MATERIALS AND METHODS

Six (6) apparently healthy adult male migrant cattle egrets line-trapped at the University of Ibadan Teaching and Research Farm were used for this study. The birds were euthanized by lethal injection using a combination of xylazine (10 mg/kg) and ketamine (100 mg/kg). They were then decapitated at the level of the atlanto-occipital joint. The organs in the head were removed. The tongue was carefully dissected from the mouth and eyes were removed from the socket by stitching the upper eye lid with the lower one and the pulling the eye by the thread as described by Olopade et al. (2011), then carefully dissect around the rim of the socket. The heads were then de-fleshed as much as possible using scalpel blade. Cold water maceration method of Ekeolu et al. (2016) was then used to prepare the skull and the mandible. Briefly, the egrets were soaked in cold water with ammonium solution and sodium hydroxide overnight to remove grease and soften the connective tissues; meninges (dura mater) of the brain, and muscular attachment on the bones of the head. The solution was changed daily, removing the dissolved brains and tissues. This was repeated for a week and extraneous tissues on the bones were picked using hand (thumb) forceps, after brushing the muscles fibers and connective tissues attached to the bones with sponges. Then, each skull and mandible were then washed in clean water and air dried. Digital Vernier caliper was engaged in taking the linear measurement of the various bones of the skull, the volume was measured by filling the cranium up with grains. The quantities of grains that fill up each cranium were quantified in a measuring cylinder in millimeters. Figures 1 and 2 show the *B. ibis* skull with illustrations of methodologies employed in the measurement, and Tables 1-6 show the morphometric indices of measured parameters. Measured parameters are concisely defined below.

Definition of Parameters. Cranium and foramen magnum

1. Cranium top length **(CTL)** - dorsal cranial length, anterior tip of occipital condyle to the craniofacial hinge (beginning of the upper jaw).
2. Cranium top breadth **(CTB)** - dorsal cranial breadth, distance between the lateral orbital borders.
3. Cranium back **(CB)** - length from the lateral sides of the occipital crest
4. Whole length of cranium to beak length **(LCBL)** - dorsal anterior tip of occipital condyle to anterior tip of premaxillary (beak)
5. Height of cranium **(CH)** - dorsal frontal surface to post orbital process
6. Cranium flat top **(CFT)** - dorsal frontal length
7. Foramen magnum length **(FML)**

8. Foramen magnum breadth **(FMB)**

Eye socket

9. Left eye socket length **(ESLL) -** orbital length of left eye
10. Left eye socket breadth **(ESLB**) - orbital breadth of left eye
11. Right eye socket length **(ESRL)** - orbital length of right eye
12. Right eye socket breadth **(ESRB**) - orbital breadth of right eye
13. Eye socket and beak breadth **(ESBB**) - highest point of orbit to articular surface of lower jaw
14. Eye socket and upper beak length **(ESBL)** – post-frontal process of orbit to tip of premaxillary of upper jaw
15. Eye socket to nose length (upper) **(ESNL)** – post-frontal process of orbit to nasal length end

Beak and nose

16. Beak whole length- upper jaw **(BLU)** - craniofacial hinge to tip of premaxillary.
17. Beak whole length- lower jaw **(BLL)** - articular to tip of lower jaw
18. Beak breadth lower beak- lower jaw **(BBL)** - height of dentary.
19. Gap between lower beak **(BLG)** - posterolateral width at the jaw articulation and the width between the middle of the lower jaw.
20. Upper beak breadth **(UBB)** - breadth of craniofacial hinge to tip of upper jaw.
21. Beak flat top **(BFT)** - dorsal surface of the craniofacial hinge and dorsal beak width.
22. Nose breadth **(NB)** - nasal breadth
23. Nose length **(NL)**- nasal length
24. Nose to end of upper beak **(NUB)** - nasal length to end of premaxillary of the upper jaw

All numerical data generated from the morphometric studies were subjected to statistical analysis using student's t-test and correlation analysis with the use of GraphPad Prism (GraphPad Prism 5.04, GraphPad Software, Inc., La Jolla, CA, USA) and expressed as mean±standard deviation (SD). Values of $p<0.05$ were considered statistically significan

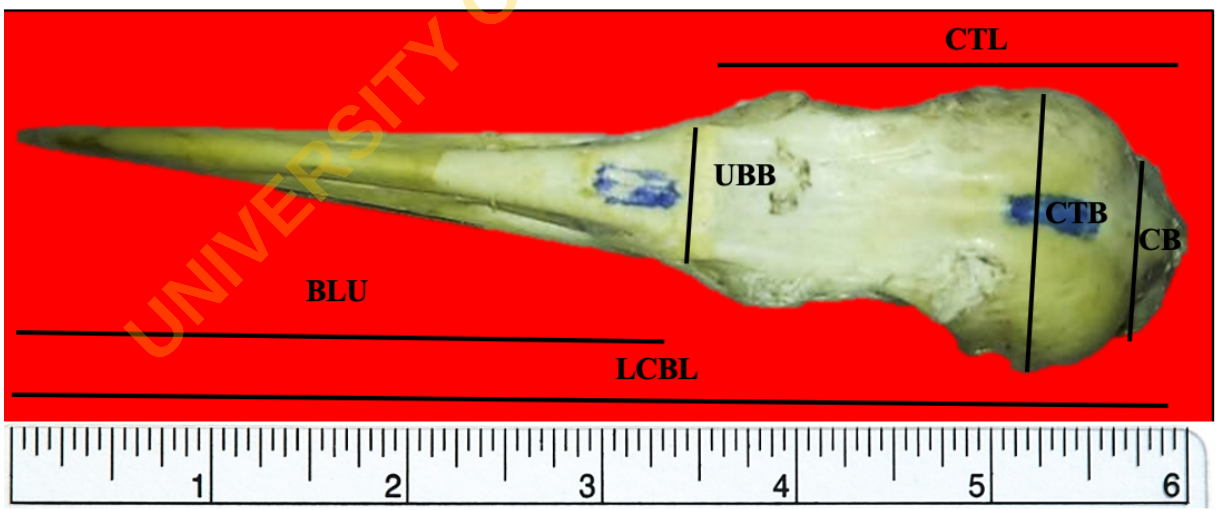

Fig. 1: dorsal view of the skull and beak of the cattle egret with illustrations of some of the measured parameters: Beak whole length- upper jaw **(BLU)** - craniofacial hinge to tip of premaxillary, whole length of cranium to beak length **(LCBL)** - dorsal anterior tip of occipital condyle, upper beak breadth **(UBB)** - craniofacial hinge to tip of upper jaw, cranium top- length **(CTL)** - dorsal cranial length, anterior tip of occipital condyle to the craniofacial hinge (beginning of the upper jaw) ,cranium top-breadth **(CTB)** - dorsal cranial breadth, distance between the lateral orbital borders, Cranium back **(CB)** - length from the lateral sides of the occipital crest

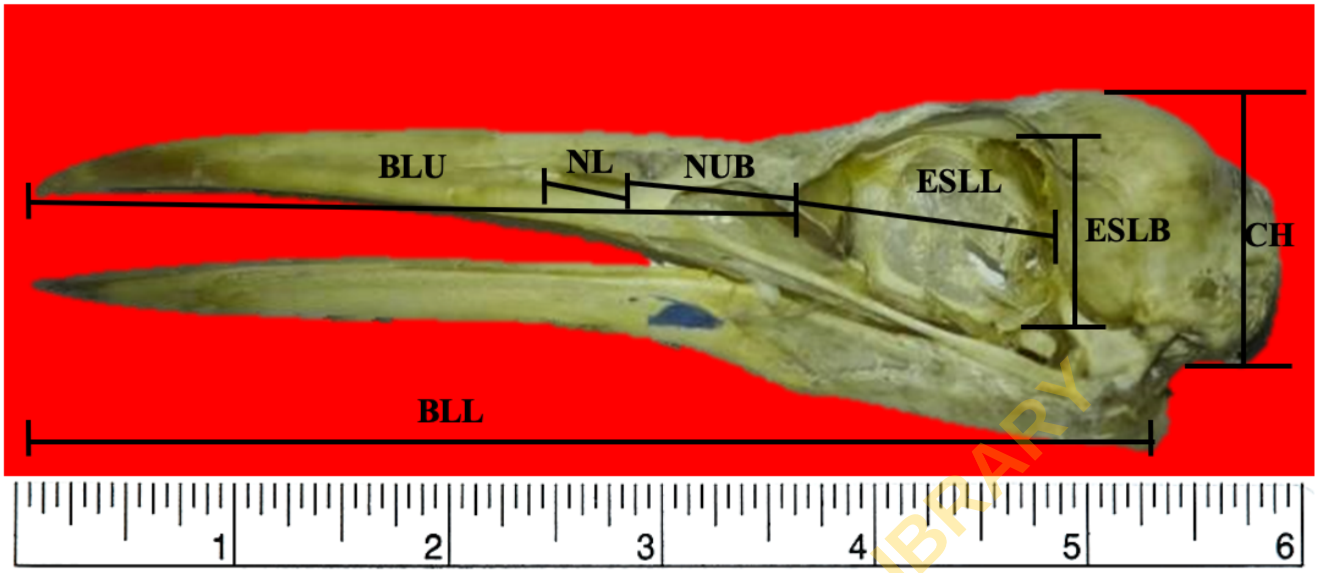

Fig. 2: Left lateral view of the skull and beak of the cattle egret with illustrations of some of the measured parameters: Upper beak length **(BLU)** - craniofacial hinge to tip of upper jaw; nose length **(NL)** - nasal length; nose to end of upper beak **(NUB)** – nasal length to end of premaxillary of the upper jaw; eye socket left length **(ESLL)** - orbital length; eye socket left breadth **(ESLB)** - orbital breadth; height of cranium **(CH)** - dorsal frontal surface to post orbital process; Beak whole length- lower jaw **(BLL)** - articular to tip of lower jaw

RESULTS

Cranium and foramen magnum

The skull of the cattle egret has the splanchnocranium comprising mainly the bones of the face and the neurocranium in which the brain is lodged. A prominent orbital cavity clearly demarcates the two parts. As shown in Table 1, the cranium breadth (CTB) which is a measure of the distance between the lateral orbital borders is lower than the cranium length (CTL), measured from the tip of the occipital condyle to the craniofacial hinge (34.48±1.714 *vs* 52.92±1.800). The foramen magnum length (FML) is slightly higher than the breadth (FMB) (19.17±0.753 *vs* 18.83±0.753). The height of cranium (CH) value (33.92±0.970) was similar to cranium flat top (CFT) (32.32±1.933). The whole length of cranium to beak length **(LCBL)** measured (112.17±2.463) while the length of the cranium from the lateral sides of the occipital crest (CB) measured 31.83±0.753). With Pearson's correlation, there was a strong positive correlation of CTL *vs* LCBL (0.940) and CTL *vs* FML (0.824), as shown in Table 2.

Table 1: Mean±SD of cranium indices (n=6)

Parameters	Mean±SD
CTL	52.92±1.800
CTB	34.48±1.714
CB	31.83±0.753
LCBL	112.20±2.463
CH	33.92±0.970
CFT	32.32±1.933
FML	19.17±0.753
FMB	18.83±0.753

Table 2: Pearson's correlation coefficient values (r) of cranium indices (n=6)

Parameters	**CTL**	**CTB**	**CB**	**LCBL**	**CH**	**CFT**	**FML**	**FMB**
CTL		0.576	-0.307	0.940	0.024	0.635	0.824	-0.307
CTB	0.576		0.199	0.387	0.131	0.665	0.111	-0.654
CB	-0.307	0.199		-0.575	-0.434	0.497	-0.647	-0.412
LCBL	0.940	0.387	-0.575		0.070	0.392	0.953	-0.090
CH	0.024	0.131	-0.434	0.070		-0.186	0.023	-0.434
CFT	0.635	0.665	0.497	0.392	-0.186		0.259	-0.740
FML	0.824	0.111	-0.647	0.953	0.023	0.259		0.059
FMB	-0.307	-0.654	-0.412	-0.090	-0.434	-0.740	0.059	

Eye socket

Eye socket indices and Pearson's correlation coefficients are shown in Table 3 and 4, respectively. The eye sockets of the cattle egrets are lodged in the splanchnocranium. Right eye socket length **(ESRL)** was slightly larger than the left eye socket length **(ESLL**) (36.17±5.636 vs 35.92±4.128) while value of the left eye socket breadth (**ESLB**) was very similar right eye socket breadth **(ESRB**) (31.00±2.757 vs 31.67±2.160). With Pearson's correlation, there was a strong positive correlation between ESLB and ESRB (0.940), and also a strong positive correlation was found between ESLL and ESRL (0.981). The ESBB had a negative correlation with the ESRB (-0.133) and a very low negative correlation with the ESRL (-0.078), while a low positive correlation was recorded with the ESLB and ESLL (0.148 *vs* 0.060). ESBL was negatively correlated with ESLB (-0.474) and ESRB (-0.521) while it has strong positive correlations with ESLL (0.874) and ESRL (0.817).

Table 3: Mean±SD of eye socket indices (n=6)

Parameters	**Mean±SD**
ESLB	31.00±2.757
ESLL	35.92±4.128
ESRL	36.17±5.636
ESRB	31.67±2.160
ESBB	36.33±1.966
ESBL	96.50±2.757
ESNL	58.00±2.074

Table 4: Pearson's correlation coefficient values (r) of eye socket indices (n=6)

Parameters	**ESLB**	**ESLL**	**ESRL**	**ESRB**	**ESBB**	**ESBL**	**ESNL**
ESLB		-0.782	-0.862	0.940	0.148	-0.474	-0.560
ESLL	-0.782		0.981	-0.845	0.060	0.874	0.888
ESRL	-0.862	0.981		-0.865	-0.078	0.817	0.830
ESRB	0.940	-0.845	-0.865		-0.133	-0.521	-0.625
ESBB	0.148	0.060	-0.078	-0.133		0.037	0.184
ESBL	-0.474	0.874	0.817	-0.521	0.037		0.971
ESNL	-0.560	0.888	0.830	-0.625	0.184	0.971	

Beak and nose

Beak and nose indices and Pearson's correlation coefficients are shown in Table 5 and 6, respectively. There was a low positive correlation between the upper beak length (BLU) and the upper beak breadth (UBB) (0.290). Similar values were recorded for nasal breadth (NB) (17.50±0.894) and nasal length (NL) (22.83±1.472), with moderate positive correlation (0.632). The NL was higher than the NB (22.83±1.472 *vs* 17.50±0.8944) with a positive correlation. Meanwhile, very similar values was recorded between the BLG and BBL (20.70±0.7014 *vs* 20.43±0.5989) with low insignificant correlations (0.224). Also, the NB was recorded to have strong positive correlations with the BLU (0.822) and BLL (0.867), respectively.

Table 5: Mean±SD of nose and beak indices (n=6)

Parameters	Mean±SD
BLU	73.67±1.633
BLL	102.6±3.353
BBL	20.43±0.599
BLG	20.70±0.701
UBB	20.85±1.350
BFT	21.53±0.963
NB	17.50±0.894
NL	22.83±1.472
NUB	63.83±3.296

Table 6: Pearson's correlation coefficient values (r) of nose and beak indices (n=6)

Parameters	BLU	BLL	BBL	BLG	UBB	BFT	NB	NL	NUB
BLU		0.901	-0.518	-0.733	0.290	0.187	0.822	0.555	0.322
BLL	0.901		-0.345	-0.591	0.604	0.371	0.867	0.672	0.581
BBL	-0.518	-0.345		0.357	0.109	0.005	-0.597	-0.424	0.398
BLG	-0.733	-0.591	0.357		0.042	0.021	-0.335	-0.562	-0.476
UBB	0.290	0.604	0.109	0.042		0.872	0.629	0.659	0.503
BFT	0.187	0.371	0.005	0.021	0.872		0.534	0.725	0.220
NB	0.822	0.867	-0.597	-0.335	0.629	0.534		0.684	0.153
NL	0.555	0.672	-0.424	-0.562	0.659	0.725	0.684		0.426
NUB	0.322	0.581	0.398	-0.476	0.503	0.220	0.153	0.426	

DISCUSSION

The nomenclature here adopted in this study for the anatomical descriptions was as described in the *Nomina Anatomica Avium* by Baumel et al. (1993). While there is information on the gross morphological descriptions skulls of the cattle egrets (Rezk 2015), there is presently a dearth on information concerning morphometric parameters of the skull. For this purpose, some of the craniofacial measurements were investigated. This study also shows the correlative relationship of cranial, orbital, beak and nasal parametric indices.

Morphometric data (i.e. absolute or relative size of particular interest) are useful barometers of functional morphology (Saber and Gummow 2014; Oyelowo et al. 2017), and we present for the first time, data on the craniofacial indices of cattle egret. In the morphology of avian skulls, their striking spectrum of shapes and relative sizes of the facial skeleton are very evident (Zusi 1993). Thin plates of bone that are formed from connective tissue or cartilaginous templates form the head (König, Korbel, and Hans-Georg 2016). Birds differ critically in the way the neurocranium is structured which is most visible in the extreme inclination of the nuchal plane in any species (Marugán-Lobón and Buscalioni 2004; 2006).

The frontal and maxillary processes of the premaxillary bone form the boundary of the bony nostril, and the delicate nasal bone, while the caudodorsal angle of the nostril is formed by the premaxillary and maxillary processes of the nasal bone (König, Korbel, and Hans-Georg 2016). The nasal bones, which are thin flat bones form the dorsolateral boundary of the nasal cavity and form part of the upper beak (Rezk 2015).

In the present study, we showed the cranium breadth and length of the male *B. ibis* to be 34.48±1.714 and 52.92±1.800, respectively. Darwish et al. (2006) report the length and height of the skull of *E. ibis* as 10.6 and 2.4; *C. coturnix* as 4.1 and 1.4; *M. gallopavo* as 10.1 and 3.2; *A. anser* as 12.5 and 4.4 and *A. atthis* as 9.1 and 1.9, respectively. It is highly expected that the length and breadth of the skull will affect the cranial capacity *vis a vis* the brain capacity. Brain capacity/size in relationship to intelligence in school children (Estabrooks 1928) and animals (Hiecks and Dougherty, 2013) have been studied and in wombats and wallabies could reflect their intelligence for getting food and water, managing territory, offences and in defence (Saber and Gummow 2014) which could also be the case with cattle egret.

Concerning the foramen magnum, our report showed the length and breadth to be 19.17±0.753 and 18.83±0.753, respectively. Burdan et al. (2012) reported the mean values of the foramen length for Eastern-European adult males versus female (human) (37.06 ± 3.07 vs. 35.47 ± 2.60 mm), breadth (32.98 ± 2.78 vs. 30.95 ± 2.71 mm) were significantly higher in males than in females with a significant, positive correlation between foramen length and breadth. The foramen magnum is an important anatomical opening in the base of the skull through which the posterior cranial fossa communicates with the vertebral canal and it also related to a number of pathological conditions including tumors, and occipital dysplasia.

It has been reported that avian species have a very large bony orbit that lies between the neurocranium and splanchnocranium (Nickel, Schummer, and Seiferle 1977; Dyce, Sack, and Wensing 2010) and the position and size varying within the various species reflecting the divergence of their behavior and mode of locomotion (Wiedersheim 1909; Darwish et al. 2006). In this present study, we showed that the mean length and breadth of the right orbit of cattle egret to be 36.17±5.636 and 31.67±2.160, respectively and the mean length and breadth of the left orbit to be 35.92±4.128 and 31.00±2.757, respectively. Earlier, Darwish et al. (2006) reported same parameters in various avian species; *E. ibis* as 2.3±0.03 and 1.7±0.07; *C. coturnix* as 1.3±0.05 and 1.0±0.07; *M. gallopavo* as 3.6±0.07 and 2.3±0.07; *A. anser* as 3.0±0.01 and 2.6±0.07 and *A. atthis* as 2.1±0.04 and 1.4±0.07 for length and height of the orbit, respectively.

Evidence had shown that the relative visual field depends on the position and direction of the orbit, the degree of projection of the eye ball beyond the orbital rim and the movement of the animal head (Ibrahim et al. 1990, 1992). Specifically, Ibrahim et al. (1992) mentioned that the relative height of the orbit to the total height of the skull is more dorsal in cattle (63.4-100%)

compared to rabbit (39.4-100%), dog (56-92%) and sheep (46.95-82.6%).

We suggest that the position, height and length of the orbit in relation to the beak in cattle egret strongly influences its vision and feeding habits. This is so as Wiedersheim (1909) had showed a relationship between the size and position of the eyes as well as the orbit and the shape of the beak.

Avian beaks are remarkable for their diversity in shape and size and provides elegant illustrations of the process and power of natural selection (Darwin 1859; Badyaev 2010; Badyaev et al. 2008). Even small differences in beak morphology including its length and width can critically affect what foods are accessible to individual (Boag and Grant 1981; Benkman and Lindholm 1991; Temeles and Kress 2003) and how they can defend themselves against ectoparasites (Badyaev et al. 2008). Because beaks are under such strong selection pressure, recently, great interest in understanding the mechanisms that control their morphology have been on the increase (Handel et al. 2010) with less attention on its morphometrics especially in cattle egret.

The conformation of the facial skeleton is influenced considerably by the shape and mobility of the beak (König, Korbel, and Hans-Georg 2016). Changes in key morphometric proteins can result to broad diversity of beak shapes (Schneider and Helms 2003; Wu et al. 2006; 2004; Campàs et al. 2010), thus affecting its morphometrics. Beak morphometrics can be used to characterize normal and abnormal beak and also to monitor the development of beak deformities in individual birds through time (Handel et al. 2010). Important information that can be obtained from beak morphometrics could include crossed bite which occurs when the upper and lower beaks are laterally offset from each other and overbite which occurs when upper beak is longer than the lower beak (Handel et al. 2010) among others. In this present study, we showed strong correlations between the ESLL and ESRL; BLU and BLL; BLU and NB; BLL and NB, respectively. This could have potential clinical implications. Earlier, Handel et al., (2010) showed that in adult Black capped chickadees, the upper beak ranged from 6.0 to 8.5mm from nares to tip and average slightly longer in males than in females and the gonys of the lower beak ranged from 6.0 to 7.5mm not differing by sex, similar to our findings herein reported.

Recent studies of the genetic control of beak development have found that two signaling molecules (bone morphogenic protein 4 and calmodulin) play key roles in determining the depth, width and length of beak (Wu et al., 2004, Abzhanov et al., 2006, Grant et al., 2006). These proteins are active during embryonic craniofacial development and control the outgrowth of the beak primordia (Handel et al. 2010).

In conclusion, our findings have provided baseline information on the Craniofacial Indices of the Male Cattle Egret (*Bubulcus ibis*), which would be of benefit in understanding morphofunctional and paleontological studies of this rodent.

CONFLICT OF INTEREST: The authors declare that there is no conflict of interest with regards to the publication of this article.

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