

**AGE RELATED CHANGES IN EXTERNAL ANATOMY, AND SONG-BASED GENDER IDENTIFICATION IN LOCAL GUINEA FOWLS (NUMIDA MELEAGRIS)****Abdul-Rahman, I. I^{1*} and Weseh, A².**¹University for Development Studies, Ghana. ²University for Development Studies, Tamale.

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SUMMARY

There is hardly any research data on the external anatomy of guinea fowls. Three hundred and sixty local guinea fowls, involving 180 each of males and females were studied, and documented the developmental variations in the external anatomy of these birds from hatching until adulthood. The study also explored the call-based approach as an alternative sexing method in these birds. Externally, all the birds changed from brownish black-stripped down feathers at hatching to white regular spotted or pearl feathers by 8th week of age (WOA). Both sexes began to emit their respective calls from 8 WOA, while moulting of the neck feathers started between the 4th and 6th WOA and completed between 12 and 16 WOA. All external biometric traits increased significantly ($P < 0.0001$) from first WOA and plateaued at different ages. Wing span and tail length plateaued at 16 WOA, abdominal and pelvic inlet widths stabilized at 20 WOA, head-bill length, width, neck and body lengths stabilized from 24 WOA, cere and wattle lengths, as well as body weight continued to increase until the end of the study. Significant development in most external biometric traits was accomplished from 24 WOA, while the type of call emitted could be used to discriminate between the two sexes at 100% level of accuracy from 8 WOA.

Keywords: Guinea fowl; phenotypic changes; morphometry; sexing; call type

INTRODUCTION

The first data to be considered biometric in ornithology was morphometric measurements of birds (Hernández *et al.*, 2010). External measurements used in ornithology have been depicted and explained in detail (Hernández *et al.*, 2010). Many aspects of birds, including differences in size among populations, sex determination, wing morphology, and body mass - body size relationships have been studied using biometry (Hernández *et al.*, 2010). Mostly, in birds, body size is directly proportional to body mass. It is essential to determine the value of body mass, as it may mirror the plane of nutrition of the bird (Hernández *et al.*, 2010). Variation in body condition of birds is an enormously interesting subject in evolutionary ecology, and a precise understanding of this allows us to validate theories on bird adjustments to varied ambient conditions. It is not uncommon for the size of the populations to differ slowly all through their geographical distribution in a single species. Through morphometric scrutiny, we can relate biometric differences to ambient conditions and deduce possible causes to explain them (Hernández *et al.*, 2010). For instance, the climate change encountered over time may affect the changes in body size of birds through changes in factors such as environmental unpredictability (Jakober and Stauber, 2000). Issues affecting endangered species and needing urgent attention may be found and resolved through analysis of size variations in bird populations. The guinea fowl is a monomorphic bird, and this makes sex identification very challenging, even for experienced ornithologists (Abdul-Rahman *et al.*, 2015). In the past these birds have been sexed using differences in morphometric traits (Umosen *et al.*, 2008; Abdul-Rahman *et al.*, 2015), examination

of sex chromosomes and the vents (Abdul-Rahman *et al.*, 2015). Umosen *et al.* (2008) concluded that adult female and male guinea fowls may be distinguished based on wattle size, shape and angle of attachment in the upper jaw, while Abdul-Rahman *et al.* (2015) concluded that juvenile birds could be reliably sexed using eight external biometric traits in stepwise discriminant function analysis. Considering that sexing using morphometric traits may involve more laborious measurements and estimation of a discriminant function by skilled individual, while molecular sexing requires resources/facilities to successfully conduct, it will be difficult for the largely smallholder, and uneducated farmers to apply these techniques, hence the need to explore the song-based sexing approach as an alternative means of sexing the birds by farmers. Several bird species have also been sexed successfully using the call-based approach, including the Cuban whistling duck *Dendrocygna arborea* (Volodin *et al.*, 2009), orange-bellied fruit-dove *Ptilinopus iozonus* (Baptista and Gaunt, 1997) and white-napped crane *Grus vipio* (Swengel, 1996). Presently, biometric studies in guinea fowl in Ghana has been limited to the reports of Awotwi (1975) in juvenile birds, and this will need further studies. The objective of this work, therefore, was to provide a record of age related variations in external anatomy of guinea fowls found in the northern region of Ghana from hatching until adulthood and explore the call-based approach in sex identification in these birds. The data obtained will serve as a baseline data for comparison with guinea fowls found in other parts of the country/continent.

MATERIALS AND METHODS

Experimental site

The work was carried out at the Poultry facility of the Department of Animal Science of the University for Development Studies, Nyanpkala, Tamale, Ghana (latitude 9° 69'N and longitude 0° 83'W). Vegetation, temperatures, humidity and rainfall pattern and amounts were as reported by Abdul-Rahman *et al.* (2016).

Animals and Management

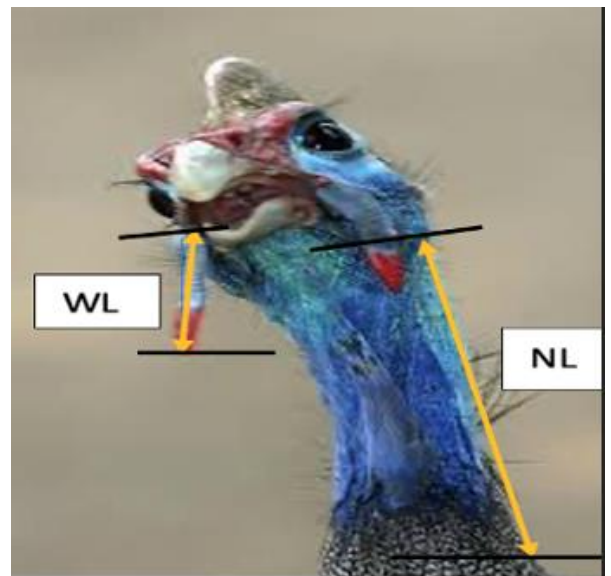
Three hundred and sixty local guinea fowls (*Numidae meleagris*) of the pearl variety, involving 180 each of females and males were studied. Brooding of keets was for 6 weeks, and followed the procedure previously described by Abdul-Rahman *et al.* (2017). Water and feed were provided *ad libitum*. The ages at which starter, grower and layer rations were fed, as well as their respective crude protein and metabolizable energy compositions were previously described by Abdul-Rahman *et al.* (2021), and supplied by Agricare Ghana Limited (a commercial feed supplier). A lighting programme similar to the one employed by Abdul-Rahman *et al.* (2015) was used.

Experimental Procedure

Forty birds (Twenty per sex) per age group were studied. Wingspan, body height and length were recorded to the nearest 0.1 cm, while all other variables were noted to the nearest 0.1 mm. The underlisted biometric variables were considered from 1-32 WOA, at 4-week interval. Landmarks defining the measurements of the various biometric variables have been illustrated in Figure 1.

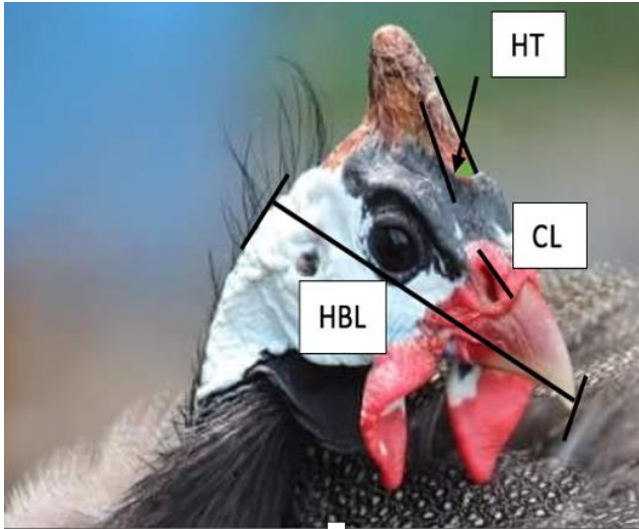


Width of head: This was measured by placing the calipers on the processus postorbitalis on either side of the head.



Wattle length: This extends from the site of attachment to the tip of the wattle. The longest length was taken using a ruler.

Length of neck: This stretched from the occipital condyle to the site where the neck articulates with the trunk and was measured with a flexible tape.



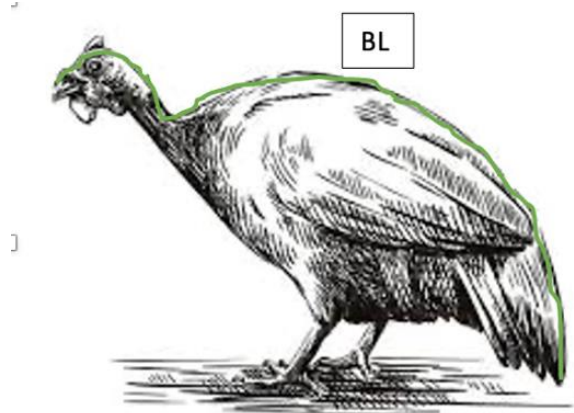
Head-Bill length: This extends from the tip of the bill to the back of the occipital bone and was measured with a pair of calipers.

Length of cere: This is a swelling on top of the upper bill, and was measured with a ruler from the culmen or edge of the cere border to the end of the thickening, towards the tip of the upper bill.

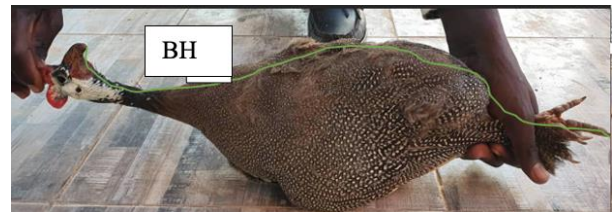
Helmet thickness: The helmet was enclosed in between the calipers from left to right at the base, and not front to back.

Wingspan: This stretched from the scapulohumeral joint to the remotest feather on the phalanges on each side (i.e., left and right wings).

Body weight: The bird's live weight taken with Mettler electronic balance before all other measurements. Weights were noted to the nearest 0.1g.



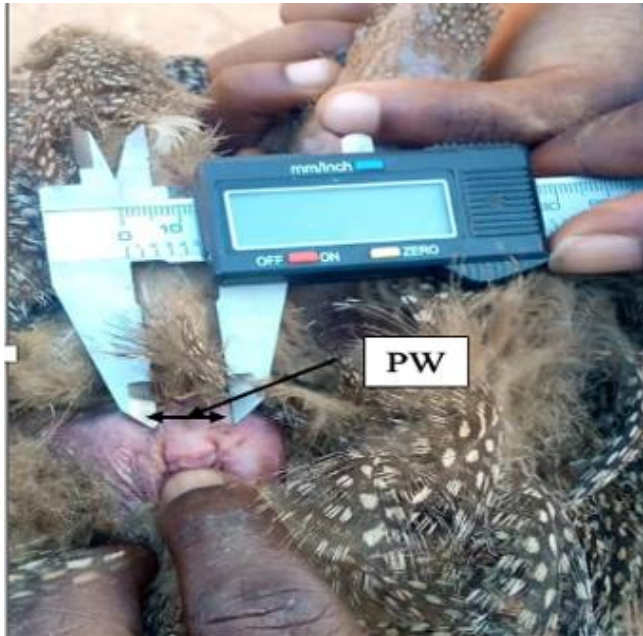
Length of body: This extends from the tip of the bill to the tip of the remotest tail feather and was taken with a flexible tape.



Body height: The bird was laid on its back with the head and neck flattened out gently, and the hind limbs stretched. This was followed by measuring the distance between the tip of the helmet and the tip of the claws, with the aid of a flexible tape.



Width of abdomen: The distance between the two pubic bones measured with calipers. The widest distance was taken.



Pelvic inlet width: This is the distance between the tips of the 2 pubis on either sides measured with a caliper.

Fig 1: Landmarks for measurements of external anatomical variables in guinea fowls. Note: body height (BH), Abdominal width (AW), Pelvic inlet width (PW), Wattle length (WL), Neck length (NL), Head width (HW), Head-Bill length (HBL), Helmet Thickness (HT), Cere length (CL), body length (BL).

Also, general changes in the phenotypic appearance of the bird such as changes in colour, when moulting of the neck feathers started and ended, and when both sexes began to make their characteristic calls were observed and recorded. There were both continuous observation and the use of Closed-Circuit Television (CCTV) Cameras (CP PLUS). The CCTV cameras were mounted in each pen and connected to Digital Video Recorder (DVR) for recording and data storage. Footages

were subsequently reviewed to determine the age at which birds began to emit their characteristic sounds. The birds were subsequently sacrificed (at 32 WOA) by cervical dislocation and sexes confirmed retrospectively using the gonads. All procedures used were in line with approved guidelines for the ethical treatment of animals.

Statistical analysis

Data were evaluated for normality of variance by Shapiro-Wilk's W test, and homogeneity of variance using the Levene's test. The condition of homogeneity of variances were not met, and therefore age related variations in external anatomical variables in guinea fowls were analysed using Kruskal-Wallis test and medians separated using Mann-Whitney U test. All comparisons were done at 5% level of significance.

RESULTS

At hatching, keets had down feathers, appeared brownish with black stripes and markings, with the ventrum being lighter than the rest of the body. They also had red to orange shanks. The down feathers were gradually replaced by purple grey feathers with white stripes from the end of the 2nd WOA, and by the 5th WOA, all the brown feathers had been replaced. Towards the end of the 3rd WOA, the white stripes gradually gave way to white regular spots or pearl, and this was complete in all birds by 8 WOA (Fig. 2).

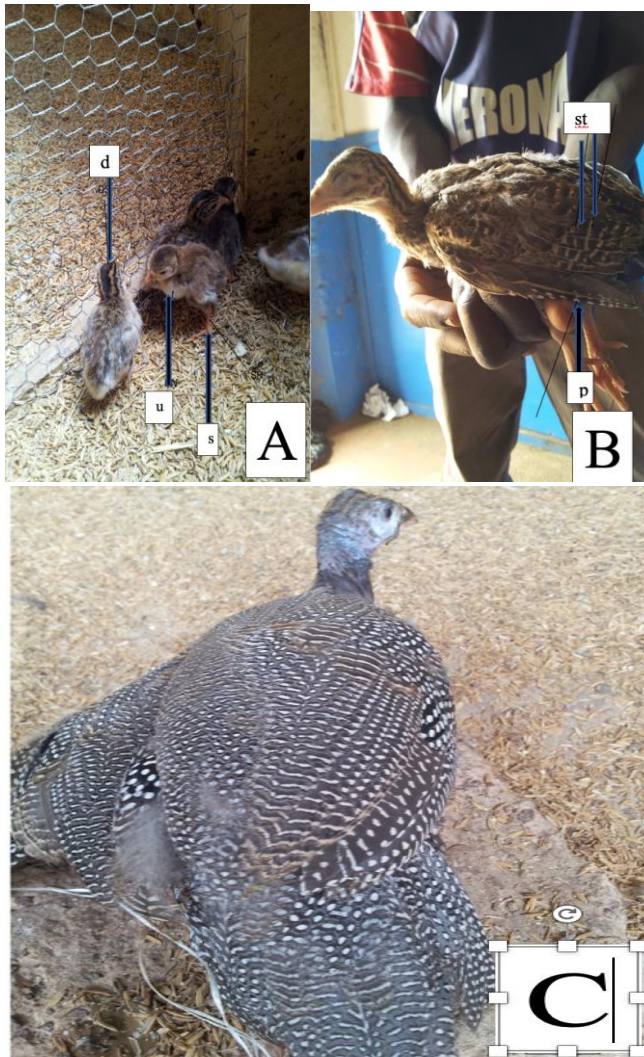


Fig. 2: Day (A), 3-week (B) and fully pearled eight-week (C) old guinea fowls. Note the dark stripes and markings (d) on the head, the lighter underpart (u) and orange shanks (s) in day old keets. In 3-week-old keets, note the appearance of white stripes (st) on the purple grey feathers and gradual conversion of these stripes into white regular spots/pearl (p) commencing from the edges of the last primary feathers.

At this age, both sexes began to emit their respective calls. Males emitted one syllable shriek ‘back’, which was repeated quickly, while females emitted two syllable shriek ‘come-back’ ‘come-back’. The reliability of using this difference in call

types/songs to distinguish between the two sexes was 100%, as all birds from 8 WOA onwards emitted the respective call types. In both sexes, feathering started a week after hatching, while moulting of the neck feathers started between the 4th and 6th WOA, and ended between the 12th and 16th WOA. Moulting usually started from the nape and proceeded in both directions. The helmet first appeared as a thickening on the crown in both sexes at 4 WOA while the wattle was first spotted on the 2nd WOA. Whitish coloration of the face was first seen at 7 WOA in both sexes.

Age related changes in head-bill length and width in local guinea fowls irrespective of sex are shown in Figure 3. Head-bill length differed significantly among age groups (Kruskal-Wallis test $X^2 = 329.747$, $df = 8$, $P < 0.0001$). Head-bill length increased significantly ($P < 0.05$) from first until 24 WOA. Between 24 and 28 WOA, no change occurred. There was, however, a significant increase from 28 to 32 WOA. Similar pattern was observed in the growth of head width (Kruskal-Wallis test $X^2 = 315.173$, $df = 8$, $P = 0.001$).

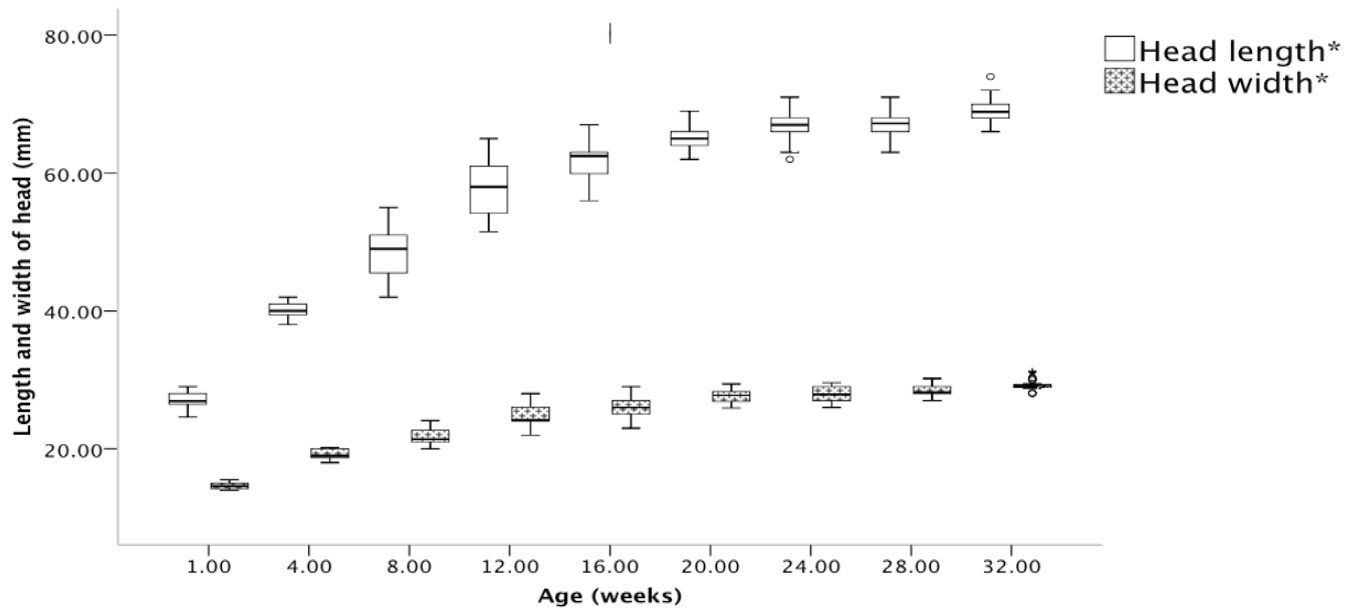


Fig 3: Age related changes in head-bill length and head width in local guinea fowls
 *Medians (Interquartile range) within a category across age differ significantly ($P < 0.05$; see text)

In this case, however, growth ceased between 20 and 24 WOA and resumed from 24th WOA until the end of the study. Cere and wattle lengths showed a similar pattern of growth. There were significant increases in cere (Kruskal-Wallis test $X^2 = 331.356$, $df = 8$, $P < 0.0001$) and wattle (Kruskal-Wallis test $X^2 = 339.120$, $df = 8$, $P < 0.001$) lengths from one to 32 WOA (Fig. 4). There was, however, no detectable wattle in some birds until 4 WOA. The helmet appeared as a thickening on the crowns of both sexes during the first 8 weeks after hatching. From 8 WOA it was consistently measurable, and it thickened significantly (Kruskal-Wallis test $X^2 = 308.092$, $df = 8$, $P < 0.0001$) each month, until 28 WOA. Thereafter no significant increase was noticed (Fig. 4).

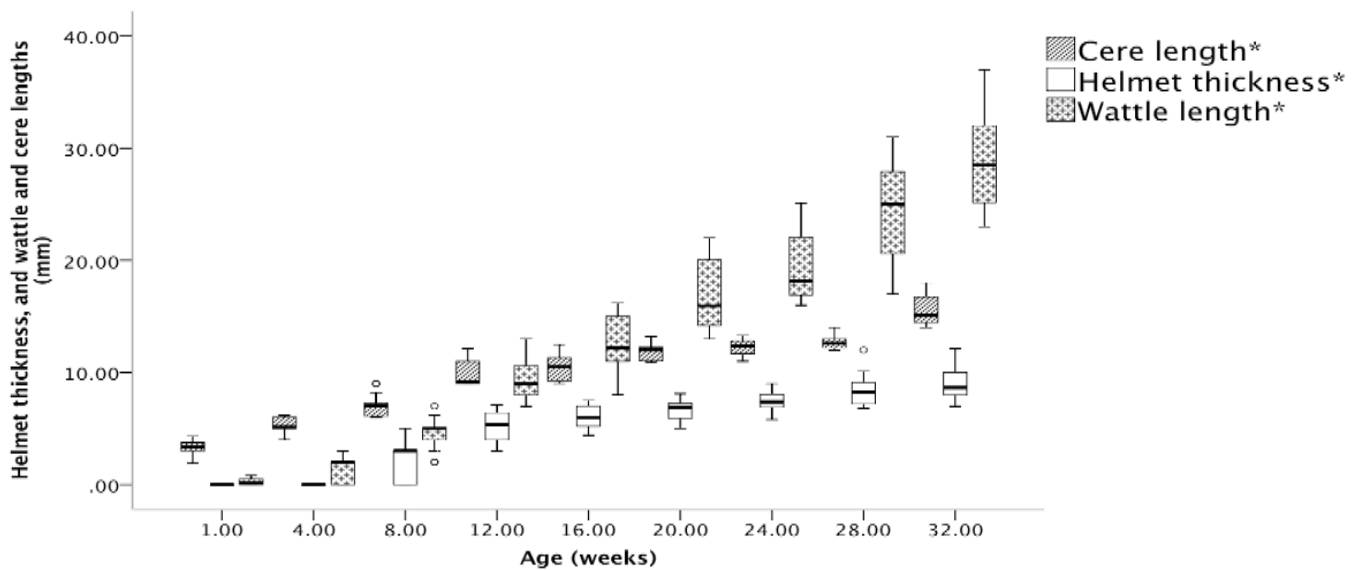


Fig 4: Developmental changes in helmet thickness, cere and wattle lengths in local guinea fowls
 *Medians (Interquartile range) within a category across age differ significantly ($P < 0.05$; see text)

Significant monthly increases were recorded in neck (Kruskal-Wallis test $X^2 = 334.457$, $df = 8$, $P < 0.0001$) and body (Kruskal Wallis test $X^2 = 329.047$, $df = 8$, $P < 0.0001$) lengths between hatching and 24 WOA, and between 28 and 32 WOA (Fig. 5). Body height on the other hand increased significantly (Kruskal-Wallis test $X^2 = 335.071$, $df = 8$, $P < 0.0001$) between hatching {11.5 (11.0-11.7) cm} and 28 WOA {43.6 (42.2-45.5) cm} at 4 week intervals, and then remained unchanged ($P > 0.05$) up to the end of the study (Fig. 5). Wingspan had a slightly different growth pattern from the other biometric traits studied. This trait rapidly increased monthly (Kruskal-Wallis test $X^2 = 314.553$, $df = 8$, $P < 0.0001$) until 16 WOA, and remained constant ($P > 0.05$) thereafter (Fig. 5).

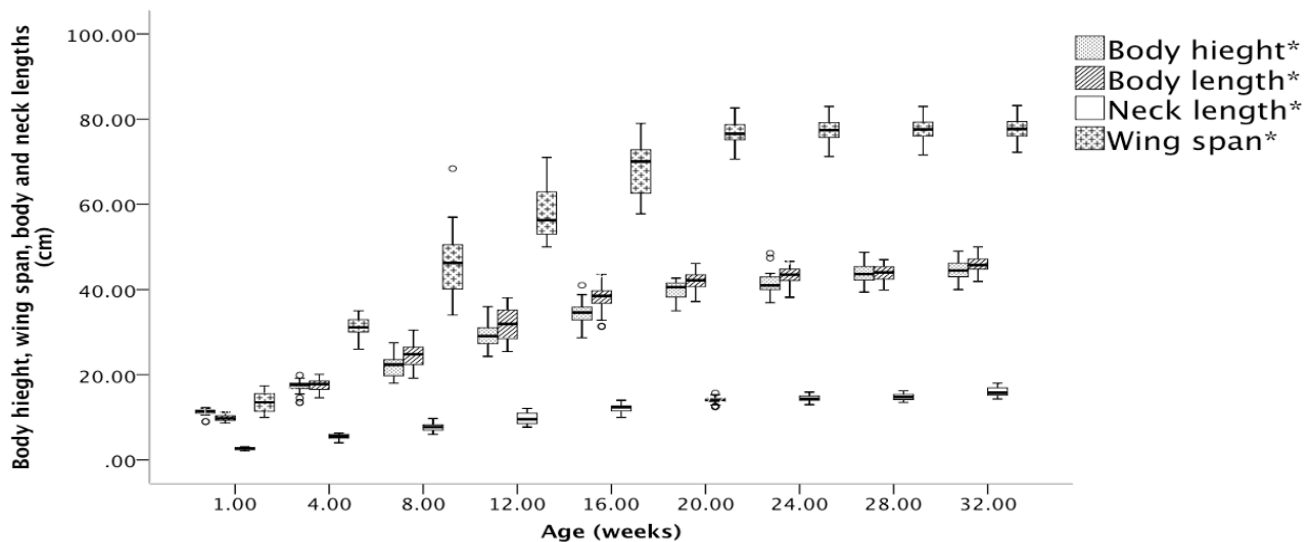


Fig 5: Age related changes body height, wing span, body and neck lengths in local guinea fowls.
 *Medians (Interquartile range) within a category across age differ significantly ($P < 0.05$; see text)

Abdominal width increased rapidly (Kruskal-Wallis test $X^2 = 303.456$, $df = 8$, $P < 0.0001$) between hatching and 20 WOA, monthly. While no significant differences were noticed in abdominal width between subsequent age groups, cumulatively, there was a significant ($P < 0.05$) increase in width between 20 and 28 weeks, and 24 and 32 weeks (Fig. 6). Similar pattern of growth was observed for the width of pelvic inlet. This increased significantly (Kruskal-Wallis test $X^2 = 234.333$, $df = 8$, $P < 0.0001$) between hatching and 20 WOA, at monthly intervals, and remained unchanged ($P > 0.05$) thereafter (Fig. 6). Tail length on the other hand rose significantly (Kruskal-Wallis test $X^2 = 268$, $df = 8$, $P < 0.0001$) until 16 WOA, and remained there until 32 WOA (Fig. 6).

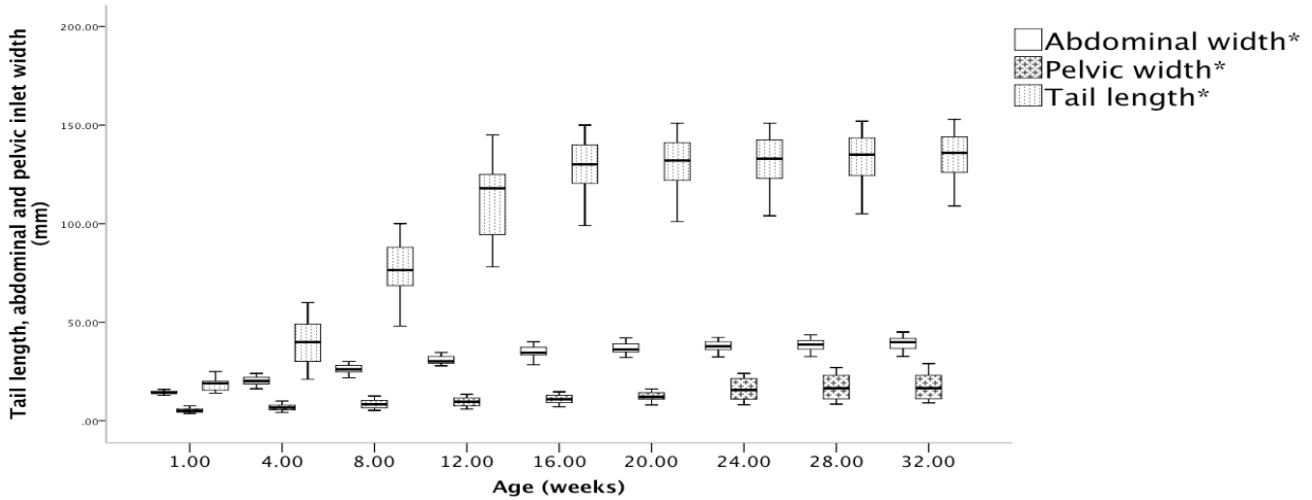


Fig 6: Age related changes in tail length, abdominal and pelvic inlet width in local guinea fowls. *Medians (Interquartile range) within a category across age differ significantly ($P < 0.05$; see text)

Body weight also rose significantly (Kruskal-Wallis test $X^2 = 339.241$, $df = 8$, $P < 0.0001$) between one and 32 WOA, at 4-week intervals (Fig. 7).

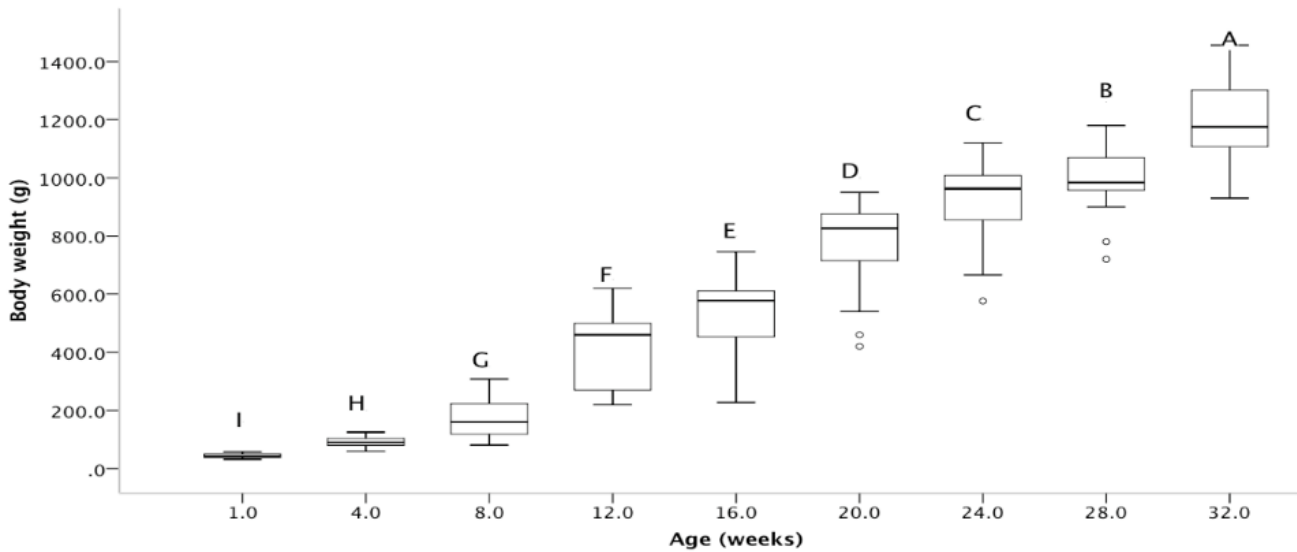


Fig 7: Age related changes in the body weight of local guinea fowls *Medians (Interquartile range) having no letter in common are significantly different ($P < 0.05$)

DISCUSSION

Gross changes noticed in the morphological traits of guinea fowls in this study were similar to earlier reports (Awotwi, 1975). In these birds, by 8 WOA, the final plumage colouration distinguishing growing guinea fowls from keets had been established. Abdul-Rahman *et al.* (2015) also reported that differences in phalli sizes between males and females emerged at 8 WOA. In the present study, males and females also began to emit their characteristic calls from 8 WOA. It appears that at this age, the formation of most external qualitative features are complete in the local guinea fowl, and therefore, except changes in size resulting from growth, no qualitative changes in these features may be expected.

The higher level of accuracy (100%) noted in the use of male and female specific types of call for distinguishing between the two sexes have also been reported in other bird species, including Cuban Whistling Duck *Dendrocygna arborea* (Volodin *et al.*, 2009), Willow Ptarmigan *Lagopus lagopus* (Martin *et al.*, 1995), Orange-bellied Fruit-dove *Ptilinopus iozonus* (Baptista and Gaunt, 1997), Swinhoe's Storm-petrel *Oceanodroma monorhis* (Taoka and Okumura, 1990), White-napped Crane *Grus vipio* (Swengel, 1996), Fulvous Whistling Duck *Dendrocygna bicolor* (Volodin *et al.*, 2009) and White-faced Whistling Duck *Dendrocygna viduata* (Volodin *et al.*, 2005; 2009). In all these birds, the sexing reliability was 100% due to strong vocal differences between sexes (Volodin *et al.*, 2009). However, a few studies reported lower level of reliability in some birds [Brown Skua *Catharacta antarctica* (Janicke *et al.*, 2007), Black-legged Kittiwake *Rissa tridactyla* (Mulard *et al.*, 2009), and Kea *Nestor notabilis* (Schwing *et al.*, 2012)]. The call-based approach to sexing is a substitute to the frequently used intrusive sexing methods requiring capturing of

birds and blood sampling for gene extraction, body measurements and cloaca inversion, thereby avoiding potential trauma to the bird (Volodin *et al.*, 2015). In the voices of monomorphic birds, sex differences may arise in a number of ways, including differences in method of vocal production (Niemeier, 1979), morphology and size of vocal apparatus (Livezey, 1991; 1995), construction of vocal apparatus and type of calls/songs (Volodin *et al.*, 2015). The guinea fowl may therefore be sexed based on differences in type of call between the two sexes from 8 WOA. Almost all the external biometric traits increased in size from hatching until 24 WOA, from which point most of them tended to stabilise, apart from tail length and wingspan both of which tended to stabilise from 16 WOA. This age also coincided with the age at which moulting of the neck feathers ended in these birds. The implication of these findings is that all significant developments of the feathers in local guinea fowls completes at 16 weeks from hatching. Beyond this age therefore, no further growth in feathers in all parts of the body may be expected. Many aspects of birds have been studied using biometry. For instance, biometry has been used for sex determination in guinea fowls *Numida meleagris* (Abdul-Rahman *et al.*, 2015), Mute Swans *Cygnus olor* (Brown *et al.*, 2003), Coscoroba Swans *Coscoroba coscoroba* (Calabuig *et al.*, 2011), Balearic Shearwaters *Puffinus mauretanicus* (Genovart *et al.*, 2003), Common Wood Pigeons *Columba palumbus* (O'Huallachain and Dunne, 2010) and Blue-fronted Amazons *Amazona aestiva* (Berkunsky *et al.*, 2009). Variations in size among populations, wing morphology and body mass-body size relationship have also been studied using biometry (Hernandez *et*

al., 2010). Morphometrics also facilitate subspecies differentiation in birds (Hernandez *et al.*, 2010). The inferences made from applying biometric characteristics are usually validated through genetic analysis. Considering the semi-wild and relatively undeveloped nature of the local guinea fowls to date, biometric studies have a bigger role in highlighting the differences existing between guinea fowls found in different parts of the country/continent, and this may serve as a clue for further molecular work to enable the classification of these birds into breeds/strains. Presently, scrutiny of genes found in nuclear and or mitochondrial DNA has ensured enormous level of precision when defining different population taxonomic categories (Hernandez *et al.*, 2010). The data obtained in the present study serves as a baseline information for comparison with future work on biometric characteristics in guinea fowls in other parts of the country/sub-region. Additionally, such study when repeated on guinea fowls in Northern Ghana after a prolonged period, may clearly help reveal the effect of climate change and other factors on body size in these birds. This is particularly useful in locations where guinea fowls are kept as game birds.

CONCLUSION

Differences in type of call could be used to reliably discriminate between male and female guinea fowls from 8 WOA. Significant development of most external anatomical biometric traits in the guinea fowl was accomplished from 24 WOA, except feather development, which ended at 16 WOA. Formation of external qualitative features also seem to complete by 8 WOA.

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Conflicts of interest: No conflict of interest to declare.

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