Exploring skull shape variation and allometry across different chicken breeds

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Abstract

This study investigates skull shape variation and allometry among three different chicken breeds: Broiler, Lohman Brown, and Leghorn. Geometric morphometrics analysis was employed to analyse skull morphology, focusing on facial bones and the neurocranium. The study aims to understand how skull shape differs between these breeds and how it relates to size variation. Results show significant differences in skull morphology among the chicken breeds. Following PCA analysis, it was observed that PC1 explained 21.7% of the total variation. The PC1 values of Broiler chickens were notably lower compared to other breeds, indicating distinct morphological differences in their cranial shape. Increasing PC1 values corresponded to a more rounded head shape, with individuals possessing high PC1 values exhibiting a higher neurocranium. In contrast, Lohman Brown and Leghorn chickens show similarities in skull shape, with a more elongated appearance. Broiler chickens were found to be the smallest among the breeds studied, with statistical analysis confirming their distinguishability based on centroid size. In contrast, Lohman Brown and Leghorn chickens exhibited similar sizes, with no significant difference between them. Allometric analysis reveals that skull shape changes with size, particularly in the neurocranium and facial bones. These findings suggest that evolutionary adaptations and breeding practices have influenced the skull morphology of these chicken breeds. Overall, this study provides insights into the skull shape variation and allometry of different chicken breeds, highlighting the importance of considering both genetic and environmental factors in understanding morphological diversity in poultry.

Introduction

The skull morphology of poultry, including chickens, turkeys, ducks, and other domesticated birds, is a fascinating aspect of avian anatomy. The skull of poultry is characterized by its lightweight yet sturdy structure, optimized for efficient feeding, vocalization, and protection of vital organs (3, 9, 19). Key features of poultry skull morphology include the shape and size of the beak, which is composed of a keratinous sheath covering the upper and lower mandibles. The overall shape of poultry skulls varies among breeds, with distinct adaptations to their respective habitats and feeding strategies (13, 14).

The skull morphology of poultry reflects their evolutionary adaptation to diverse ecological niches and behavioural repertoires. Studying the anatomy of poultry skulls provides valuable insights into their evolutionary history, ecological adaptations, and functional morphology, contributing to our understanding of avian biology and evolution (9, 20). By studying skull morphology, scientists can better understand how birds have adapted to their environments. In this study, the researchers examined the morphological structure of the skulls of three different chicken breeds that were raised in the same geographic area. Broiler chickens, one of the breeds studied, are primarily raised for meat consumption, and their body structure develops rapidly.

Geometric morphometrics is a powerful tool used in veterinary anatomy and skull studies to analyse and quantify shape and size variations in anatomical structures, including skulls (6, 25). This method combines the principles of geometry with statistical analysis to study the form and development of organisms (1, 4, 22). In veterinary anatomy, geometric morphometrics allows researchers to precisely quantify and analyse complex shapes, such as the skull, by digitizing anatomical landmarks or curves on the structure of interest (8). The coordinates of these landmarks are then analysed using geometric and statistical methods to explore shape variations among individuals, populations, or breeds (10, 22). Skull studies benefit greatly from geometric morphometrics due to the intricate and diverse shapes of skulls across different species and breeds. Overall, geometric morphometrics has become an essential tool in veterinary anatomy and skull studies, providing a quantitative framework for analysing shape and size variations in anatomical structures and advancing our understanding of animal morphology and evolution (5).

Traditionally, collecting landmark data in geometric morphometry has been a time-consuming and labor-intensive manual process, prone to observer bias (15, 17). Recent technological advancements have introduced automated methods, particularly in volumetric imaging, improving efficiency and reducing bias (11, 12). One such method, Automated Landmarking through Point Cloud Alignment and Correspondence Analysis (ALPACA), automates the application of a draft landmark file across all study samples. In this study, ALPACA was used to ensure standardized data collection.

Skull morphology plays a crucial role in understanding the evolutionary history and taxonomic relationships of bird breeds. Variations in skull shape and structure can help researchers classify birds into different groups and gain insights into their evolutionary adaptations. Additionally, the shape of the skull is closely linked to a bird's feeding behaviour, diet, and ecological niche (13, 20). The researchers aimed to investigate skull variations among these chicken breeds, which have different intended uses and nutritional requirements. They also aimed to explore differences in skull shape among different subspecies with the same diet (Lohman Brown and Leghorn chickens). This study underscores the significance of skull morphology in understanding the evolutionary and functional traits of bird breeds, especially regarding their feeding behaviors and environmental adaptations. By analyzing skull variations across different chicken breeds, the researchers offer important insights into the diversity and evolution of avian skull morphology.

Materials and Methods

Samples: In this study, a total of 32 skulls were utilized, comprising 11 Broiler chickens, 11 Lohman Brown chickens, and 10 Leghorn chickens. The Broiler chickens, representing meat breeds, were of the Ross 308 breed and aged 2.5 months, while the Lohman Brown and Leghorn chickens, representing laying breeds, were aged 15 months. All specimens were sourced from individuals without any observed pathological conditions, and notably, all samples were female. The study samples were sourced from the university's slaughterhouse. As the samples were collected from slaughterhouse materials, ethics committee permission was not required for their use in the study.

After collection, the skulls were dissected in the anatomy laboratory to extract muscles and skin. Following this, the skulls underwent boiling in water, with Broiler chickens boiled for an average of 20 minutes and Lohman Brown and Leghorn chickens boiled for 1.5 hours. Subsequently, all samples were soaked in hydrogen peroxide for 20 minutes to eliminate fat from the bones. These procedures yielded skulls ready for 3D scanning.

Modelling and Data Collection: The skulls of the samples were 3D modelled using the Shining 3D EinScan SP 3D scanner. During the scanning process, fixed scanning was conducted utilizing a rotary table, with a dot distance of 0.2 mm. Following the completion of the scanning process, the acquired data was processed utilizing EXScan software for mesh operations, and the resulting models were saved in PLY format for subsequent analysis.

To streamline the landmarking process and ensure consistency across the dataset, the study employed the ALPACA technique within the Slicer program (version 5.2.2) to process the initial draft landmark set across all 3D models (Figure 1) (18). This automated approach applied the draft landmark set to all samples (82 landmarks).

Geometric Morphometry and Statistical Analysis: Principal Component Analysis (PCA) was used to identify patterns and reduce dimensionality in the skull shape data (2). PCA was applied to the landmark data obtained from the skulls to determine the main axes (principal components) of shape variation. Graphical visualization of these components helped researchers understand how skull shapes differed among Broiler chickens, Lohman Brown, and Leghorn chickens. The 3D models of the skulls were used to visualize the negative and positive limits of the principal components, showing how the skulls deformed based on these values. ANOVA was used to assess the statistical differences in principal components between breeds, with the Bonferroni test applied due to unequal group sizes.

Centroid size, a measure of overall size, was calculated based on the landmark configuration. Procrustes distance, indicating the distance of samples from the average shape, was also calculated for each breed. These measures allowed for the evaluation of size and shape differences between individuals, with ANOVA used to detect statistical differences in centroid size between breeds.

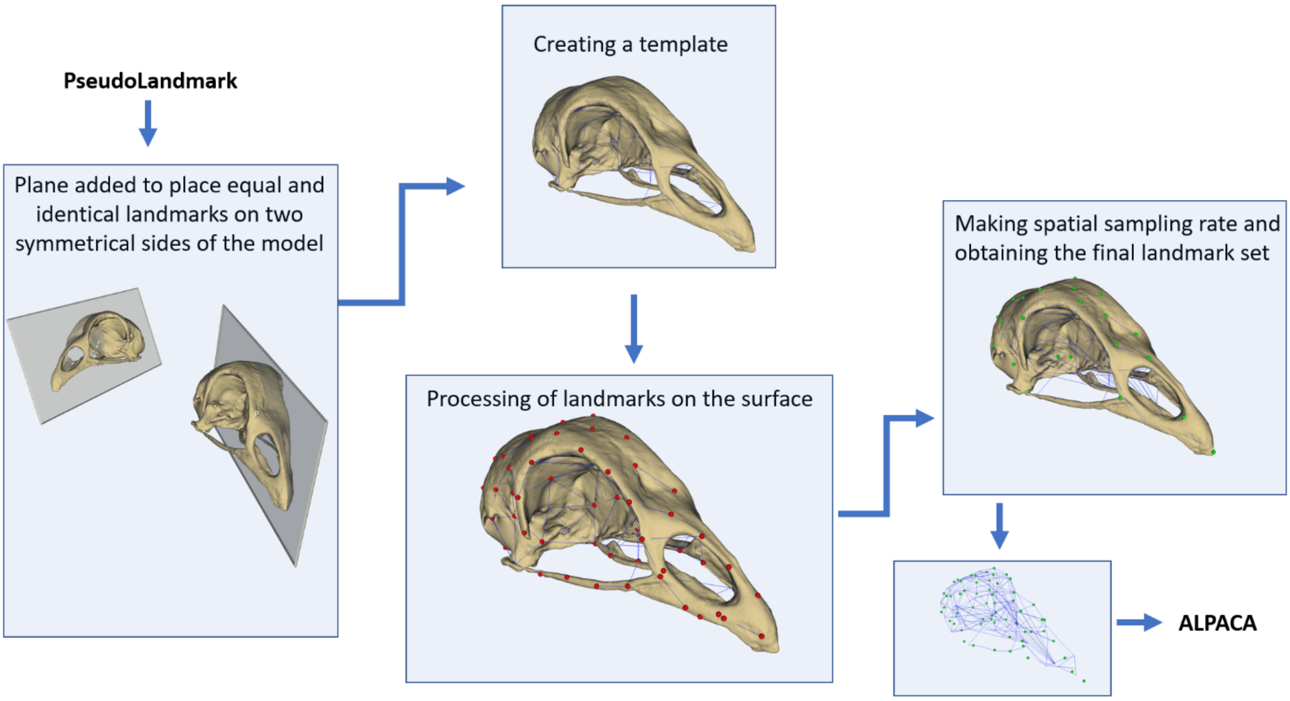

Figure 1. Draft Landmark creation processes.

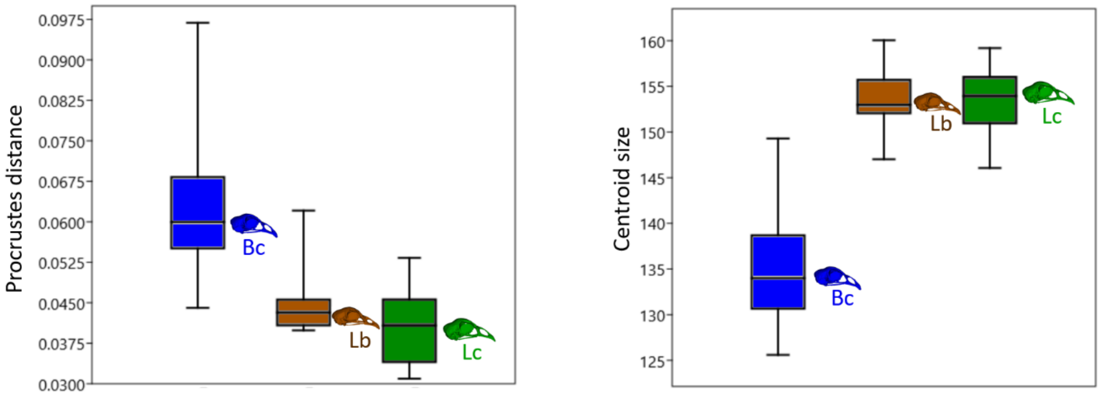

Figure 2. Boxplot with variation in Procrustes distance and centroid size values for breeds. The darker horizontal line is the median, the margins of the boxes represent the percentiles (25 and 75), and the extensions of the bars represent maximal and minimal values for skull groups.
Broiler chickens (Bc); Lohman Brown (Lb); Leghorn chicken (Lc).

The study aimed to assess the allometric effect, examining how shape changes with size, using multivariate regression analysis. By regressing centroid size on the principal component explaining the highest variation, researchers investigated whether there was a consistent pattern of size-related shape change across breeds.

Results

Procrustes distance and centroid size distributions are presented in Figure 2. The analysis revealed intriguing insights into the morphological variations among chicken breeds. The standard deviation of Procrustes distance values for Broiler chickens was notably higher than that of other breeds, indicating a wider range of shape variation within the Broiler population.

In terms of size, Broiler chickens were found to be the smallest among the breeds studied. Statistical analysis confirmed that Broiler chickens could be distinguished from other breeds based on centroid size ($P \leq 0.05$). In contrast, Lohman Brown and Leghorn chickens exhibited similar sizes, with no statistically significant difference between these two breeds.

Following PCA analysis, it was observed that PC1 explained 21.7% of the total variation, while PC2 and PC3 accounted for 13.7% and 8.6%, respectively. The PC1 values of Broiler chickens were notably lower compared to other breeds, indicating distinct morphological differences in their cranial shape. The PC1 values of Lohman Brown and Leghorn chickens were closer to each other. Statistical analysis confirmed that Broiler chickens could be distinguished from other breeds based on their PC1 values (P≤0.05).

For PC2, Lohman Brown and Leghorn chicken breeds were significantly differentiated from each other (P≤0.05), while Broiler chickens were not significantly separated by PC2. Broiler chickens showed greater variation than the other breeds in both PC1 and PC2 (Figure 3).

Increasing PC1 values corresponded to a more rounded head shape, with individuals possessing high PC1 values exhibiting a higher neurocranium. In contrast, individuals with low PC1 values, such as Broiler chickens, had thinner, longer skulls. As PC1 values increased, the upper beak became blunter and shorter in shape.

The most significant shape change associated with PC2 occurred in the upper beak. Increasing PC2 values led to a downward variation in the shape of the upper beak. At a negative PC2 value, the upper beak was shaped at the orbital level. This distinct beak shape, characterized by a positive PC2 value, allows for the morphological differentiation of Leghorn chickens from other breeds. Broiler chickens exhibited considerable variation in their PC2 values, suggesting a lack of distinctive features in their beak shape compared to other breeds. Therefore, PC2 values may not be as useful in morphologically differentiating Broiler chickens.

With increasing PC3 values, the upper beak became more distinct in shape, and the head widened. These findings underscore the importance of considering multiple PC axes in morphological analyses to capture the full range of variation present in chicken breeds (Figure 4).

The relationship between size and the principal component that had the most significant impact on the total variation was investigated, revealing the presence of allometry. A statistically significant effect of size on PC1 was identified (Wilks' lambda: 0.3291, F: 61.17, P≤0.05). As size increased, chickens exhibited a more rounded skull shape, along with a higher neurocranium and a blunter upper beak.

However, it was noted that the relationship between size and PC2 was not statistically significant (Wilks' lambda: 0.999, F: 0.029, P: non-significant). This indicates that while size influenced the morphology captured by PC1, it did not have a significant impact on the variation represented by PC2 (Figure 5).

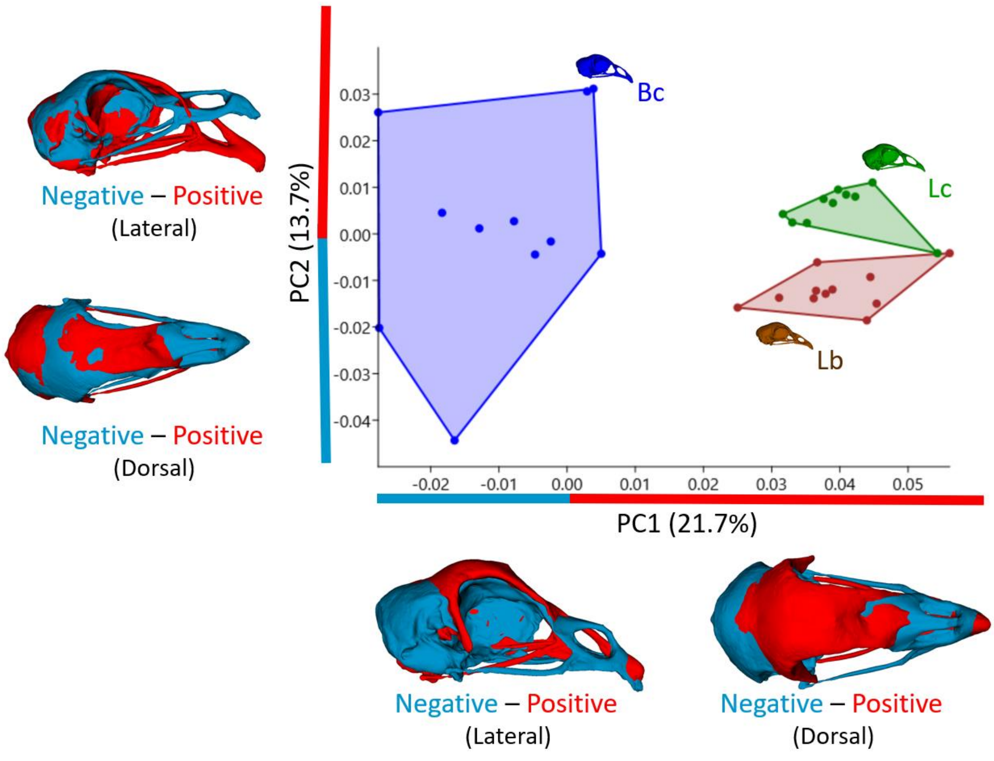

Figure 3. Principal component analysis scatter plot comparing skull morphology of three breeds. Models describing skull shape between the negative and positive values of PC1 and PC2 from lateral and dorsal view.
Broiler chickens (Bc); Lohman Brown (Lb); Leghorn chicken (Lc).

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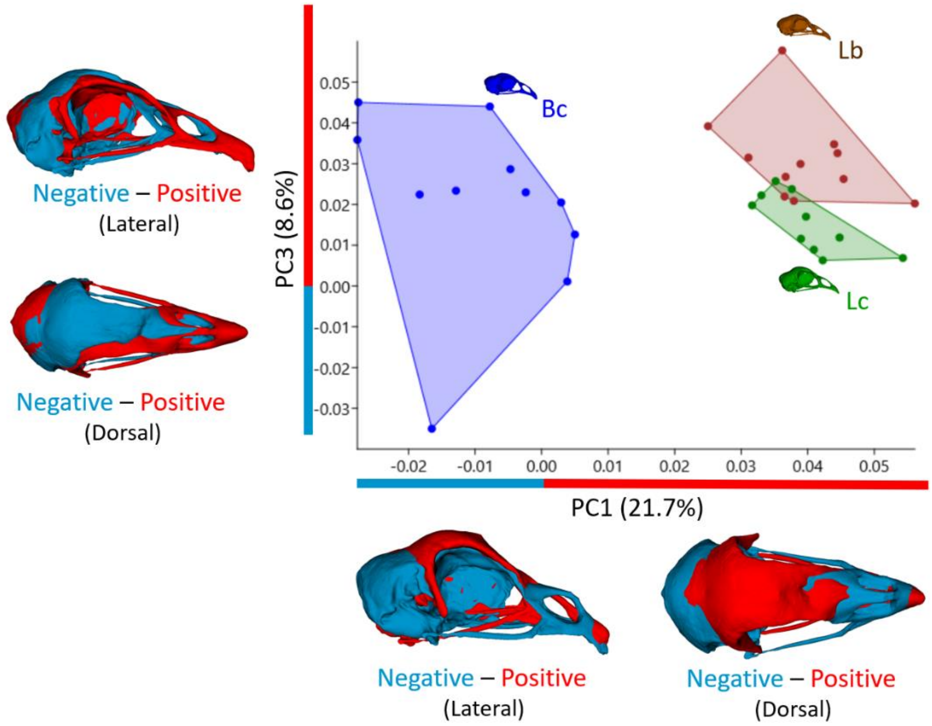

Figure 4. Principal component analysis scatter plot comparing skull morphology of three breeds. Models describing skull shape between the negative and positive values of PC1 and PC3 from lateral and dorsal view.
Broiler chickens (Bc); Lohman Brown (Lb); Leghorn chicken (Lc).

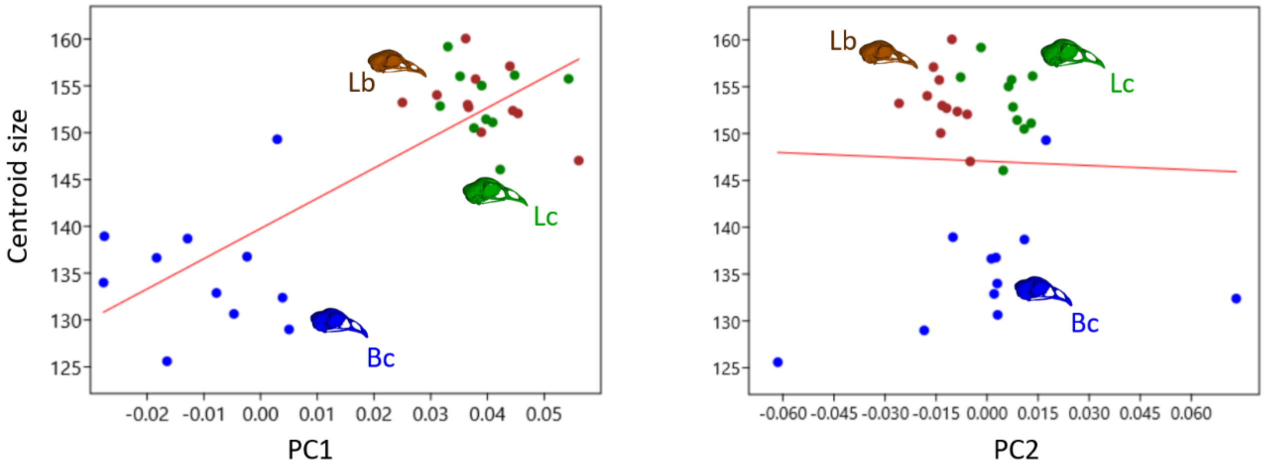

Figure 5. Allometry for skulls.
Broiler chickens (Bc); Lohman Brown (Lb); Leghorn chicken (Lc).

Discussion and Conclusion

Overall, the results of this study provided valuable insights into the morphological variations and allometric effects among chicken breeds, demonstrating the importance of considering size and shape differences in understanding their evolutionary and selective processes. The presence of allometry, as indicated by the significant effect of size on PC1, suggests that skull shape changes with size across chicken breeds. PC2 and PC3 were instrumental in morphologically distinguishing the skulls of Lohman Brown and Leghorn chickens. Broiler chickens were found to be the smallest among the breeds studied, with statistical analysis confirming their distinguishability based on centroid size. In contrast, Lohman Brown and Leghorn chickens exhibited similar sizes, with no significant difference between them.

In his study, Stange et al. (24) conducted a comparison of skull morphology variations in chickens and wildfowl animals using three-dimensional geometric morphometrics and multivariate statistics. The study highlighted the cranial vault as the most variable part of the chicken skull, formed by dermal and neural crest-derived bones. This current study, focusing on three different chicken breeds (Broiler, Lohman Brown, and

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Leghorn), yielded results similar to those of Stange et al. (24). The analysis revealed that increasing PC1 values corresponded to a more rounded head shape, with individuals possessing high PC1 values exhibiting a higher neurocranium. This morphological difference allowed Broiler chickens, used for meat production, to be distinctly separated from the other breeds. Furthermore, Stange et al. (24) noted that the centroid size of meat breeds is typically higher than that of many egg breeds. However, in this study, the size of Broiler chickens used for meat production was found to be lower than that of egg breeds. While both studies emphasized the significance of the cranial vault in terms of shape variation, there are contrasting findings regarding size differences between meat and egg breeds. The contrasting findings regarding skull morphology and size in chickens from different studies suggest that there may be diverse evolutionary adaptations occurring in chickens across various ecological regions and dietary contexts. In support of this idea, a study by Sophian et al. (23) demonstrated that chickens from different regions exhibit distinct morphological characteristics. These adaptations could be driven by factors such as environmental pressures, available food sources, and breeding practices specific to each region or population. Further research comparing chickens from different ecological regions and with varying diets could provide valuable insights into the evolutionary processes shaping skull morphology and size in these birds.

The skull is composed of facial bones and the neurocranium, and the morphology of these bone groups helps to determine the overall shape of the skull (7). For example, in the woodcock, the long and straight beak is accompanied by a downward orientation of the face, creating a bent or angled appearance to the skull. On the other hand, many other birds also have a long and straight beak, but their facial skeleton is aligned with the main axis of the cranium, resulting in a more elongated skull shape (13). Marugán-Lobón's study on a large sample group of 76 species of modern birds found that these facial differences among bird skulls coincide with changes in the doming of the cranial vault and the orientation of the occiput, which connects the head to the neck. Although Marugán-Lobón's study encompassed a wide range of bird species, this current study focused on only three different chicken breeds (Broiler, Lohman Brown, and Leghorn). However, even with a smaller sample size, this study found that the angled view of the chicken beak, especially when directed downwards at a positive PC2 value, supports Marugán-Lobón's hypothesis. Similarly, a long, straight beak, as indicated by a positive PC3 value, gives the skull a longer appearance. Increasing the number of samples in future studies may further validate these observations and provide more insight into these morphological developments within breeds.

The beak morphology of birds is a classic example of dietary adaptation and environmental influence. Studies on birds have demonstrated that the shape of the beak can be significantly affected by environmental factors (16, 27). This indicates that beak morphology may exhibit different adaptations due to environmental conditions and also differ within the same breeds. For instance, Szara et al. (26) conducted a study on African Penguins and found that individuals from different regions, despite belonging to the same breeds, had varying bill shapes. This suggests that environmental factors play a crucial role in shaping beak morphology within a breed. In the context of this study, the observed distinctive features in beak structure between chicken breeds may be attributed to environmental or dietary factors. To verify this hypothesis, further research could be conducted, which includes detailed analysis of the environmental and nutritional characteristics of the study samples. This would help to establish a clearer understanding of the relationship between beak morphology, environmental factors, and dietary adaptations in chicken breeds.

One constraint of our study is the exclusive inclusion of female avian from the selected breeds. This decision was primarily driven by the fact that the two breeds under investigation are predominantly used for egg production, hence the preference for female specimens. While some studies have worked with smaller bird cohorts, it's important to acknowledge that our study comprised minimal group sizes (21). Despite this limitation, our research methodology and calculations yielded valuable insights aligned with our study objectives.

Understanding the intricate nuances of skull morphology is paramount in unravelling the evolutionary tapestry and taxonomic affinities of avian breeds. It serves as a pivotal tool for researchers to delineate birds into distinct groups, shedding light on their adaptive trajectories over time. Furthermore, the configuration of the skull intimately correlates with a bird's dietary preferences, foraging behaviours, and ecological niche. This study delved into the morphological intricacies of three chicken breeds cohabiting in the same geographic locale. Notably, the broiler chickens, renowned for their rapid growth and meat production, were juxtaposed against Lohman Brown and Leghorn breeds, primarily prized for their egg-laying prowess. By scrutinizing skull variations among breeds with divergent purposes and dietary requisites, the researchers sought to glean insights into the nuanced adaptations sculpted by development. Moreover, they ventured into discerning subtle disparities in skull morphology among subspecies, notwithstanding their shared dietary regimen. In essence, this investigation

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underscores the pivotal role of skull morphology in unravelling the evolutionary narratives and functional adaptations of avian species, particularly in elucidating their dietary strategies and ecological adaptations.

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Ethical Statement

This study does not present any ethical concerns.

Conflict of Interest

The authors declared that there is no conflict of interest.

Author Contributions

AK and OG conceived the idea and planned the manuscript. AK, and BU contributed to sample preparation. OG and CB have made significant scientific support and also contributed to the interpretation of the results. All authors provided significant contributions by giving feedback and help shape the manuscript.

Data Availability Statement

The data supporting this study's findings are available from the corresponding author upon reasonable request.

Animal Welfare

The authors confirm that they have adhered to ARRIVE Guidelines to protect animals used for scientific purposes.

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