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Comparative analysis of head shape in Sahiwal and Holstein Friesian crossbred cattle in India by using geometric morphometrics

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ABSTRACT

Holstein Friesian cattle (*Bos taurus*), despite their high dairy productivity, show limited adaptation to tropical environments compared with indigenous zebu cattle (*Bos indicus*). In India, extensive crossbreeding has produced Holstein Friesian crossbred cattle with improved adaptability; however, the morphological consequences of this taurine–zebu admixture remain insufficiently documented. The present study assessed cranial shape variation between Sahiwal (*Bos indicus*) and Holstein Friesian crossbred cattle using geometric morphometric methods. Head morphology of 51 adult female cattle (32 Sahiwal and 19 Holstein Friesian crossbreds) was analyzed using 44 two-dimensional landmarks from dorsal and lateral photographs. Following Generalized Procrustes Superimposition, shape variation and inter-breed differentiation were examined using Principal Component Analysis (PCA), Canonical Variate Analysis (CVA), Discriminant Function Analysis (DFA), and Procrustes Analysis of Variance (Procrustes ANOVA). PCA revealed structured cranial shape variation, with the first three components explaining more than 60% of total variance in both views. Procrustes ANOVA indicated no significant differences in centroid size between breeds. In contrast, cranial shape differed significantly between Sahiwal and Holstein Friesian crossbred cattle in both dorsal and lateral views ($P < 0.0001$), with stronger differentiation evident in the lateral profile. CVA and DFA demonstrated clear inter-breed separation, supported by high Mahalanobis distances and significant permutation tests. These findings indicated that cranial shape retains clear signatures of taurine–zebu divergence despite crossbreeding, with lateral head morphology providing the strongest discriminatory signal. The findings showed that differences in head shape reflect adaptation, domestication, and function, highlighting phenotypic diversity in indigenous and crossbred cattle under tropical conditions.

Keywords: Cattle, Craniofacial morphology, Geometric morphometrics, Holstein Friesian crossbred, Procrustes ANOVA, Sahiwal

Holstein Friesian cattle are high-yield dairy animals that thrive in temperate climates with intensive management, whereas indigenous zebu cattle are better adapted to tropical and subtropical conditions, handling heat, diseases, and low-input environments more effectively (Vaccaro 2002).

In India, long-term crossbreeding between indigenous cattle and Holstein Friesians has aimed to combine high milk yield with tropical adaptability (Sarang *et al.* 2024). This has resulted in widely reared Holstein Friesian crossbreds that perform better in tropical conditions than pure Holsteins while producing more milk than most indigenous breeds (Barwa *et al*. 2021) . However, detailed phenotypic and morphological characterization, especially of cranial traits, remains limited.

In cattle, skull shape is closely associated with feeding behavior, respiration, horn development, thermoregulation, and breed identification (Güzel *et al*. 2025, Turgut 2025, Xu *et al.* 2025). Head shape reflects genetic, developmental, and environmental factors, making it a useful marker for studying breed differences and the effects of crossbreeding.

Traditional cranial measurements capture basic size and angles but miss subtle shape variations (Casanova 2015). Geometric morphometrics (GMM) overcomes this by analyzing homologous landmarks while preserving spatial relationships (Zelditch, 2012), enabling detailed, biologically meaningful visualization of skull shape differences (Adams *et al*. 2013). GMM methods have been widely used to study head shape variation in domestic animals like sheep, goats and horses (Drake *et al.* 2017); (DemiRciOğlu *et al.* 2020); (Liuti and Dixon 2020); (Alhaddad *et al.* 2024), helping to distinguish breeds (Meng *et al.* 2018), detect sexual dimorphism (Kiliçli

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et al. 2025), and identify population-level morphological patterns (Robinson and Visona-Kelly 2025). However, despite cattle's economic and genetic importance, studies comparing head morphology across different cattle breeds, especially crossbreds, are still limited.

The present study compared cranial shapes of Sahiwal cattle and Holstein Friesian crossbreds using landmark-based GMM. Analysis of dorsal and lateral cranial landmarks revealed breed-specific shape differences and morphological variation, highlighting the effects of long-term crossbreeding. The results can aid in breed identification, conservation, and assessment of phenotypic diversity in crossbreeding programs.

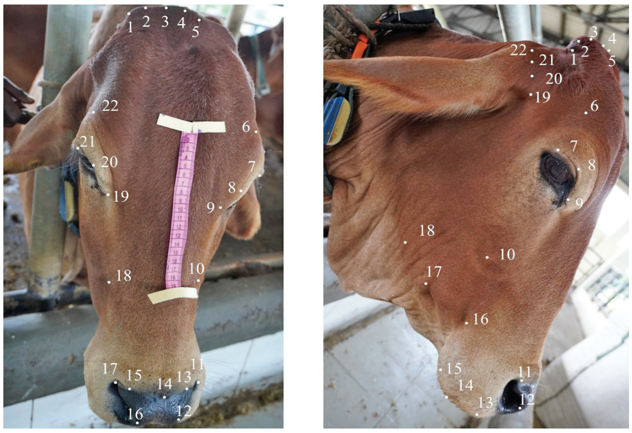
MATERIALS AND METHODS

Samples: The study was conducted on 51 adult live cattle, comprising 32 Sahiwal and 19 Holstein Friesian cross breeds. To minimize confounding variables, only female cattle were included, and all were maintained on a uniform feeding regimen to eliminate dietary effects. The head region was selected for morphometric assessment. A total of 102 high-resolution photographs were captured, with each animal photographed twice—once from the dorsal surface and once from the lateral surface.

The Sahiwal cattle were sourced from the Rashtriya Krishi Vikas Yojana (RKVY), Faculty of Veterinary and Animal Sciences, Institute of Agricultural Sciences, Rajiv Gandhi South Campus, Banaras Hindu University, Barkachha, Mirzapur, Uttar Pradesh, India. The Holstein Friesian crossbred cattle were obtained from a small private farm near Mirzapur, Uttar Pradesh, India. All experimental protocols and animal handling procedures were conducted in strict accordance with the institutional Animal Ethics guidelines. To reduce morphological variability associated with horns, the cornual region was excluded from analysis.

Image acquisition: Photographs were taken using a Sony ZV-E10L digital camera, maintaining a fixed distance of 60 cm from the subject to ensure isometric scaling. Animals were gently restrained to prevent head movement during image capture. All images were stored in JPEG format and transferred to a computer for digital processing.

Landmark selection and digitization: A total of 44 two-dimensional landmarks were designated, with 22 landmarks on the dorsal head surface and 22 on the lateral (right) head surface (Figure 1). Landmarks were chosen based on clearly identifiable cranial features, ensuring anatomical homology and repeatability.



(a) (b)

Fig. 1. Anatomical landmarks (1–22) positioned on the surface of the head to delineate its overall outline and capture shape variation for morphometric analysis. (a) Dorsal view of the head (b) Lateral view of the head.

Dorsal Surface Landmarks:

- Landmarks 1–5: Outline of frontal eminence (torus frontalis)
- Landmark 6: Left widest point of head near eye orbit and ear
- Landmarks 7–9: Upper outline of left eye orbit
- Landmark 10: Left facial tuberosity
- Landmarks 11–12: Upper and lower margins of left nostril
- Landmarks 13–15: Upper left, central, and right margins of muzzle
- Landmarks 16–17: Lower and upper right nostril points
- Landmark 18: Right facial tuberosity
- Landmarks 19–21: Upper outline of right eye orbit
- Landmark 22: Right widest point of head near eye orbit and ear

- Lateral Surface Landmarks (right side):
  - Landmarks 1–5: Outline of forehead
  - Landmark 6: Widest point of head near eye orbit and ear
  - Landmarks 7–9: Upper outline of eye orbit
  - Landmark 10: Union of malar and maxilla
  - Landmarks 11–12: Upper and lower margins of nostril
  - Landmarks 13–15: Outline of mouth
  - Landmarks 16–18: Outline of lower jaw
  - Landmarks 19–22: Upper outline of ear

Digital processing and morphometric analysis: Landmark digitization was performed using TPSDig2 (version 2.32), with landmarks manually placed in a consistent, predetermined sequence. Coordinate matrices were exported and converted into TPS file format using TPSUtil (version 1.83) (Rohlf, 2015). All configurations were subsequently imported into MorphoJ (version 1.08.02) for statistical analysis (Klingenberg, 2011) Prior to shape analysis, a Generalized Procrustes Superimposition was applied to eliminate non-shape variation through translation, scaling, and rotation. This normalization isolated pure shape variables by aligning all specimens into a common coordinate framework. GMM analyses were conducted to investigate patterns of shape variation and differentiation between breeds. Principal Component Analysis (PCA) was employed to identify the major axes of shape variation within the dataset. Discriminant Function Analysis (DFA) was used to assess the accuracy of breed classification based on shape variables. Canonical Variate Analysis (CVA) facilitated the visualization of inter-breed shape differences in multivariate space. Additionally, Procrustes Analysis of Variance (Procrustes ANOVA) was performed to statistically evaluate the significance of shape variation among groups. All statistical analyses and visualizations, including deformation grids, wireframe diagrams, and morphospace scatterplots, were carried out using the MorphoJ software environment.

RESULTS AND DISCUSSION

Principal Component Analysis (PCA): PCA of dorsal head shape showed that the first three principal components accounted for 63.79% of total shape variation, with PC1 alone explaining 38.05%. Shape variation along PC1 primarily reflected coordinated changes in orbital spacing, nostril aperture and width of frontal eminence whereas PC2 and PC3 captured variation in muzzle breadth, facial tuberosity, orbital outline and nasal region configuration. Although some overlap between breeds was observed, specimens tended to be distributed along PC1 and PC2 according to species, indicating structured breed-related dorsal shape variation. In the lateral head shape, PCA revealed a more evenly distributed variance structure, with the first three PCs accounting for 61.42% of total variation. Lateral PC1 primarily described variation in craniofacial profile, including forehead slope and facial elongation, while PC2 and PC3 reflected differences in orbital outline and mandibular contour (lower jaw) and outline of ear. Separation between breeds was more pronounced in lateral PCA morphospace, suggesting that lateral cranial landmarks captured stronger morphological contrasts

Table 1. Principal Component Analysis (PCA) of the first five principal components (PC1–PC5) in Sahiwal and Holstein Friesian (HF) crossbred cattle

Dorsal Surfaces				Lateral Surfaces			
PC	Eigenvalues	% Variance	Cumulative %	PC	Eigenvalues	% Variance	Cumulative %
1.	0.00214039	38.053	38.053	1.	0.00266061	26.270	26.270
2.	0.00096567	17.168	55.221	2.	0.00214579	21.187	47.456
3.	0.00048195	8.568	63.790	3.	0.00141437	13.965	61.421
4.	0.00041941	7.456	71.246	4.	0.00095765	9.455	70.876
5.	0.00032269	5.737	76.983	5.	0.00071793	7.088	77.965
Total variance: 0.00562474				Total variance: 0.01012809			

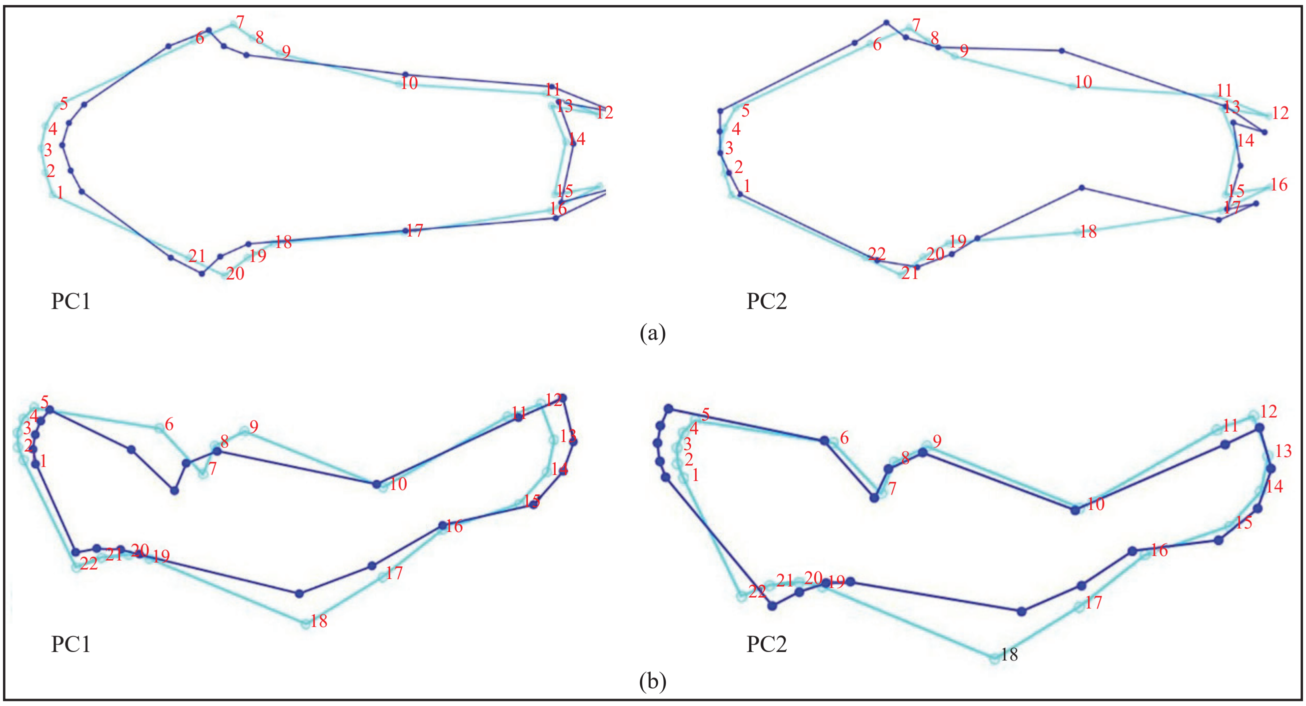
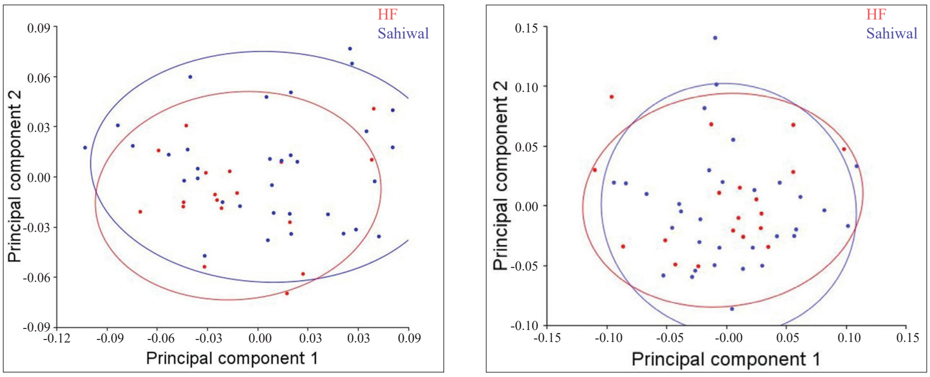


Fig. 2. Wireframe graphs derived from first two Principal Component Analysis (PCA), illustrating mean head shape variation of Sahiwal and Holstein Friesian (HF) cross breed cattle. (a) Mean head shape variation across dorsal surface. (b) Mean head shape variation across lateral surface. The green wireframe represents the mean head shape, while the blue lines indicate shape deviations along the principal components derived from PC1 and PC2.



(a) (b)

Fig. 3. Principal Component Analysis (PCA) scatterplot showing craniofacial shape variation in Sahiwal and Holstein Friesian (HF) cross breed cattle based on the first two principal components (PC1 and PC2). Coloured ellipses represent 95% confidence intervals for each group: HF (red), Sahiwal (blue). (a) Dorsal surface (b) Lateral surface

Table 2. ANOVA – Centroid Size analysis revealed no significant differences in centroid size between breeds. Procrustes ANOVA for shape analysis showing a significant species effect on the dorsal surface, with an even stronger species effect observed on the lateral surface.

	View	Effect	SS	MS	df	F	P (param.)	Pillai tr.	P (param.)
ANOVA Centroid Size	Dorsal	Individual	122617.381248	122617.381248	1	0.16	0.6889		
		Residual	37055764.249911	756240.086733	49	-	-		
	Lateral	Individual	885331.146722	885331.146722	1	0.99	0.3248		
		Residual	43850471.050291	894907.572455	49	-	-		
Procrustes ANOVA	Dorsal	Individual	0.02063603	0.0005159007	40	3.67	<.0001	0.95	0.0044
		Residual	0.27561249	0.0001406186	1960	-	-	-	-
	Lateral	Individual	0.09551823	0.0023879559	40	9.43	<.0001	0.96	0.0023
		Residual	0.49627645	0.0002532023	1960	-	-	-	-

associated with crossbreeding (Table 1, Figure 2, Figure 3).

Procrustes Analysis of Variance (Procrustes ANOVA): Procrustes ANOVA demonstrated no significant differences in centroid size between Sahiwal and Holstein Friesian crossbred cattle in either dorsal or lateral views, indicating that observed differences were independent of size and not driven by allometric effects. In contrast, shape variation differed significantly between breeds in both views. For the dorsal dataset, the species effect on shape was significant ($F = 3.67$, $P < 0.0001$; Pillai's trace = 0.95, $P = 0.0044$). The lateral dataset showed an even stronger species effect ($F = 9.43$, $P < 0.0001$; Pillai's trace = 0.96, $P = 0.0023$). These results provided a robust statistical confirmation that breed-specific cranial shape differences existed, with lateral head morphology exhibiting greater discriminatory power than dorsal morphology (Table 2).

Canonical Variate Analysis (CVA):CVA further clarified inter-breed separation by maximizing between-group variance relative to within-group variation. In both dorsal and lateral datasets, a single canonical variate (CV1) accounted for 100% of the between-group variation, reflecting the two-group design. For the dorsal view, CVA revealed significant separation between breeds (Goodall's $F = 3.67$, $P = 0.0052$; Pillai's trace = 0.9536, $P = 0.0051$). The Mahalanobis distance (9.30) and Procrustes distance (0.0421) indicated consistent but moderate dorsal shape divergence. In the lateral view, inter-breed separation was stronger, as evidenced by higher test statistics (Goodall's $F = 9.43$, $P < 0.0001$; Pillai's trace = 0.9598, $P = 0.0021$), a larger Mahalanobis distance (9.91), and a greater Procrustes distance (0.0895). Visualization of CV1 deformation patterns indicated pronounced differences in craniofacial profile, forehead inclination, muzzle projection, and mandibular configuration between Sahiwal and Holstein Friesian crossbred cattle (Table 3, Figure 4).

Discriminant Function Analysis (DFA): DFA confirmed

Table 3. Results of Canonical Variate Analysis (CVA) showing inter-breed shape differentiation

View	Eigenvalues (CV1)	Goodall's F	P-value	Pillai's Trace	P-value	Mahalanobis Distance	Procrustes Distance
Dorsal	20.55	3.67	0.0052	0.9536	0.0051	9.30	0.0421
Lateral	23.91	9.43	<0.0001	0.9598	0.0021	9.91	0.0895

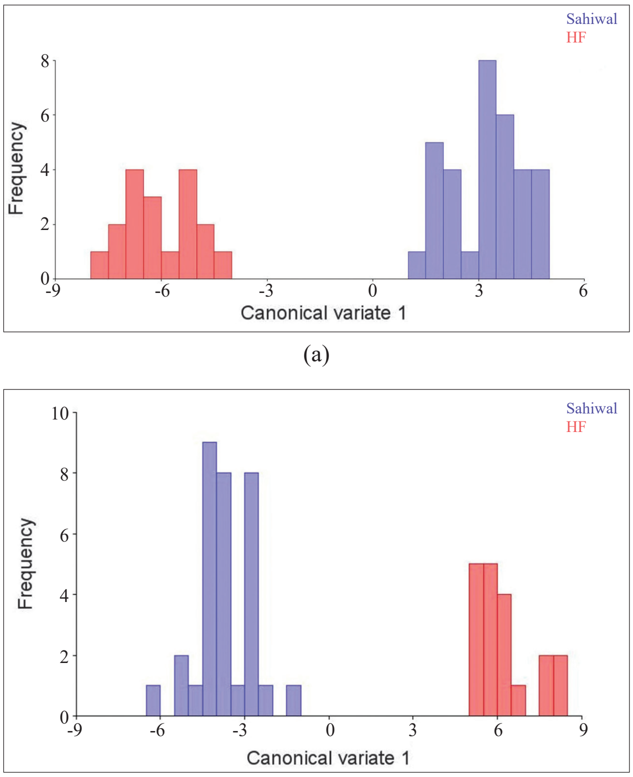


Fig. 4. The histogram shows the distribution of Canonical Variate Analysis (CVA) scores illustrating cranial shape differentiation between Sahiwal and Holstein Friesian crossbreed (HF) cattle based on dorsal (a) and lateral (b) views.

the strong discriminatory capacity of cranial shape variables. In both dorsal and lateral views, For the lateral head view, cross-validated accuracy was 72.5%, with higher correct classification for Sahiwal cattle (81.3%) than for HF crossbreds (57.9%). For the dorsal view, cross-validated accuracy was 70.6%, again with higher accuracy for Sahiwal (75.8%) than HF crossbreds (61.1%). The relatively higher misclassification rate among HF crossbreds likely reflects greater morphological heterogeneity arising from their mixed taurine–zebu genetic background. Permutation tests associated with DFA were highly significant for both views, confirming that the observed discrimination was not due to chance (all $P \leq 0.005$) (Figure 5).

The present study provided robust multivariate evidence that cranial shape differed significantly between Sahiwal (*Bos indicus*) and Holstein Friesian crossbred cattle, despite the absence of detectable differences in centroid size. By integrating PCA, CVA, DFA, and Procrustes ANOVA across dorsal and lateral head views, the results consistently demonstrated that shape not size is the primary axis of phenotypic divergence, with lateral craniofacial morphology emerging as the most informative perspective. This finding was consistent with previous morphometric studies in skull of Holstein and Simmental breed of cattle, where breed-related variation was often more strongly expressed in cranial shape than in overall size (Gündemir *et al.* 2025). These findings have important implications for understanding heat adaptation, domestication history, and functional craniofacial anatomy in taurine zebu hybrid cattle reared under tropical conditions.

Indigenous zebu cattle such as Sahiwal are well recognized for their superior adaptation to hot and humid environments. Heat tolerance in *Bos indicus* cattle is multifactorial, involving physiological, integumentary, and morphological traits. Craniofacial morphology constitutes an underexplored but functionally relevant component of this adaptive complex. The pronounced lateral head shape differences observed in this study particularly involving forehead inclination, facial profile, muzzle projection, and mandibular configuration likely reflect adaptive traits associated with thermoregulation and respiratory efficiency (Hansen 2004); (Aggarwal and Upadhyay 2013).

A more sloping forehead and elongated facial profile, characteristic of zebu cattle, may facilitate enhanced airflow through the nasal passages, thereby improving evaporative cooling during respiration. Additionally, differences in muzzle length and nasal region geometry may influence nasal surface area and heat dissipation capacity. The stronger discriminatory signal detected in the lateral view supports the notion that profile-related craniofacial traits are closely linked to environmental adaptation, whereas dorsal traits, such as frontal width and orbital spacing, capture subtler aspects of morphological divergence. The broader muzzles, expanded nasal apertures, and wider orbital regions observed in Sahiwal cattle may reflect functional and evolutionary adaptations related to their zebu lineage, which is historically adapted to tropical climates and harsher environments (Rege and Tawah 1999). Conversely, the comparatively narrower and more compact craniofacial profile of Holstein Friesian crossbred cattle reflected the taurine lineage, which has undergone intensive selection for dairy productivity rather than adaptation to heat or feed constraints (Miglior *et al.* 2017).

Holstein Friesian crossbred cattle displayed intermediate cranial shapes, with greater within-group variability and higher misclassification rates under cross-validation, particularly in DFA. This morphological heterogeneity, perhaps, reflects segregation of taurine and zebu alleles affecting craniofacial development, as well as ongoing selection pressures imposed by tropical environments. The persistence of zebu-like cranial features in crossbreds suggested that adaptive morphology may be preferentially retained under thermal stress, even when crossed with high-producing taurine breeds.

The observed cranial shape divergence must also be interpreted in the context of cattle domestication and evolutionary history. *Bos indicus* and *Bos taurus* cattle originated from distinct domestication events and subsequently evolved under markedly different ecological and management regimes. Zebu cattle were shaped by prolonged exposure to tropical climates, natural selection, and extensive pastoral systems, whereas taurine cattle evolved largely under temperate conditions with increasing human-mediated

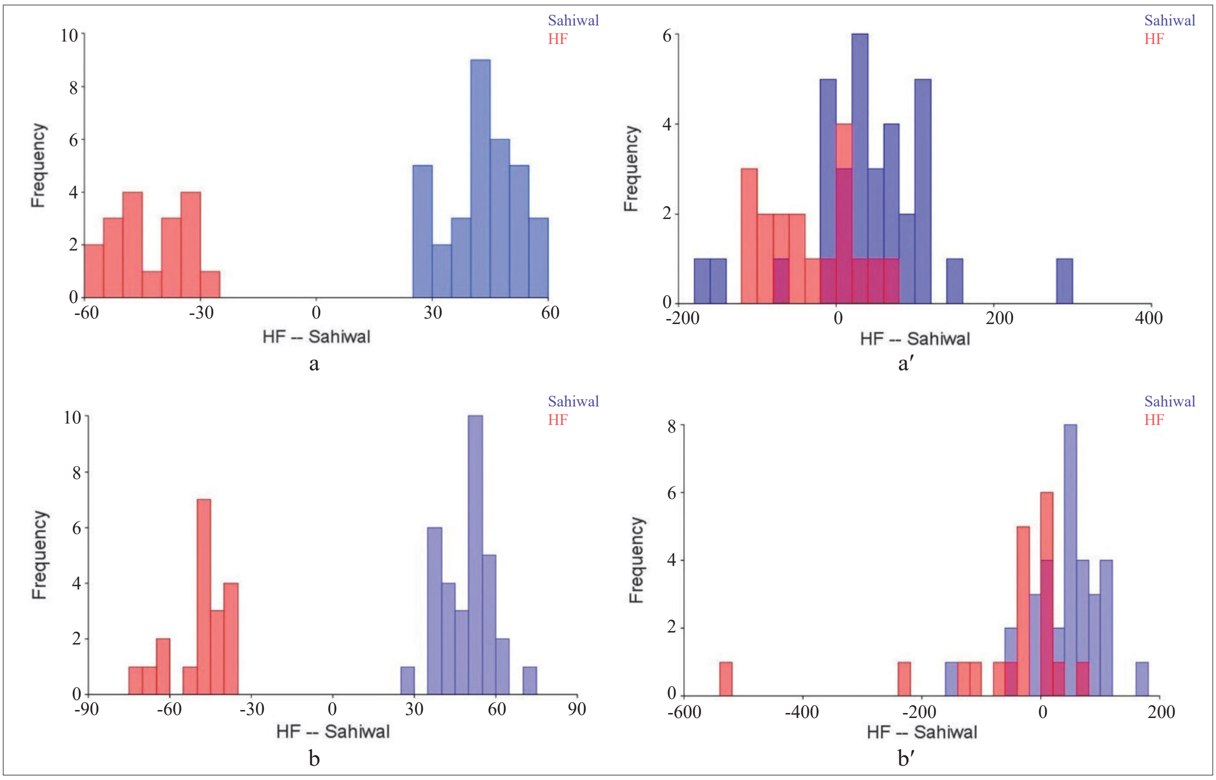


Fig. 5. Histogram showing the distribution of discriminant function analysis (DFA) scores for HF crossbreed (red) and Sahiwal (blue). Discriminant scores (a) and cross-validation scores (a′) are shown for the dorsal surface, while discriminant scores (b) and cross-validation scores (b′) are shown for the lateral surface.

selection for productivity (Felius *et al.* 2014).

Cranial morphology is a conservative yet informative phenotypic trait that integrates genetic background and developmental constraints. The significant Mahalanobis distances and Procrustes distances observed in CVA and DFA indicated that breed-specific cranial shape signatures remain distinct, even after decades of crossbreeding. Importantly, the relatively small absolute Procrustes distances, particularly in the dorsal view, suggest that crossbreeding has not produced extreme morphological divergence, but rather subtle, coordinated shape shifts that are nonetheless statistically consistent. The greater separation observed in lateral cranial morphology implies that domestication-related differences in facial profile and jaw architecture are among the most persistent markers of taurine versus zebu ancestry. These traits may be under stronger genetic control or subject to functional constraints that limit complete homogenization through crossbreeding. Similar findings have been reported in studies of caprine (Sarma 2006) and bovine morphometrics (Gambo *et al.* 2019); (Özkan *et al.* 2019), where lateral perspectives often capture more functionally relevant and breed-specific traits. As such, cranial shape provides a valuable phenotypic window into the legacy of domestication history within contemporary crossbred populations.

From a functional anatomical perspective, the head integrates multiple systems, including feeding, respiration, sensory perception, and social signaling. Differences in jaw depth, muzzle outline, and facial curvature observed along major axes of variation likely influence bite mechanics, grazing behavior, and forage handling—traits that differ between indigenous and exotic cattle breeds adapted to distinct feeding ecologies.

The high apparent classification accuracy achieved in DFA, coupled with moderate cross-validated accuracy, further suggests that cranial shape contains strong discriminatory information, while also reflecting biological reality—namely, greater morphological variability in crossbred cattle. This balance between discrimination and overlap aligns with expectations for hybrid populations and highlights the utility of cranial morphometrics for breed characterization without oversimplifying complex genetic backgrounds (Boettcher *et al.* 2015). This may be attributable to individual variation, management-related effects (Shamsuddin, 2011), or potential introgression between zebuine and taurine lineages in crossbreeding contexts (Siddiqui *et al.* 2024).

In conclusion, the present study established cranial geometric morphometrics as a powerful and biologically informative approach for evaluating morphological consequences of taurine–zebu crossbreeding. By linking head shape variation to adaptation, domestication, and function, these findings contribute to a deeper understanding of phenotypic diversity in Indian cattle and support the use of shape-based traits in sustainable breeding and conservation strategies.

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