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#### Morphometric Analysis of the Skull in the Holstein Cow: A Computed Tomography Study

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#### ABSTRACT

Most craniometric studies have been conducted on dry skulls. This study aims to identify the craniometric characteristics of the skull in Holstein cows using Computed Tomography (CT) imaging. Fourteen Holstein cow heads were utilized, scanned via CT, and images were processed with the DICOM Viewer software program. Seventeen craniometric measurements (13 extracranial, 4 intracranial) were obtained through the program's multiplanar reconstruction tool, and 14 indexes were calculated based on these morphometric data. In Holstein cow, total length was 519.4 ±21.7 mm, basal length was 472.1 ±22.2 mm, viscerocranium length was 288.4 ±17.4 mm. Further, the greatest frontal breadth was 225.4±8.5 mm, while the length of the cranial cavity1, length of the cranial cavity2, maximum width, and maximum height of the cranial cavity were 140.5 ±6.4, 116.8 ±4.3, 103.3 ±4.4 and 96.6 ±4.7 mm, respectively. Skull index was 43.4±1.3, facial index was 78.3±3.7, basal index was 47.8±1.8, foramen magnum index was 83.1  $\pm$ 3.2, cranial cavity index1 was 73.6  $\pm$ 4.6, and length-width index1 was found to be 136.3  $\pm$ 8.1. This study provides initial reference data on the morphometric properties of the Holstein cow skull, derived through a reproducible measurement protocol. These findings offer valuable insights for veterinary anatomists, radiologists, clinicians, and researchers in terms of both the data and methodology presented. Craniometric data may assist in diagnosing head region pathologies, pre-surgical planning (such as trepanation, dehorning, and facial surgery), and in applications of regional anesthesia. Additionally, these findings have potential future applications in assessing skull morphology changes related to breed and gender, and in correlating skull dimensions with meat and milk production data.

Keywords; Bovine, Cephalometry, Craniology

#### Holstein Sığırında Kafatasının Morfometrik Analizi: Bilgisayarlı Tomografi Çalışması

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#### ÖΖ

Craniometrik çalışmaların büyük çoğunluğu kuru kafatası üzerinde gerçekleştirilmiştir. Bu çalışmanın amacı, Holstein sığırında Bilgisayarlı tomografi (BT) görüntüler üzerinde kafatasının craniometrik özelliklerini belirlemektir. Bu çalışmada toplam 14 adet dişi Holstein sığır başı kullanıldı. Başlar BT ile tarandı ve görüntüler DICOM Viewer yazılım programına aktarıldı. Programın multiplanar reconstruction aracı kullanılarak toplam 17 kraniyometrik (13 ekstracranial-4 intracranial) ölçüm gerçekleştirildi ve bu morfometrik veriler kullanılarak 14 adet index hesaplandı. Holstein sığırında total uzunluk 519.4±21.7, basal uzunluk 472.1±22.2, viscerocranium uzunluğu 288.4±17.4, frontal genişlik 225.4±8.5 mm iken, cavum cranii uzunluğu1, cavum cranii uzunluğu2, cavum cranii'nin maksimum genişliği ve yüksekliği sırasıyla 140.5±6.4, 116.8±4.3, 103.3±4.4 ve 96.6±4.7mm idi. Skull index 43.4±1.3, facial index 78.3±3.7, basal index 47.8±1.8, foramen magnum index 83.1±3.2, cavum cranii index1 73.6±4.6 ve uzunluk-genişlik index1 136.3±8,1 olarak belirlendi. Holstein sığırında tekrarlanabilir bir ölçüm protokolü ile kafatasının morfometrik özelliklerine ait ilk referans niteliğinde veriler elde edildi. Araştırma sonuçları hem sunulan veriler yönüyle hem de metodoloji vönüvle veteriner anatomistler, radvologlar, klinisvenler ve diğer arastırmacılara favda sağlavabilir. Kraniometrik bilgi, baş bölgesinde şekillenebilecek patolojilerin tanısında, cerrahi öncesi planlamada (trepanasyon, boynuz kesimi ve yüz bölgesi cerrahisi vb.), bölgesel anestezi uygulamalarında katkı saylayabilir. Ayrıca bulgular gelecekte ırk ve cinsivete bağlı kafatası morfolojisinin gelişimsel değerlendirilmesinde, etçi ve sütçü ırklarda kafatası boyutları arasındaki ilişkinin tanımlanmasında kullanılabilir.

Anahtar kelimeler: Sığır, Kraniyoloji, Sefalometri

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#### **INTRODUCTION**

The morphological and morphometric characteristics of the skull reflect the influence of evolutionary modifications, as well as genetic and environmental factors on individuals (Getty 1975; Hanken 1993; Zelditch et al. 2004). Over about 250 years of craniometric studies, researchers have investigated the origins of domestic animals, explored intra- and intersimilarities and differences, species identified developmental anomalies and variations, and classified animals based on skull size and shape (Wilckens 1876; Grigson 1974; Bartosiewicz 1980a; Evans and Christensen 1979). Craniometry has been applied to determine the typology of skulls found in archaeological contexts (Onar et al. 2012), in the development of stereotactic devices for central nervous system examination (Saito et al. 2004), and in the field of veterinary forensic science (Toledo González et al. 2020). Additionally, endocranial volume estimations have been made for certain individuals within the orders Carnivora (Finarelli 2006) and Artiodactyla (Finarelli 2011; Balcarcel et al. 2021) using models based on specific external skull measurements.

Morphometric measurements were carried out on the dry skull in European bison (Krasıñska et al. 2008; Szara et al. 2023), water buffalo (Özkan et al. 2019), wild cattle (Grigson 1978; Brudnıckı et al. 2012; Balcarcel et al. 2021), domestic cattle (Grigson 1974; Parés Casanova and Jordana i Vidal 2008; Özkan et al. 2019), Simmental and Holstein Cattle (Çakar et al. 2024), native Asian cattle (Hayashi et al. 1981; Hayashi et al. 1988), Korean and Indonesian cattle (Nishida et al. 1983), Zebu cattle (Grigson 1980; Bökönyi 1997), Hungarian grey (Bartosiewicz 2006; Kőrösi 2013), Niata cattle (Veitschegger et al. 2018), Kuri cattle (Gambo et al 2015; Gambo et al. 2019), hybrids cattle (Krasinska 1988) and on the head region in some live cattle (Cabezas Congo et al. 2019; Lomillos and Alonso 2020; Neves et al. 2021). As a result of the studies, craniometric characteristics were identified in various animal species, revealing dimensional variations between breeds and sexes. However, most craniometric research in cattle to date has utilized dry skull samples.

The skull presents a complex anatomical structure that houses the brain, sensory organs, and components of the respiratory and digestive systems. CT imaging is highly effective for assessing intricate structures like the head, diagnosing bone tissue pathologies, and evaluating conditions related to paranasal sinus diseases and skull trauma (Stieger-Vanegas and Hanna 2022; Turgut et al. 2023). Recently, veterinary-specific CT devices capable of visualizing multiple body regions in large animals such as equidae and ruminants in a standing position have been developed. These devices enable efficient morphometric analyses and clinical evaluations on the acquired images (Stewart et al. 2021; Brounts et al. 2022). However, the accurate evaluation of these images also requires comprehensive radiological and morphometric data specific to the region of interest in various animal species.

The Holstein cattle breed is globally prevalent and holds substantial economic value due to its milk and meat yield capabilities, along with its high adaptability to various environments. Males of this breed are typically slaughtered at around 14-16 weeks or 12-15 months of age to meet meat production demands, as they exhibit rapid growth, while some are reared as breeding bulls. Female Holsteins, which are slower to mature, are raised until about 7-9 years due to their significant milk yield, thus constituting the majority of the Holstein population (ESK 2024, FOA 2024). Skull structure in cattle varies based on breed and gender, with meat breeds like Holsteins exhibiting shorter, wider skulls and dairy breeds characterized by longer, narrower skulls (Sasimowski 1987). Skull shape in cattle is influenced by factors such as brain development, sinus formation, and cornual process development (Barone 1999; Nickel et al. 1986) and continues to evolve with age (Bartosiewicz 1980a, 1980b; Kőrösi 2013; Neves et al. 2021). Skull shape serves as a key criterion for breed classification, with skull indices providing important data for defining morphological types. Thus, morphometric data on the head and other body regions in Holstein cows remain relevant. While general morphological characteristics of cattle skulls have been extensively documented, morphometric data specific to Holstein cows are limited, with no available intracranial measurements. This study aims to define the craniometric features of the Holstein cow skull using CT imaging. The resulting data may support determinations of skull morphology related to breed and gender, aid in intra- and inter-species craniometric datasets, assist in diagnosing bonerelated pathologies, inform surgical planning for trepanation and dehorning, enhance regional anesthesia applications, and support veterinary forensic sciences in the analysis of animal-related evidence in criminal cases involving animals.

#### MATERIAL and METHODS

#### Animals

The study utilized 14 healthy Holstein cow heads with blunted horns, averaging an age of  $5.4 \pm 1.7$  years (range 1.5–8.0), obtained from a slaughterhouse in Konya, Turkey. Following clinical assessments conducted by the slaughterhouse veterinarian, the heads of the slaughtered animals were selected randomly. The ages of the animals were verified by inspecting their ear tags as well as assessing the condition and wear of their permanent incisors and canines (Schummer et al. 1979; Barone 1997). All procedures were conducted under the approval of the Ethics Committee of Selçuk University Faculty of Veterinary Medicine, Experimental Animal Production and Research Center, under decision number 2024/077.

#### **CT** Scans

The heads were scanned using an MSCT device (Siemens Dual Source, Somatom Definition Flash, Germany) positioned perpendicular to the hard palate, with settings of 140 kV, 475–500 mAs, a  $512 \times 512$  matrix, and a slice thickness of 0.6 mm. Scanning occurred within 24 h post-slaughter to minimize postmortem alterations, with the heads stored in cold conditions between slaughter and scanning. Axial reformat data sets of 1 mm thickness for the 14 animals that met the study's criteria (free from head and bone-related diseases, asymmetric development, and anomalies) were archived in Digital Imaging and Communication in Medicine (DICOM) format.

# Extracranial and Intracranial Linear Measurements

The RadiAnt DICOM Viewer (Medixant, Poland) software was utilized to acquire morphometric parameters. DICOM data sets for each animal were transferred into the software, and linear measurements were conducted on the images using the Multiplanar Reconstruction (MPR) tool. Each linear measurement was repeated three times at the bone window setting (window level 300 HU and window width 2800 HU), and the arithmetic mean of the results was calculated. Measurement values were recorded in millimeters (mm). Anatomical structures were named following classical anatomy references (Sisson 1975; Nickel et al. 1986) and the Nomina Anatomica Veterinaria (NAV 2017). All craniometric measurements were visualized using Adobe Photoshop CC 2015.5 (Version: 25.5.0, Adobe Systems, San Jose, CA, USA).

To characterize the general head morphometry of the animals in this study, 14 indices were derived using data from 17 linear measurements, including 13 extracranial and 4 intracranial parameters. In extracranial measurements, bone reference points were established according to Von den Driesch (1976) (Figure 1). Reference points for intracranial measurements-comprising height, width, and two lengths-were defined by the author. For this purpose, the sagittal plane was aligned to pass through the midmedian plane, the dorsal plane through the nasion, and the transverse plane through the base of the hypophyseal fossa. Six points where these planes intersected the cranial cavity served as bone reference points for three intracranial measurements (height, width, and length 2). In the measurement of length 1 (LCC1), the basion and the intersection of the dorsal plane with the ethmoidal crest (crista galli) on the sagittal image were used as reference points (Figure 2). A total of 31 parameters, comprising both extra- and intracranial measurements along with calculated indices, are presented in Table 1 and Figures 1 and 2.



Figure 1. The extracranial measurements on CT image in Holstein cow. a) Sagittal CT section image. b-d) Dorsal CT section images. c) Transverse CT section image. A, Akrokranion; B, Basion; Ect, Ectorbitale; N, Nasion; O, Opisthion; Ot, Otion; P, Prosthion. See to section material and methods for details of abbreviations used in measurements.



Figure 2: The intracranial measurements on CT image in Holstein cow. a) Sagittal CT section image. b) Dorsal CT section images. c) Transverse CT section image. A, Akrokranion; B, Basion; N, Nasion; O, Opisthion. See to section material and methods for details of abbreviations used in measurements.

Parameters	Definition of measurements and indices	Description of measurements and indices	Figure
Extracranial measurements (mm)			
TL	Total length	Acrocranion-prosthion	Figure 1/a
CL	Condylobasal length	Aboral border of the occipital condyles-prosthion	Figure 1/b
BL	Basal length	Basion-prosthion	Figure 1/a
NL	Neurocranium length	Basion-nasion	Figure 1/a
MFL	Median frontal length	Acrocranion-nasion	Figure 1/a
GFB	Greatest frontal breadth	Ectorbitale-ectorbitale	Figure 1/d
VL	Viscerocranium length	Nasion-prosthion	Figure 1/a
GHOR	Greatest height of the occipital region	Basion-highest point of the intercornual ridge in the median plane	Figure 1/a
LHOR	Least height of the occipital region	Opisthion-highest point of the intercornual ridge in the median plane	Figure 1/a
GBOC	Greatest breadth of the occipital condyles		Figure 1/b,d
GMB	Greatest mastoid breadth	Otion-otion	Figure 1/d
GBFM	Greatest breadth of the foramen magnum		Figure 1/c
HFM	Height of the foramen magnum	Basion-opisthion	Figure 1/c
Intracranial measurements (mm)			
LCC1	Length 1: Lenght of the cranial cavity1	The most rostral point of ethmoidal crest to basion	Figure 2/a
LCC2	Length 2: Lenght of the cranial cavity2	The most rostral point of ethmoidal crest to the vermiform impression	Figure 2/a,b
MHCC	Maximum height of the cranial cavity	Hypophyseal fossa to internal lamina of frontal bone	Figure 2/a,c
MWCC	Maximum width of the cranial cavity	Greatest width distance between internal wall of parietal bones	Figure 2/b,c
Indices			
51		GFB/1L x 100	
FAI	Facial index	GFB/VLX100	
FKI DI	Prontal index	GFD/MFL x 100	
DI		GFD/DLX100	
	Cranicl activity index 1	NWCC / LCC1 = 100	
CCI2	Granial cavity index 1	NWCC / LCC2 × 100	
CCI2	Granial cavity index 2	MUCC / LCC2 x 100	
CCIA	Cranial cavity index 5	MHCC / LCC1 x 100	
	Granial cavity index 4	MHCC / LCC2 X 100	
	Length length index 1	MFL/VL*100	
	Length length index 2	LCC1 / 1L **100	
	Length length index 3	LCC2 / 1L*100	
	Length-width index 1		
LW12	Length-width index 2	LUU2/MWUU*100	

#### **Statistical Analysis**

The SPSS software (version 29.0, Armonk, NY: IBM Corp. USA) was employed for statistical analysis. Descriptive statistics, including mean, standard deviation, minimum, and maximum values, were provided for both categorical and continuous variables. Pearson's correlation coefficient was used to assess relationships between two continuous variables, with p-values of p<0.05 and p<0.01 considered statistically significant. To evaluate the reliability of the three repeated measurements, 95% confidence intervals were calculated using the intraclass correlation coefficient (ICC), where an ICC above 0.75 is considered ideal. This coefficient is

acknowledged to range between 0 and +1 (Lee et al. 1989; McGraw and Wong 1996).

#### RESULTS

The statistical analysis in this study revealed a significant agreement among the three measurement sets (p<0.05). The 13 extracranial measurements, which were conducted for the first time on CT images of the bovine head, demonstrated high reproducibility (ICC: 0.879-0.996, mean: 0.965). Additionally, four intracranial measurements (LCC1, LCC2, MWCC, MHCC), defined by the author with novel reference points, also displayed strong reproducibility on bovine CT images (ICC: 0.887-0.949, mean: 0.920). Intracranial measurements, specifically, were effectively obtained from sagittal (height and two lengths), transverse (height and width), and dorsal (width and length) images at consistent levels on the MPR screen (Figure 2). These measurements clearly identified the cranial cavity's highest, widest, and longest points.

The study presents the results of 13 extracranial and 4 intracranial measurements conducted on multiplanar CT images of 14 Holstein cows, as shown in Table 2, along with the 14 calculated indices detailed in Table 3. Table 4 provides the relationships among the craniometric measurements and their associations with age, while Table 5 outlines the interrelationships among the indices. A cranial length of  $519.4 \pm 21.7$  mm and a maximum cranial width of  $225.4 \pm 8.5$  mm were observed in Holstein cows (Table 2).

A positive correlation was observed among the craniometric measurements exclusively between age and the VL value (p: 0.03, r: 0.582). Positive correlations were identified in the extracranial measurements, while intracranial and extracranial measurements exhibited correlations specifically between LCC1 and BL (p: 0.03, r: 0.574), LCC2 and GHOR (p: 0.01, r: 0.648) and LHOR (p: 0.01, r: 0.661), as well as MHCC and MFL (p: 0.003, r: 0.729) (Table 4). Analysis of the relationships among the indices revealed both positive and negative correlations, except for FMI. Notably, LWI1 showed a negative correlation with all indices except LWI2 (p: 0.007, r: 0.687) (Table 5).

Table 2. The morphometric data related to the craniometric measuraments
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Authors. Year	Specimen	Age	N-Sex	TL	CL	BL	NL
Krasınska et al., 2008*	Bison (European bison)	5-27 y 5-22 y	152-F 154-M			447.0±12.6 471.8±14.3	
Özkan et al.,2019*	Water buffalo (B. Bubalis L)	3-7 y	15 F	472.0±45.8(410.7-528.4)	482.3±45.9(420.8- 541.8)	450.1±45.0(389.7-505.2)	
Grigson 1974*	Cattle (Bos taurus L.)	2 y ≤	18-F 17-M	-		414.8±31.8 429.4±30.1	
Grigson 1978*	Cattle (Bos primigenius)	2 y ≤	24-F 60-M	_		511.6±18.1 564.8±22.9	
Grigson 1980*	Cattle (Bos indicus L.)	2 y ≤	23-26 F,M	-		407.7±62.9	
Bartosiewicz 1980*	Cattle (Red Pied- Fleckvieh)	0-2 m 1-7.7 y	21-F,M	263.4±27.3 484.9±27.2		236.7±27.1 437.8±28.1	132.3±18.1 215.5±10.0
Hayashi et al.,1981*	Cattle (Sumatra)	4 y ≤ 1-3 y	9-F 1-M	417.4±15.8		381.8±10.6	
Nıshıda et al.,1983*	Cattle (Korean)	4 y ≤ 2-3 y	8-F 8-M	474.2±9.8 484.9±26.2		423.6±15.0 433.5±19.9	
	Cattle (Banteng)		10-F 3-M	441.1±9.9 501.0±7.8		415.3±11.2 449.7±10.0	
	Cattle (Bali)	-	10 F 8 M	391.3±13.7 424.9±19.6		383.4±11.5 405.1±21.8	
Hayashi et al.,1988*	Cattle (Madura)	4 y ≤	8-F	411.3±17.8		381.3±19.6	
	Cattle (Aceh)	-	9-F	417.4±15.8		381.8±10.6	
	Cattle (Leyte)	_	6-F	405.7±21.4		365.0±22.2	
	Cattle (Korean)		8-F	474.3±9.8		423.6±15.0	
Bartosiewicz 2006*	Cattle (Hungarian grey)	2-16 y	30-F 15-M	497.2±16.2 538.7±28.0		447.2±13.6 481.8-23.7	
Parés Casanova & Jordana i Vidal 2008*	Cattle (Bos taurus L.)	2.5 y <	502-F 76-M	533.0±37.1 579.1±49.2			
Körösi 2013*	Cattle (Hungarian grey)	2-16 y	46- F(cow) 25-M(ox) 5-M(bull)	495.1 (454.1-555.9) 542.8 (462.1-579.5) 516.9 (462.2-541.0)	477.7 (446.5-524.5) 516.7 (451.5-547.5) 495.0 (455.8-515.2)	447.2 (415-490.0) 486.5 (459.6-518.8) 466.6 (421.0-483.5)	243.1 (218.6-270.0) 260.0 (240.0-280.0) 257.5 (240.0-270.0)
Cabezas Congo et al., 2019**	Cattle (Criollo)	Adult	198-F 19-M	456.2±29.2 446.3±11.92			
Gambo et al.,2019*	Cattle (Kuri cattle)	9 m-10 y	15-F 15-M	498.7±47.5 503.8±67.5	466.2±32.6 470.7±41.6	468.3±32.3 478.7±46.1	
Özkan et al.,2019*	Cattle (Bos taurus L.)	3-7 y	20-F	592.5±15.9(499.7-558.1)	519.6±15.9(485.7- 543.4)	486.2±15.8(455.0-512.9)	
Lomillos and Alonso 2020**	Cattle (Lidia)	4-6 y	80-F 184-M	471.0±31.0 491.0±34.0			
Neves et al., 2021**	Cattle (Jersey)	1-11 m 16-24 m 25-58 m	18-F 17-F 13-F	308.3±54.8 432.9±17.6 441.5±13.4			
Çakar et al., 2024***	Cattle (Holstein) Cattle (Simmental)	12-14 m	25M 29M	490.6±28.1 485.9±24.3	475.6±25.1 467.4±19.3	445.2±24.4 435.1±20.3	
The present study****	Cattle (Holstein cow)	1.5-8 y	14-F	519.4±21.7(467.9-544.0)	506.9±21.5(453.8-	472.1±22.2(417.8-505.1)	235.3±8.5(219.9-

 $\frac{1}{541.0} = \frac{1}{541.0} + \frac{1}{222.2} +$ 

Table 2-Continuation. The morphometric data related to the craniometric measuraments

Authors. Year	Specimen	Age	N-Sex	GHOR	LHOR	GBOC	GMB	GBFM	HFM
Özkan et al.,2019*	Water buffalo (B. Bubalis L)	3-7 y	15 F	177.5±11.9 (155.0-191.7)	168.4±12.5 (140.6-184.5)	98.4±5.7 (89.2-107.8)	199.2±22.4 (167.7-226.8)	40.8±5.4 (29.5-56.2)	34.5±3 (29.0-4
Grigson 1974*	Cattle (Bos taurus L.)	2 y ≤	18-F 17-M	144.4±10.0 157.7±12.0		97.2±9.0 108.3±7.8			
Grigson 1978*	Cattle (Bos primigenius)	2 y ≤	24-F 60-M	190.7±13.3 222.5±12.0		116.8±6.0 137.0±6.3			
Grigson 1980*	Cattle (Bos indicus L.)	2 y ≤	23-26 F, М	118.0±17.4	114.4±26.3	87.5±15.0			
Hayashi et al.,1981*	Cattle (Sumatra)	4 y ≤ 1-3 y	9-F 1-M	116.9±10.4					
Nıshıda et al.,1983*	Cattle (Korean)	4 y ≤ 2-3 y	8-F 8-M	145.5±5.4 153.0±10.8					
	Cattle (Banteng)		10-F 3-M	$148.9\pm6.4$ 165.0±4.4					
	Cattle (Bali)	-	10 F 8 M	$140.2\pm5.2$ $164.0\pm14.7$					
Hayashi et al.,1988*	Cattle (Madura)	4 y ≤	8-F	138.5±6.0					
	Cattle (Aceh)	-	9-F	116.9±10.4					
	Cattle (Leyte)	_	6-F	133.8±5.7					
	Cattle (Korean)		8-F	145.5±5.4					
Bartosiewicz 2006*	Cattle (Hungarian grey)	2-16 y	30-F 15-М	-	117.1±6.2 125.1±6.2		224.7±10.5 247.2±16.1		
Körösı 2013*	Cattle (Hungarian grey)	2-16 y	46- F(cow) 25- M(ox) 5- M(bull)	154.8 (114.0- 171.2) 168.4 (149.3- 200.5) 170.9 (146.5- 188.6)	120.5 (104.7- 156.7) 127.5 (115.8- 159.7) 129.4 (114.3- 151.2)	108.1 (92.4- 129.0) 125.6 (110.3- 198.6) 122.6 (117.9- 129.6)	223.7 (202.3- 258.6) 251.8 (218.8- 275.0) 271.8 (227.3- 288.6)	40.0 (30.7- 51.6) 42.0 (27.7- 64.3) 32.8 (26.5- 35.6)	38.0 (3 43.2) 47.1 (3 45.7) 34.8 (3 36.3)
Gambo et al.,2019*	Cattle (Kuri cattle)	9 m-10 v	15-F 15-M	_ ^	101.3±6.7 104.3±12.3	99.9±8.4 108.7±8.5		38.5±3.8 36.3±3.4	39.7±3 39.5±3
Özkan et al.,2019*	Cattle (Bos taurus L.)	3-7 y	20-F	170.3±7.5 (156.6-184.7)	131.3±7.3 (114.7-144.9)	113.3±5.7 (103.2-129.9)	231.5±11.3 (205.4-245.5)	42.7±3.0 (36.6-50.3)	38.9±2 (34.6-4
Çakar et al., 2024***	Cattle (Holstein) Cattle (Simmental)	12-14 m	25M 29M	158.6±9.3 157.6±12.5	122.2±9.6 122.1±10.8	109.7±7.3 112.8±7.5	210.9±16.9 212.2±15.3	40.1±4.5 40.4±5.8	39.4±3 38.9±4
The present study****	Cattle (Holstein cow)	1.5-8 y	14-F	162.7±8.2 (146.8-173.6)	124.3±7.6 (112.2-134.6)	113.8±6.7 (105.0-125.0)	224.4±10.3 (199.4-236.8)	47.3±2.4 (45.1-52.4)	39.3±2 (35.5-4

**Abbreviations:** GHOR, Greatest height of the occipital region; LHOR, Least height of the occipital region; GBOC, Greatest breadth of the occipital condyles; breadth of the foramen magnum; HFM, Height of the foramen magnum; LCC1, Lenght of the cranial cavity1; LCC2, Lenght of the cranial cavity2; MWCC, Max height of the cranial cavity; F, Female; M, Male; y, years; m,months; \* Skull study; \*\* Head study; \*\*\* Skull-surface scan; \*\*\*\*CT scan-cadavers study. Mean ± Skull study; \*\*\* Skull-surface scan; \*\*\*\*CT scan-cadavers study.

Table 3. The morpAuthors. Year	phometric data relat Specimen	ted to the Age	Indices N- Sex	SI	FAI	FRI	BI	FMI	CCI1	CCI2	CCI3	CCI4	I
Al-Sagaw and Elmougy 2002*	Camel (Malha)	2-3 у 6-7 у	15-M 15-M	41.1±0.6 45.1±0.6	72.3±1.6 79.8±1.6								
Yahaya et al., 2012*	Camel (One-Humped)	Adult	15-F 15-M	-				107.4±6.3 109.3±4.4					
Yahaya et al., 2012*	Camel (One-Humped)	2-3 у	6-F 6-M	40.9±0.6 41.1±0.4	96.5±1.4 96.2±1.4			$\begin{array}{c} 104.3 \pm \ 1.6 \\ 102.8 \pm \ 3.2 \end{array}$					
Zhu 2012*	Tibetan Gazelle (Procapra Picticaudata)	_	10-M	43.2±0.4	116.4±1.2								
Yılmaz et al., 2020***	Gazelles (Gazella subgutturosa)	Adult	5-F 4-M	42.1±2.5 41.5±1.9				98.2±3.5 86.2±4.5					
Choudhary and Singh 2015*	Indian Blackbuck (Antelope cervicapra)	Adult	6-F,M	$45.9 \pm 0.04$ $46.4 \pm 0.04$				98.7					
Kataba 2015*	Goat (Capra hircus)	18 m ≤	15-F 15-M	54.4±3.4				88.5±5.6					
Karimi et al., 2011*	Sheep (Mehraban Sheep)	Adult	8	53.6±3.3	85.4±1.9								
Ömer and Alpak 2012*	Sheep (Kwww.k.sheep)	1 y	20-F 20-M	47.0±1.1 47.4±2.2	81.7±2.8 83.6±5.5								
Gündemir et al., 2020*	Sheep (Bardhoka sheep)	Adult	13-F 12-M	41.7±1.7 41.5±2.4				94.5±6.9 93.7±9.7					
Özkan et al., 2019*	Water buffalo (Bubalis bubalis L.)	3-7 у	15-F	43.0±1.8 (39.6-46.0)	74.7±5.7 (66.0-84.7)	94.9±6.3 (83.7-106.7)	45.1±2.0 (42.1-48.3)	85.0±6.7 (78.2-104.6)					0.79 (0.67
Parés Casanova and Jordana i Vidal 2008*	Cattle (Bos taurus L.)	2.5 y <	502-F 76-M	44.3±3.6 47.2±3.7									
Cabezas Congo et al., 2019**	Cattle (Criollo)	Adult	198-F 19-M	43.1±7.0 36.5±3.6									
Gambo et al.,2019*	Cattle (Kuri cattle)	9 m-10 y	15-F 15-M	-				103.7±11.3 109.2±10.9					
Özkan et al., 2019*	Cattle (Bos taurus L.)	3-7 y	20-F	43.2±1.5 (39.8-46.0)	76.9±3.0 (70.5-81.5)	98.3±6.8 (89.1-113.9)	47.1±1.4 (44.3-50.5)	91.3±7.7 (79.9-108.6)					0.79: (0.66
Neves et al., 2021**	Cattle (Jersey)	1-11 m 16-24 m 25-58 m	18-F 17-F 13-F	85.7±6.1 78.15±2.5 77.0±3.1									
Lomillos and Alonso 2020**	Cattle (Lidia)	4-6 y	80-F 184-M	44.6±6.2 (26.8-58.1) 50.6±4.3 (27.3-61.6)									
Çakar et al., 2024****	Cattle (Holstein) Cattle (Simmental)	12-14 m	25M 29M	43.6±2.5 44.9±2.4	60.1±3.7 58.6±4.9	96.0±5.9 96.4±6.4	48.1±2.8 50.1±3.0	99.3±13.6 98.2±16.8					83.6 86.4
The present study*****	Cattle (Holstein cow)	1.5-8 y	14-F	43.4±1.3 (41.9-46.0)	78.3±3.7 (72.3-86.1)	96.2±3.4 (90.4-102.5)	47.8±1.8 (44.4-50.5)	83.1±3.2 (78.2-89.4)	73.6±4.6 (68.0-84.9)	88.5±3.7 (84.1-94.1)	68.9±4.5 (62.5-76.2)	82.8±3.7 (78.8-90.2)	81.5: (71.2

**Abbreviations:** SI, Skull index; FAI, Facial index; FRI, Frontal index; BI, Basal index; FMI, Foramen magnum index; CCI1, Cranial cavity index1; CCI2, Cranial Cranial cavity index4; LLI1, Length-length index1; LLI2, Length-length index 2; LLI3, Length-length index3; LWI1, Length-width index1; LWI2, Length-width i \*\*\*\*Skull-surface scan, \*\*\*\*CT scan-cadavers study; F, Female; M, Male; y, years; m,months. Mean ± SD (minimum-maximum)

Table 4. The relationship between extra-intracranial measurements and age (N=14).

	Age	TL	CL	BL	NL	VL	MFL	GMB	GBOC	GFB	GHOR	LHOR	LCC1	LCC2	MWCC	MHCC	GBFM
TL																	
CL		,884**															
BI		,903**	,971**														
DL		,562*	,613*	,690**													
NL	582*	886**	920**	929**													
VL	,502	,000	,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,	,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,													
MFL		,088															
GMB		,663**	,573*				,553*										
<b>CDOO</b>								,614*									
GBOC		749**	637*	631*		647*	564*	813**	650*								
GFB		,7 12	,057	,001		,017	,501	,015	,050								
GHOR		,568					,645										
LHOR							,605*				,952**						
LIIOK				,574*													
LCC1				-							648*	661*					
LCC2											,010	,001					
MWCC																	
MHCC							,729**										
MITCC																	
GBFM									<b>1</b> 0**	- <b>- - *</b>							**
HFM									,670**	,572*							,/4 <sup>/</sup> **

\*, p< 0.05; \*\*, p< 0.01 Abbreviations: TL, Total length; CL, Condylobasal length; BL, Basal length; NL, Neurocranium length; VL, Viscerocranium length; MFL, Median frontal length; GMB, Greatest mastoid breadth; GBOC, Greatest breadth of the occipital condyles; GFB, Greatest frontal breadth; GHOR, Greatest height of the occipital region; LHOR, Least height of the occipital region; LCC1, Lenght of the cranial cavity1; LCC2, Lenght of the cranial cavity2; MWCC, Maximum width of the cranial cavity; MHCC, Maximum height of the cranial cavity; GBFM, Greatest breadth of the foramen magnum; HFM, Height of the foramen magnum.

Table 5. The relationship between the indices (N	=14).
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	FMI	SI	FAI	FRI	BI	CCI1	CCI2	CCI3	CCI4	LLI1	LLI2	LLI3	LWI1
FMI													
SI													
FAI		,782**											
FRI													
BI		,858**	,855**										
CCI1			<b>,</b> 573*		,634*								
CCI2						,676**							
CCI3						,689**							
CCI4								,743**					
LLI1			,816**	-,654*	,541*	,613*		,571*					
LLI2								-,644*					
LLI3			,592*								,590*		
LWI1			-,601*		-,653*	-,997**	-,687**	-,705**		-,650*			
LWI2						-,676**	-1,000**						<b>,</b> 687**

\*, p< 0.05; \*\*, p< 0.01

Abbreviations: FMI, Foramen magnum index; SI, Skull index; FAI, Facial index; FRI, Frontal index; BI, Basal index; CCI1, Cranial cavity index1; CCI2, Cranial cavity index2; CCI3, Cranial cavity index3; CCI4, Cranial cavity index4; LLI1, Length-length index1; LLI2, Length-length index 2; LLI3, Length-length index3; LWI1, Length-width index1; LWI2, Length-width index2.

#### DISCUSSION

This study provides pioneering descriptive data on the morphometric characteristics of the Holstein cow skull using CT imaging. Indices were calculated based on specific morphometric measurements of the skull, with mean±SD values of craniometric measurements and indices detailed in Tables 2 and 3 alongside comparisons to existing literature. The craniometric data for Holstein cows were compared with data from other members of the *Bovidae* family (Table 2), while indices were contrasted with those from the order *Artiodactyla* (Table 3). Despite variations in nomenclature used for certain linear measurements in the literature, the measurement reference points align well with those used in the current study.

A review of previous studies indicates that craniometric measurements are generally larger in males within species of the *Bovidea* family (Table 2). Similarly, index values tend to be higher in males across several *Artiodactyla* species, including camels (Al-Sagair and Elmougy 2002; Yahaya et al. 2012), sheep (Ömer and Alpak 2012), and cattle (Parés Casanova and Jordana i Vidal 2008; Gambo et al. 2019; Lomillos and Alonso 2020) (Table 3).

In this study, the CL and BL in Holstein cows were 506.9 ±21.5 mm and 472.1 ±22.2 mm, respectively. The CL in Holstein cows was found to be greater than in other breeds, except for domestic cattle (Özkan et al. 2019) and male Hungarian greys (Körösi 2013), while the BL exceeded that of other female breeds, with exceptions including Bos primigenius (Grigson 1978) and domestic cattle (Özkan et al. 2019) (Table 2). Parés Casanova and Jordana i Vidal (2008) recorded a mean TL of  $533.0 \pm 37.1$  mm in various domestic cattle and 559.7 ±28.7 mm in the Friesian breed (n = 38 females), whereas Özkan et al. (2019) reported a TL of 592.5 ±15.9 mm in domestic cattle. In contrast, this study found a TL of 519.4 ±21.7 mm in Holstein cows, and a TL of 490.59 ±28.08 mm in Holstein bulls was reported by Çakar et al. (2024). When evaluating data from the literature on female cattle breeds (Bartosiewicz 1980; Hayashi et al. 1981; Nıshıda et al. 1983; Hayashi et al. 1988; Bartosiewicz 2006; Körösi 2013; Cabezas Congo et al. 2019; Gambo et al. 2019; Lomillos and Alonso 2020; Neves et al. 2021), it was noted that TL values tended to be lower than those in Holstein cows (Table 2). Previous studies largely conducted measurements on dry skulls, though some utilized fresh skulls or live animals, with methods involving metric rules, threads, calipers, hauptner measuring canes, non-flexible measuring tapes, or photogrammetric equipment. These variations in cranial measurements across cattle breeds are likely influenced by breed-specific size differences and the methodological variations mentioned above.

Upon reviewing studies within the Bovidae family, it was noted that research specifically addressing NL measurements is relatively scarce (Table 2). In Holstein cows, the mean NL measurement  $(235.3 \pm 8.5 \text{ mm})$  was found to be larger than that of the Red Pied-Fleckvieh (Bartosiewicz 1980) but smaller than the Hungarian Grey (Körösi 2013). Körösi (2013) reported an MFL value of 235.9 mm in Hungarian Grey cattle, while Özkan et al. (2019) recorded 233.5 mm in domestic cattle. The MFL value from this study in Holstein cow (234.4 ±9.2 mm) aligns closely with values from Hungarian Grey cattle and domestic cattle (Grigson 1974, 1978, 1980; Hayashi et al. 1981; Nıshıda et al. 1983; Hayashi et al. 1988; Bartosiewicz 2006; Cabezas Congo et al. 2019; Gambo et al. 2019; Neves et al. 2021; Cakar et al. 2024), indicating similar average measurements across these breeds (Table 2). To fully elucidate the impacts of dimensional differences observed between species on these animals, further examination of genetic, environmental, and production traits is recommended.

Parés Casanova and Jordana i Vidal (2008) reported skull width measurements of 235.3 ±14.2 mm in various domestic cattle and 234.8 ±11.5 mm in the Friesian (black and white) breed. In the present study, the skull width of Holstein cows was measured at 225.4  $\pm$ 8.5 mm. A review of literature on other cattle breeds (Bartosiewicz 1980; Hayashi et al. 1981; Nıshıda et al. 1983; Hayashi et al. 1988; Bartosiewicz 2006; Körösi 2013; Cabezas Congo et al. 2019; Gambo et al. 2019; Özkan et al. 2019; Lomillos and Alonso 2020) indicates that Holstein cows generally have lower mean skull width values than female European bison (Krasınska et al. 2008), domestic cattle (Özkan et al. 2019), and Jersey (Neves et al. 2021). Furthermore, Parés Casanova and Jordana i Vidal (2008) documented a facial length of  $400 \pm 20.5$ mm in the Friesian breed, whereas the Holstein cow in this study exhibited a facial length of 288.4  $\pm 17.4$ mm, surpassing most other female cattle breeds reported in the literature (Bartosiewicz 1980; Nishida et al. 1983; Hayashi et al. 1988; Körösi 2013; Cabezas Congo et al. 2019; Gambo et al. 2019; Neves et al. 2021) except for domestic cattle (Parés Casanova and Jordana i Vidal 2008; Özkan et al. 2019) (Table 2). The observed differences in skull width and facial length between Holstein cows and Friesians may stem from genetic and environmental factors contributing to dimensional variations, as well as potential methodological disparities.

In this study, the GHOR value for Holstein cows was determined as 162.7  $\pm$ 8.2 mm (Table 2). This value was higher than those recorded for other female individuals in the literature, with the exception of *Bos primigenius* (190.7  $\pm$ 13.3 mm) (Grigson 1978) and *Bos taurus* (170.3  $\pm$ 7.5 mm) (Özkan et al. 2019) (Table 2). The LHOR values reported by Özkan et al. (2019) were 168.4  $\pm$ 12.5 mm for water buffalo and 131.3  $\pm$ 7.3 mm for domestic cattle, while in Holstein cows, the LHOR was 124.3  $\pm$ 7.6 mm, exceeding

values reported for other female cattle breeds (Grigson 1980; Bartosiewicz 2006; Körösı 2013; Gambo et al. 2019), with the exception of water buffalo and domestic cattle. The GBOC value in Holstein cows closely matched that of domestic cattle (Özkan et al. 2019), and the GMB value was similar to that of the Hungarian grey (Bartosiewicz 2006). Additionally, the GBFM value in Holstein cows had a higher mean compared to buffalo and cattle data in the literature (Brudnickı et al. 2012; Körösi 2013; Gambo et al. 2019; Özkan et al. 2019). The HFM value in Holstein cows (39.3 ±2.1 mm) was comparable to Kuri cattle values (Gambo et al. 2019), aligning closely with literature reports (Körösi 2013; Özkan et al. 2019). For the cranial cavity measurements—LCC1 (140.5  $\pm$ 6.4 mm), LCC2 (116.8 ±4.3 mm), MWCC (103.3 ±4.4 mm), and MHCC (96.6  $\pm$ 4.7 mm)—no directly comparable data were available in the literature (Table 2).

In this study, an SL value comparable to index values reported for Holstein cows ( $43.4 \pm 1.3$ ), Tibetan Gazelle (Zhu 2012), water buffalo, domestic cattle (Özkan et al. 2019), and Criollo cattle (Cabezas Congo et al. 2019) was obtained. The FAI value in Holstein cows (78.3  $\pm$  3.7) was lower than those of camels (Yahaya et al. 2012), gazelles (Zhu 2012), and sheep (Karimi et al. 2011; Ömer and Alpak 2012), but higher than values reported for water buffalo, domestic cattle (Özkan et al. 2019), Holstein bulls, and Simmental bulls (Cakar et al. 2024). Özkan et al. (2019) documented FRI values of 94.9  $\pm$ 6.3 for water buffalo and 98.3  $\pm$ 6.8 for domestic cattle, while Çakar et al. (2024) reported values of 96.0 ±5.9 and 96.4  $\pm$ 6.4 for Holstein and Simmental bulls, respectively. In the current study, the FRI value in Holstein cows was positioned between water buffalo and domestic cattle, measuring 96.2  $\pm$  3.4. The BI value in Holstein cows (47.8 ±1.8) closely aligned with the domestic cattle measurement of  $47.1 \pm 1.4$ (Özkan et al. 2019). Relative to literature values (Yahaya et al. 2012; Kataba 2014; Choudhary and Singh 2015; Gambo et al. 2019; Özkan et al. 2019; Gündemir et al. 2020; Yılmaz et al. 2020; Çakar et al. 2024), Holstein cows exhibited the lowest FMI value  $(83.1 \pm 3.2)$  among Artiodactyla samples. Cakar et al. (2024) recorded LLI1 values of  $83.6 \pm 6.1$  and 86.4  $\pm$ 7.7 in Holstein and Simmental bulls, respectively. This study also found that the LLI1 value in Holstein cows was similar to values for water buffalo and domestic cattle (Özkan et al. 2019). For other indices, including CCI1, CCI2, CCI3, CCI4, LLI2, LLI3, LWI1, and LWI2, no comparable data were found in the literature (Table 3).

Craniological studies spanning about 250 years have highlighted both interspecies similarities and skull variations within the *Bovidae* family, noting age- and gender-related changes. It has been observed that cranial dimensions decrease as cattle breeds transition from wild *Bos primigenius* to domesticated *Bos taurus* (Grigson 1978). Balcarcel et al. (2021) reported a

25.6% reduction in brain size in domestic cattle, as measured by some extracranial dimensions, compared to the aurochs. Craniometric analysis on wild Banteng cattle and five Asian local cattle breeds (Bali, Madura, Aceh, Leyte, Korea) suggested that Bali cattle could be a domesticated form of Banteng due to their close morphological relationship (Hayashi et al. 1988). When the hybrids between European bison and domestic cattle were compared with their parents, an increase in the skull size of the hybrids was observed (Krasinska 1988). In a study examining the changes in European bison skulls over time (from 1950 to the present), a year-related decrease in skull size and an increase in skull height in male individuals were detected in almost all of the skulls examined (Szara et al. 2023). Gender differences in skull measurements in European bison were notable between ages 1 and 3 but stabilized after age 5 (Krasıñska et al. 2008). In neurocranium ontogeny, cattle measurements generally decrease, except for neurocranium length, with larger changes observed in the viscerocranium (Bartosiewicz 1980a, 1980b). Hungarian grey cattle exhibit age-related morphological changes in facial and frontal bones (Kőrösi 2013). In Jersey cattle (1-58 months), total head length, cranial and nasal length, and cranial width increased with age, while index values decreased (p<0.05) (Neves et al. 2021). In this study, a positive correlation was found solely between age and viscerocranium length (VL) in Holstein cows (p: 0.03, r: 0.582) (Table 4). Morphometric assessments in Lidia cattle (4–6 years) indicated a mesocephalic head in males (50.6) and a dolichocephalic head in females (44.6) (Lomillos and Alonso 2020). Criollo cattle (adults) also displayed a dolichocephalic cranial type (M: 36.5, F: 43.1) (Cabezas Congo et al. 2019). Holstein and Polish Holstein-Friesian breeds have been classified as dolichocephalic and fall under the primigenius cranial type (Gulinski 2021). Similarly, in the current study, female Holstein cows (1.5-8 years) exhibited a dolichocephalic head structure, consistent with female Lidia and Criollo cattle, as indicated by the calculated skull index (43.4) (Table 3). These morphological differences across species are likely due to genetic, environmental, and productivity factors.

#### CONCLUSIONS

Despite limitations such as the restricted age range and number of female animals from a single species and the absence of morphometric data on the cranial cavity of the species, this study offers the first comprehensive reference data on craniometric features in Holstein cows, which had not been previously documented in the literature. These findings could serve as valuable resources for radiological and clinical studies, forensic science, investigations of sexual dimorphism, and zooarchaeological research. Multidisciplinary studies are needed to investigate craniometric features across various developmental stages and genders, with findings evaluated from clinical, anatomical, and biological perspectives.

**Conflict of interest:** The author has no conflicts of interest to report.

**Authors' Contributions:** NT contributed to the project idea, design and execution of the study. NT contributed to the acquisition of data. NT analysed the data. NT drafted and wrote the manuscript. NT reviewed the manuscript critically. Author has read and approved the finalized manuscript.

Ethical approval: This study was carried out at Selçuk University. This research was approved by The Selçuk University Faculty of Veterinary Medicine Experimental Animal Production and Research Center Ethics Committee (SUVDAMEK, Ref No: 2024/077, Tarih: 05/2024)

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