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RESEARCH ARTICLE

Determination of Breed-Specific Differences in Intracranial Volume in Awassi and Kangal Sheep Using Stereology and Computerized Tomography Methods

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Abstract

The main objective of the study was to observe the intracranial volume differences between Kangal and Awassi sheep using computerized tomography and Cavalieri principle and to observe the compatibility of the methods used. The intracranial volumes of the craniums of six adult Kangal sheep and six adult Awassi sheep used in the study were calculated using models taken from computerized tomography sections and the Cavalieri principle performed on the models and compared. The findings show that there are significant intracranial differences and high correlation between both sexes. In addition, it has been observed that computed tomography and volume calculations performed with the Cavalieri principle are compatible. The study shows that computed tomography and Cavalieri principle methods are effective tools in morphometric analysis and provide new information about the morphological characteristics of different animal breeds. The results show that these methods can be used as mutually supportive alternatives in future studies and can contribute to the morphological analysis of animal species.

Keywords: Computed tomography, intracranial volume, sheep

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INTRODUCTION

Sheep breeding has an important place in global livestock production (Savas et al 2020). Meat and milk productivity in sheep is directly affected by the genetic characteristics of the breed (Aksoy 2008). Known for its robust structure, high meat yield and adaptation to cold climates, Kangal sheep have a long, narrow head and a long neck (Akcapinar 1982, Turkmen and Cak 2021). In contrast, Awassi sheep, which stand out with its high milk yield and ability to thrive in hot climates, is one of the most preferred breeds for milk production. They are especially valuable in Middle Eastern countries due to its economic and ecological importance (Gursoy et al 1993, Ustuner 2007, Talafha and Ababneh 2011).

Although Kangal sheep are not as widely known as Kangal dogs, they belong to the Akkaraman sheep breed and are well known and preferred by breeders (Yilmaz 2007). Kangal sheep are a local Akkaraman variety and represent the largest species in terms of body size, height and weight (Soysal et al 2004). Rams are generally 100-110 cm tall and ewes are around 75,9 cm tall (Yilmaz et al 2011). Lactation is 140-150 days, milk yield in lactation is 120-150 kg. The coat of Kangal Sheep is dirty white. It is possible to see small black dots around the nose, eyes and feet (Akcapinar 1982). There are no horns in sheep, while rams have 10% horns. Its wool is of higher quality than the Akkaraman breed, its structure is thinner and more curved. Its nose structure is different from the Akkaraman sheep (Orkiz et al 1984). The thin tail tip forms an "S" shaped fold over the fatty main part (Garip and Arslan 2021).

The Awassi sheep breed is mainly raised in the Southeastern Anatolia region (Gursoy et al 1993, Ustuner 2007, Talafha and Ababneh 2011). Şanlıurfa, Gaziantep, Kilis and Hatay are among the provinces where it is raised (Ertugrul et al 2009). It is an animal that lives in a climate where it feels the effect of hot weather less than other sheep breeds. It does not need to go out to pasture on hot summer days like other breeds (Darcan and Guney 1997). Awassi sheep are white, with brown heads and legs. The Awassi breed is called yellow head in some places. The existing yellow color in the sheep breed can dominate a color towards black (Karaca 2021). In the sheep breed, females are hornless and males are horned (Kaymakci 2013). The Awassi sheep breed differs in live weight in the females and males. The female Awassi weighs 45 to 55 kg. Male Awassi rams weigh 60 to 90 kg (Degen and Benjamin 2003).

One of the most important points in measuring intracranial volume is that it has an important place in clinical applications such as the diagnosis and monitoring of neural abnormalities. In addition, it is a method that can be used to evaluate differences based on race and gender (Rushton 1994). Intracranial volume makes it possible to measure healthy brain size (Davis and Wright 1977), sex differences, inter-individual variability in head size (Scahill et al 2003). It also serves as a valuable tool for assessing morphological differences due to race and sex (Rushton 1994, Schofield et al 1995). Measurements were obtained using cross-sectional radiological imaging techniques combined with the Cavalieri principle. The results obtained with computerized tomography (CT) may not always provide the most accurate measurement, but the Cavalieri method provides the closest result to the correct result in estimating organ volume with its minimum error rate and mathematical reliability (Canan et al 2002, Odaci et al 2003, Ordu 2015). This approach has been applied extensively to analyze cranium sizes and changes in regional brain volumes (Hansen et al 2015). Studies have shown that female sheep generally have longer craniums than males, while meat-oriented breeds tend to have larger cranium structures compared to dairy-oriented breeds (Yilmaz and Demircioglu 2020, Duro et al 2021). Moreover, cranium shape and size are influenced by geographical factors reflecting species-specific adaptations. As a result, cranial morphology plays an important role in understanding animal behavior and distinguishing population differences (Thomason et al 2001, Elbroch 2006). In previous studies, morphometric measurements such as body weight, cranium length, thorax depth, chest width, trunk length, withers length and coccyx height were recorded in Awassi sheep (Ozbeyaz et al 2018). Additionally, geometric morphometric analysis of the craniums and mandibles of male and female Awassi sheep was performed (Demircioglu et al 2021). However, although previous studies have examined morphological characteristics such as body weight, cidago height, body length, chest circumference and shin circumference in Kangal and Awassi sheep, no research has been found on intracranial volume (Yalcin and Aktas 1969).

In this study, it is aimed to determine the volumes of the craniums of Kangal and Awassi sheep mathematically using three-dimensional images obtained from computerized tomography scans and to reveal the statistical differences between these two breeds. At the same time, the accuracy of the methods used will be compared with the data obtained using the Cavalieri principle and the degree of accuracy will be revealed. It is expected that the numerical data obtained and the accuracy of the study will make significant contributions to the literature when compared with similar studies conducted with other sheep and goat breeds.

Material and Methods

The sheep used in the study were supplied from private slaughterhouses in Şanlıurfa province and its surroundings. The study was approved by the ethical decision taken by the Harran University Animal Experiments Local Ethics Committee (Date and Decision No: 2024/008/04). The craniums of six adult Kangal sheep and six adult Awassi sheep were used in the study. The gender of the sheep was not taken into consideration and the craniums of these animals were used for intracranial volume measurement. The craniums used in the study were scanned using a 64-detector Multi-Detector Computed Tomography (MDCT, General Electronic Revolution, USA) device with 0,625 mm slice thickness, 80 kV, 200 mA and 639 mGy settings. The data obtained via raw DICOM were transferred to the MIMICS 20.1 software. The intracranial space boundaries in each section were defined manually using the "Edit Mask" option in the software interface. The intracranial space of each animal was modeled in 3D using the "Calculate Part" option in the software. Volume values were recorded via the "Properties" option in the software interface. Cavalieri principle was applied using tomographic slices for intracranial volume estimation (Figure 1). Systematic random sampling method was used to select 12 tomographic slices for each sheep cranium. ImageJ software was used for volume estimation. An area of 144 mm² was selected via the "Grid" option in the ImageJ software interface. The number of points in each

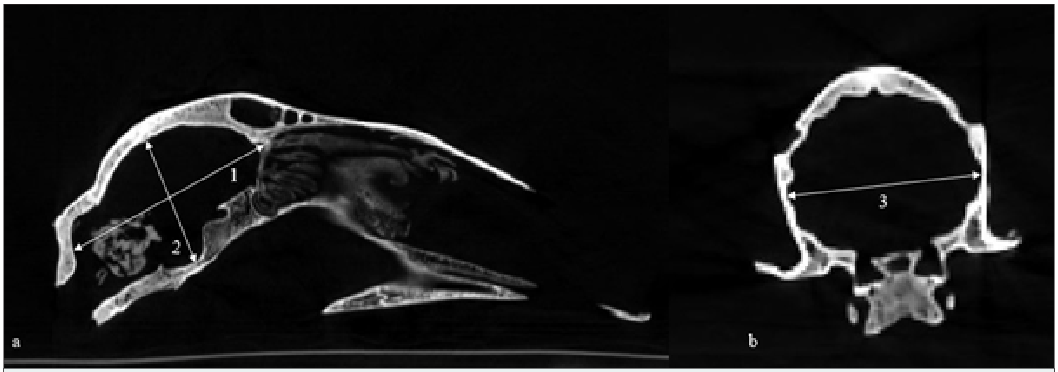

Figure 1. Cranial cavity morphometric measurements. (1) Maximum length of the cranial cavity (MLCC), (2) Maximum height of the cranial cavity (MHCC), (3) Maximum width of the cranial cavity (MWCC)

slice was recorded. To use the intracranial volume the formula ‘’V = ∑p × t × a (p)” was used (Figure 2).

Results

The findings showed that the two methods yielded quite similar and consistent results.

The mean intracranial volume values obtained by both methods were similar, with 140.17 cm³ found by computed tomography and 140.23 cm³ by the Cavalieri principle. Examination of the minimum and maximum values revealed measurements ranging from 104.24 to 160.09 cm³ with computed tomography and from 106,14 to 157.24 cm³ with the Cavalieri method, demonstrating that the results of both techniques were comparable. The similarity of the standard deviations (16.49 for CT; 15.26 for Cavalieri) supports the homogeneous distribution of data for both methods. Furthermore, the p-value of 0.993 indicates that there is no statistically significant difference between the two methods. In addition, an extremely high correlation coefficient of 0,998 shows that the results of the two measurement techniques strongly corroborate each other and exhibit a high level of consistency (Table 1).

The mean intracranial volume obtained by the CT method was measured as 127.82±14.06 cm³ in Awassi sheep and 152.52±5.90 cm³ in Kangal sheep. When the Cavalieri principle was applied, intracranial volumes were calculated as 129.01±13.43 cm³ in Awassi sheep and 151.45±5.48 cm³ in Kangal sheep. In both methods, it was determined that Kangal sheep have a larger intracranial volume than the Awassi breed. The p values obtained in both methods (p=0.003 and p=0.004) were less than 0,005, indicating that the difference in intracranial volume between the two breeds was statistically significant. Furthermore, the low coefficient of error (CE=0.031) obtained with the Cavalieri method supports the reliability and reproducibility of this technique (Table 2).

According to Table 3, there are significant differences between the two sheep breeds in terms of cranial dimensions. In particular, the significant findings observed in MWCC (maximum width of the cranial cavity) and related index parameters revealed that the Kangal breed has a more developed intracranial width structure compared to the Awassi breed.

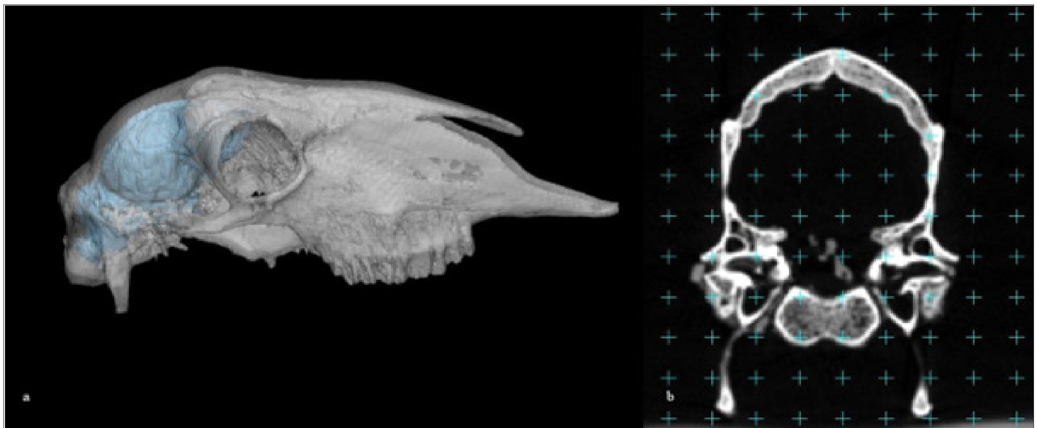

Figure 2. 3D model of the intracranial cavity (a), Point- counting grid (b).

Table 1. Intracranial volumes obtained by two methods (cm^3)							
Method	n	Minimum	Maximum	Mean	Std. dev	p	Correlation
Computed tomography	12	104.24	160.09	140.17	16.49	0.993	0.998
Cavalieri's principle	12	106.14	157.24	140.23	15.26		

Discussion

This study aimed to measure the total intracranial volumes of Kangal and Awassi sheep using three-dimensional models obtained by computerized tomography scans and stereological methods and to compare the accuracy and similarity of the obtained models according to the Cavalieri principle.

In a study aiming to compare the intracranial volumes of goats and Tuj sheep, three-dimensional models taken from computerized tomography section images and two different volume measurement estimation methods based on the Cavalieri principle using these models, it was revealed that there were intracranial volume differences between the goat and sheep species and at the same time, it was found that the methods used were compatible with each other and took their place in the literature (Kocyigit et al 2024f). In another study using four different methods, the Cavalieri principle was compatible with the stereological methods used in intracranial volume calculations and mathematically supported the use of these methods (Sahin et al 2007). Intracranial measurements calculated with computerized tomography method also showed that stereological techniques provide fast, objective and reliable estimation of total intracranial volume (Mazonakis et al 2004). In a bone defect study comparing Cavalieri principle and three-dimensional reconstruction, bone defect volume measurements were taken and these values were compared with real volume measurements and smaller results were obtained (Altay et al 2018).

In one study, intracranial volume of Hamdani sheep was measured in stereological and computerized tomography models and 129.54±12.16 cm^3 was obtained, while it was measured as 131.55±9.43 cm^3 only in computerized tomography models. It has been observed that there is a high correlation between the Cavalieri principle and these methods and that they support each other for a significant change between the methods used (Kocyigit et al 2024c). In another methodological study on the metacarpus of Hamdani sheep, measurements were taken using digital caliper measurements, photometric analysis and three-dimensional software, and it was found that some parameters were similar to each other and did not create a statistically significant difference, while some parameters were statistically significant (Guzel et al 2022). In a study where the intracranial volume of gazelles (Gazella subgutturosa) was calculated using stereological and computed tomography methods, no difference was found between the two methods (p>0.05) and the reliability of the methods was determined (Demircioglu et al 2021). As a result of the statistical comparison of intracranial volumes calculated using the stereological method and computed tomography (CT)–based three-dimensional models in Akkaraman and Kangal Akkaraman sheep, no statistically significant difference was detected between the two methods (p > 0.05) (Bas-Ekici and Besoluk 2024). Similar to these results, a study on red deer (Cervus elaphus) (Logan and Clutton 2013) measured skull volume using computed tomography and the bead method and obtained values of 370±39 cm^3 and 362±37 cm^3, respectively, showing that both methods gave compatible results. In the study where the intracranial volume values of Kıvırcık sheep were calculated and a value below 1% was found, this result was achieved by using the stereological point counting technique (Haluk and Alpak 2012). While the error coefficient was calculated as 0.29 in the Cavalieri principle-based method used in a study on Tuj sheep and goats, it was found to be 0.031 in our study, which showed that the measurement methodology was reliable and repeatable, and showed that it was similar to the Tuj sheep and goat study in this sense (Kocyigit et al 2024f). In another study, orbital

Table 2. Calculation of intracranial volume in Awassi and Kangal sheep breeds using two methods (cm^3)								
Methods	Gender	n	Minimum	Maximum	Mean	Std. dev	p	CE
Computed tomography	Awassi	6	104.24	143.23	127.82	14.06	0.003**	
	Kangal	6	145.41	160.09	152.52	5.90		
Cavalieri's principle	Awassi	6	106.14	142.13	129.01	13.43	0.004**	0.031
	Kangal	6	144.14	157.24	151.45	5.48		

Intracranial volumes calculated using both methods showed significant differences between the breeds (p<0.005). *Correlation is significant at the p<0.05. **Correlation is significant at the p<0.01.

Table 3. Parameters of intracranial space volume (mm)

Parameter	Gender	n	Minimum	Maximum	Mean	Std. dev	p
MLCC	Kangal	6	93.20	101.87	97.94	3.84	0.055
	Awassi	6	84.98	98.62	92.53	4.76	
MWCC	Kangal	6	60.41	66.34	63.83	2.03	0.003**
	Awassi	6	48.06	60.58	55.94	4.62	
MHCC	Kangal	6	57.52	94.35	66.23	13.96	0.155
	Awassi	6	51.65	61.16	57.09	4.10	
Index 1	Kangal	6	62.63	68.35	65.30	2.35	0.038*
	Awassi	6	53.64	64.74	60.48	4.36	
Index 2	Kangal	6	1.46	1.60	1.53	0.06	0.048*
	Awassi	6	1.54	1.86	1.66	0.12	

MWCC: Maximum width of the cranial cavity, MHCC: Maximum height of the cranial cavity, MLCC: Maximum length of the cranial cavity. Index 1 (Cranial cavity index): maximum width of the cranial cavity x 100 / maximum length of cranial cavity Index 2 (Length width index): maximum length of cranial cavity / maximum width of the cranial cavity.*Correlation is significant at the p<0.05. **Correlation is significant at the p<0.01.

volume measurements of gazelles, sheep and goats were determined by computerized tomography and no significant difference was found between goats and sheep in terms of right orbital volume ($p>0.05$), but a significant difference was found between gazelles and sheep ($p<0.05$) (Kocyigit et al 2024b). In a study conducted on Van cats, it was revealed that MWCC (maximum width of the cranial cavity) MHCC (maximum height of the cranial cavity) and MLCC (maximum length of the cranial cavity) parameters did not differ between the sexes and it was concluded that the effect of sex on cranium morphology was limited in this breed (Kocyigit et al 2024d). In an MRI-based study conducted on the sheep brain, it was determined that the corpus callosum was longer in male sheep, whereas its surface area was wider in females (Aydogdu et al 2021). In a study on the cranium of Kagani goats, an average value of 113 cm^3 was reported using mustard seeds (Sarma 2006). Intracranial volume in Rambouillet sheep was presented as 116±0.433 cm^3 (Suri et al 2003), while this value was revealed as 128±7.93 cm^3 in Tuj sheep (Kocyigit et al 2024f). In our study, the measurements obtained with computerized tomography were calculated as 152.52±5.90 cm^3 for Kangal sheep and 127.82±14.06 cm^3 for Awassi sheep. According to these results, the intracranial volume of the Kangal sheep was found to be larger than that of the Rambouillet sheep, the Tuj sheep and the Kagani goat, while the Awassi sheep was larger than that of the Rambouillet sheep and the Kagani goat, but close to the Tuj sheep. The intracranial volume of the red deer was found to be larger than both the Tuj and Awassi sheep (Logan and Clutton 2013, Kocyigit et al 2024f). In a study conducted on the Saanen goats according to the Cavalieri principle, the intracranial volume was measured as 423.6±48.2 cm^3 (Tohidifar et al 2020). In this study conducted on Kangal sheep and Awassi sheep, measurements were taken according to the Cavalieri principle and the intracranial volume measurements of Kangal sheep and Awassi sheep were measured as 151.45±5.48 cm^3 and 129.01±13.43 cm^3, respectively.

Accordingly, the intracranial volumes of the Kangal and Awassi sheep are smaller than those of the Saanen goats. In a study conducted on Van cats, thoracic morphometry and lower respiratory tract were modeled three-dimensionally using computed tomography and significant it was concluded that there were differences between the sexes by looking at the measurement result of the transverse diameter of the thoracic cavity ($p<0.05$), the minimum width of the apertura thoracis cranialis and the height of the thoracic cavity ($p<0.005$) (Kocyigit et al 2024e). In a study examining sella turcica parameters in gazelles, goats and sheeps, significant differences were determined between sheep and gazelles and gazelles and goats ($p<0.005$) (Kocyigit et al 2024a). In our study, similar findings were found in measurements made with both methods, and significant differences were found between sheep breeds in terms of intracranial volume ($p<0,005$). In a study conducted on Van cats, the cranium volume was measured as 28.17±1.19 cm^3 in males and 26.99±0.57 cm^3 in females, and the difference between the gender was found to be statistically significant (Kocyigit et al 2024d). In a study conducted on Awassi sheep, radiometric measurement values of the metapodia were examined, and statistically significant sexual dimorphism was determined at certain measurement points of the metacarpus and metatarsus ($p<0.001$) (Kahraman et al 2022). In a study where Index 1 and Index 2 were measured on groups of dogs of different ages, it was concluded that the indexes did not create a statistically significant difference between the groups (Onar et al 2002). Our study also did not reveal any statistically significant difference ($p>0.05$). The aim of this study was to determine the differences between two sheep breeds by intracranial volume measurements obtained using computed tomography and Cavalieri principle and to determine whether the two methods are compatible. The results obtained are largely consistent with previous studies (Onar et al 2002, Kocyigit et al 2024a, Kocyigit et al 2024d, Kocyigit et al 2024e). In our study, intracranial differences were detected between Kangal and Awassi sheep coming from different geographical regions for meat and milk production. A strong correlation was observed between the measurements obtained with computed tomography and Cavalieri principle.

Conclusion

In conclusion, the findings of these studies indicate that computed tomography and stereological methods are effective and reliable tools for morphometric analysis.

Declarations

Competing Interests

Authors declares that there are no conflicts of interest related to the publication of this article.

Availability of Data and Materials

The data that support the findings of this study are available on request from the corresponding author.

Ethical Statement

The study was approved by the ethical decision taken by the Harran University Animal Experiments Local Ethics Committee (Date and Decision No: 2024/008/04).

Author Contributions

Motivation/Concept: BK, NK, MOD; Design: BK, NK, MOD; Control/Supervision: BK, NK, MOD; Data Collection and Processing: BK, NK, MOD; Analysis and Interpretation: BK, NK, MOD; Literature Review: BK, NK, MOD; Writing the Article: BK, NK, MOD; Critical Review: BK, NK, MOD

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