Geometric analysis of mandible using semilandmark in Hamdani and Awassi sheep

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ABSTRACT

The aim of this study is to determine whether or not the breed and sex factors have an effect on the shape in the mandibles of Hamdani and Awassi sheep. A total of 31 mandibles were used. The samples were analyzed via geometric morphometric methods by using semilandmark. In the study, it was determined that the first principal component accounted for 36.52% of the total shape difference. According to principal component analysis, samples were clustered significantly in terms of breed; whereas, they were not clustered in terms of sex. In terms of the first principal component, the places where the shape differences were concentrated were the attachment sites of teeth to the mandible, between the second molar and ramus mandibulae, the processus coronoideus and the angulus mandibulae. The Mandibulae of Hamdani sheep had a higher body than the mandible of Awassi sheep. The mandibulae of male sheep was more voluminous than the mandible of female sheep, especially in the body area. Consequently, it is thought that the data obtained as a result of the study would serve as a reference for the ruminant mandible remains obtained from archaeological excavations.

Introduction

Hamdani sheep are reared in wide geography including primarily Northern Iraq as well as Iran and Southeastern Türkiye (2). This sheep is a strain of Karadi sheep. Karadi sheep is the largest size of a local breed raised in Iraq. Hamdani sheep are distinguished by a fat tail, white and wide-body, long ears, black-brown head, and high legs from other sheep breeds (1). Awassi sheep is a sheep breed that is widely reared in Türkiye, Iraq, Israel, Jordan, and Syria (28). This fat-tailed sheep breed has a medium-sized body covered with matte white wool, a brown and narrow head and medium-sized floppy ears (1).

Shape analysis is performed by using the geometric morphometry (GM) method based on a statistical analysis of landmark(LM) coordinates (3, 19, 29). Landmarks are the biological homolog points among the samples. Many structures cannot be examined by using classical landmarks, as landmark locations along the fold or surface cannot be homologized according to the individuals. Semilandmarks (SLM) enable quantifying two- or three-dimensional homologous curves and surfaces and analyzing them together with traditional landmarks (9).

In recent years, numerous studies have been conducted to reveal the shape differences of cranium or mandible via the geometric morphometric method in different species such as wolf (11), dog (23), quail (30), turkey (10), sheep (6), goat (5), and deer (17). However, no study comparing two different sheep breeds via semilandmarks was encountered. Thus, the aim of this study is to determine whether or not the breed and sex factors have an effect on the shape of the mandible over the samples of Hamdani and Awassi sheep.

Materials and Methods

Samples: In the study, heads of a total of 31 sheep including 12 (6 female, 6 male) Hamdani sheep and 19 (9 female, 10 male) Awassi sheep were used. They were older than one year old. After the materials were collected from the slaughterhouses in Siirt and Şanlıurfa, they were boiled and macerated. Attention was paid to ensure that the materials used in the study did not have any pathological and clinical conditions in the tooth and bone tissue. This study was approved by the Harran University Animal Experiments Local Ethics Committee (28.03.2022/01-13).

Imaging and Digitization: Left mandibles were photographed laterally by using a camera (18x55 lens, Canon Eos, 600D, Japan) in order to keep the focus (first molar tooth) on the same plane (camera resolution 890x1065 pixels). The distance between the lens and the material was detected as 30 cm. Among the photographs in the format of JPG were recorded on the computer, 10 homolog landmarks (Figure 1) and 132 semilandmarks were marked by using TpsUtil (Version 1.79) (27) and TpsDig2 (Version 2.31) (25) program, respectively. Thus, the *x* and *y Cartesian* coordinates of the general shape of the mandible were considerably determined, together with the homologous anatomical points (14) and the short-distance points (semilandmarks) between these points. Before the statistical analysis, a verification test was done in TpsSmall (Version 1.34) (26) program for semilandmarks. Accordingly, uncentred correlation and root mean square error values were detected as 1.000000 and 0.000006, respectively. These results revealed the accuracy of the semilandmarks.

Landmarks: LM1=SLM132: Oral caudodorsal end point of alveoli dentales of I4, LM2=SLM14: Rostroventral edge of PM1, LM3=SLM21: Caudoventral edge of PM3, LM4=SLM32: Caudoventral edge of M3, LM5=SLM60: Dorsal edge of processus coronoideus, LM6=LM72: Medioventral point of incisura mandibulae, LM7=SLM78: Caudal end point of condylus mandibulae, LM8=SLM90: Caudoventral corner of angulus mandible, LM9=SLM96: Incisura vasorum facialium, LM10=SLM130: Aboral rostroventral end point of alveoli dentalis of I1 (I: incisiv, PM: Premolar, M: Molar).

Statistical analysis: In the mandible photographs, General Procrustes Analysis (superimposition) was conducted due to the differences such as size, position, and direction (29). PAST (Version 4.02) (12) program was used for this analysis. By using the same program, principal components analysis (PCA) was performed on the new coordinates obtained as a result of the Procrustes Analysis, and the components were calculated according to the breed and sex factors for the total shape variation. MorphoJ (14) program was used to analyze at which landmarks the shape differences are concentrated (PCA), proximity degree of individuals (Classical Cluster), allometry and grouping characteristics (Canonical variance analysis-CVA).

Results

Table 1 shows the results of the principal components analysis conducted on the semilandmark coordinates detected in the sheep mandible. Accordingly, the first principal component (PC1) explained 36.52% of the total shape difference and the first three principal components (PC1+PC2+PC3) explained 63.822% of the total shape difference. Among the principal components, a significant point of inflexion was observed between PC3 and PC4. The distribution of the samples according to PC1 is shown in the graph in Figure 2. Accordingly, the individuals were clustered significantly in terms of breed. However, the samples did not show any clustering in terms of sex.

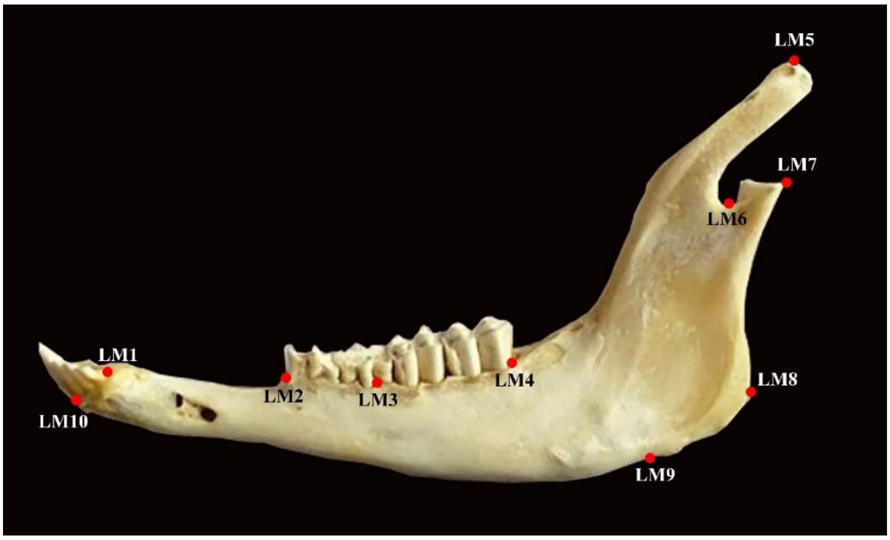

Figure 1. Landmarks for the mandible.

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Table 1. Results of the principal component analysis, PC: principal component.

PC	Eigenvalue	% Variance	PC	Eigenvalue	% Variance
1	0.000956539	36.521	**16**	1.67996E-05	0.64142
2	0.000435134	16.614	**17**	1.43954E-05	0.54962
3	0.000279896	10.687	**18**	1.17449E-05	0.44843
4	0.000182373	6.9631	**19**	1.0266E-05	0.39196
5	0.000163238	6.2325	**20**	9.81998E-06	0.37493
6	0.000106542	4.0679	**21**	7.04139E-06	0.26884
7	8.27837E-05	3.1607	**22**	6.94329E-06	0.2651
8	6.40332E-05	2.4448	**23**	5.6035E-06	0.21395
9	5.68579E-05	2.1709	**24**	5.54077E-06	0.21155
10	4.13401E-05	1.5784	**25**	5.04472E-06	0.19261
11	3.85708E-05	1.4727	**26**	4.65318E-06	0.17766
12	3.32541E-05	1.2697	**27**	4.43753E-06	0.16943
13	2.63544E-05	1.0062	**28**	3.35491E-06	0.12809
14	2.30908E-05	0.88162	**29**	2.65676E-06	0.10144
15	1.82981E-05	0.69863	**30**	2.52151E-06	0.096273

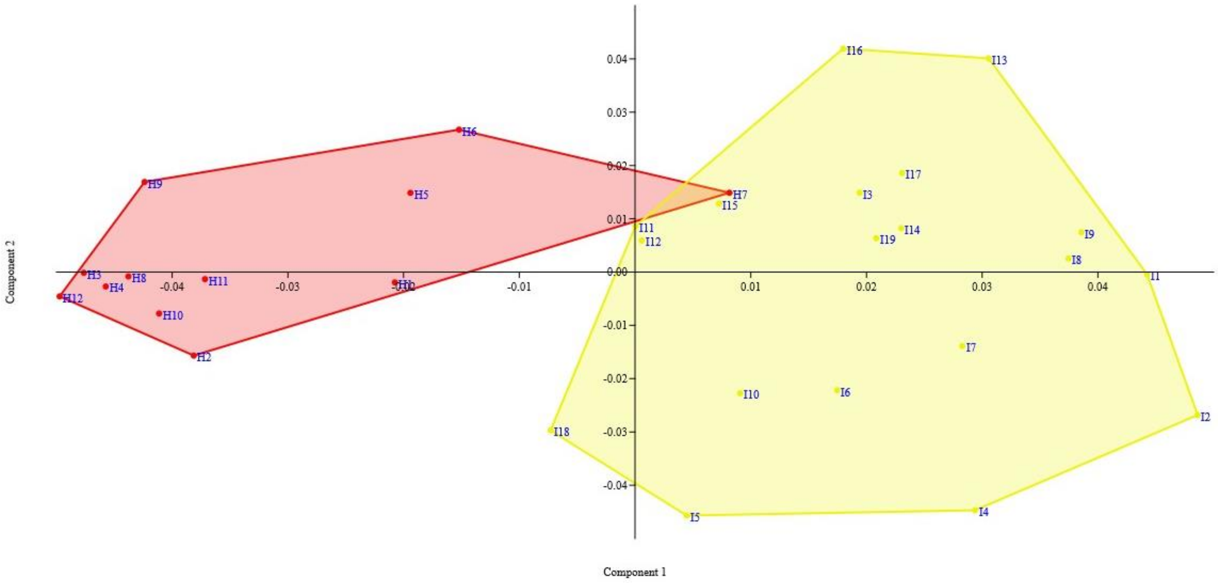

Figure 2. The distribution of individuals on the graph is based on the first principal component, Red: Hamdani (H), Yellow: Awassi (I), Individuals H1-H6 and I1-I9 are Female, H7-H12 and I10-I19 are Male.

Figure 3 shows the graph obtained as a result of the test performed on the Procrustes coordinates in order to show the proximity of the samples. While the samples were mostly grouped according to the breed factor, no significant proximity occurred according to the sex factor.

Regression analysis of shape over the centroid size (PCs) determined that 8.6402% of shape oversize was estimated according to the breed and 16.1168% according to the sex. This was significant at a confidence interval of 95% for both breed and sex (P: 0.0030 for the breed, P: 0.0002 for sex). In terms of breed factor, 38.8077% of the shape identified by PC1 was estimated by size (P: 0.0002). The shape identified by PC2 and estimated by size was smaller and insignificant in terms of this factor (6.1267%, P = 0.1733). The same values were determined as 38.2229% (P: 0.0004) and 7.8924% (P: 0.1309) for PC1 and PC2 according to the sex factor. According to these results, it was determined that the shape variations of the mandible according to the breed and sex factors used in the study did not depend on the size and thus there was no significant allometric component.

Figure 4 shows graphs showing at which semilandmarks the shape differences are concentrated. The locations where the shape differences concentrated in terms of PC1 are the attachment sites of teeth to the jaw bone (SLM128-132, SLM12-32), section starting from the

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second molar tooth to the ramus mandibulae (SLM31-49), tip and caudal edge of processus coronoideus (SLM58-68), and angulus mandible including incisura vasorum facialium (SLM84-109). Among these concentration areas, the most significant semilandmark range was between SLM101-111 and SLM30-42. Thus, the high PC1 ventral edge defines the mandibles as having a significant convex line. PC1 also defines the mandibles with a shape deformity on the dental arch as of the last molar tooth and on the anterior margin of the ramus mandible. In terms of PC2, shape differences have completely concentrated on the mandibular dental arch level and the continuation of this arch towards ramus mandibulae (SLM1-43). Thus, PC2 only defines the mandibles with significant ventrocaudal (glenoid) edge at the anterior margin of the ramus mandibulae.

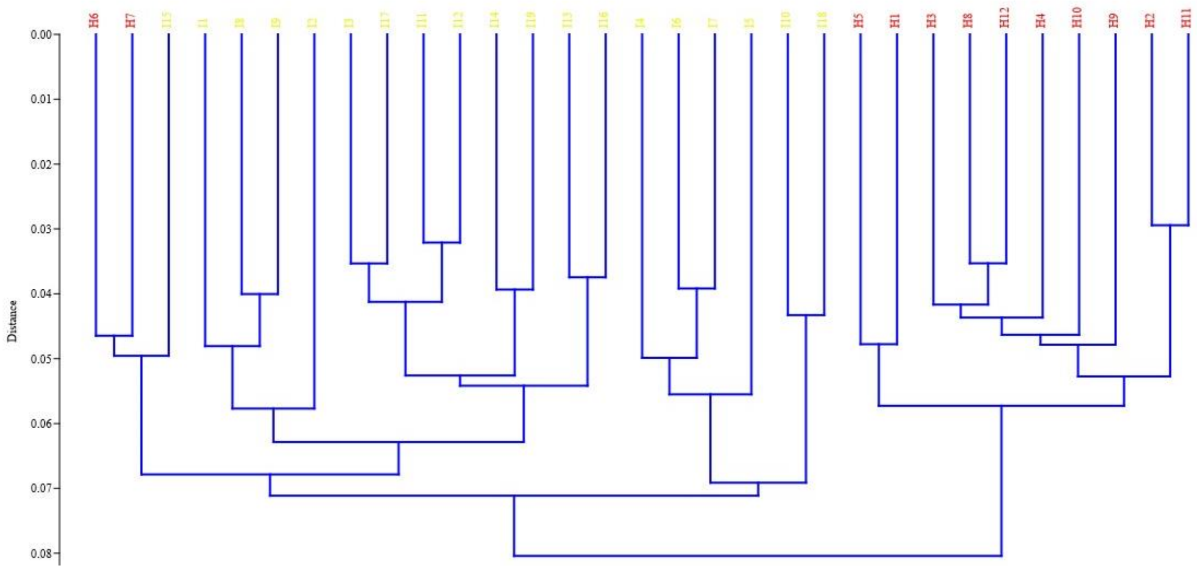

Figure 3. Graphical representation of hierarchical closeness of individuals, Red: Hamdani (H), Yellow: Awassi (I), H1-H6 and I1-I9 individuals are female, H7-H12 and I10-I19 individuals are male.

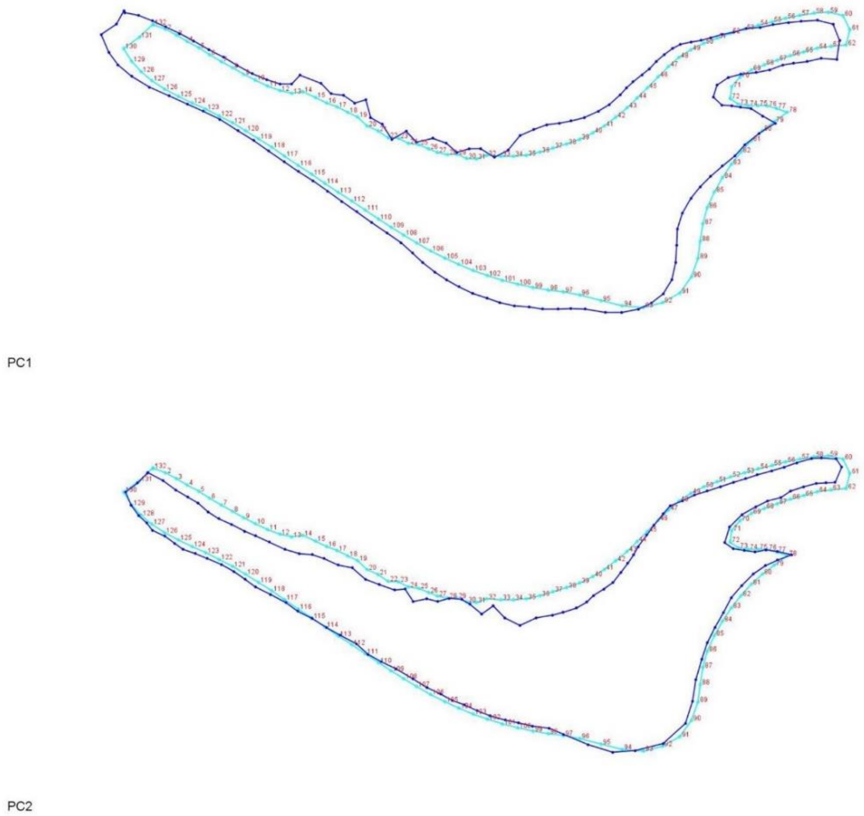

Figure 4. Wireframe graphical representation of shape differences concerning PC1 and PC2. Dark blue represents the positive bounds of principal component scores.

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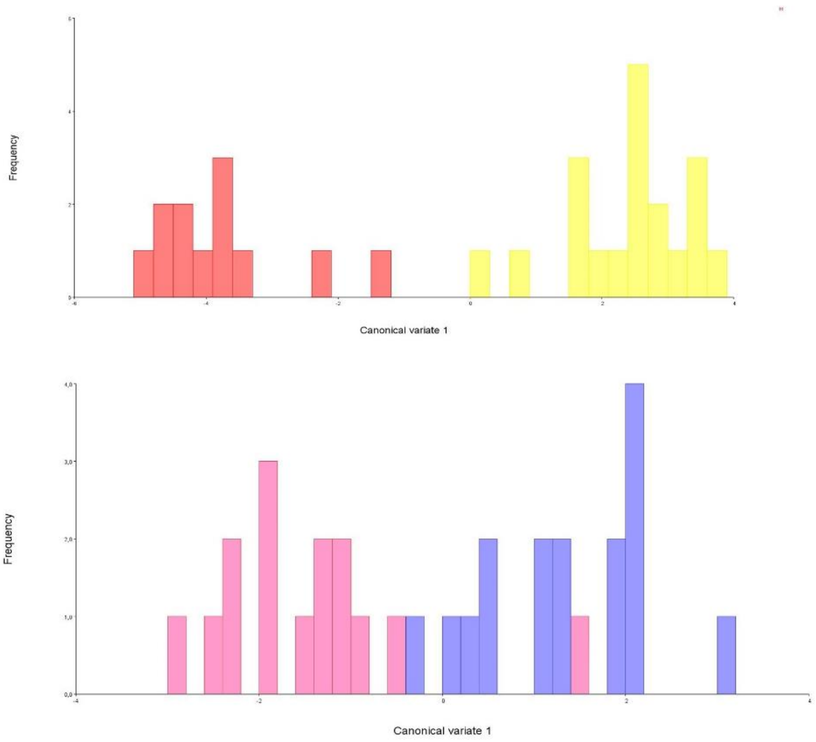

Figure 5. Wire-frame warp and frequency graphs of the mandible by breed (A) and sex (B). Red is Hamdani, yellow is Awassi for the breed. Pink is female and blue is male for sex.

Canonical variance analysis defined the sheep mandibles in a canonic variable in terms of breed or sex factors. Shape variations according to CV1 were similar to the anatomical points defined according to PC1. Table 2 shows Mahalanobis and Procrustes distances values according to breed and sex. Accordingly, distribution difference in terms of breed (<0.0001) showed a significant superiority compared to sex (0.1496).

Table 2. Mahalanobis distances (MD), Procrustes distances (PD) and P-value for PD (from permutation tests, 10000 permutation rounds) between sheep mandibles.

Breed			Sex		
MD	PD	P-value	MD	PD	P-value
6.1825	0.0543	<0.0001	2.7481	0.0224	0.1496

The wire-frame warp graph of sheep mandibles in Figure 5 shows the shape differences and frequencies in terms of breed and sex. While the frequencies were homogeneously distributed according to breed, no homogeneous distribution was found according to sex. In the breed-based comparison, it was found that the mandible of Hamdani sheep had a higher body than the mandible of Awassi sheep. In the sex-based comparison, the mandible of male sheep was found to be more voluminous than female sheep, especially in the body area.

Discussion and Conclusion

The development process of the mandible depends on various factors such as the growth hormones, growth factors, breed, and mechanical stress (13). In the study, the sheep mandible depending on breed and sex (hormonal) factors were analyzed by using semilandmarks. The most significant limitation of the study was the failure to standardize all the factors affecting the development of the mandible. This limitation can be neglected according to the scientific study methodology.

In their study, Pares-Casanova (21) examined allometry in the mandible of domestic sheep. In the present study, it was stated that the first three principal components explained 77.5% of the total shape variation. In the study, the mandible shape differences were primarily caused by the extraction of molar teeth with age and allometry in the margo ventralis . In the present study, the first three principal components explained 63.822% of the total shape variation. The shape differences were most significant in the arcus dentalis (premolar, molar), the section starting from the second molar tooth towards ramus mandibulae,processus coronoideus and angulus mandible. In addition, most of the shape variations according to the breed and sex factors of the sheep did not depend on the size and no significant allometric component was found. The researcher (21) suggested that the points showing variation in the mandible may be associated with a certain morphofunctional difference since they correspond to the adhesion sites of significant

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masticatory muscles. The findings obtained in the present study also support this opinion.

Sexual dimorphism is common in different animal groups including goats and sheep (24). Rensch's rule defines the sexual dimorphism model by asserting that for large-sized species there is often a more significant male body size than female (7, 24). Demiraslan et al. (5) stated that sexual dimorphism was present in the mandibles of Honamlı and hair goats. Likewise, it has been highlighted that sexual dimorphism is not seen in the mandibles of Anatolian wild (32) and Awassi sheep (6).

Understanding the differences related to sex is important to learning ecology, behavior, generational mobility, and evolution (15). In the literature (18), it is stated that sexual dimorphism is important in sheep. Also, it is important to extensively analyze the features other than the cranium and mandible shape and the presence of the horn in terms of sexual dimorphism (6). In the present study, the curves affecting the general shape of the sheep mandible were analyzed by semilandmarks in order to see the details. In Iranian fallow deer (17) and Awassi sheep (6), the significant differences in the mandibles of male and female individuals are present at angulus mandibulae and molar teeth arch levels. In the current study, shape differences by sex were mostly concentrated on margo ventralis mandibulae and molar teeth arch level.

In Anatolian Wild and Akkaraman sheep, first-degree shape differences took place at LM1, 3, 8, 9 and 10 (32). In Honamlı and hair goats, first-degree shape differences were observed at LM4, 7, 8, 9, and 10. In both studies, as in the current study, the first-degree differences represented arcus dentalis, processus coronoideus, and angulus mandible. This data showed that shape differences of the mandible by species or breed factor concentrated on specific points.

The geometric morphometric method can be used to reveal the phylogenetic relationships from the cranium or mandible of current mammalian forms (16). The data of this study can be used for estimation of the morphologic properties, fauna determination, or some socio-economic implications in ancient period mammals (4, 8, 20, 22). As a result of the study, it was detected that the general shape of the sheep mandible was significantly affected, especially from the breed factor by using semilandmark. In addition, detailed shape analysis was performed on the mandibles of Hamdani and Awassi sheep, which are also remarkable in terms of geographical proximity. Consequently, it is thought that the data obtained as a result of the study would serve as a reference for the ruminant mandible remains obtained from archaeological excavations, especially in Mesopotamia region.

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Conflict of Interest

The authors declared that there is no conflict of interest.

Author Contributions

YD and ID conceived and planned the experiments. YD and ID carried out the experiments. YD and ID planned and carried out the simulations. BCG contributed to sample preparation. YD, ID and BCG contributed to the interpretation of the results. YD, ID and BCG took the lead in writing the manuscript. All authors provided critical feedback and helped shape the research, analysis and manuscript.

Data Availability Statement

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

Ethical Statement

This study was approved by the Harran University Animal Experiments Local Ethics Committee (28.03.2022/01-13).

Animal Welfare

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

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