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Parentage Verification and Genetic Characterization of Dromedary Camels Using Molecular Markers

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This study assesses the use of ten microsatellite markers for parentage testing in Saudi Arabian dromedary camel population. A total of 216 camels from various local breeds were investigated, employing microsatellite markers endorsed by the International Society for Animal Genetics (ISAG). Multiplex PCR with fluorescently labeled primers was utilized for marker amplification, followed by capillary electrophoresis on a genetic analyzer for analysis. Data analysis was conducted using Genepop, CERVUS, and GenAlEx software packages. Across the markers, 59 alleles were amplified, with nine loci exhibiting polymorphism. Allelic diversity ranged from 2 (in LCA65 and YWLL29) to 16 (in LCA99), averaging 6.444 alleles per locus. The population demonstrated a mean expected heterozygosity (He) of 0.46, with values ranging from 0.05 to 0.83. Polymorphic Information Content (PIC) values ranged from 0.049 to 0.812, with an average of 0.4667. Notably, the findings indicate a low probability of identity (2.4E-06) and high parentage exclusion probabilities, both for one parent (0.98) and for both parents (0.999999). These results suggest that the selected microsatellite markers are robust and suitable for parentage testing in dromedary camel.

Keywords: microsatellite; dromedary camel; parentage verification; camel breeding

INTRODUCTION

Dromedary camels, renowned for their cultural significance and multifaceted economic contributions, play pivotal roles in transportation, food production, and sports across various regions, particularly in the Middle East (Ahmad et al. 2010; Gagaoua et al. 2022; Yam & Khomeiri, 2023). Their domestication, traced back to approximately 3000 B.C. in the Arabian Peninsula, underscores their historical and contemporary importance within the livestock sector (Burger, 2016; Yam & Khomeiri, 2023). Camel racing, a prominent industry in the Middle East and beyond, further highlights their enduring cultural relevance and economic value (Khalaf, 1999a).

In the Kingdom of Saudi Arabia, where numerous camel competitions are held, the prestige associated with winning top honors translates into substantial monetary rewards, elevating the market worth of champion camels and their progeny (Bornstein, 2021; Khalaf, 1999b). Consequently, ensuring accurate determination of parentage is essential for maintaining the integrity of camel breeding programs and safeguarding against fraudulent claims (Al-Swailem et al. 2008).

In this context, parentage testing emerges as a crucial tool for verifying the biological relationships between individuals, facilitating informed decision-making in breeding programs, and preserving genetic diversity within dromedary camel populations. Within the field of genetic research, microsatellites, also known as Short Tandem Repeats (STRs), have garnered widespread acclaim for their utility in genetic characterization and parentage analysis(Oliveira et al. 2006).

Microsatellite markers are short, repetitive DNA sequences characterized by variable lengths among individuals. The inheritance of these markers follows a Mendelian co-dominant pattern, rendering them particularly amenable to polymerase chain reaction (PCR)-based studies. The discriminatory power of microsatellites allows for precise determination of genetic relatedness among individuals by comparing the unique allelic patterns present in their DNA (Jarne & Lagoda, 1996; Oliveira et al. 2006; Zane et al. 2002).

Numerous studies, such as those by (Misrianti et al. 2022), (Lai et al. 2022), (Xu et al. 2022) and (Sartika et al. 2023), underscore the efficacy of microsatellite markers in parentage testing across various species. Specifically within dromedary camels, microsatellite markers have demonstrated high accuracy in delineating genetic

relationships and establishing parentage with confidence (Mahmoud et al. 2020; Prasad et al. 2014; Sharma et al. 2020; Spencer & Woolnough, 2010). By leveraging the robustness of microsatellite-based analyses, this study aims to evaluate the suitability of selected markers for parentage testing in Saudi Arabian dromedary camels, thereby contributing to the advancement of genetic research and breeding practices in this economically and culturally significant species.

MATERIALS AND METHODS

2.1. Sampling and DNA extraction:

Sampling and DNA extraction procedures were conducted on a total of 216 dromedary camels, comprising representatives from various indigenous breeds, as well as 21 sires, 48 dams, and 147 progenies. Blood samples were obtained for genetic analysis. Specifically, DNA extraction was performed using the Maxwell RSC Blood DNA Kit (Promega, USA - Cat. No. AS1400), with 300 µl of whole blood utilized for this purpose.

2.2. Microsatellite genotyping:

The dromedary camels under study were subjected to genotyping utilizing a selection of ten microsatellite (STR) markers, as recommended by the International Society for Animal Genetics (ISAG) as shown in Table 1. This panel of STR markers, crucial for parentage testing in camels, was meticulously designed, developed, and employed within the Genome Laboratory of the Ministry of Environment, Water, and Agriculture in Riyadh, Saudi Arabia. To facilitate efficient genotyping, the STR marker panels were organized into four sets of fluorescently labeled primers. Analysis of the microsatellite markers was conducted using the ABI 3500XL genetic analyzer (Applied Biosystems, Thermofisher Scientific, Waltham, MA, USA).

2.3. PCR amplification and fragment analysis

PCR amplification was conducted in a reaction volume of 25 µl, comprising 1.0 µl of genomic DNA, 1.8 µl of forward and reverse primer mix, 12 µl of Basic PCR Master Mix, and 0.2 µl of SmarTaq polymerase. The amplification protocol was executed using a programmable thermal cycler (Bio-Rad Laboratories, Inc., USA) with the following cycling conditions: initial denaturation at 96°C for 10 minutes, followed by 32 cycles of denaturation at 96°C for 30 seconds, annealing at 60°C for 40 seconds, elongation at 72°C for 1 minute, and a final extension at 72°C for 10 minutes, followed by cooling to 4°C. Subsequent to amplification, PCR products were mixed with Hi-Di Formamide (Applied Biosystems, USA) and a Genescan Liz-500 size standard (Applied Biosystems, USA). The mixture was subjected to denaturation at 95°C for 2 minutes, followed by immediate placement on ice for 3 minutes. Fragment separation was accomplished via capillary electrophoresis utilizing an ABI 3500XL Genetic Analyzer (Applied Biosystems). Finally, fragment analysis was performed using GeneMapper® Software 5 (Applied Biosystems).

Table 1: Characteristics of the microsatellite loci

Locus	Primer sequences (5' – 3')	size range (bp)	Accession No/ reference
LCA19	F: TAAGTCCAGCCCCACACTCA R: GGTGAAGGGGCTTGATCTTC	75 – 85	Penedo *et al.*, (1998)
LCA56	F: ATGGTGTTTACAGGGCGTTG R: GCATTACTGAAAAGCCCAGG	125 – 139	AF091122
LCA66	F: GTGCAGCGTCCAAATAGTCA R: CCAGCATCGTCCAGTATTCA	231 – 255	Penedo *et al.,* (1998)
LCA37	F: AAACCTAATTACCTCCCCCA R: CCATGTAGTTGCAGGACACG	129 – 141	AF060105
LCA99	F: CAGGTATCAGGAGACGGGCT R: AGCATTTATCAAGGAACACCAGC	232 – 334	La Manna *et al.,* (2011)
LGU49	F: TCTAGGTCCATCCCTGTTGC R: GTGCTGGAATAGTGCCCAGT	214 – 262	Sarno *et al.,* (2000)
LCA65	F: TTTTTCCCCTGTGGTTGAAT R: AACTCAGCTGTTGTCAGGGG	160 – 172	AF091124
LCA8	F: GCTGAACCACAATGCAAAGA R: AATGCAGATGTGCCTCAGTT	228 – 240	Penedo *et al.,* (1998)
YWLL44	F: CTCAACAATGCTAGACCTTGG R: GAGAACACAGGCTGGTGAATA	85 – 114	Lang *et al.,* (1996)
YWLL29	F: GAAGGCAGGAGAAAAGGTAG R: CAGAGGCTTAATAACTTGCAG	203 – 215	Mehta *et al.,* (2007)

Statistical analysis:

Genetic data from ten microsatellite loci within the Saudi Arabian dromedary camel population underwent rigorous statistical analysis utilizing various software tools. Key population genetic parameters such as the number of alleles per locus (No), effective number of

alleles (Ne), allelic frequencies, observed heterozygosity (Ho), expected heterozygosity (He), probability of identity, sibling identity, and the combined power of exclusion (CPE) were computed using GenAlEx 6.503 software (Peakall & Smouse, 2012).

Polymorphic Information Content (PIC) and likelihood ratios for accurate parentage assignment were determined employing CERVUS version 3 software (Kalinowski et al. 2007; Marshall et al. 1998). This facilitated the assessment of correct parentage assignment for each offspring by calculating likelihood ratio (LOD) scores for potential parents and determining the two most probable parents based on delta scores (Δ). Parentage assignment was undertaken with a confidence level of 95%.

Furthermore, GenAlEx 6.503 (Peakall & Smouse, 2012)was utilized to estimate probabilities of identity (PI) and exclusion (PE) based on allele frequencies, crucial for assessing genetic diversity and parentage certainty within the population. Finally, Genepop version 4.8.3 (Rousset, 2008) provided a comprehensive analysis of diversity metrics, contributing to a deeper understanding of the genetic structure and diversity of the dromedary camel population under investigation.

RESULTS

3.1. Allele frequency and polymorphism

Genotyping analysis was conducted on 216 dromedary camels utilizing ten microsatellite loci, revealing nine polymorphic loci and one monomorphic locus (LCA19) within the studied cohort (Table 2). The polymorphic loci exhibited a range of allele numbers, from 2 in LCA65 and YWLL29 to 16 in LCA99, with a mean value of 5.9. Effective Alleles (Ne) varied from 1.053 in LCA65 to 5.88 in LCA99, averaging 2.58 across all loci.

Observed heterozygosity (Ho) levels spanned from 0.04 in LCA65 to 0.78 in LCA99, with a mean value of 0.39 for polymorphic loci, while expected heterozygosity (He) values ranged from 0.05 in LCA65 to 0.83 in LCA99, averaging 0.46. Polymorphic Information Content (PIC) values ranged from 0.049 in LCA65 to 0.812 in LCA99, with a mean value of 0.42 (Table 2).

Four loci demonstrated heightened degrees of polymorphism, notably YWLL44, LCA66, LGU49, and LCA99, with PIC values ranging from 0.558 to 0.812. Additionally, four loci, YWLL29, LCA56, LCA37, and LCA8, exhibited intermediate PIC values ranging from 0.274 to 0.386, while LCA65 demonstrated a low degree of polymorphism with a PIC value of 0.049 (Table 2).

The Combined Probability of Identity (CPI) was computed to assess the likelihood of two individuals sharing the same DNA profile by chance. CPI values were estimated to be 4.8×10^{-6}, 1.5×10^{-5}, and 1.6×10^{-3} for 9, 7, and 4 loci, respectively, indicating a low probability of random genetic identity. Moreover, CPI values were calculated to be 6.4×10^{-3}, 1.1×10^{-2}, and 8.7×10^{-2} for 9, 7, and 4 loci, respectively, in scenarios where all individuals are full siblings (Table 2).

Notably, two genetic loci, LCA37 and LCA56, displayed significant deviations from the Hardy-Weinberg Equilibrium (HWE), while the remaining loci adhered to expected HWE patterns post Bonferroni adjustments. The frequency of null alleles ranged from 0.0236 (LGU49) to 0.7097 (LCA37), with an average frequency of 0.126 (Table 2).

Table 2: Polymorphism statistics of microsatellite loci.

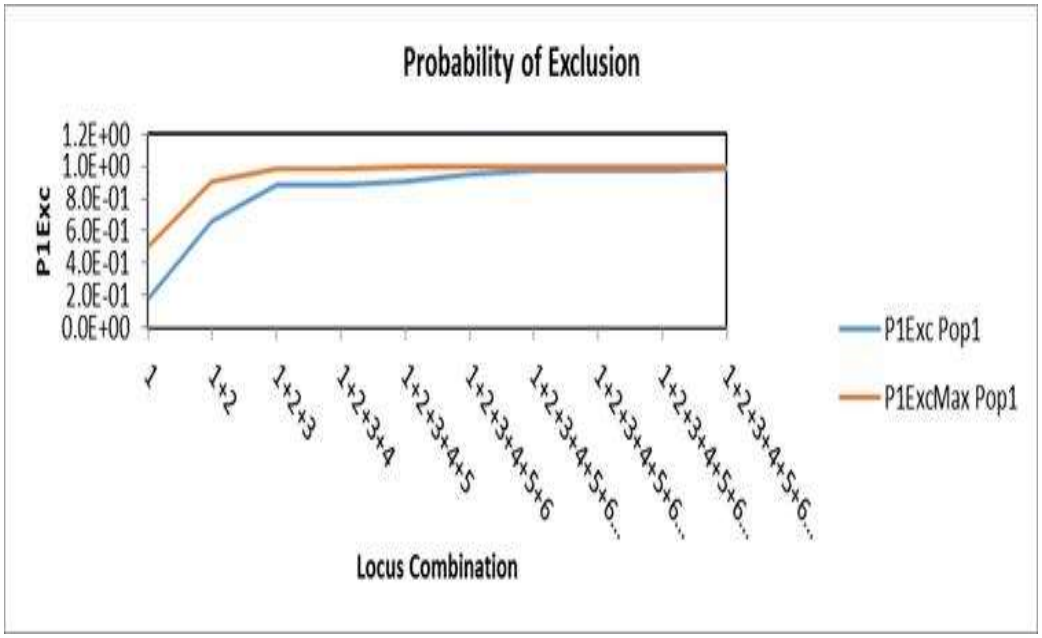
Locus	Na	Ne	Ho	He	PIC	F(Null)	HW
LCA37	4	1.664	0.069	0.399	0.341	0.7097	***
LGU49	11	4.451	0.736	0.775	0.746	0.0236	NS
LCA99	16	5.882	0.782	0.830	0.812	0.0325	NS
LCA56	3	1.655	0.324	0.396	0.326	0.1084	*
LCA66	7	3.995	0.685	0.750	0.708	0.0443	NS
YWLL44	8	2.603	0.528	0.616	0.558	0.0793	NS
LCA65	2	1.053	0.042	0.050	0.049	0.0850	NS
LCA8	5	1.998	0.472	0.499	0.386	0.0290	NS
YWLL29	2	1.486	0.310	0.327	0.274	0.0266	NS
Mean±SE	6.444±1.6	2.754±0.5	0.439±0.1	0.516±0.1	0.4667±0.1	0.126±0.1	

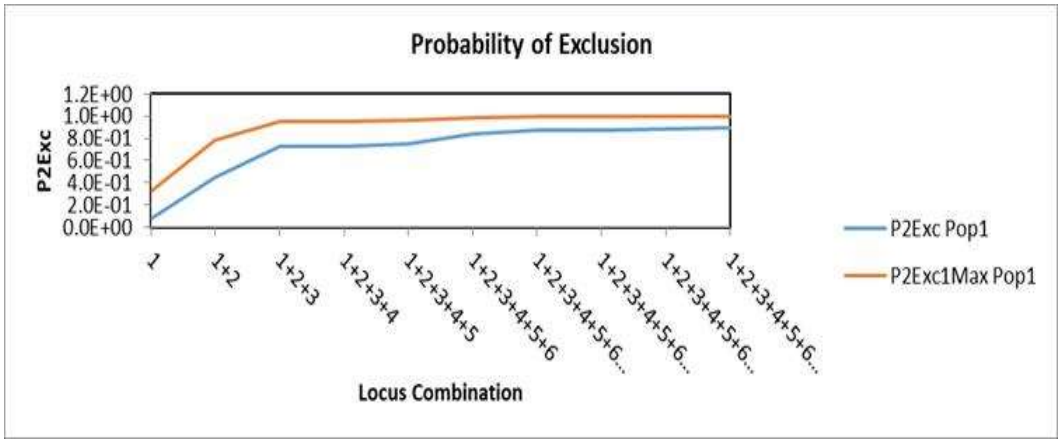
The robustness of the microsatellite markers for parentage analysis was evident through the observed high exclusion probabilities and genetic variability. The PE1 values (denoting one known parent) across the 9 loci ranged from 0.024 (LCA65) to 0.676 (LCA99), averaging at 0.317 (Table 3). Conversely, the mean PE2 values

(indicating no known parents) stood at 0.2 across the 9 loci, with individual values spanning from 0.001 (LCA65) to 0.508 (LCA99). The relatively higher PE1 values compared to PE2 suggested that additional maternal information aids in the exclusion of erroneous parentage. Notably, the average PE3 value was 0.444 for the 9 loci, with a range from 0.047 (LCA65) to 0.856. Significantly, the combined exclusion probability for combinations of up to 8 markers peaked at 0.998 for PE3, affirming parentage with a high degree of certainty (Figure 1)

Tabe3: Probability of identity (PI) and probability of exclusion (PE) of microsatellite markers

Locus	PI	PE1	PE2	PE3
LCA37	0.419	0.1840	0.0797	**0.2945**
LGU49	0.080	0.5818	0.4030	**0.7735**
LCA99	0.047	0.6766	0.5089	**0.8566**
LCA56	0.435	0.1684	0.0783	**0.2650**
LCA66	0.104	0.5223	0.3458	**0.7053**
YWLL44	0.205	0.3636	0.2071	**0.5364**
LCA65	0.904	0.0244	0.0013	**0.0470**
LCA8	0.364	0.1993	0.1248	**0.3009**
YWLL29	0.506	0.1368	0.0535	**0.2196**





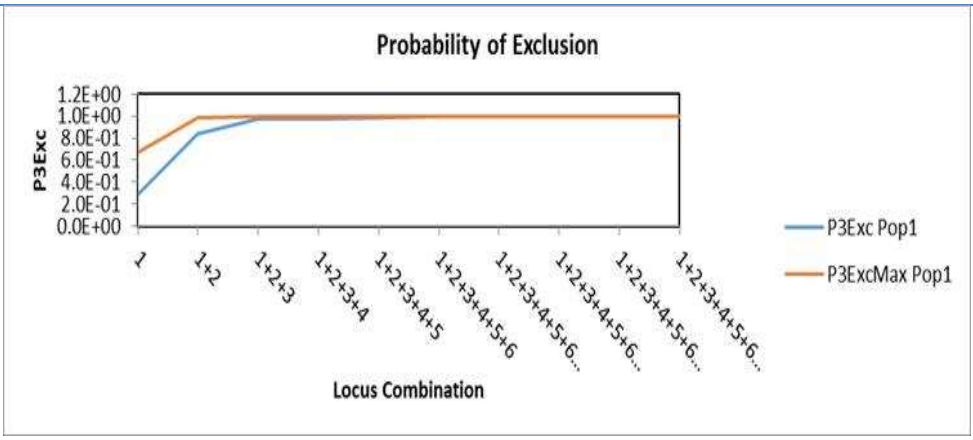


Figure 1: Probability of Exclusion at (a)PE1, (b)PE2, and (c)PE3 levels of microsatellite markers used for parentage verifications in dromedary camels.

In terms of maternity, LOD values for microsatellite pairs varied from 0.896 to 8.27, while delta values ranged from 0.296 to 8.06, accurately assigning mothers. For paternity, LOD values for microsatellite pairs ranged from 1.09 to 7.489, with corresponding delta values spanning from 0.05 to 7.489, indicating precise assignment of offspring to their fathers. Furthermore, the combined non-exclusion probability for parent pairs approached zero (0.00119), underscoring a dependable approach for correctly establishing the parentage of offspring with their respective sires and dams.

DISCUSSION

Parentage testing for dromedary camels in the Kingdom of Saudi Arabia has become of great importance due to the significant role that dromedary camels play in the country, particularly regarding food, racing, and exhibitions (Al-Swailem et al. 2008; Bornstein, 2021; Khalaf, 1999b). Parentage testing provides crucial information that contributes significantly to the breeding programs of dromedary camels and legal aspects related to buying and selling distinguished camels with high market value(Al-Swailem et al. 2008).

Therefore, it was essential to test the power of the markers used in the parentage testing to achieve a more accurate genotype and more reliable results. Most camel populations have been researched using markers ranging from 3 to 23 loci (Vijh et al. 2007) whereas most research has been conducted using 10–15 microsatellite loci (Vijh et al. 2012). Among the 10 loci selected for the present study, 5 loci were common with Emirati, African, Bactrian, and Australian camels (Spencer & Woolnough, 2010) , 3 with Indian camels (Vijh et al. 2012), 5 with Iranian camels (Hedayat-Evrigh et al. 2018), All loci were common with the study on Qatari camels (Radwan et al. 2020).

The range of alleles per locus (2–16) demonstrated the rich genetic diversity of the sampled population. The mean number of alleles (6.4) was higher than that of Bactrian (4.35), lower than that of Australia (10.59), and similar to that of Emirati (6.06) and African (6.00) (Spencer et al. 2010). It was found to be comparatively higher than Omani (5.4), and Pakistani (3.9), by (Hashim et al. 2014). Furthermore, it was found to be higher than that of Qatari camels in the same loci (5.8) and lower than that in all loci (8.13) by (Radwan et al. 2020).

The mean Ho value (0.439) was nearly equivalent to that reported for Australian (0.45), Emirati (0.452), African (0.463) (Spencer et al. 2010), Tunisian (0.46) (Ould Ahmed et al. 2010), and Bactrian camels (0.402) (Ming et al. 2019), and lower than those reported for Iranian (0.74) dromedaries (Hedayat-Evrigh et al. 2018), and Qatari (0.555) (Radwan et al. 2020).

The estimated mean He (0.516) value was near that reported for Australian (0.530), Emirati (0.531), and lower than those reported for African (0.646) (Spencer & Woolnough, 2010) Tunisian (0.60) (Ould Ahmed et al. 2010), Iranian (0.86) dromedaries (Hedayat-Evrigh et al. 2018), Bactrian (0.543) (Ming et al. 2019), and Qatari (0.562) (Radwan et al. 2020).

The average PIC value (0.4667) was nearly equivalent to that reported for Emarati (0.4908), Australian (0.4946), and Bactrian (0.4807) by (Spencer et al. 2010), and slightly lower than that of Qatari camels by (Radwan et al. 2020).

The polymorphic information content (PIC) values of these loci, especially in markers like YWLL44, LCA66, LGU49, and LCA99, highlighted their high polymorphism.

Notably, the locus LCA19 was monomorphic. Deviations from the Hardy-Weinberg Equilibrium (HWE) were observed in two loci (LCA37 and LCA56) even after Bonferroni corrections, which may be due to genotyping errors and reduced heterozygosity, and the frequency of the null allele in these two loci was high.

The high frequencies of null alleles lead to high rates of genotyping errors in heterozygotes, resulting in incorrect exclusions of dam-offspring or sire-offspring pairs, and, according to (Marshall et al. 1998), should not be used for paternity testing as they tend to have reduced

heterozygosity. The high probability of exclusion (PE) values demonstrated the successful use of the chosen microsatellite markers for parentage testing in dromedary camels.

The probability of exclusion (PE) is a measure of the ability of a certain panel of markers to identify genetic paternity, excluding all other candidates (Zhang et al. 2010). The effectiveness of the selected microsatellite markers in parentage testing was confirmed by high probability of exclusion (PE) values, which ranged from 0.024 to 0.676, emphasizing the efficacy of the marker panel in differentiating between potential sires. The LOD values in maternity and paternity analysis, along with the delta values, underscored the reliability of the markers in correctly assigning parentage.

The combined non-exclusion probability for a parent pair approaching zero (0.00119) is a testament to the robustness of the chosen loci in accurately matching offspring with their sires and dams. The higher PE1 values compared to PE2 further highlight the added value of maternal information in excluding false sires.

The information gained from this study has significant implications for dromedary camel breeding programs in Saudi Arabia. The identified polymorphic microsatellite markers provide a powerful tool for accurate parentage testing and are essential for maintaining and enhancing desirable genetic traits within the camel population. The markers with high polymorphic information content (PIC) values, such as YWLL44, LCA66, LGU49, and LCA99, can be particularly valuable for maximizing genetic diversity.

CONCLUSIONS

In conclusion, it can be concluded that a multiplex microsatellite panel consisting of 10 loci has been successfully validated for its use in paternity studies in dromedary camels. It is a fast, robust, reliable, and economic tool to verify the parentage as well as to assign the putative sire to daughters. This study suggests the need to expand the number of microsatellite loci to more loci recommended by ISAG to increase the reliability of parentage assignment.

Author contributions

Conceptualization, M.H.A.; M.A.A.; A.A., and F.M.A; validation, M.A.A., S.Y.W., A.A. and F.M.A.; formal analysis, F.M.A., A.A.,S.Y.W. and A.A.F.M.; investigation, F.M.A., and M.A.A.; resources, M.H.A., and F.M.A.; writing—original draft preparation, F.M.A., A.A.F.M., A.A., and M.A.A.; writing—review and editing, M.H.A. and A.F.A.; supervision, M.H.A. and A.F.A.; project administration, M.H.A.; funding acquisition, M.H.A., and F.M.A. All authors have read and agreed to the published version of the manuscript.

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Institutional Review Board Statement

The animal study protocol was approved by the Research Ethics Committee of the King Saud University, Riyadh, Saudi Arabia (Ethic Reference No: KSU-SE-23-93).

Informed Consent Statement

Not applicable.

Data Availability Statement

All of the relevant data are presented in the paper

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Conflict of interest

The authors declare no conflict of interest.

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