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Integrating morphometric traits, body scores, and SNP markers to evaluate dromedary camels for production traits

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Abstract The investigation presents a pragmatic methodology for assessing camels based on morphometric characteristics, body scores, and genomic information. The study comprised 87 dairy camels, including 62 Pakistani and 25 Iranian she-camels, which were assessed by three experts using 23 morphometric measurements, with scores out of 100 being awarded. In addition, genetic evaluation was conducted using markers associated with growth traits, with 60 SNPs being genotyped in 22 elite male calves. Pin width, depth, and chest circumference were found to be significantly greater in Pakistani she-camels (mean body score of 76.71 ± 12.31) compared to Iranian she-camels (mean body score of 57.12 ± 10.03) ($p<0.05$). The two she-camel groups were separated into distinct clusters based on principal component analysis of the morphometric characteristics. Moreover, the genetic analysis of SNPs (25% of the investigated records) confirmed that genetic differentiation between the two groups in terms of growth traits was significantly different ($p<0.05$). As a practical approach, the male calves were first evaluated based on maternal body score and milk ability; they were then evaluated based on growth-related SNPs. The findings suggest that morphometric data and body scores can be a suitable approach for evaluating dromedaries for milk production in extensive and semi-extensive systems. Additionally, the selection of male camels based on genetic markers related to growth traits can be effective due to natural mating.

Keywords: body score, dromedary, growth, morphometric traits, SNP

Introduction

Camels possess inexhaustible prospects for forthcoming human sustenance and medical demands of their unparalleled attributes. Their meat, milk, and products have been recognized to offer diverse health advantages (Ali et al. 2019; Kadim et al. 2022). Camels exhibit an exceptional genetic capacity for milk synthesis, characterized by an extended lactation period. This feature emphasizes the relevance of camel milk production, particularly in arid regions where camels constitute the exclusive source of milk (El-Hanafy et al., 2023). Estimating daily milk production of camels in extensive and semi-extensive systems poses a significant challenge. This is due to the diverse factors influencing camel milk production, such as age, breed, management conditions, nutrition, and lactation stage (Faraz et al., 2021). Although camels are an

essential food source in arid and semi-arid regions, their milk production potential has not been fully exploited until recently (Nagy et al., 2022). Under the same harsh conditions, dromedary camels produce more milk for longer than other animals. The produced milk is a primary food, nutrition, and income security source for some pastoralists throughout the year (Oselu et al., 2022). Due to its unique characteristics, the camel is considered an animal with unlimited potential for future human food and medical needs; therefore, it is necessary to investigate its production potential in its natural habitat, as the camel has an excellent genetic potential for producing milk with a more extended lactation compared to other ruminants (Bitaraf Sani et al., 2023). Camel milk production, especially in desert areas where camels are the only source of milk, is of great importance (Bitaraf Sani et al., 2022). Because of the breeding system in rangeland, estimating the daily milk production of camels in extensive and semi-extensive systems is very difficult. The phenotypic and performance traits of dairy camels hold immense significance in their evaluation, wherein body scores serve as a crucial parameter for breeding programs, as posited by Dioli et al. in 2016. The primary focus of camel breeders for genetic enhancement is on producing a dual-purpose animal conducive to meat and milk production (Dioli et al., 2011). The camel population of Iran has decreased in the last sixty years, and most imported camels are entered unofficially from neighboring countries.

On the other hand, camel owners in Iran tend to use them to improve their herds genetically. To introduce certification, it is necessary to introduce male camels based on morphometric traits and genetic markers for crossbreeding to increase the milking ability of Iranian dromedaries using Pakistani bull camels and production of Pakistani-Iranian F1. Due to difficulties in recording and phenotyping camels, using body condition score is a good criterion for evaluating dromedaries in extensive systems. BCS correlates to milk production and growth traits, so using them effectively improves performance. On the other hand, using genetic markers can effectively address the challenges of camel breeding. Understanding genetic factors influencing economic traits is crucial for accurate Selection and genetic improvement. Growth traits, especially birth and weaning weight and gain per day, are economically critical traits and necessary for planning a successful camel breeding program (Bitaraf Sani et al., 2021). The genetic markers reported by Bitaraf Sani et al. (2021) can effectively improve growth-related traits. Using the GBS technique, they genotyped ~15K SNPs for dromedaries and identified a list of SNPs correlated with birth weight, body weight, and growth (Bitaraf Sani et al., 2021). We endeavored to assess camels utilizing morphometric features and genomic information to identify the most superior bull camels for future breeding. So far, very few studies have been reported on the use of morphometric and genomic data for the evaluation of camels, and this is the first report that utilizes body scores and genetic markers to evaluate camels.

Methods and materials

All animal procedures were performed per the guidelines and regulations proposed by the Animal Science Research Institute of Iran. All experimental protocols were approved by the Animal Science Research Institute of Iran under ASRI-34–64-1357–005-970,180. Blood samples were collected from the jugular vein in EDTA tubes during qualified veterinary treatment on the pasture within the framework of governmental programs aimed at animal identification, health monitoring, and parentage confirmation of the dromedary populations included in our study. No other kind of tissue was used in this study. Camels were kept in closed area at night, which is called Garch. The animal identification was inferred via three-digit ear tags. We constructed a portable weighting scale, consisting of 13 pieces of iron, a digital scale for 2000 kg, and one chain crane. Body weight and morphometric measurements were recorded at the morning before grazing on the pasture.

The present study was conducted within the arid regions of Iran, encompassing Yazd and South Khorasan provinces. The vegetative cover of the rangelands in these specific provinces chiefly consists of shrubs, namely *Seidlitzia* rosmarinus, *Haloxylon*, *Alhagi*, *Tamarix*, and *Zygophyllum*. Due to the rangeland carrying capacity, dry and semi-dry climatic conditions, and specific plant species, Yazd and South Khorasan provinces are considered favorable for camel husbandry.

Phenotypic evaluation

The evaluation of the 62 Pakistani-origin dairy camels in South Khorasan province in Iran (32.8653°N 59.2164°E) was conducted in 2021, wherein the she-camels aged between 9 to 14 years with a mean of 11.5 years. The camels were fed twice a day. The daily diet included 2.7 kg *Alfalfa* hay, 2 kg *Alhagi camelorum*, 2 kg Wheat straw, 3.4 kg Concentrate, 0.5 kg Barely grain, 0.5 kg Wheat barn, and 0.1 kg Salt. Days in milk (DIM) ranged from 303.25 ± 63.13 to 457.25 ± 63.14 days. Body score was estimated using 23 measurements considering a maximum score of 100 including height at withers (4), body weight (5), head size (2), neck (2), front legs front view (3), front legs side view (3), chest width (4), chest girth (4), chest callosity (2), hump (1), lumbar area angle (2), rump width (3), rear legs rear view (3), rear legs side view (3), feet (2), fetlocks (4), tail (3), udder (25), teats distance between each other (10), placement (10), color (1), and hair distribution (1). The body score, with a maximum of 100, was determined by three experts (Bitaraf Sani et al., 2022). This research aimed to introduce certified Pakistani male camels, which will be used for crossbreeding with native Iranian camels. Therefore, it was necessary to compare two populations of Iranian and Pakistani camels to determine the diversity and its effect on heterosis. This comparison

was conducted using four body measurements, including shoulder height, pin width, udder depth, and udder circumference, estimated in 62 Pakistani-origin dairy she-camels and 25 Iranian she-camels aged between 9 and 14 years.

Marker assistant selection

Marker-assisted Selection (MAS) is an indirect selection process where a trait of interest is selected based on a marker (morphological, biochemical, or DNA/RNA variation) linked to a trait of interest. All 62 calves were evaluated based on maternal body score. Among 29 male calves, we selected 22 of the best males based on maternal BS for MAS. MAS was made using the 60 associated SNPs to evaluate camel potential for growth traits in 22 male calves. These SNPs were associated with the three traits (birth weight, daily gain, and body weight) (p-value < 0.002) and MAS based on these SNPs can be effective for body growth (Bitaraf Sani et al., 2021). Blood samples were collected using EDTA-containing tubes and were immediately chilled and transferred to the laboratory of Beyan Gene Pars in Shiraz for DNA extraction and genotyping, where they were stored at 25°C before DNA extraction. The Nucleospin Blood kit (MACHEREY-NAGEL, Germany), a mini kit for DNA extraction from blood, was used to extract DNA. The extracted DNA's quantity and quality were assessed using the NanoDrop One™ spectrophotometer (ThermoScientific™, USA) and stored at -20°C until further use. To design specific primers, sixty mutant-specific and sixty wild-type specific primers were created using Allele ID 7.5 software (PREMIER BiosoftTM, USA), and the specificity of each set of primers was verified using primer-BLAST (https://www.ncbi.nlm.nih.gov/tools/primerblast/). To enhance the primers' specificity, an extra mismatch was defined as the second or third nucleotide from the 3'OH end of each genotyped primer (see Table 1 in the supplementary materials). The Multiplex-ARMS PCR reaction was performed using Multiplex TEMPase 2x Master Mix (amplicon, Denmark). Different sets of 10X primers were prepared based on the Qiagen® Multiplex PCR kit. The PCR reactions were performed in 50 µl, containing 25 µl Multiplex TEMPase 2x Master Mix, 5 µl of 10X primer mix, 100 ng of genomic DNA, and up to 50 µl RNase/DNase-free water. The PCR profile was as follows. The preliminary denaturation procedure commenced at a temperature of 95°C for 15 minutes, succeeded by 35 amplification cycles consisting of denaturation at 95°C for 30 seconds, annealing at a temperature ranging from 55 to 65°C for the same duration of 30 seconds, and extension at 72°C for a length of 50 seconds. The ultimate extension was completed at 72°C for 10 minutes, utilizing the Applied Biosystems' Veriti 96-Well Fast Thermal Cycler. The polymerase chain reaction (PCR) process was optimized for the deoxyribonucleic acid (DNA) specimens that embodied the sought-after single nucleotide polymorphisms (SNPs). The segregation pattern was appraised using diverse multiplex arrays, agarose gel electrophoresis, and conventional staining techniques.

Statistical Procedure/ Method

First, all 62 calves were evaluated based on maternal body scores, and then the top 22 male calves were chosen for genetic assessment. Marker assistant selection of growth traits was computed through the anticipated impact of the 60 SNPs. The genomic breeding values (GBVs) were subsequently categorized into four classifications: weak (-2.5 to -0.11), moderate (-0.11 to 2.40), good (2.40 to 4.93), and very good (4.93 to 30.29) based on the likelihood of achievable SNP genotype combinations. These classifications were attained by utilizing at least 1,000,000 iterations in R, as detailed in the Supplementary Material. Ultimately, we assessed the frequency distribution of the 60 SNPs in two populations of Pakistani and Iranian camels. In order to compare the morphology of Pakistani and Iranian camels, four measurements, including shoulder height, pin width, udder depth, and udder circumference, were compared through an Independent samples t-test using SPSS version 22. Also, admixture analysis and Principal Component Analysis (PCA) of two populations were utilized by SNPs using the ggfortify package in R version 3.5.3 (Tang et al., 2016).

Results and discussion

Phenotypic evaluation

The shoulder height of Pakistani camels was higher than that of Iranian camels (p<0.05). The pin width, depth, and circumference of the udder were larger in Pakistani camels (p<0.05) (Table 1). Additionally, udder circumference and depth were correlated to milk production (Bitaraf Sani et al., 2022). Because of difficulties in accessing test day records of milk, using morphometric data is a good criterion for evaluating dromedaries in extensive systems.

Table 1. Mean and standard deviation of body measurements (cm) in camels

group	N	shoulder height	pin width	udder depth	udder circugfmference
IR	25	173.75 ± 11.80	34.90 ± 5.48	19.54 ± 6.42	52.55 ± 14.32
PK	62	183.00 ± 12.54	65.84 ±7.14	23.12 ±4.08	65.70 ± 12.09
P value		<0.05	<0.05	<0.05	<0.05

IR: Iran, PK: Pakistan

Principal component analysis of body measurements showed that Iranian dromedaries and Pakistani-origin dairy camels are in separate groups and have different morphologies. The first principal component (61.27%) and the second principal component (20.81%) explained

more than 83% of the variance of the physical biometric traits (Bitaraf Sani et al., 2022b).

The average body scores in Iranian camels (57.12 ± 10.30) and Pakistani-origin camels (76.71 ± 12.31) were estimated, indicating that the body scores of Pakistani camels are more suitable for milk production than dromedaries of the central desert of Iran ($p<0.05$). The body scores of Pakistani-origin dairy camels ranged from 52.70 to 93. The frequency distribution of body scores showed that 15.31% of Pakistani-origin camels had scores between 50 and 70. On the other hand, 52.46% of the Pakistani camels had scores between 70 and 90, and 39.16% had scores above 90. The body scores of Iranian camels ranged from 41 to 77. The small body size of Iranian dromedaries is a crucial adaptation to the harsh environmental conditions of food scarcity and high temperatures. This feature enables them to regulate their body temperature better and conserve energy, making them well-suited to survival in arid regions (Ehsaninia, 2022). Improvements in genetics, nutrition, and management practices can significantly increase growth and productivity rates. By implementing these improvements, farmers and breeders can optimize the performance of their livestock and increase their yields and profitability (Kadim et al., 2022). The results of our study suggest that body score is vital to consider when selecting camels. Farmers and breeders can make more informed decisions about which camels are best suited for milk production using these criteria. Conducting phenotypic evaluations and assessing diversity within the populations is crucial for the registration, preservation, and genetic improvement of existing resources (Alhajer et al., 2021). By doing so, targeted breeding programs can be developed to produce suitable genetic reserves for various objectives, similar to what is currently available for other livestock species such as cows, sheep, and goats. Previous research on collecting standardized phenotypic and biometric data in camels (Alhaddad & Alhajeri, 2019; Alhaddad et al., 2019) offers a promising pathway for future research progress. By continuing to gather and analyze such data, researchers can gain a deeper understanding of camel genetics and develop more effective breeding programs to enhance their productivity and overall performance.

The phenotypic and genetic findings of this study align with existing literature on camel milk production and farming practices, particularly in Pakistan. Our study reveals that Pakistani camels have superior body scores and morphological traits compared to Iranian dromedaries, which are known for their resilience to extreme environmental conditions (Ehsaninia, 2022). This aligns with the work of (Ahmad et al., 2012), who observed that male camels constitute a lesser percentage of the total herd composition, with female camels being the primary contributors to milk production ($p < 0.05$). Specifically, (Ahmad et al., 2012) reported a mean daily milk yield of 8.17 L, a value that correlates closely with the results of our study, which showed that the body scores of Pakistani camels were more suited to milk production.

Furthermore, (Ahmad et al., 2012) found that parity and stage of lactation have a significant influence on milk composition, including protein and lactose content. This finding complements our results, where body score and udder measurements also contributed significantly to milk production performance. In the context of selective breeding, both studies suggest that enhancing traits like body score and udder depth should be prioritized when selecting for high-yielding milk camels.

Marker assistant selection

Genomic evaluation was based on 60 SNPs related to growth traits. The minor allele frequency (MAF) exhibited an average of 0.19 in Pakistani camels and 0.18 in Iranian camels (as presented in Table 2). The heterozygote frequency in Pakistani and Iranian camels was 0.22 and 0.19, respectively. Furthermore, the TajimaD statistic in Pakistani camels suggested a higher degree of relatedness among the samples (+ 0.18).

Table 2. Genetic statistics of Pakistani and Iranian camels based on SNPs

Genetic statistics	PK	IR
MAF	0.19	0.18
Hetrozygote	0.22	0.19
TajimaD	0.18	1.39
Tetha	0.26	0.19
R Square	0.06	0.03

The admixture analysis conducted on Pakistani and Iranian camels revealed a noteworthy genetic differentiation between the two populations, as shown in Figure 1. Furthermore, the principal component analysis (PCA) exhibits an apparent clustering effect, as depicted in Figure 2 PC1 and PC2 together explained ~50 % of the variation. These findings conclusively establish that Pakistani and Iranian camels possess distinct genetic characteristics and must be treated as separate populations in all future genetic studies. Such valuable insights are paramount for efficiently managing and conservating these indispensable livestock species. The study results provide irrefutable evidence that camels from Pakistan and Iran exhibit discernible genetic traits and should be considered distinct populations in all subsequent genetic research endeavors. These insightful findings are important for effectively managing and preserving these vital livestock species.

Additionally, the genetic differentiation observed in both (Ahmad et al., 2012) and our study—through genomic evaluations of SNPs related to growth traits—emphasizes the importance of using advanced genetic tools to improve camel productivity. By applying marker-assisted selection in breeding programs, the effectiveness of improving milk yield and quality could be accelerated, as demonstrated by the distinct genetic characteristics of Pakistani and Iranian camels. The findings further corroborate this, suggesting that targeted

genetic and management interventions could optimize the potential of camels in Pakistan.

Yaqoob et al (2007) suggests that milk production in Pakistani camels can be enhanced under semi-intensive and intensive farming conditions. This supports our recommendation that selective breeding and improved management strategies can increase milk production in Iranian camels. Specifically, targeted breeding programs incorporating high-yielding Pakistani camels could enhance the genetic potential of Iranian camels for dairy purposes.

These findings underscore the importance of genetic selection in enhancing the productivity of dairy camels. Yaqoob et al (2007) also stresses the need to develop breeding strategies aimed at increasing milk yield. Our findings support this perspective, as we observed that camels with larger udder dimensions and higher body scores exhibited better milk production performance.

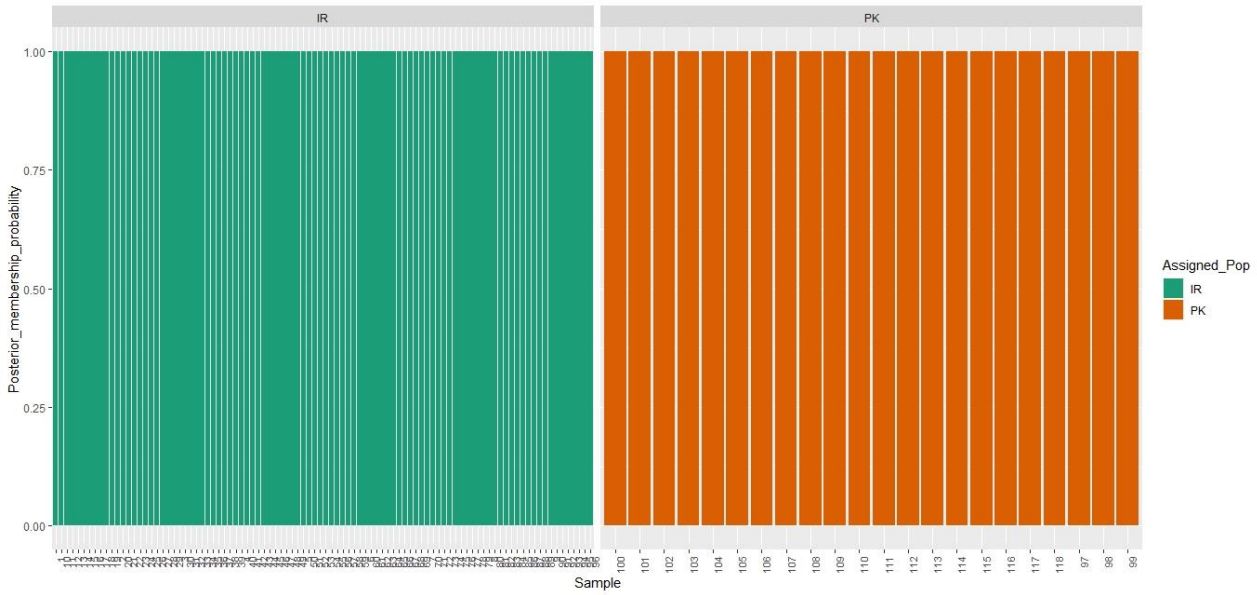

Figure 1 Admixture analysis of Pakistani and Iranian camels using the studied SNPs (PK: Pakistani and IR: Iranian camels)

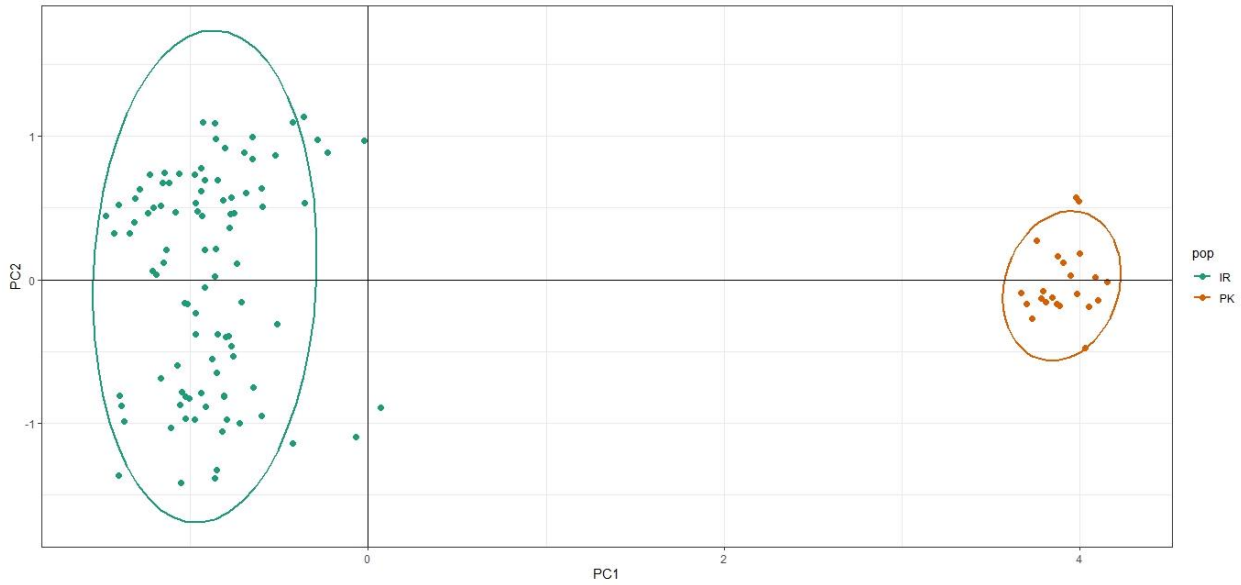

Figure 2 principal component analysis (PCA) of Pakistani and Iranian camels using the studied SNPs (PK: Pakistani and IR: Iranian camels)

Assessment of male calves using phenotype and genotype evaluation

The presented data in Table 3 pertains to the genomic breeding values (GBV) for growth trait, maternal body score, and dam age of 22 male dromedary camels. The GBVs of the growth trait were calculated using a

combination of 60 SNPs. Upon inspection, it was observed that camels numbered 513, 510, and 556 had particularly favorable GBV for growth and were consequently deemed as the best candidates for crossbreeding based on their physical appearance and the expert opinion of the breeder (Table 3). These select camels possess substantial potential as breeding sires for milk production, given that they are provided with appropriate nutritional and management support.

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Table 3. ID, GBV, Body score, and age of dams and calves

ID	GBV	Body score dam's	Dam age(years)	Male calf age (months)
504	Very good	82.00	12	26.60
513	Very good	90.50	11	27.90
507	Very good	56.00	9	26.50
510	Very good	60.50	11	27.23
525	Very good	80.00	14	28.43
556	Very good	79.50	10	27.96
516	Very good	81.00	13	34.43
508	Very good	75.80	12	28.10
554	good	83.00	11	28.26
530	good	63.90	13	28.33
532	good	65.00	12	28.70
502	moderate	54.70	13	25.73
540	moderate	65.00	11	27.90
550	moderate	89.50	12	27.23
538	weak	88.00	10	26.26
501	weak	92.00	11	29.70
559	weak	52.00	10	27.96
561	weak	88	12	26.20
549	weak	79.50	11	27.36
515	weak	92.00	10	28.53
519	weak	92.00	13	28.16
512	weak	90.00	9	28.80

Conclusion

Using morphometric records and assessing body scores can serve as valuable techniques in camel breeding selection and substitution programs. These criteria can effectively assess dromedaries in extensive and semi-intensive systems. The Selection of camels based on the depth of their udders, the circumference of their chests, and their respective body scores can significantly enhance milk production. Furthermore, measuring the chest and abdominal circumference can be valuable for further selecting camels to augment milk production. All in all, the use of morphometric data presents an essential approach for the enhancement of productivity and sustainability within camel herds.

The mean values of body scores, encompassing height, hip width, chest circumference, and udder depth, exhibited a higher magnitude in Pakistani camels than in their Iranian counterparts. Moreover, Principal Component Analysis (PCA) aptly captured and delineated the observed dissimilarities between the two populations. While Iranian camels are renowned for their superior resilience to environmental stress and disease immunity, mating with high-yielding milk sires can be an efficacious approach to augment milk production in native Iranian camels. Nevertheless, conducting protracted selective programs that target the traits pertinent to milk production, like average milk production and body scores in Iranian camels, can also prove instrumental in enhancing their genetic potential and engendering high-yielding milk sires sans crossbreeding. Genetic evaluation predicated on markers that utilize growth-related Single Nucleotide Polymorphisms (SNPs) evinced genetic differentiation between the two populations. In this study, male calves were chosen based on maternal body scores. Subsequently, those showcasing the desirable genetic potential for body growth were selected to propagate, given that mating is natural in camel breeding.

We conclude that selective breeding programs integrating superior traits of Pakistani camels could significantly improve the milk yield of Iranian camels. Additionally, implementing genetic selection programs alongside strategic nutritional and managerial interventi-

ons can contribute to the sustainable development of the camel dairy industry.

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Conflict of interests

No potential conflict of interest was reported by the authors.

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Ethics declarations

All animal procedures were performed strictly with the guidelines and regulations proposed by the Animal Science Research Institute of Iran. All experimental protocols were approved by the Animal Science Research Institute of Iran under ASRI-34–64-1357–005-970,180. All experiments were carried out following the recommendations in the ARRIVE guidelines (https://arriveguidelines.org/).

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