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Research Article/Araştırma Makalesi

Investigation on allele and genotype frequencies of ADH1C and FASN genes in three cattle breeds in Türkiye

Türkiye'de yetiştirilen üç sığır ırkında ADH1C ve FASN genlerinde allel ve genotip frekanslarının araştırılması

Eymen DEMİR[1]

[1]Akdeniz University, Faculty of Agriculture, Department of Animal Science, Antalya, Türkiye

[1]https://orcid.org/ 0000-0003-4507-7426

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Address for Correspondence:
Eymen DEMİR
e-mail:
eymendemir@akdeniz.edu.tr

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ABSTRACT

Improvement of selection programs based on candidate genes for meat yield and quality is an efficient approach for overcoming the current dilemma between the increasing human population and the decreasing population size of farm animals. Being known to be associated with meat yield and quality in cattle, *ADH1C* and *FASN* genes were investigated across three cattle breeds reared in Türkiye namely East Anatolian Red (EAR), South Anatolian Yellow (SAY), and Holstein Friesian (HF) in this study. For this purpose, 37 animals per breed were genotyped via the allele-specific polymerase chain reaction (AS-PCR) technique. The distribution of allele frequencies significantly differed between HF and native Turkish breeds. C allele frequency ranged from 0.014 (EAR) to 0.311 (HF) while T allele frequency varied between 0.689 (HF) and 0.986 (EAR) in *ADH1C* polymorphism. C and T allele frequencies were calculated as 0.068 and 0.932, respectively, in SAY breed. G was the most frequent allele across all cattle breeds regarding *FASN* gene variation. The lowest (0.014) and highest (0.365) A allele frequency were detected in EAR and HF breeds, respectively, while G allele frequency ranged from 0.027 (EAR) to 0.635 (HF). Compared to native breeds, HF had a higher heterozygosity. A large part of the total genetic variation (67%) was attributed to differences within individuals. Variations of *ADH1C* and *FASN* genes turned out to be informative enough to distinguish native Anatolian cattle breeds from HF via genetic distance-based phylogenetic analysis. No animals with superior genotypes for the *ADH1C* and *FASN* genes were observed in EAR, while two animals with AA genotype were detected for the *FASN* gene in the SAY breed. These findings imply that for the time being, these genes do not seem efficient for marker-assisted selection (MAS) studies while desired genotypes may be developed via suitable mating programs for long-term production. Further studies may focus on screening native Turkish cattle breeds regarding other meat yield and quality-related traits to develop selection strategies.

Key Words: ADH1C, AS-PCR, FASN, genetic variant, polymorphism

ÖZ

Et verimi ve kalitesinin iyileştirilmesi için aday genler temelli seleksiyon programlarının geliştirilmesi, artan insan nüfusu ile azalan çiftlik hayvanı populasyonu arasındaki mevcut ikilemi aşmak için etkili bir yaklaşımdır. Sığırlarda et verimi ve kalitesiyle ilişkili

olduğu bilinen *ADH1C* ve *FASN* genleri Doğu Anadolu Kırmızısı (DAK), Yerli Güney Sarısı (YGS) ve Siyah Alaca (SA) olarak bilinen ve Türkiye'de yetiştirilen üç farklı sığır ırkında incelenmiştir. Bu amaçla, her ırktan 37 hayvan allel spesifik polimeraz zincir reaksiyonu (AS-PZR) tekniğiyle genotiplendirilmiştir. Allel frekans dağılımı Türkiye yerli ırkları ile SA arasında önemli şekilde farklılık göstermiştir. *ADH1C* polimorfizmi bakımından C allel frekansı 0.014 (DAK) ile 0.311 (SA), T allel frekansı ise 0.689 (HF) ile 0.986 (DAK) aralığında değişmiştir. YGS ırkında C ve T allel frekansı sırasıyla 0.068 ve 0.932 olarak hesaplanmıştır. *FASN* gene polimorfizmi bakımından bütün populasyonlarda en çok görülen allel G bulunmuştur. En düşük (0.014) ve en yüksek (0.365) A allel frekansı sırasıyla DAK ve SA ırkında tespit edilirken, G allel frekansının 0.027 (DAK) ile 0.635 (SA) aralığında değiştiği belirlenmiştir. Yerli ırklarla kıyaslandığında, SA ırkında daha fazla heterozigotluk belirlenmiştir. Toplam genetik varyasyonun büyük bir kısmı (%67) bireyler arasındaki farklılıktan kaynaklanmıştır. Genetik mesafe temelli filogenetik analiz yoluyla *ADH1C* and *FASN* genlerindeki varyasyonların yerli Anadolu sığırlarının SA ırkından olan farklılığını ortaya koymada yeterince bilgi verici olduğu ortaya çıkmıştır. DAK ırkında *ADH1C* ve *FASN* genleri için arzu edilen genotipe sahip herhangi bir hayvan tespit edilemezken YGS ırkında AA genotipine sahip iki hayvanın olduğu belirlenmiştir. Bu bulgular mevcut durumda bu genlerin marker destekli seleksiyon (MDS) çalışmaları için etkili olmamakla birlikte uzun vadede uygun çiftleştirme programları sayesinde arzu edilen genotiplerin elde edilebileceğini göstermektedir. Gelecekte yapılacak çalışmalarda, seleksiyon stratejilerinin geliştirilmesi amacıyla Türkiye yerli sığır ırklarının et verimi ve kalitesiyle ilgili diğer özellikler açısından taranması üzerinde durulabilir.

Anahtar Kelimeler: ADH1C, AS-PZR, FASN, genetik varyant, polimorfizm

Introduction

The trend of increasing human population and decreasing population size in local farm animals is one of the major concerns among scientists (Aby et al. 2014; Eusebi et al. 2019). Therefore, cattle breeding remains an indispensable part of the agricultural sector, providing valuable food resources such as milk and meat. Cattle breeding is practiced at diverse production systems across almost all continents to obtain valuable food resources. As mentioned by Demir and Argun Karsli (2024), native cattle breeds are reared by smallholder farmers at a small scale in Türkiye whereas commercial companies prefer cosmopolitan cattle breeds for large-scale production. Among native Anatolian cattle breeds, East Anatolian Red (EAR) survives in a limited geographic zone of the east part of the country covering Erzurum, Kars, and Ardahan provinces (Çobanoğlu and Ardıçlı 2022) while South Anatolian Yellow (SAY), reared for both milk and meat production, was reported to be well-adapted to mountainous areas (Demir et al. 2021). On the other hand, Holstein Friesian (HF) is the most preferred cosmopolitan cattle breed by the farmers to produce milk and meat. The fact that cosmopolitan cattle breeds are advantageous in economically important traits has led to a significant decrease in the population size of local Anatolian breeds (Argun Karslı 2024). However, local populations are known to be well-adapted to a specific environment and create opportunities to shape selection programs against diverse environmental stressors. Indeed, a recent study using 211.119 bi-allelic single nucleotide polymorphisms (SNPs) obtained by next-generation sequencing (NGS) confirmed that several genes related to survival traits such as visual modality (*LGSN*), olfaction (*MOXD2*, *OR4C1F*, and *OR4C1E*), and immune response (*TRBV3-1* and *CLDN10*) have become fixed in cattle breeds reared in Türkiye (Demir et al. 2023a).

Meat yield and quality are primarily considered in selection practices by farmers to increase the profitability and sustainability of long-term production (Hozáková et al. 2020). However, these traits show quantitative inheritance meaning that they are influenced by several environmental factors and controlled by numerous genes (Raza et al. 2020). For example, feeding practices may affect meat yield while meat processing methods and storage conditions significantly impact meat quality (Grigoletto et al. 2020). On the other hand, variations in some major genes may cause differences among individuals in a certain population regarding these traits. These variations create opportunities for farmers to select advantageous genotypes for

breeding programs called marker-assisted selection (MAS) (Brito et al. 2021). In MAS studies, it is essential to investigate genetic variations of genes which are previously been confirmed to be related to meat yield and quality via affordable and accurate molecular genotyping methods. For example, Ward et al. (2012) discovered a novel SNP described as c.-64T>C in the promoter region of alcohol dehydrogenase 1 C (*ADH1C*) via polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP) which turned out to have a significant impact on intramuscular fat trait in Angus-cross steers. Another PCR-RFLP-based study revealed that g.17924G>A variation in fatty acid synthase (*FASN*) gene was directly linked to the concentration of some fatty acids in meat such as oleic and palmitic acid in Hanwoo cattle breeds (Bhuiyan et al. 2009). Moreover, Rempel et al. (2012) genotyped 620 cattle via 33 mutations belonging to several genes in which a variation occurred in the position of 52183794 overlapping with the *FASN* gene was reported to significantly affect meat traits such as body weight, average daily gain, and hot carcass weight. Although known variations of the genes related to meat yield and quality could be screened via several molecular genotyping methods, RT-PCR, PCR-RFLP, and direct sequencing may not be affordable by smallholder farmers due to requiring expensive chemical reagents, professional labor, equipment, and time (Lee et al. 2022). However, allele-specific PCR (AS-PCR), relying on the amplification of mutant and wild alleles with minimal chemical reagents requirement, is one of the fastest and most accurate techniques to investigate known variations. Therefore, Lee et al. (2022) have designed AS-PCR protocols to genotype cattle breeds for several genes including *ADH1C* and *FASN* to identify superior genotypes for meat yield and quality. Although this protocol seems cost-effective and simple, it has not been utilized to genotype native and cosmopolitan cattle breeds reared in Türkiye. Hence, this study aims to screen *ADH1C* and *FASN* gene variations in EAR, SAY, and HF breeds via the AS-PCR technique. Besides these variations were evaluated in terms of whether they were informative enough to distinguish native and cosmopolitan cattle breeds via genetic distance-based molecular approaches.

Materials and Methods

Sampling and DNA isolation

A total of 111 cattle were chosen from at least three representative herds of EAR (n=37), SAY (n=37), and HF (n=37) sampled from populations reared in Erzurum, Şanlıurfa, and Antalya provinces, respectively. An oral interview was carried out with farmers to select unrelated animals. Blood samples collected from the jugular vein were subjected to a salting-out method to isolate DNA (Miller et al. 1988). 1% agarose gel electrophoresis was employed to assess the success of the DNA isolation stage.

AS-PCR genotyping

By using specific primer combinations recommended by Lee et al. (2022), c.-64T>C and g.17924G>A polymorphisms of *ADH1C* and *FASN* genes, respectively, were investigated across three cattle breeds reared in Türkiye (Table 1).

Table 1. An overview of mutation and primer sequences of the studied polymorphisms in the AS-PCR protocol

Gene	SNP	Amplicon	Expected band sizes (bp)	Primer combinations	
				Forward	Reverse
ADH1C	c.-64T>C	Control region	762	**ADH1C-OF:** ACTGGTGTCTGATTTCTCTGTTGTGAAG	**ADH1C-OR:** AGAATTCCAGTTGAGCTATTCCAGATCC
		Variant allele (T)	492	**ADH1C-OF:** ACTGGTGTCTGATTTCTCTGTTGTGAAG	**ADH1C-IR:** TTACAGACTTACAGGCTCTTCCCTGTTAAA
		Wild allele (C)	330	**ADH1C-IF:** AATCTGTGCAATCTATCTCTTGTATGTCCC	**ADH1C-OR:** AGAATTCCAGTTGAGCTATTCCAGATCC
FASN	g.17924 G>A	Control region	830	**FASN-OF:** GGGAAATCCGGCAGCTCACAATCCACAA	**FASN-OR:** GTGTAGGCCATCACGAAGGTGTGCGAGC
		Variant allele (G)	507	**FASN-OF:** GGGAAATCCGGCAGCTCACAATCCACAA	**FASN-IR:** GGCCATAGGTGGGGATGCTGAGCTTTGC
		Wild allele (A)	377	**FASN-IF:** CACCACCGTGTTCCACAGCCTGGACA	**FASN-OR:** GTGTAGGCCATCACGAAGGTGTGCGAGC

As highlighted in Table 1, different primer combinations were utilized to amplify the variant and wild type for two polymorphisms. Additionally, control regions at 762 and 830 bp length for the *ADH1C*, and *FASN* genes were amplified for all genotypes to confirm that targeted regions were monitored. An optimized PCR reaction (5 µl template DNA, 10 pmol/µl each primer, 12,50 µl EcoTech 2X Master Mix, and 5,50 µl ddH_2O) and cycler program (30 s at 95 ºC for pre-denaturation followed by 35 cycles of 30 s at 95 ºC for denaturation, 30 s at 61 ºC for annealing, and 30 s at 72 ºC for extension) were used to amplify expected bands. The final extention stage was optimized at 72 ºC for 5 min. %3 agarose gel electrophoresis was used to separate PCR fragments for genotyping animals.

Statistical analysis

Allele and genotype frequencies, observed (H_O) and expected (H_E) heterozygosity as well as chi-square ($\chi 2$) based Hardy-Weinberg equilibrium (HWE), were calculated by Popgene v.1.32 (Yeh et al. 1997). Analysis of molecular variance (AMOVA) test was carried out with the option of 999 permutations by GenAlex software (Peakall and Smouse 2012) to categorize sources of total genetic variation. Nei's standard genetic distance among breeds was estimated via GenAlex software (Peakall and Smouse 2012). The genetic distance matrix was processed in MEGA 11 software (Kumar et al. 2008) to construct a Neighbour-Joining (NJ) tree per breed.

Results and Discussions

Agarose gel electrophoresis-based genotyping revealed that all animals carried 762 bp and 830 bp length fragments for the *ADH1C* and *FASN* genes, respectively, indicating that targeted genomic regions were amplified during the AS-PCR process (Figure 1).

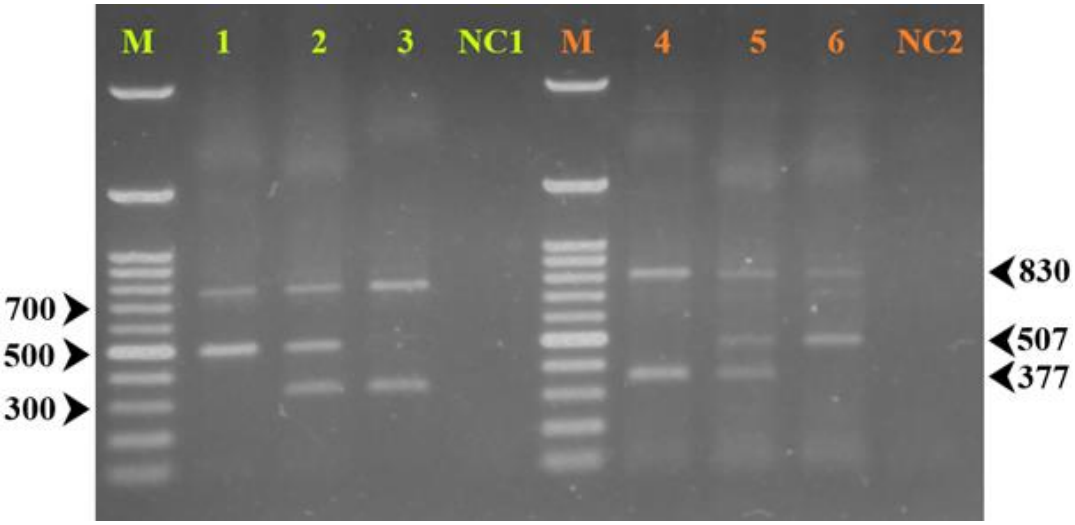

Figure 1. Agarose gel (3%) image of some representative amplified PCR fragments for *ADH1C and FASN* polymorphisms in three cattle breeds.

M: molecular weight marker (100 bp); **1-3:** TT (762 and 492 bp), CT (762, 492, and 330 bp), and CC (762 and 330 bp) genotypes for the *ADH1C* gene; **NC1:** negative control for the *ADH1C* gene; **4-6:** AA (830 and 377 bp), AG (830, 507, and 377 bp), and GG (830 and 507 bp) genotypes for the *FASN* gene; **NC2:** negative control for the *FASN* gene.

The results of allelic diversity and genetic variability parameters were summarised in Table 2 in which T and G alleles turned out to be represented with higher frequency across all breeds in terms of the *ADH1C* and *FASN* polymorphisms, respectively. However, allelic distribution significantly differed between native Anatolian cattle populations and the HF. Indeed, no animals with CC genotype were detected in EAR and SAY breeds leading to a low frequency of the C allele (0.014 and 0.068) in terms of the *ADH1C* polymorphism. 2 and 5 animals were recorded as CT genotype in EAR and SAY populations, respectively. On the other hand, 4 and 14 animals carried CC and CT genotypes for the *ADH1C* gene in the HF breed. Similar results were also detected for the *FASN* gene polymorphism in which the frequency of the A allele was higher in the HF breed (0.365) compared to Anatolian cattle breeds (0.014-0.135) (Table 2). No animals with the AA genotype were detected in the EAR breed while only 2 animals carried the AA genotype in the SAY breed. 1 and 6 animals turned out to be heterozygous for the *FASN* gene in EAR and SAY breeds, respectively. On the other hand, 7 and 13 animals were recorded as AA and AG genotypes, respectively, in the HF breed. Due to conserving a higher number of heterozygous individuals, the HF breed showed higher heterozygosity for both the *ADH1C* (H_O = 0.405) and *FASN* (H_O = 0.351) polymorphisms compared to native Anatolian cattle breeds (Table 2).

Table 2. A summary of allele frequencies and genetic variability of the *ADH1C* and *FASN* genes in EAR, SAY, and HF cattle breeds

Gene	***ADH1C***					***FASN***				
Parameter	**Allele frequency**		**Genetic variability**			**Allele frequency**		**Genetic variability**		
Breed	C	T	H_O	H_E	χ^2	A	G	H_O	H_E	χ^2
EAR	0.014	0.986	0.027	0.027	0.007^{ns}	0.014	0.986	0.027	0.027	0.007^{ns}
SAY	0.068	0.932	0.135	0.126	0.194^{ns}	0.135	0.865	0.162	0.234	3.470^{ns}
HF	0.311	0.689	0.405	0.428	0.107^{ns}	0.365	0.635	0.351	0.463	2.165^{ns}

EAR: East Anatolian Red: **SAY:** South Anatolian Yellow: **HF:** Holstein Friesian; **H_O:** Observed heterozygosity; **H_E:** Expected heterozygosity, **χ^2:** Chi-square test value; **ns:** non-significant deviation from HWE ($\chi^2_{0.05;1}$: 3.84).

AMOVA analysis categorized total genetic variation into three levels such as among populations, among individuals, and within individuals in which a large part of it (67%) was attributed to within individuals (Table 3). The differences between breeds corresponded to 19% of the total genetic variation, which was in agreement with the genetic differentiation value (F_{ST}) of 0.195.

Table 3. Summary of AMOVA analysis across EAR, SAY, and HF cattle breeds

SV	df	SS	MS	EV	%
Among populations	2	8.423	4.212	0.053	19
Among individuals	108	27.784	0.257	0.036	13
Within individuals	111	20.500	0.185	0.185	67
Total	**221**	**56.707**	-	**0.274**	**100**

SV: source of variation; **df:** degree of freedom; **SS:** sum of squares; **MS:** mean square; **EV:** estimated variance.

The lowest genetic distance (0.546) was detected between EAR and SAY, while the highest value (1.927) was observed between HF and SAY. Similarly, EAR and SAY were clustered together in NJ tree analysis while HF constituted a separate branch (Figure 2). This confirms that even two genes related to economically important traits are sufficient to genetically distinguish native Anatolian cattle from cosmopolitan breeds.

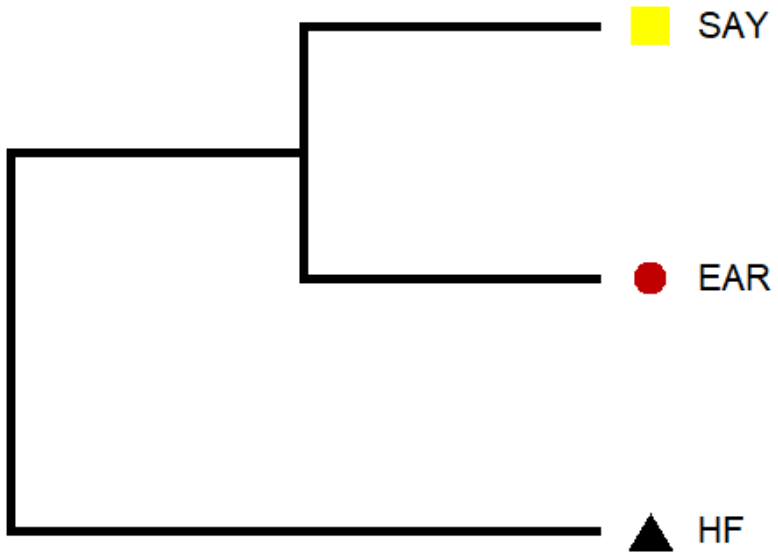

Figure 2. NJ tree-based phylogenetic analysis at breed level
EAR: East Anatolian Red: **SAY:** South Anatolian Yellow: **HF:** Holstein Friesian.

The variations of the *ADH1C* (Ward et al. 2012) and *FASN* (Bhuiyan et al. 2009; Rempel et al. 2012) genes and their effects on phenotype were clarified by several studies. Ward et al. (2012) investigated the impact of vitamin A restriction together with the *ADH1C* variation in 130 Angus steers in which animals with TT genotype had 23% higher intramuscular fat compared to animals with CC genotype in the treatment group without vitamin A supplementation. Moreover, unsupplemented vitamin A animals with the TT genotype were reported to show 24% greater intramuscular fat compared to vitamin A-supplemented animals with the TT genotype (Ward et al. 2012). The number of animals with CC, CT, and TT genotypes for Angus steers was reported to be 30 (0.230), 50 (0.385), and 50 (0.385), respectively (Ward et al. 2012). On the contrary, studying the effects of the *ADH1C* variations on meat-related traits, Peng et al. (2017) reported that animals with CT genotype are advantageous over TT genotypes in terms of eye muscle area, marbling, and carcass weight in 60 Korean native steers. The frequency of TT and CT genotypes was reported as 85% and 15%, respectively, while no animals with CC genotype

were observed (Peng et al. 2017). The results of the current study show similarities with the findings reported in the literature. As known, beef cattle are expected to carry advantageous genotypes for meat yield and quality compared to native cattle breeds. As observed in native Korean steers (Peng et al. 2017), no animals with CC genotype were detected in two Anatolian cattle breeds (EAR and SAY) in this study. Besides, a small number of animals turned out to carry CT genotypes (2 and 5 animals from EAR and SAY) which is the desired genotype for meat yield and quality. On the other hand, the CT genotype detected in Angus breed by Wang et al. (2012) was also observed in the HF breed reared in Türkiye. Similar to the *ADH1C* polymorphism, the *FASN* variations also significantly differed between HF and native Anatolian breeds in which no animals with the AA genotype were detected in the EAR breed while only 2 samples carried the AA genotype in the SAY breed. The A allele frequency ranged from 0.014 (EAR) to 0.135 (SAY) in Anatolian cattle while a higher frequency (0.365) was detected in the HF breed. Similarly, a higher AA genotype frequency (0.299) was reported in HF breed raised in China (Zhou et al. 2023). These results demonstrate that local cattle breeds adapted to specific environmental conditions cannot compete with breeds specialized for beef production in terms of meat yield and quality. However, as seen in this study, local breeds may conserve advantageous alleles and/or genotypes at low frequencies. This fact creates significant opportunities to detect and subject animals with desired genotypes for MAS studies to improve meat yield and quality, while it seems to take longer times of intensive breeding practices.

In this study, the highest proportion of the total genetic variation (67%) was detected within individuals by AMOVA analysis, which was consistent with previous studies. For example, Demir and Balcioglu (2019) assessed the genetic diversity and population structure of three native and HF cattle breeds via 20 microsatellite markers, in which the highest part of total genetic variation (80.068%) was attributed to the within individuals. Another study based on 22 microsatellite loci confirmed that 88.90% of the total genetic variation could be explained by the differences within individuals in five native Turkish cattle breeds (Öner et al. 2019).

It is known that denser genetic data have a significant potential to distinguish local cattle populations from cosmopolitan breeds at a molecular level. Indeed, several studies based on microsatellite and single nucleotide polymorphism (SNP) demonstrated that native Turkish cattle breeds were genetically different from cosmopolitan cattle breeds via several phylogenetic analyses (Demir and Balcioglu 2019; Karayel and Karsli 2022; Demir et al. 2023b). Similarly, in this study, genetic variations of two genes (*ADH1C* and *FASN*) were found enough to distinguish native Anatolian cattle from HF breed while genome-wide genetic data seems required to reveal the genetic distinctiveness of SAY and EAR breeds (Demir et al. 2022). Indeed, a recent study utilizing 211.119 SNP highlighted that EAR was genetically distinct from the other native Turkish cattle breeds (Demir et al. 2023b).

Conclusions

In this study, two previously known variations in *ADH1C* (c.64T>C) and *FASN* (g.17924G>A) genes were investigated via AS-PCR technique in EAR, SAR, and HF cattle breeds. The desired genotypes regarding meat-related traits were detected at sufficient frequencies in HF breed, while no animals with CC genotype for *ADH1C* and AA genotype for *FASN* genes were detected in EAR and SAY cattle breeds. This finding supports the idea that the genome of native Turkish cattle breeds has been mainly shaped by environmental challenges while desired genotypes are maintained in cosmopolitan cattle breeds due to ongoing selection studies. The current results imply that the *ADH1C* and *FASN* genes do not seem to be effective in improving meat yield and quality traits in Anatolian cattle breeds due to low genetic variability, while a larger sample size

covering more geographic locations has the potential to obtain more heterozygous animals. Alternatively, the current populations could be monitored in terms of other meat-related genes in further studies.

Ethical Statement

Blood samples used in this study were previously collected during routine visits of qualified veterinarians. Therefore, no ethical permission was required to carry out this study.

Conflict of interest

The author declares no conflict of interest.

Author contributions

ED conceptualized the study, developed the methodology, validated the results, and wrote the original draft.

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