Genetic Variability of *FABP4* c.328 G>A (rs110652478) Polmorphism and Its Association with Slaugter Weight and Carcass Traits in Aberdeen Angus and Hereford Bulls Imported into Turkey

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Abstract

The study aimed to determine the genotypic distribution of the c.328 G>A (rs110652478) polymorphism in the bovine fatty acid-binding protein 4 (*FABP4*) gene and to determine its effect on slaughter weight in Aberdeen Angus and Hereford bulls imported into Turkey. A total of randomly selected 95 bulls were used in this study. Bulls were housed for fattening in semi-open pens for approximately nine months with the same management procedures. Genotyping of the *FABP4* c.328 G>A (rs110652478) polymorphism, located in exon 3, was performed by the PCR-RFLP method. Allele and genotype frequencies were calculated by the standard procedures and the Hardy-Weinberg equilibrium was tested by comparing expected and observed genotype frequencies using chi-square and Fisher's exact tests. Indices of genetic diversity were calculated based on the allelic frequencies. The least-squares method as applied in a GLM procedure was used to test the association between *FABP4* c.328 G>A and phenotypic traits including slaughter weight, hot and cold carcass weight, chilling loss, dressing percentage, and carcass pH. Results revealed that the most frequent genotype was GG (49.18% and 52.94% for Angus and Herefords, respectively) and the minor allele frequency (A allele) was 0.32 in the total population. The genotype distribution was in agreement with Hardy Weinberg equilibrium (P>0.05). The moderately low levels of genetic variability observed in this study may be caused as a result of eventual inbreeding. PIC levels suggest that *FABP4* c.328 G>A can be a mildly informative genetic marker for the tested population. Association analysis indicated that the effect of the *FABP4* on slaughter weight was statistically significant (P<0.05) in the total population. However, this association was not substantiated in breed-specific analyzes. There was no significant effect of the marker on any remaining phenotypic traits. Consequently, it is important to emphasize that analysis of *FABP4* c.328 G>A may provide valuable, but limited, information on imported beef cattle raised in Turkey conditions.

Keywords: Beef cattle, *FABP4*, single nucleotide polymorphism, marker-assisted selection

Introduction

In recent years, parallel to biotechnology developed in the field of molecular genetics, in particular, the opportunity to conduct more detailed research at the molecular level has enabled the development of new methods and increased availability. Traditional genetic improvement of livestock, using the information on phenotypes and pedigrees to predict breeding values, has been very successful. However, breeding values should be able to predict more accurately by using genomic data between animals.[1] Moreover, genetic selection methods have the ability to reduce the generation interval which is a crucial point in livestock, especially for cattle breeding. Concerning beef cattle, it is worth noting that the selection based on the traits that can be detected after slaughter (or traits that emerge in the later stages of animals' life) with classical selection methods requires a long period and can lead to economic losses. Thus, molecular genetic studies allow many traits to be evaluated when the animal is still alive and that these data can be used in early selection.[2,3]

The use of genetic markers increases the frequency of desired genes in herds based on the selection in breeding programs. In this context, many candidate genes have been identified as potentially associated with the specific traits analyzed. Among them, fatty acid-binding protein [4]

(*FABP4*), which was mapped to BTA14, is one of the most important genes in cattle breeding. The main function of this gene is to play a regulative role in lipid metabolism associated with changes in lipid hydrolysis and intracellular fatty acid.[4] It is also essential for glucose homeostasis through interaction with peroxisome proliferator-activated receptors.[5] The protein encoded by the *FABP4* gene (GenBank accession number: NC_007312) is a cytoplasmic protein that binds long-chain fatty acids and other hydrophobic ligands.[6] Taken together, this gene has been suggested as a functional and positional candidate gene for the evaluation of fat synthesis dynamics in cattle.[7-9] Although *FABP4* gene shows its main effects on meat quality,[5,10] it is also of great importance in terms of some carcass characteristics.[2,11,12] The c.328 G>A polymorphism (rs110652478) in the bovine *FABP4* gene is located on exon 3 and it induces valine to methionine substitution at position 110.[8,9] This SNP has been associated with desirable increases in marbling scores and meat quality grades.[2,8]

In Turkey, the supply and demand balance in beef has been tried to be regulated mostly by the importation of live animals or carcasses. There is limited information about the genetic characteristics and/or structure of the imported beef cattle. Even their breed purity can be controversial. Although genetic analysis has become more widespread in dairy cattle, this approach is mostly not adopted in beef cattle in Turkey. Indeed, the genomic evaluation will provide significant insights into consistent selection strategies for not only raising cattle but also for selecting individuals with a superior performance before importation into Turkey. Therefore the main objective of this study was to evaluate the genetic diversity of *FABP4* c.328 G>A (rs110652478) polymorphism in Aberdeen Angus and Hereford bulls. Moreover, their slaughter weights and some carcass characteristics were evaluated following a fattening period in Turkey conditions.

Materials and Methods

Animals and sampling

A total of 95 Aberdeen Angus (n=61) and Hereford (n=34) bulls from a commercial farm located in the South Marmara region (40°15'28.04"N and 29°30'50.18"E) of Turkey were used. The cattle were transported from Brazil to Bursa province of Turkey. All cattle were housed for fattening in semi-open pens for approximately nine months under the same environmental conditions and management procedures (Figure 1). At the end of the fattening period, the animals were slaughtered at a commercial slaughterhouse based on standard practices. During exsanguination, 4 mL of the blood sample was obtained into a sterile K3EDTA tube (Vacutest Kima, SRL, Italy) from flowing blood.

Figure 1. Animal material and environmental conditions in the present study

Phenotypic traits

The slaughter weight (SW) was measured before slaughter following a period of 12 h deprived of feed, but with full access to water. Hot carcass weight (HCW) was recorded after conventional slaughter processing and it was measured without removing the subcutaneous fat and keeping the kidney and pelvic fat. The carcasses were chilled at 4°C for approximately 24 h after which the cold carcass weights (CCW) were obtained. Carcass weights after chilling were likewise measured to determine the chilling loss (CL). The dressing percentage (DP) was estimated considering HCW. Carcass ph (24 h postmortem) was measured in the m. longissimus thoracis between 12th and 13th ribs using a calibrated pH probe (Testo 205 pH meter, Lenzkirch, Germany).

DNA extraction and the genotyping

In this study, a 565 bp fragment in the exon 3 region of the *FABP4* gene was amplified. Genomic DNA was isolated from white blood cells using standard techniques 13. The concentration and quality of DNA were detected by a NanoDrop spectrophotometer (NanoDrop 2000c, Thermo Scientific, Wilmington, DE, USA). The genotyping of the SNP was carried out by the PCR-RFLP method in a total of 25 μL reaction volume. 3 μL genomic DNA sample (~60 ng) was used as a template. The reaction contained

12.50 μL PCR master mix (OneTaq Quick-Load 2x MM with Standard Buffer, New England BioLabs Inc., Ipswich, Cat#M0486S, USA), 1 μL (0.5 μM) of each primer, and 8 μL of nuclease-free water (Thermo Scientific). Oligonucleotide sequences (5' to 3') were ACCCCTATGAT-GCTATTCCACA as the forward primer and ATACG-GTTCACATTGAGAGGGA as the reverse primer 8. The amplification reaction was as follows: initial denaturation step at 95°C for 4 min, 35 cycles of denaturation at 94°C for 1 min, annealing at 60°C for 1 min, and extension at 72°C for 1.5 min, followed by a final extension step at 72°C for 5 min. 15 μL of PCR product (if verified) was digested with 10 U of *Cvi*AII (isoschizomer of *Nla*III) restriction enzyme (New England BioLabs, Cat#R0640S), with 10x buffer (New England BioLabs) by incubating at 25 °C for 16h. All PCR-RFLP reactions were performed in a thermal cycler (MyGenie 96 thermal block, Bioneer Corporation, South Korea). The resulting RFLP fragments were separated on 3% agarose gel by electrophoresis with 1× TBE buffer and were stained using ethidium bromide (2 μg mL-1). Gel photos were taken under UV light using a gel documentation system (DNR-Minilumi, DNR Bio-Imaging Systems, Israel).

Statistical analysis

The allelic and genotypic frequencies were estimated according to Falconer and Mackay.[14] The Hardy–Weinberg equilibrium (HWE) was tested using the chi-square goodness-of-fit test. However, Fisher's exact test was used to evaluate HWE because of the low numbers of individuals per genotype (genotype counts below 5).

Indices of genetic diversity including effective allele numbers (Ne) and the polymorphism information content (PIC) were calculated based on allele frequencies using appropriate formulas described by Botstein et al.[15] and Nei and Roychoudhury[16] as follows:

$$Ne = 1 / \sum_{i=1}^{n} P_i^2$$

$$PIC = 1 - \left(\sum_{i=1}^{n} P_i^2 \right) - \sum_{i=1}^{n-1} \sum_{j=i+1}^{n} 2P_i^2 P_j^2$$

where:
Pi was the frequency of the ith allele,
n was the number of alleles.

Theoretical heterozygosity (Hthe) was calculated as:

$$H_{the} = 1 - \Sigma(p^2 + q^2)$$

The Fixation Index (FIS) was estimated from the values of Hthe and experimental (Hexp) heterozygosities using the following formula:

$$F_{IS} = (H_{the} - H_{exp}) / H_{the}$$

Theoretical homozygosity (E) was calculated as:
The level of possible variability realization (V%) was estimated according to Crow and Kimura[17] as follows:

$$E = \sum p_i^2$$

where:

$$V = \frac{1 - E}{1 - \frac{1}{N}} \times 100$$

E= expected homozygosity
N= number of individuals in a population regarding a particular locus

The least-squares method as applied in a general linear model (GLM) procedure of Minitab software v19.1.1 was used to test the significance of the association of *FABP4* c.328 G>A with slaughter weight and carcass traits. The selected statistical model is as follows:

$$Y_{ijklm} = \mu + A_i + S_j + G_k + I_l + e_{ijklm}$$

where:
Yijklm = the studied traits,
μ = the overall mean,
Ai = the fixed effect of slaughter age (i = 14-16 months),
Sj = the fixed effect of slaughter season (j=spring and summer),
Gk = *FABP4* genotypes (k=AA, GA, and GG),
Il = two-way interactions,
eijklm = random error.

Tukey's multiple comparison test was used; if significant effects were detected. Two-way interactions were statistically non-significant (P>0.05), thus, they were excluded from the model and will not be considered further.

Additive and dominance effects were estimated according to Falconer and Mackay[14] as follows:
The degree of dominance=d/a
where:
Additive effect (a) = the difference between the means of

two homozygous divided by two

Dominance effect (d) = the heterozygote's deviation from the mean of the homozygotes

Overdominance can be indicated when d is greater than + a or less than – a.

Results

PCR-RFLP patterns

The electrophoresis pattern of PCR amplification for the *FABP4* gene is shown in Figure 2. The cleavage of a 565 bp PCR product (Fig 3) by *Cvi*AII yielded two fragments of 233 bp and 230 bp and was diagnostic for the AA genotype. It is important to note that these fragments appear to be a single band because they are very close to each other. The GG genotype was characterized by 463 bp whereas three bands, 463 bp, 233 bp, and 230 bp, were indicative for the heterozygous genotype (Figure 3).

Allelic and genotypic distributions and population genetic indices

The frequency of GG genotype in the *FABP4* gene was found to be remarkably high in both Aberdeen Angus (49.18%) and Hereford (52.94%) bulls, as shown in Table
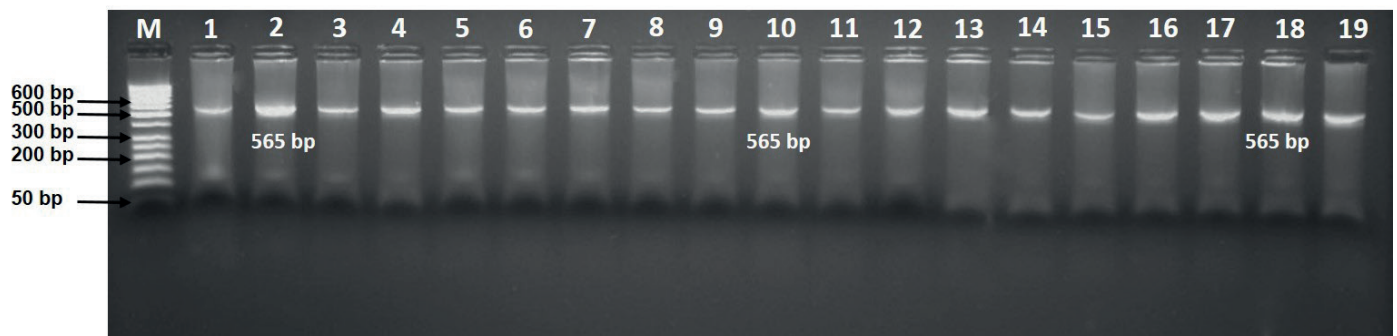

Figure 2. The electrophoresis pattern of PCR amplification (565 bp) for c.328 G>A polymorphism within the bovine *FABP4* gene
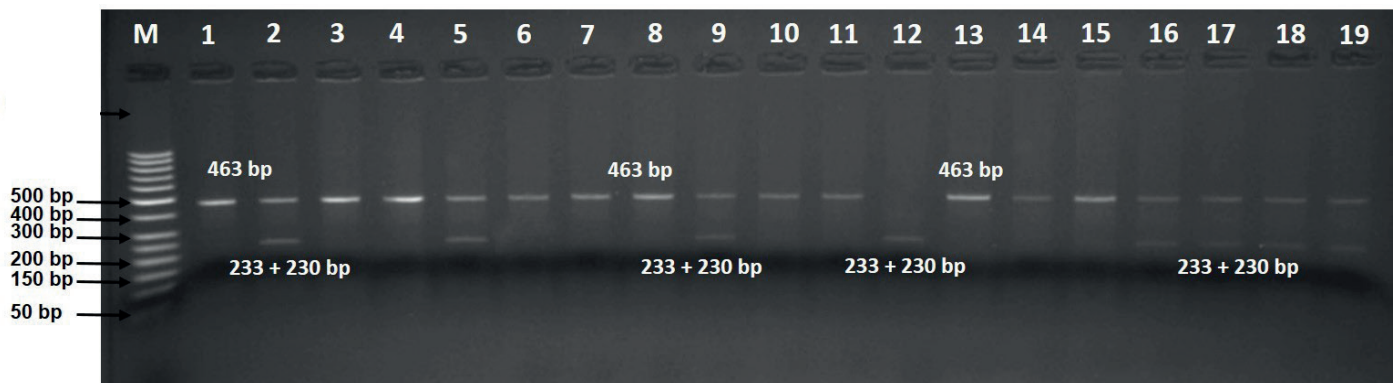
M: marker

Figure 3. The electrophoresis pattern of restriction enzyme digestion with CviAII for c.328 G>A polymorphism within the bovine *FABP4* gene.
Line 12: AA genotype; Lines 1, 3, 4, 6-8, 10, 11, 13-15: GG; and Lines 2, 5, 9, 16-19: heterozygous genotype.
M: Marker.

Table 1. Genotype and allele frequencies of the SNP c.328 G>A of the bovine *FABP4* gene in Aberdeen Angus and Hereford bulls.

Breed	n	Genotype frequencies (%)*			Allele frequencies	
		AA	GA	GG	A	G
Aberdeen Angus	61	14.75 (9)	36.07 (22)	49.18 (30)	0.33	0.67
Hereford	34	11.77 (4)	35.29 (12)	52.94 (18)	0.29	0.71
Total	95	13.68 (13)	35.79 (34)	50.53 (48)	0.32	0.68

1. Moreover, 48 out of 95 cattle were GG genotype carriers. For both breeds, the predominant allele was the G (0.68 in total). Only 9 and 4 cattle carry the AA genotype in the *FABP4* c.328 G>A polymorphism in Aberdeen Angus and Hereford bulls, respectively. This resulted in a low frequency of the A allele (0.32 in total).

cy of the A allele (0.32 in total).

HWE testing revealed that the population was determined to be compatible with the equilibrium (P>0.05). The population genetic indices are presented in Table 2. Ho was higher than 0.50 while the PIC was higher than 0.25 for both Aberdeen Angus and Hereford breeds. A considerable high level of FIS (~0.71) was observed in Hereford bulls. Besides, Herefords had a higher V% compared to Aberdeen Angus bulls. As shown in Table 2, an admissible

Table 2. Genetic diversity at the *FABP4* gene in Aberdeen Angus and Hereford bulls.

Breed	H_{the}	Ho	Ne	PIC	F_{IS}	V%	Locus HWE test
Aberdeen Angus	0.4422	0.5578	1.7928	0.3444	0.5025	44.93	Equilibrium (*P*>0.05)
Hereford	0.4118	0.5882	1.7001	0.3270	0.7086	42.42	Equilibrium (*P*>0.05)
Total	0.4352	0.5648	1.7705	0.3405	0.2188	43.98	Equilibrium (*P*>0.05)

Table 3. Least square means for *FABP4* c.328 G>A genotype effects on slaughter weights (kg) in Aberdeen Angus and Hereford bulls.

Breed	Genotype			*P*-value
	AA	GA	GG	
Aberdeen Angus	581.10±14.60	594.99±9.00	565.88±7.81	**0.054**
Hereford	595.89±29.97	599.34±18.91	555.49±14.01	0.137
Total	581.62±14.01[ab]	591.27±8.51[a]	558.99±7.11[b]	**0.013**

Table 4. Least square means for *FABP4* c.328 G>A genotype effects on carcass traits in Aberdeen Angus bulls.

Trait	Genotype			*P*-value
	AA	GA	GG	
Hot carcass weight (kg)	312.88±9.42	301.69±6.45	300.89±7.27	0.487
Cold carcass weight (kg)	307.83±9.43	296.30±6.46	295.74±7.28	0.476
Chilling loss (kg)	5.05±0.21	5.38±0.14	5.15±0.16	0.299
Dressing percentage (%)	54.28±0.77	55.15±0.53	54.05±0.59	0.311
Carcass pH at 24 h	5.54±0.09	5.53±0.05	5.56±0.04	0.897

Ne level (>0.70) was found in the studied population.

Marker effects on trait means

Concerning the association between the *FABP4* genotypes and the SW, levels of significance are reported in Table 3. Heterozygous animals had higher SW compared to alternative genotypes. However, this association was substantiated in only the total cattle population (P<0.05). As shown in Table 4, the association of the *FABP4* genotypes with carcass weights, CL, DP, and pH (24h) was found to be non-significant.

Discussion

In genetic association studies, the evaluation of population genetics characteristics plays a key role in achieving reliable and confidential results. These parameters can provide valuable information on the strength and conformity of the genetic marker regarding the selected phenotypes. Moreover, they display the breeding properties of the selected animal population, such as eventual inbreeding.[9,18] Based on the results of the χ2 test, genotype frequencies of the SNP were in compliance with HWE in both breeds.

Table 5. Additive and dominance effects of the marker with the significant association in the studied population (n=95).

Trait	Additive effect	Dominant effect	Overdominance
Slaughter weight	11.315	20.965*	+

Besides moderate levels of population genetic indices were observed for He, Ne, and PIC (Table 2). According to the interpretation by Botstein et al.[15], PIC can be classified into three main groups as follows: PIC>0.50 (high polymorphism), 0.25<PIC<0.50 (moderate polymorphism), and PIC<0.25 (low polymorphism). Thus the *FABP4* marker in this study can be evaluated as mildly informative for Aberdeen Angus and Hereford cattle. On the other hand, undesirable levels of FIS and V% were found in Hereford bulls. The main reason is that the sample size was rather limited in Herefords. Moreover, the Ho level was the highest in this breed (~0.6).

Importation of cattle has become a frequently used method to maintain the balance between supply and demand in Turkey. However, farms generally do not perform genotypic analyses on beef cattle to be imported.[19] This can lead to controversial situations about the origin and genotypic status of animals and even their breed purity. Breed registries have been established for livestock species to maintain the purity of breeds and to document the ancestry of animals. However, a significant number of animals are unregistered with no or incomplete pedigree data and uncertain ances-

tral breed origin.[20] The SNP rs110652478 has been suggested to be monomorphic in Creole, Hereford, Limousin, and Brahman breeds.[6] However, in this study, we observed all three genotypes in Herefords (even if there was an unbalanced genotypic distribution). Genetic assessment can prevent controversial results and inconsistencies between literature (previously reported genotypic distributions) and the observed results in the field. There are also newly developed genetic tools for determining the breed purity of cattle.[20]

In the last few decades, the main focus on animal breeding in many countries has gradually changed from conventional phenotype-based evaluation to molecular genetic analysis. These molecular techniques are rather confidential and effective for selecting animals with superior performance in specific desirable traits. In the present study, the association of *FABP4* c.328 G>A with slaughter weight and certain carcass traits was tested in Aberdeen Angus and Hereford bulls. The *FABP4* c.328 G>A genotype effect was strongly significant for slaughter weight (P=0.01), with the heterozygous animals associated with increased slaughter weight (591.27±8.51 kg) concerning total cattle population. The GA genotype carriers had +9.65 kg and 32.28 kg higher slaughter weights compared to the AA and GG animals, respectively. The corresponding estimates indicated significant overdominance (Table 5). This interpretation was not substantiated in breed-specific evaluation, although heterozygous animals were heavier than alternative genotype carriers. But it is important to note that the association of *FABP4* with slaughter weight approached statistical significance (P=0.054). Although *FABP4* is a leading genetic marker for meat quality traits, especially marbling[2,5,8], some papers are indicating a potential effect of this gene on live and carcass weight. In this context, Lee et al.[21] showed that the *FABP4* microsatellite (3237AT) marker had a significant effect on live weight in Hanwoo. A SNP located in intron 1 of the *FABP4* (g.2834C>G) was associated with desirable increases in live weight, hot carcass weight, and chilled carcass weight.[2] On the other hand, in Holstein bulls, a potential novel effect of *FABP4* gene polymorphisms on average daily weight gain was shown by Ardicli et al.[9] This gene was associated with carcass weight in various commercial beef cattle populations. Mizoshita et al.[22] demonstrated that a quantitative trait loci (QTL) for carcass weight in purebred Japanese Black (Waygu) is close to the *FABP4* gene on BTA14. Moreover, Lee et al.[12] suggested that the genetic polymorphisms of the *FABP4* gene (g.3473T>A and 2774G>C) are significantly associated with carcass weight in Hanwoo (Korean Cattle). They also found that the CAG haplotype effect for carcass weight was larger than the largest single-locus effect of g.3473A>T. In

this study, non-significant differences among *FABP4* genotypes were found for hot and cold carcass weights. In a similar vein, studies conducted on different cattle breeds have reported that *FABP4* may have limited influences on live weight and carcass traits.[23-25] In some cases, unbalanced distribution of the alleles, limited sample sizes, and remarkably low frequencies of alleles may be the potential reasons for the non-significant phenotype-genotype interactions. [23,24] On the other hand, the genetic composition of the selected breeds and/or polygenic effects associated with the expression of the studied traits are important factors to be considered. FABPs (especially *FABP4* and FABP5) have been proposed as potential candidate genes for obesity as they are located within a QTL region.[8] From a broader point of view, *FABP4* would be potentially relevant to elucidate the influence of this genomic region concerning live weight and carcass traits.

The bovine *FABP4* gene was hypothesized to be a genetic factor influencing intramuscular fat deposition and subcutaneous fat in muscle.[5,8,26-29] It has been suggested to be one of the major metabolic indicators of intramuscular fat deposition ability.[28] Cho et al.[11] reported that *FABP4* c.220A>G polymorphism in exon 2 significantly affects BFT in Korean cattle. Ardicli et al.[2] demonstrated that g.3691G>A × g.2834C>G interaction was effective on the BFT in Holstein bulls. In the present study, however, no association of *FABP4* marker with BFT was observed. Moreover, there was no significant effect of this gene on CL, DP, and pH (24h). As suggested by Barton et al.[28] and Aviles et al.[26], the effects of genetic markers are often breed-specific and they are not eligible for all cattle populations. Furthermore, it is worth noting that, the sample size for association analyzes in this study was rather limited because crossbreds and the animals with missing information were excluded from the genetic analyses to prevent unreliable results. Thus, studies performed with larger populations may be needed for confirmation.

Evaluation of genotype-phenotype interactions based on economically important traits will provide significant insights into Turkey's livestock breeding. Genetic interpretation is crucial not only in cattle raised in Turkey but also in cattle to be imported.[19] The important point to note here is that marker-assisted selection (MAS) has advantages compared to conventional phenotype-based selection methods. However, MAS cannot provide a widespread use of DNA information in animal breeding. The main reason was that many functional traits in livestock are much more complex than expected. In traits under polygenic inheritance, small effects are usually ignored and make it difficult to evaluate.[30] Genome-wide approaches have become more common in many European countries and the USA. Unfortunately, Turkey is far behind in terms of genomic applications in animal breeding. Hence genetic studies in Turkey should be encouraged to provide a more profitable and sustainable cattle breeding management, even if they may have preliminary suggestions compared to studies conducted by the developed countries with high budgets.

Conclusions

This study focused on the c.328 G>A polymorphism located on exon 3 of the bovine *FABP4* gene and its genetic diversity and effects on slaughter weight and certain carcass traits. The heterozygous genotype was significantly associated with increased slaughter weight and seemed to be desirable in the evaluation of the total population. This association was not confirmed in breed-specific analyzes. Moreover, there was no association of the *FABP4* with hot and cold carcass weight, chilling loss, dressing percentage, and carcass pH. Consequently, it can be interpreted that *FABP4* c.328 G>A marker may provide valuable, but limited, information on imported beef cattle raised in Turkey conditions.

Acknowledgment

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