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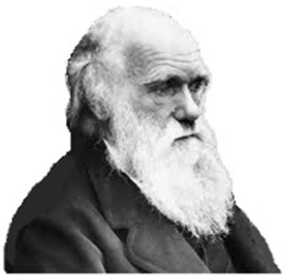

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Effect of *DGAT1 K232A* mutation and breed on milk traits in Cattle Populations of Ethiopia

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ABSTRACT

Substitution mutation (K232A) has been reported to explain variation in milk traits in *diacylglycerol acyl-CoA acyltransferase 1* (*DGAT1*) gene. The objective of this study was to investigate and analyze allele and genotype frequency and associations between K232A and milk traits in cattle populations of Ethiopia. Boran-Holstein produced higher daily milk yield than the others ($P < 0.05$) and Boran and Begait breeds produced milk with higher fat and protein content ($P < 0.05$). Horro presented higher content of milk lactose than the studied breeds ($P < 0.05$) and Boran, Begait and Horro breeds produced milk with higher content of SNF. Allele K and A and genotypes AA, KA and KK were detected, the highest allele and genotype frequencies were K and KK, respectively. DGAT1 protein variant K232 was high in Horro, Boran and Fogera cattle ranged (0.50–0.97). The KK and KA genotypic frequencies observed ranged from 0.50-0.94 and 0.03-0.50 for native cattle breeds, respectively. KK and KA genotype had statistically significant association with fat and lactose content, respectively, whereas genotype AA has the greatest association with average daily milk yield. The KK cows produced more milk fat (6.35±0.05) and KA more lactose (5.6±0.21), respectively, and AA cows produced more average daily milk yield (10±0.21 L/day). The association study confirms that DGAT1 K232A marker had significant effects on daily milk yield, milk fat and lactose content in the investigated cattle. These results suggested that *DGAT1* marker may be evaluated to achieve various commercial goals in Ethiopian cattle production.

***Keywords*:** Association analysis, Ethiopian cattle, genotypic frequency, K232A, Milk composition

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1. INTRODUCTION

The sector of cattle agriculture in the tropics faces challenging factors of growing productivity and profitability, comparative to the size of the human population. Ethiopia is a port to a large variety of cattle populations that experience an assortment of harsh ecological conditions.

Milk volume increment has started to be the first breeding goal of dairy cattle worldwide [1]. However, newer breeding objectives, notably for milk composition traits, are known in response to the demand for a healthier human diet, and the milk-pricing system has shifted from a standard amount to its composition, touching the farm financial gain directly [2]. Local breeds' performance levels can be increased through genetic improvement in milk performance traits and efficient genetic improvement requires genetic information on variation and its effects on milk production. It is known that selection increases the frequency of alleles with a positive effect on a given attribute [3].

Identifying and validating genetic markers for milk production traits is the first and most important step in establishing a marker assisted breeding program. As a result, improving productivity through genetic selection is a common goal for many animal breeding programs around the world [1, 4, 5]. Several genes affecting economically important traits in livestock species have been localized within quantitative trait loci (QTL) dispersed throughout the genome.

In cattle breeds, the *diacylglycerol O-acyl transferase 1*(*DGAT1*) gene encodes acyl coenzyme A: diacylglycerol acyltransferase, a protein involved in fat metabolism [6-9]. The important role of this gene in the metabolism of milk fat makes it an interesting candidate for the genetic variation of milk characteristics. The mutation that results in a lysine to alanine substitution at position 232 (*K232A*) has been linked to differences in the kinetics of the enzymes encoded by the two allelic variants [6]. Grisart *et al*.[6] in particular, demonstrated that the lysine variant, which represents the "wild type" and is designated as the K allele, is distinguished by a higher maximum rate of reaction(Vmax) of the enzyme in synthesizing triglycerides when compared to the alanine variant (A allele), thereby increasing the fat percentage in the animal's milk.

The distribution of the allele frequencies of the DGAT1 *K232A* mutation has been studied in various Holstein populations and other dairy cattle breeds, and the effects of *DAGT1* polymorphisms on milk production traits have been researched [10-14].The lysine variant, in particular, has been linked to higher fat yield and fat and protein percentages, whereas the alanine variant has been linked to higher milk and protein yield [15, 16]. The diallelic DGAT1 effect on milk production traits could be explained in part by the presence of multiple alleles at the *DGAT1* locus or other mutations in closely related genes [17, 18]**.** Information on the effect of DGAT1 *K232A* on the daily milk yield and milk composition of different cattle breeds is scarce in African indigenous cattle breeds.

The DGAT1 *K232A* genotypes showed significant effects on milk composition traits in Sudanese Kenana, Butana, Butana-Holstein and Benin cattle breeds [19-22]. Before the aforementioned marker is used for the genetic improvement of Ethiopian cattle productivity, its effect should be clearly examined. Ethiopia is endowed with indigenous cattle such as the Boran, Begait, Horro and Fogera breeds that have promising production potential [23, 24]. A recent study revealed the polymorphic nature of the DGAT1 gene in cattle populations in Ethiopia [25], which requires further study on the influence of DGAT1 *K232A* on milk

composition in cattle populations in Ethiopia. Such study would be useful for gathering knowledge on nutritional value of indigenous cow milk for the dairy industry in Ethiopia and for the opportunity to improve cow's milk composition with regards to human health.

Therefore, the present study aimed to estimate the milk composition variation and to determine the allele and genotypic frequencies of the DGAT1 K232A mutation and to estimate the effect of the two allelic variants on daily milk yield and milk composition of cattle populations of Ethiopia.

2. MATERIALS AND METHODS

Sample collection and DNA extraction

The animal study was reviewed and approved by Ethical Review Board of Adama Science and Technology University (certificate reference number RECSoANS/BIO/07/2021). Written informed consent was obtained from the owners for the participation of their animals in this study. A total of 92 animals comprising of four indigenous cattle (17 Boran, 16 Begait, 18 Fogera, 17 Horro and 24 Boran-Holstein (75 % F1 HF * (HF*Boran) cows were sampled from state owned farms in Ethiopia.

Boran cattle are predominantly distributed in the semi-arid and arid areas of Southern Ethiopia, Northern Kenya and South Western Somalia and are large East African zebu cattle known for their fast growth, high milk yield and heavy muscling that needs calm environment [23]. Horro cattle breed is widely distributed in South Western and West Ethiopia and is one of the Sanga x Zebu intermediate types and is suited for milk, draft power and meat [23, 26, 27]. Fogera cattle breed is found in the North West highlands of Ethiopia and mainly reared for drought and dairy production and belongs to the Zenga group which is the cross of Sanga and Zebu [23, 24]. The Begait breed is grouped under large East African Zebu [23] and used primarily for milk and meat production [24, 28].

Blood and milk samples were only obtained from randomly selected and sampled lactating cows. Studied animals were raised on natural grazing without concentrate supplementation and they were sampled at the same period eliminating the effect of season. 4ml blood samples were collected from the tail head of each animal under aseptic conditions and gently mixed with ethylene diamine tetraacetic acid (EDTA) anticoagulant placed into an ice box containing ice. Extraction of genomic DNA was carried out using salting out extraction procedurę [29]. The quality of the DNA and its concentration were assessed via NanoDrop1000 and electrophoresis in 1.5% agarose gels. Those DNA samples with good quality and quantity were considered for amplification and sequencing.

PCR Amplification and Sequencing of *DGAT1* Gene region

Based on the reference sequence (AJ318490) the primers were designed to amplify 278bp fragment enclosing the p.Lys232Ala mutation: forward, 5'-AAGGCCAAGGCTGGTGAG-3'; reverse, 5'-GGCGAAGAGGAAGTAGTAG-3' through primer 3 plus software [30]. PCR was carried out in a total volume of 25 µL containing, 5X PCR buffer (5 µl), 1.5 mM $MgCl_2$ (3 µl), 10 MmdNTP's mix (1 µl), forward primer 70 pmol/µl (0.5 µl), reverse primer 70 pmol/µl (0.5 µl), genomic DNA 25 ng/ µl (2 µl), Taq DNA polymerase 5 U/µl (0.3 µl) and DNAase free water (12.7 µl). The optimized thermal profile include an initial denaturation at 94 °C for 3 minutes, 30 cycles of denaturation at 94 °C for 1 minutes, annealing at 57 °C for 45 seconds,

elongation at 72 °C for 1 minute and a final extension at 72 °C for 7 minutes. Finally, the PCR products were visualized post electrophoresis on 1.7% agarose gel in TAE buffer followed by GelRed staining. The PCR products were sequenced on ABI 3730 sequencer (Applied Biosystems, Foster City, CA, USA) through Sanger dideoxy chain termination method at Konkuk University of south Korea (Seoul). The sequences were analyzed and deposited to GenBank with the following accession numbers (ON262825-ON262849).

Early morning and late afternoon 50 ml of milk sample were collected from each animal in clean, dry, grease free and labeled milk collection vials. The milk sample was collected during mid-milking stage to avoid sampling fluctuations in milk composition data. No milk collection was carried out from the animals which were in terminal or early stage of lactation. For milk composition analyses milk samples supplemented with milk preservative tablets (Microtab II) stored at 4 °C cold chain were sent to Holeta dairy research laboratory.

Test-day milk fat percent, protein percent, lactose percent and solid not fat percent were determined in milk samples using Lactoscan ultrasonic milk analyzer (Milkotronic Ltd).

Data Management and Statistical analysis

Prior to analysis, all the chromatograms were visualized and sequences fragments were edited using Bio-edit version 7.0.5.3 and aligned by clustalX2 software package[31]. Genotype and allele frequencies were calculated using Power Marker (version 3.25) (Liu & Muse, 2005). Standard error of allelic frequency was calculated as $[p\ (1-p)/2n]^{1/2}$ where n is the sample size and p is the frequency of allele (Spiess, 1977).

To investigate the effect of breed type and DGAT1 genotypes on milk components General Linear Model (GLM) was used and difference between means was separated using Tukey HSD test with SAS 9.3 statistical software package [34]. The breed type and genotype were fitted as fixed independent variables, while the observed traits: daily milk yield, fat%, protein %, SNF % and lactose % were fitted as dependent variables. The effects of fixed factors were fitted in the statistical model below:

$$Y_{ij} = \mu + B_i + G_j + (B_i \text{ X } G_j)_{ij} + \varepsilon_{ijk}$$

where, Yij is the observed trait: daily milk yield, fat%, protein %, solid not fat % and lactose %, μ is the population mean, B_i, fixed effect of i^{th} breed type (i=5), G_j fixed effect of *DGAT1* genotype (KK, KA and AA), $(B\times G)_{ij}$ is the fixed interaction effect between breed and *DGAT1* genotype and ε_{ijk} is the random residual associated with each record. The results of breed and *DGAT1* genotype effects are presented as least squares means ± Standard Error. Probability less than 0.05 was used to determine the level of significance. The phenotypes were nested within breed to obtain breed specific estimates. The Pearson correlation indices were calculated among the various milk component traits using SAS 9.3 statistical software.

3. RESULTS

Effect of breed on milk yield and composition

Least square means of milk components across breeds are reported in Table 1. Boran-Holstein crossbred produced higher daily milk yield than the others ($P < 0.05$).

Table 1. Effect of breed on DMY and milk composition traits in Ethiopian cattle.

Variables	Breed					P-Value
	Boran (17)	Begait (16)	Horro (18)	Fogera (17)	Boran-Holstein (24)	
DMY (L/day)	2.12±0.12^{ab}	2.82±0.12^{bc}	2.42±0.26^{b}	2.68±0.12^{bc}	7.21±0.10^{d}	0.00
Fat (%)	6.21±0.04^{d}	4.68±0.042^{c}	3.47±0.09^{b}	4.63±0.041^{c}	3.18±0.03^{a}	0.00
Protein (%)	3.54±0.03^{bc}	3.63±0.03^{bc}	3.39±0.08^{bc}	3.04±0.03^{a}	3.14±0.03^{a}	0.00
Lactose (%)	4.72±0.05^{b}	4.43±0.05^{a}	5.31±0.11^{c}	4.29±0.05^{a}	4.70±0.04^{b}	0.00
SNF (%)	8.99±0.09^{c}	8.86±0.09^{c}	9.35±0.18^{c}	7.90±0.08^{a}	8.57±0.08^{b}	0.00
Ash (%)	0.71±0.008^{a}	0.76±0.008^{b}	0.74±0.01^{b}	0.69±0.008^{a}	0.70±0.007^{a}	0.00

DMY: Daily Milk Yield; ^{a,b,c,d} Values within a row with different superscripts differ significantly at $P < 0.05$.

Boran and Begait breeds produced milk with higher fat and protein content, respectively, compared to the others ($p < 0.05$). On the other hand, Horro presented higher content of milk lactose than the studied breeds ($p < 0.05$). The Boran, Begait and Horro breeds produced milk with higher content of SNF in comparison to the other breeds. Statistically significant differences were observed for milk components among the studied breeds (Table 1).

Allele and Genotype frequencies of *K232A* protein variant

The allele and genotypic frequencies of the *DGAT1* gene for Boran, Begait,Horro, Fogera and Boran-Holstein cattle are presented in Table 2. Two alleles (K and A) and three genotypes (KK, KA and AA) were observed in the total population. The *DGAT1* (K) allele was observed with high frequencies in Horro (0.97), Boran (0.80), Fogera (0.78) and Begait (0.75) and moderate frequency in the Boran-Holstein (0.50) breeds, whereas the (A) allele frequency was 0.50, 0.25, and 0.22, in Boran-Holstein, Begait and Fogera cattle, respectively.

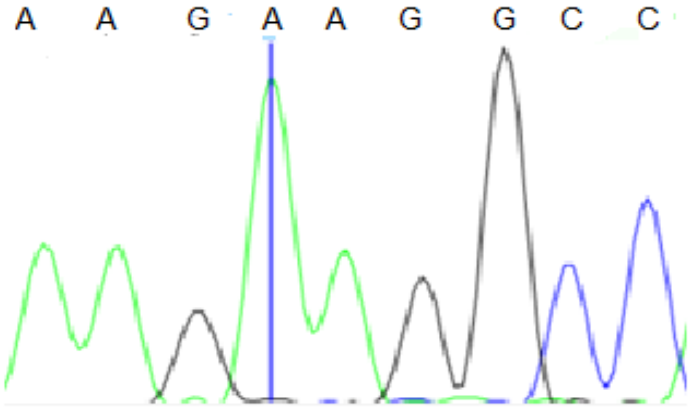
Table 2. Gene and genotypic frequencies of exon-8 of *DGAT1* gene.

Breed	N	Genotypic frequencies			Allele frequencies		SE
		KK	KA	AA	K	A	
Boran	17	0.59	0.41	--	0.80	0.20	0.07
Begait	16	0.50	0.50	--	0.75	0.25	0.08

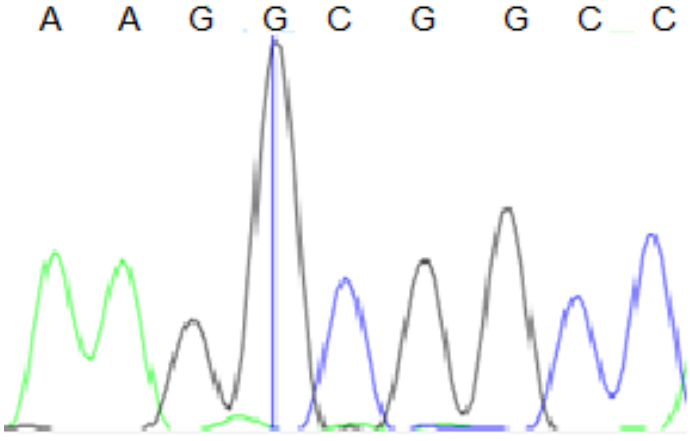
Horro	17	0.94	0.06	--	0.97	0.03	0.03
Fogera	18	0.56	0.44	--	0.78	0.22	0.07
Boran-Holstein	24	0.25	0.50	0.25	0.50	0.50	0.07
Overall	92	0.54	0.39	0.07	0.74	0.26	0.03

N; Number of animals, SE-Standard error

The *DGAT1* KK and KA genotypes were observed in all studied breeds with varying frequencies (Fig 1). The (KK) genotype (Fig. 1) was observed with high frequencies in Horro(0.94), Boran (0.59) and Fogera and relatively low frequency for Boran-Holstein(0.25) breeds, whereas the (KA) genotype frequency was the same for Begait and Boran-Holstein (0.50) cattle. The frequency of the (KA) genotype in Boran (0.20) and Horro (0.03) was lower than that in the others. The AA genotype was only seen in Boran-Holstein cattle with a frequency of 0.25 (Fig. 1, Table 2).



AA/AA (KK) Genotype



GC/GC (AA) Genotype

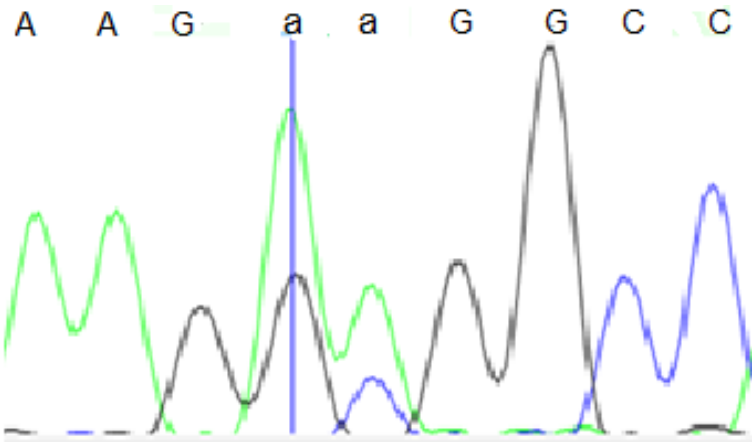


Figure 1. Sequence trace views of sequencing traces for positions10433 and 10434. The vertical line indicates nucleotide position 10433. Positions 10433 and 10434 are responsible for the *K232A* substitution. The positions of individual mutations were named according to the sequence available in the GenBank (AJ318490).

Effect of *DGAT1 K232A* on daily milk yield and composition

Analysis of the effect of *DGAT1 K232A* on daily milk yield and composition showed that the KK (lysine homozygote) and KA (lysine-alanine heterozygote) genotype had significant associations with average daily milk yield, fat and lactose percentage levels in all studied breeds(Table 3).

Cows with genotype AA (alanine homozygote) produced higher daily milk yield and milk with lower fat content. Genotype AA cows had a higher ($P < 0.05$) daily milk yield (10±0.21 L/day) than KA cows (7.62±0.15 L/day) and KK cows (4±0.21 L/day) in Boran-Holstein cows, whereas, genotype KK was significantly associated with higher milk fat and KA with higher lactose percentage (Table 3). However, no statistically significant difference ($P>0.05$) was observed for the protein (%), solid not fat (%) and ash (%) among the KK, KA and AA genotypes in all breeds.

Table 3. Associations between *K232A* genotypes and daily milk yield and composition traits (Mean±SE).

		Genotype			Genotype		
		DMY			Fat (%)		
Breed	No.Cows	AA	KA	KK	AA	KA	KK
Boran	17	-	2.40±0.19^{b}	1.84±0.16^{a}	-	6.08±0.06^{a}	6.35±0.05^{b}
Begait	16	-	2.91±0.18^{b}	2.74±0.18^{a}	-	4.46±0.06^{a}	4.9±0.06^{b}
Horro	17	-	2.65±0.51^{b}	2.20±0.13^{a}	-	3.42±0.17^{a}	3.52±0.04^{b}

Fogera	18	-	2.89±0.18[b]	2.47±0.16[a]	-	4.44±0.06[a]	4.83±0.05[b]
Boran-Holstein	24	10±0.21[c]	7.62±0.15[b]	4±0.21[a]	2.41±0.07[a]	3.12±0.05[b]	4.03±0.07[c]
		Lactose (%)			Protein (%)		
Breed	No.Cows	AA	KA	KK	AA	KA	KK
Boran	17	-	5.00±0.08[b]	4.45±0.07[a]	-	3.38±0.06	3.69±0.05
Begait	16	-	4.49±0.08[b]	4.36±0.08[a]	-	3.60±0.06	3.66±0.06
Horro	17	-	5.6±0.21[b]	5.01±0.05[a]	-	3.34±0.16	3.43±0.04
Fogera	18	-	4.34±0.08[b]	4.23±0.07[a]	-	2.99±0.06	3.11±0.05
Boran-Holstein	24	5.11±0.09[c]	4.59±0.06[b]	4.40±0.09[a]	3.12±0.06	3.03±0.05	3.28±0.06

DMY: Daily Milk Yield. Predicted means and standard error of those means were derived from the GLM. [a,b,c] Values within a row with different superscripts differ significantly at $P < 0.05$ for DMY, fat (%) and Lactose (%) traits, Whereas for protein (%) $P > 0.05$

The phenotypic correlations between milk components in Boran-Holstein cows are presented in Table 4. The correlations of fat percentage were negative between all the traits (P<0.01). DMY showed a statistically significant (P<0.01) positive correlation with lactose content and SNF (0.76) and a negative correlation with fat content (-0.96, P<0.01). The lactose percentage showed a positive correlation with DMY (0.76) and SNF (1, P<0.01) but a negative correlation with fat content -0.69, P<0.01). The SNF percentage showed a significantly positive correlation with DMY (0.76) and lactose content (1, P<0.01) and negative correlations with fat content (-0.70, P<0.01).

Table 4. Phenotypic coefficient of correlations (Pearson) for Boran-Holstein cattle (above the diagonal) and Indigenous cattle (below the diagonal)

Variable	DMY	Fat (%)	Protein (%)	Lactose (%)	SNF (%)	Ash (%)
DMY		-0.96**	-0.29	0.76**	0.76**	0.27
Fat (%)	-0.30*		0.38	-0.69**	-0.70**	-0.33
Protein (%)	-0.39**	0.29*		-0.16	-0.16	0.23
Lactose (%)	-0.08	-0.34**	0.04		1.00**	0.05
SNF (%)	-0.14	0.06	0.68**	0.68**		0.04
Ash (%)	0.03	-0.36**	0.56**	0.33**	0.56**	

DMY: Daily milk yield, SNF: Solid not fat
*P <0.05; **P< 0.01

The phenotypic correlations between milk components in indigenous cows are presented in Table 4. The fat percentage showed significantly (P<0.05) positive correlation with protein content (0.29) and negative correlation with DMY(-0.30) (P<0.05), lactose (-0.34) and ash content (-0.36) (P<0.01), where as the protein percentage showed significantly positive correlation with fat content (0.29) (P<0.05), SNF (0.68) and Ash (0.56) (P<0.01) and negative correlations with DMY (-0.39) (P<0.01).

Lactose percentage showed positive and moderate correlation (P<0.01) with SNF (0.68) and ash (0.33) but a negative correlation with fat content (-0.34, P<0.01). The correlations of SNF were positive between all the traits except for DMY (-0.14) (Table 4).

4. DISCUSSION

Effect of breed on milk yield and composition

The milk fat content of studied breeds was within the range for the milk composition standard requirement of cows (3.18-6.21%). Zebu cows can give milk containing up to 7% fat [35] . Significant differences were observed for all milk components among the studied breeds. Similar studies reported the effect of breed on milk composition in South African [36] and Benin cattle breeds [20]. Boran cows in Ethiopia presented a higher fat content (6.21%) than the values of 2.68% for Boran in South Africa and Borgou and White Fulani cows (4.78 and 4.85% respectively) in Benin [20, 36]. Thus, according to the present study Boran-Holstein cows are most favored for daily milk yield whereas Boran cows are most favored for their milk fat percent. The observed differences between breeds would therefore be due to their genetic background. Furthermore, the forage nutrient composition under natural grazing could be the reason for the variation in milk components among the cattle breeds. The investigated cattle breeds are reared in different agro-ecological zones with different floristic compositions. Cow's milk composition could be affected by forage species and variety, climate and stage of growth.

Allele and Genotype frequencies of *K232A* protein variant

The present study revealed that the lysine DGAT1 protein variant K232 was high in Horro, Boran and Fogera cattle. This is in agreement with studies in other *Bos indicus* breeds where the frequency of the lysine variant K232 ranged from 0.80 to 1 (Four African Zebu (Banyo Gudali ,Kenana, Butana and White Fulani) [17, 19, 22, 37] and six Indian cattle (Nellore, Sahiwal, Rathi, Deoni, Tharparkar, Red Kandhari and Punganur) [38, 39] and was comparable to the frequencies of K232 in Borgou (0.77) and White Fulani (0.92)cattle of Benin [21]. The higher K allele seen in those three Ethiopia breeds might reflect their exceptional adaptive value. The overall immunity of native cattle against diseases and their survival adaptation to coarse feed could be because of increased acyltransferase activity of the *DGAT1* gene that catalyzes the synthesis of retinol esters and thus regulates the synthesis of vitamin A. Manga and Riha [12] reported a favorable association of the DGAT1 'K' allele with low somatic cell count in lactating cows that partly explains the exceptional genetic resistance of native cattle against mastitis [40].

In the present study, the observed frequencies of the (K) allele (0.50–0.97) are higher than those observed (0.210–0.380) for the native Turkish breeds [38]. European *Bos taurus* breeds, with the exception of the Turkish native and Jersey breeds, showed the lowest frequency of the (K) allele, whereas the highest (K) allele frequencies were harbored by *Bos indicus* type cattle [41]. Allele fixation was not observed in this study. Fixation of the DGAT1 K allele in three different Indian Zebus (Fleckvieh, Anatolian Black, and Sahival) was found[42], while Kaupe *et al*. [38] found fixation of the DGAT1A allele in five *Bos taurus* breeds (Belgian Blue beef, Gelbvieh, Hereford, Pinzgaurer and Slavonian Syrmian).

The high proportion of the lysine variant K232 observed in the current study might contribute to the higher fat content in native cattle in Ethiopia and other *Bos indicus* breeds compared to Holstein–Friesian cross cattle. The frequency of the lysine variant K232 in the investigated Boran-Holstein cattle is similar to studies in other Holstein–Friesian populations or their crosses where it ranged from 0.21 to 0.63 [16, 43-46]. The differences in the frequency of the lysine variant K232 among breeds might result from different breeding goals and more Holstein genomic background of Boran-Holstein cattle. This could also be seen in the allele frequency of the investigated DGAT1 marker for the *K232A* polymorphism, where alanine variant 232A is the favorite variant in Holstein cattle. The A allele frequency observed in native cattle in the present study was relatively similar to the indigenous cattle of Sudan (ranging from 0.037-0.15) [37]. The A allele frequency observed in Boran-Holstein cattle was higher than the report of Li *et al*. [14] for Kiwicross cattle from New Zealand and relatively lower than the Butana-Holstein crossbred cattle in Sudan [19]. To reiterate, the low (A) allele frequency observed in native breeds of Ethiopia might be attributed to *taurus* cattle introgression.

The KK and KA genotypic frequencies observed in the present study ranged from 0.50-0.94 and 0.03-0.50 for native cattle breeds, respectively, which is comparable to most *Bos indicus* cattle [19, 20, 22, 37].

Effect of *DGAT1 K232A* on daily milk yield and composition

The association study confirmed that the DGAT1 *K232A* marker had significant effects on daily milk yield, milk fat and lactose content in the investigated cattle. The higher effects of the KK genotype on milk fat content and the higher effect of the AA genotype on average daily milk yield were in agreement with previous studies [6, 10, 16, 47-49].

Phenotypic correlation clearly indicated that the fat percentage had moderate negative correlation ($P<0.05$) and a highly negative correlation ($P<0.01$) with daily milk yield in indigenous cows and Boran-Holstein cows, respectively. The lactose percentage in indigenous and Boran-Holstein cows has a highly negative correlation with fat content. This result is in agreement with previous reports by Fox [50], who indicated strong negative relationship between lactose and fat content in milk across species. Accordingly, the lysine variant K232 decreased lactose content in milk across breeds in the present study. Therefore, before the KK, KA and AA genotypes can be used in breeding programs, the interaction effect between this gene and feed management and other environments should be studied.

5. CONCLUSIONS

Detailed information about milk composition is generally not included in breeding goals for dairy cattle production in Ethiopia and evidence for indigenous and cross-breed effects and

effects of DGAT1 *K232A* allelic variants on daily milk yield and milk composition was covered in the present study. Allele K and genotype KK were found in higher proportions in the native cattle populations. Lysine at position p232 of DGAT1 was confirmed to be the highest allele in the indigenous breed. The association study confirmed that the DGAT1 *K232A* marker had significant effects on daily milk yield, milk fat and lactose content in the investigated cattle. Further results from studies with larger populations might be useful to draw more reliable conclusions and to perform an adequate evaluation.

Acknowledgements

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Ethics approval and consent to participate

The animal study was reviewed and approved by Ethical Review Board of Adama Science and Technology University (certificate reference number RECSoANS/BIO/07/2021). Written informed consent was obtained from the owners for the participation of their animals in this study. Animal samples were obtained in compliance with local/national laws in force at the time of sampling. Data exchange was in accordance with national and international regulations, and approved by the owners. The procedure involving sample collection followed the recommendation of directive 2010/63/EU. All methods were carried out in accordance with relevant guidelines and regulations. All authors gave their informed consent prior to their inclusion in the study.

Availability of data and materials

The datasets generated and/or analysed during the current study are available in the GenBank® repository with accession numbers (ON262825-ON262849).

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