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Technical note

Prevalence of *DGAT1* K232A polymorphism in grazing Holstein cows*

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Abstract

Introduction. Determining of the K232A polymorphism, in the exon 8 of the acyl-CoA:diacylglycerol acyltransferase 1 gene (*DGAT1)* genotypic frequencies in dairy cows, and the evaluation of its deviation from the Hardy-Weinberg equilibrium, could be an indirect genetic selection footprint of animals that were selected for improved milk fat content phenotypes. **Objective.** To determine the prevalence of the K232A *DGAT1* polymorphism in Costa Rican Holstein cows. **Materials and methods.** A total of 500 Holstein cows from 50 farms in three provinces of Costa Rica (Alajuela, Cartago, and San José) were sampled from October 2018 to March 2019. Blood samples were taken from coccygeal vessels. Samples were transported under 4 ºC from farms to the Biology School Laboratory of the University of Costa Rica, where DNA extractions were performed. The K232A *DGAT1* polymorphism determination was performed through real time quantitative polymerase chain reaction (RT-qPCR), using specific primers.

Results. The genotypes of 15 samples could not be determined due to sample limitations. The frequencies of the AA, KA, and KK genotypes for the remaining 485 cows were 54.6%, 38.8% and 6.6%, respectively. There was no deviation from the Hardy-Weinberg equilibrium (chi-square = 0.03; $p > 0.05$) in the overall population. **Conclusion.** Random mating between animals for the *DGAT1* polymorphism has occurred in Costa Rica.

Keywords: milk fat; genomic selection; allele frequency; unique nucleotide.

Nota técnica

Resumen

Prevalencia del polimorfismo *DGAT1* K232A en vacas Holstein en pastoreo

Introducción. La determinación del polimorfismo K232A, en el exón 8 del gen acil-CoA:diacilglicerol aciltransferasa 1 (*DGAT1*) en vacas lecheras, y la evaluación de su desviación del equilibrio de Hardy-Weinberg, podría ser una huella de selección genética indirecta de animales que fueron seleccionados por fenotipos de contenido de grasa en leche mejorados. **Objetivo.** Determinar la prevalencia del polimorfismo K232A *DGAT1* en vacas Holstein de Costa Rica. **Materiales y métodos.** Se muestrearon 500 vacas Holstein de 50 fincas en tres provincias de Costa Rica (Alajuela, Cartago y San José) desde octubre de 2018 a marzo de 2019. Se tomaron muestras de sangre de los vasos coccígeos. Las muestras fueron transportadas a 4 ºC desde las fincas hasta el Laboratorio de la Facultad de Biología de la Universidad de Costa Rica, donde se realizaron extracciones de ADN. La determinación del polimorfismo K232A de *DGAT1* se realizó mediante reacción en cadena de polimerasa cuantitativa en tiempo real (RT-qPCR), utilizando primers específicos. **Resultados.** No se pudieron determinar los genotipos de 15 muestras debido a limitaciones de la muestra. Las frecuencias de los genotipos AA, KA y KK para las 485 vacas restantes fueron 54,6%, 38,8% y 6,6%, respectivamente. No hubo desviación del equilibrio de Hardy-Weinberg (chi-cuadrado = 0,03; $p > 0{,}05$) en la población general. **Conclusión.** En Costa Rica se ha producido apareamiento aleatorio entre animales para el polimorfismo *DGAT1*.

Palabras clave: grasa en leche; selección genómica; frecuencia alélica; nucleótido único.

Introduction

The K232A polymorphism, in the exon 8 of the *acyl-CoA:diacylglycerol acyltransferase* gene (*DGAT1*) on the bovine chromosome 14, is an alternative sequence of 2 base pairs, where the adenine-adenine nucleotides that encodes Lysine (K) amino acid are replaced by the guanine-cytosine nucleotides, shifting the resulting amino acid to Alanine (A) at the 232 amino acid position (Grisart et al., 2002). The *DGAT1* is a key gene involved in milk fat synthesis (Mu et al., 2021), highly associated with milk fat percentage and milk fat yield. It has been reported that cows with the K allele have higher values of milk fat (Fontanesi et al., 2014; Tăbăran et al., 2015). There is strong evidence, from a metanalysis which analyzed 30 studies (Mahmoudi & Rashidi, 2023), of the positive effect exerted by the K allele of *DGAT1* K232A polymorphism over milk fat content compared to the A allele, with the KK genotype yielding more than one standardized mean difference compared to the AA genotype.

The *DGAT1* K allele has been associated with more saturated fat, larger fraction of C16:0, and lesser fractions of C14:0, unsaturated C18 and conjugated linoleic acid (Schennink et al., 2007). Random mating is one of the Hardy-Weinberg equilibrium conditions, therefore, it is rational to hypothesize that if the population accomplishes the Hardy-Weinberg equilibrium, it is less likely that directional mating for selection has been applied. Hence, the fitting into Hardy-Weinberg equilibrium could be evidence of no artificial selection exerted over the studied population for the K232A *DGAT1* polymorphism (Waples & Allendorf, 2015). In populations without animal genotyping, a deviation from the Hardy-Weinberg equilibrium according to observed genotypes for K232A *DGAT1* could be used as evidence of indirect selection for milk fat content. Consequently, to define in a population the relative prevalence of genotypes for this polymorphism, could assist in determining artificial selection footprint.

There are studies regarding *DGAT1* K232A polymorphism reporting higher and lower proportions for the allelic frequencies (Scotti et al., 2010). A compilation from some studies around the world reporting allelic frequencies of the *DGAT1* K232A are described in **Table 1**.

Table 1. Allelic frequencies of the *DGAT1* K232A polymorphism reported in Holstein populations from different countries.

Tabla 1. Frecuencias alélicas del polimorfismo *DGAT1* K232A reportada en poblaciones Holstein de diferentes países.

Country	**Number of animals**[1]	**p.232K**	**p.232A**	**Reference**
Israel	1038 sires	0.15	0.85	Weller et al., (2003)
Czech	315 cows	0.19	0.81	Manga & Řiha, (2011)
Italy	43 sires + 73 cows	0.25	0.75	Scotti et al., (2010)
Czech	278 cows	0.26	0.74	Kadlecová et al., (2014)
Brazil	50 sires	0.27	0.73	Lacorte et al., (2006)
Ireland	848 sires	0.32	0.68	Berry et al., (2010)
France	2259 sires	0.37	0.63	Gautier et al., (2007)
Spain	499 cows	0.40	0.60	Tupac-Yupanqui et al., (2004)
Netherlands	1762 cows	0.40	0.60	Schennink et al., (2007)
Germany	791	0.42	0.58	Kaupe et al., (2004)
United Kingdom	571 cows	0.47	0.53	Banos et al., (2008)
Turkey	306 cows	0.53	0.47	Gurcan et al., (2019)
Poland	89 sires	0.54	0.46	Nowacka-Woszuk et al., (2008)
Germany	1291 sires	0.55	0.45	Kaupe et al., (2007)
Germany	833 sires	0.55	0.45	Thaller et al., (2003)
Poland	244 sires + 213 cows	0.57	0.43	Pareek et al., (2005)
New Zealand	1527 sires	0.60	0.40	Spelman et al., (2002)
Greece	497 cows	0.62	0.38	Oikonomou et al., (2009)
Romania	390 cows	0.73	0.27	Tăbăran et al., (2015)
Hungary	250 cows	0.84	0.16	Anton et al., (2008)

[1] Sex of the analyzed animals was not reported.

[1] El sexo de los animales analizados no fue reportado.

Costa Rica is a tropical country located in Central America, where pasture-based farms are the prevalent feeding system in dairies (Vargas-Leitón et al., 2013). Most Costa Rican specialized dairy farms (295) are organized in small productive systems (average of 46 milking cows per farm) affiliated to a milk producers cooperative (*Cooperativa de Productores de Leche Dos Pinos*) which industrializes and commercializes their milk.

The most prevalent breed in specialized dairy farms in Costa Rica is Holstein (Saborío-Montero et al., 2017, Saborío-Montero et al., 2018). Animal genotyping is not common in these systems; therefore, until now, deviation from the Hardy-Weinberg equilibrium in this population for the K232A *DGAT1* polymorphism could not be related to systematically directed artificial genetic selection. The payment in the milk industry in Costa Rica is based on total milk solids, which enforces the necessity for improving milk fat content (CNPL, 2025).

The aim of present study was to determine the prevalence of the K232A *DGAT1* polymorphism in Costa Rican Holstein cows.

Materials and methods

Location

All farms belonged to dairy producers affiliated to the *Cooperativa de Productores de Leche Dos Pinos*. Dairy farms were selected according to an equal proportion of total active farms in three provinces (Alajuela, Cartago, and San José) of Costa Rica (COOPROLE R.L., 2017). The sampling period was from October 2018 to March 2019.

Sampling

A proportional stratified sampling method was used to collect blood samples, the number of sampled cows was calculated based on the total number of Holstein cows (n = 12474) present in the three provinces with the most dairy farms in Costa Rica in the year 2017 (COOPROLE R.L., 2017). Information extracted from data records (list) indicated the total number of farms (strata) by province. The number of farms to be sampled by province was calculated proportionally. Within each farm, a random sampling (raffle from list given by the farmer) was performed. Therefore, each Holstein cow within farm had the same probability of been sampled. An expected proportion of the lysine encoding allele (K) = 0.6, confidence level of 95% and an estimated error of 0.05 was used as a sampling assumption, due to unavailable previous information of allele (K) prevalence in Costa Rica. From that information, a final sample size of 500 animals was established. Animals were selected in groups (n = 10) for each farm, for a total of 50 farms. The 50 farms were distributed proportionally to the number of farms per province (**Table 2**). In each province, farms were randomly selected until the number of farms was reached. Within each farm, 10 cows were randomly selected to be sampled.

Table 2. Population data and estimated sample size to determine the prevalence of *DGAT1* polymorphism in Costa Rican Holstein cows, years 2018-2019.

Tabla 2. Datos poblacionales y tamaño estimado de muestra para determinar la prevalencia del polimorfismo *DGAT1* en vacas Holstein costarricenses, años 2018-2019.

Province	**Population data**				**Estimated sample size**			
	Farms		**Cows**		**Farms**		**Cows**	
	n	%	*n*	%	*n*	%	*n*	%
Alajuela	203	71.7	8761	70.2	36	72.0	360	72.0
San José	46	16.3	2076	16.6	8	16.0	80	16.0
Cartago	34	12.0	1637	13.1	6	12.0	60	12.0
Overall	**283**	**100.0**	**12474**	**100.0**	**50**	**100.0**	**500**	**100.0**

At the end of the study a total of 500 blood samples (1 per cow) from 50 dairy farms (10 cows per farm) were collected. Blood samples were taken from the coccygeal vessels, using a vacutainer, double way 21”×1” needles and 7 mL sterile tubes with EDTA. The actual distribution of farms according to province was 36, 8 and 6 for Alajuela, San José, and Cartago, respectively. Samples were transported on ice from farms to a molecular genetics’ laboratory of the Biology School at the University of Costa Rica and were stored at 4 ºC for a maximum of 7 days, until being processed for DNA extraction.

DNA extraction and polymorphism detection

The DNA extraction was performed by using a standard protocol for blood DNA extraction. The DNA concentrations and their purity were measured by using a Spectrophotometer (Nanodrop 1000, Thermo Fisher Scientific, Wilmington, USA.) with ratio 260/230 around 1.8 and 2.0. PCR reactions were processed in a StepOne Real Time PCR system by Applied Biosystems$^{®}$ (California, USA). Samples were assessed for the genotype in small pools (n = 48) including two controls, a negative control -No Template Control- and a heterozygote sample).

Specific primers (F, 5-CGCTTGCTCGTAGCTTTGG-3; R, 5-CGCGGTAGGTCAGGTTGTC-3), as well as TaqMan probes to detect the allele encoding 232K and 232A (F, 5-CGTTGGCCTTCTTAC-3 and F, 5-TTGGCCGCCTTAC-3 marked as VIC (allele 1-Lys) and FAM (allele 2-Ala), respectively, were designed based on the *DGAT1* AY065621 sequence (Li et al., 2013). The cycling conditions regarding PCR pre-read were 60 ºC for 30 seconds, 94 ºC for 5 min, followed by 50 cycles of 92 ºC for 15 s and 60 ºC for 1 min.

For the analysis, the Allelic Discrimination Software® was utilized to plot and group the samples in accordance with the genotype. Additionally, all samples were manually reviewed to verify the amount of fluorescence generated by each of the probes, allowing them to confirm the genotype determined by the software.

In total, 485 animals were genotyped. The genotypes of 15 samples could not be determined due to impossibility of isolating DNA from the sample or the genotype was undetermined. The final distribution of genotyped samples according to location was 350, 76, and 59, for Alajuela, San José, and Cartago, respectively.

Hardy-Weinberg equilibrium

Genotypic frequencies, allelic frequencies, and the Hardy-Weinberg equilibrium (Waples & Allendorf, 2015) were estimated and sorted by province. A Pearson's chi-square test was performed based on the null hypothesis of no differences between observed and expected genotypic frequencies.

$$\chi^2 = \sum_{i=1}^{n} \frac{(Observed_{genotypic\ frequencies} - Expected_{genotypic\ frequencies})^2}{Observed_{genotypic\ frequencies}} \qquad \textit{Equation 1}$$

An $\alpha = 0.05$ was used for the chi-square test, which established a threshold value of 3.84 as the cut point for the obtention of statistical evidence to reject the null hypothesis.

Additional statistical analysis

Differences in genotype frequencies were analyzed using a log-linear modeling approach. Observed genotype counts were analyzed using a Poisson generalized linear model with genotype as the explanatory factor. The significance of genotype effects was assessed using likelihood ratio chi-square tests. When a significant genotype effect was detected, pairwise comparisons among genotypes were performed using Tukey-adjusted contrasts. All statistical analyses were conducted in R environment (R Core Team, 2020).

Let Y_i denote the observed count of individuals with genotype i, where $i \in \{AA, KA, KK\}$.

We assume that genotype counts follow a Poisson distribution:

$$Y_i \sim \text{Poisson}(\mu_i)$$

The expected counts μ_i are modeled using a log link function:

$$\log(\mu_i) = \beta_0 + \beta_i$$

where:

- β_0 is the intercept (baseline log-mean count).
- β_i represents the effect of genotype i on the expected count.

The R functions to perform the analysis were: *glm* from *stats* package for the generalized linear model, *loglm* from *MASS* package for the specification of the log-linear model, and *glht* from *multcomp* package for multiple comparison of the generalized linear model.

Results and discussion

Allele discrimination

The discrimination efficiency of the A or K encoding alleles for the used protocol was 97% (485 out of 500 samples). The overall *DGAT1* genotype classification is depicted in **Figure 1**. It is evident that there is a reduced amount of KK genotypes compared to AA and KA genotypes (**Figure 1**), which suggests limited milk fat yield enhancement potential due to the lysine encoding genotype (KK) in this population. Lysine encoding alleles had been associated broadly with higher milk fat content (Manga & Řiha, 2011; Khan et al., 2021).

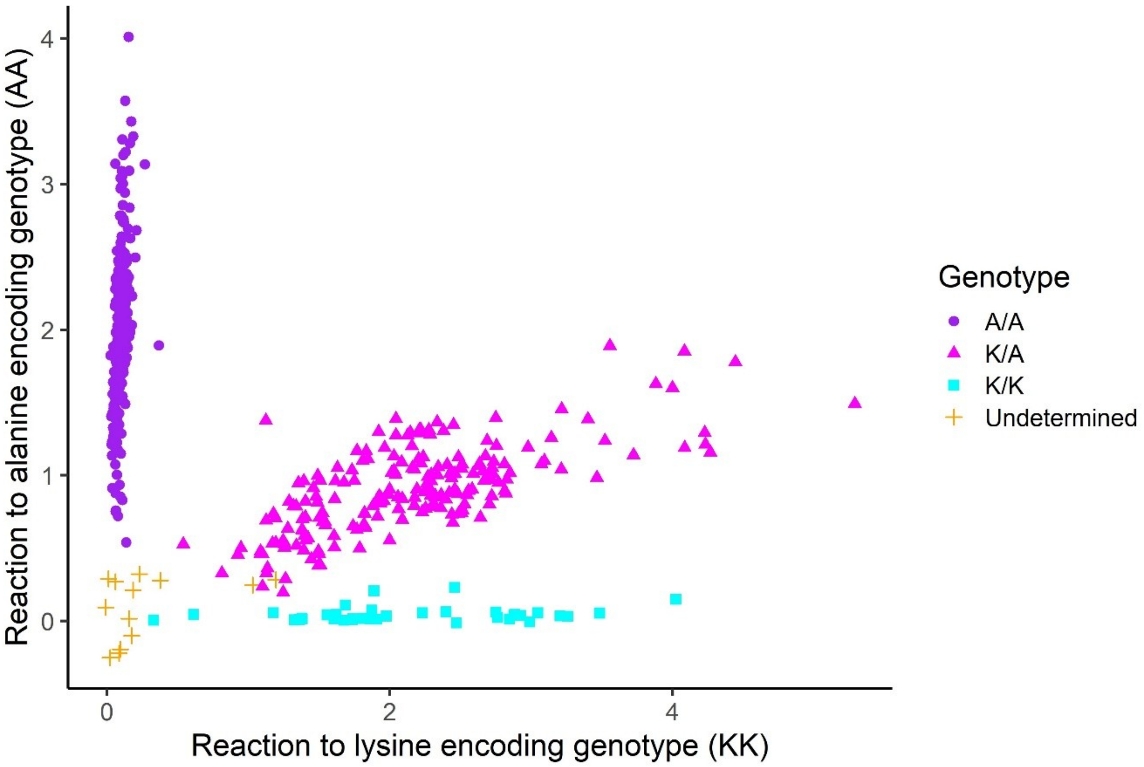

Figure 1. Country level classification (Costa Rica) of samples discriminated according to homozygous genotypes AA(•), KK(■), heterozygous genotype KA(▲) or undetermined genotype (+) according to reaction to specific encoding genotypes. Genetics and biotechnology department, Biology School, University of Costa Rica, years 2018-2019.

Figura 1. Clasificación a nivel de país (Costa Rica) de muestras discriminadas según genotipos homocigotos AA (•), KK (■), genotipo heterocigoto KA (▲) o genotipo indeterminado (+) según la reacción a genotipos codificantes específicos. Departamento de Genética y Biotecnología, Escuela de Biología, Universidad de Costa Rica, años 2018-2019.

Hardy-Weinberg equilibrium

The results, according to location, of the Hardy-Weinberg equilibrium fitting for genotypic frequencies of the *DGAT1* K232A polymorphism are described in **Table 3**.

According to the results, the genotypic frequencies at country and province levels are under the Hardy-Weinberg equilibrium. The *P* values obtained in all strata indicate that there is not enough evidence to reject the null hypothesis of equity between observed and expected frequencies.

The chi-square value between provinces is getting closer to zero as sample size increases (Cartago: chi-square = 0.16, for 59 samples; San José: chi-square = 0.12, for 76 samples; Alajuela: chi-square = 0.06, for 350 samples). This gradient is supported by the reduction of the chi-square observed at country level (chi-square = 0.03, for 485 samples).

Table 3. Hardy-Weinberg equilibrium fitting for the *DGAT1* K232A polymorphism in 485 genotyped Holstein cows according to location in Costa Rica, years 2018-2019.

Tabla 3. Ajuste del equilibrio Hardy-Weinberg por el polimorfismo *DGAT1* K232A en 485 vacas Holstein genotipadas según ubicación en Costa Rica, años 2018-2019.

Location	Genotype	Observed frequency	Genotypic frequencies	Expected frequency	Chi-square*	*p* value
Costa Rica (overall)	AA	265	0.546	265.73	0.03	0.862
	KA	188	0.388	186.53		
	KK	32	0.066	32.73		
Alajuela	AA	196	0.560	196.88	0.06	0.803
	KA	133	0.380	131.25		
	KK	21	0.060	21.88		
San José	AA	39	0.513	38.37	0.12	0.725
	KA	30	0.395	31.26		
	KK	7	0.092	6.37		
Cartago	AA	30	0.508	30.61	0.16	0.691
	KA	25	0.424	23.77		
	KK	4	0.068	4.61		

*A value of 3.84 for the chi-square test was the threshold ($p < 0.05$) used to determine deviation of genotypic frequencies from the Hardy-Weinberg equilibrium.

*Un valor de 3,84 para el test chi-cuadrado fue el umbral ($p < 0,05$) utilizado para determinar la desviación de las frecuencias genotípicas del equilibrio Hardy-Weinberg.

The mentioned chi-square gradient indicates that the larger the sample size is, the closer observed genotypic frequencies from the expected genotypic frequencies are, which agree to the Hardy-Weinberg equilibrium.

These results provide evidence that random mating between animals has occurred regarding the *DGAT1* K232A polymorphism, so that no indirect selection by milk fat content, or any other phenotype associated with this polymorphism is evidenced in this study.

Genotypic and allelic frequencies

Genotype frequencies differed significantly among genotypes (likelihood ratio χ^2, $p < 0.001$). Tukey-adjusted post-hoc comparisons revealed significant differences among all genotypes, with genotype AA occurring at the highest frequency (0.527 ± 0.029), followed by KA (0.399 ± 0.022), whereas KK exhibited the lowest frequency (0.074 ± 0.017), with $p < 0.001$ for all pairwise contrasts (**Figure 2**).

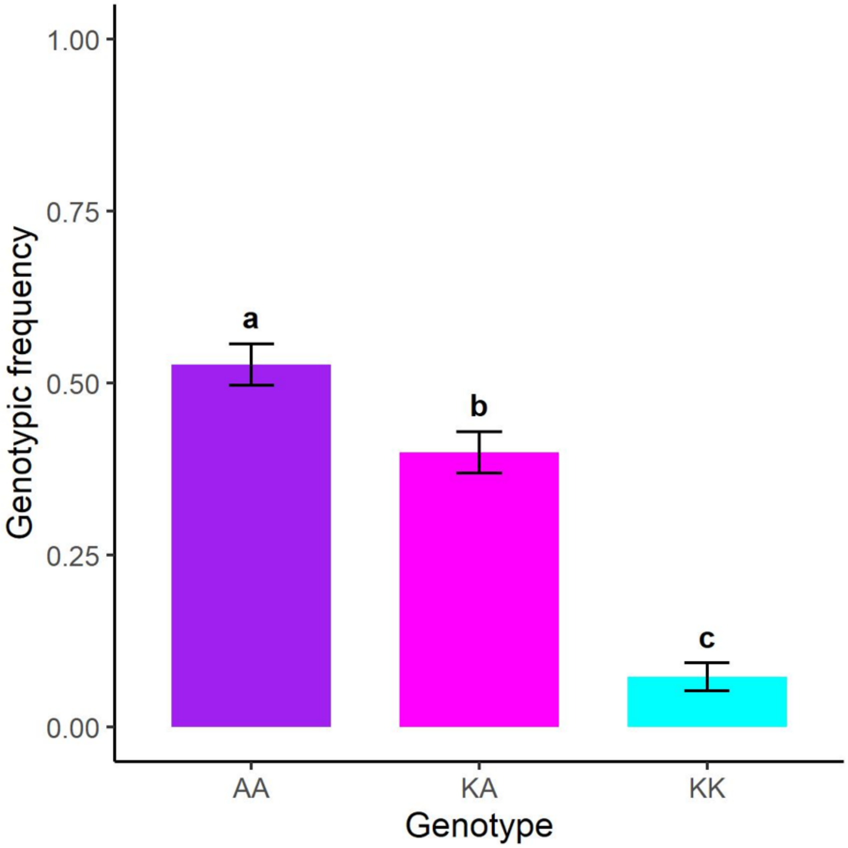

Figure 2. Genotypic frequencies for *DGAT1* polymorphism in 485 Holstein cows from three provinces (Alajuela, San José, and Cartago) of Costa Rica, years 2018-2019. The 95% confidence interval is depicted at the top of each bar (Different letters above bars indicate significant differences among genotypes based on Tukey-adjusted post-hoc comparisons, $p < 0.01$).

Figura 2. Frecuencias genotípicas para el polimorfismo *DGAT1* en 485 vacas Holstein de tres provincias (Alajuela, San José, y Cartago) de Costa Rica, años 2018-2019. El intervalo de confianza al 95% se describe arriba de cada barra (Letras diferentes sobre las columnas indican diferencias significativas entre genotipos basadas en la comparación post-hoc ajustada de Tukey, $p < 0{,}01$).

The small range of the 95% confidence interval depicts little variation in the proportion of genotypes among locations. This denotes similar and consistent proportions of *DGAT1* genotypes for the three provinces that were included in this study.

The averaged by location genotypic frequencies (AA = 0.527, KA = 0.399 and KK = 0.074) were very close to the overall genotypic frequencies (AA = 0.546, KA = 0.388 and KK = 0.066). The allelic frequencies, extracted from the overall genotypic frequencies (i.e.: A Allele freq. = overall AA freq. + 0.5 * overall KA freq.), for the K and A alleles were 0.26 and 074, respectively. These results rank the K allele frequency as the minor allele frequency in the Costa Rican Holstein population.

A wide range of frequencies for the *DGAT1* K232A allele (K) across countries has been reported (**Table 1**). Despite the K allele is the minor allele in this study, there might be an opportunity to apply artificial selection to improve milk fat content in this population, through the determination of the *DGAT1* K232A polymorphism, which would lead to higher profits for the productive system, under the current conditions of milk payment system. The lack of directed selection for the *DGAT1* K232A polymorphism in Costa Rica, as an additional selection criterion, could be attributed to the lack of systematic determination of this mayor effect polymorphisms in specialized dairy farms.

Implications

Costa Rican animal science researchers are aware of the existence and advantages of low, middle, and high-density single nucleotide polymorphisms (SNP) bead chips used around the world in animal breeding programs. However, incursion into genomic selection in dairy cattle in most developing countries is still incipient. Genes of major effect, like the one studied in this research, are considered as a link between the total lack of artificial selection, the classic kinship approach (mostly non applied in developing countries due to lack of appropriate pedigree and phenotype records) and the promise of genomic selection in the future.

Well-known major effect genes could be useful and profitable in the meantime genomic selection programs established in developing countries (Fontanesi et al., 2014). Regarding the *DGAT1* K232A polymorphism, there are studies of increase in milk fat content reporting 0.52% for each copy of the K variant (Näslund et al., 2008) and strong evidence of consistent increment of milk fat for the KK genotype across several studies (Mahmoudi & Rashidi, 2023). Therefore, there might be an opportunity to improve milk fat content in this population if the proportion of individuals carrying the KK genotype increases. This could be achieved if complementary selection for mating of KK genotype animals is performed as a breeding strategy.

This approach could lead to higher profits for the productive system due to the current milk payment structure applied in Costa Rica, which favors economically higher milk fat content milk, for instance, the last decade, a milk with 3.00% milk fat has been under-paid on average near 0.04 US$ per kg than milk with 3.82% milk fat (CNPL, 2025).

Milk fat content improvement could be driven through *DGAT1* K232A polymorphism selection within the Costa Rican Holstein population. Studies regarding the profitability of cows with favorable genotypes for the *DGAT1* K232A polymorphism must be developed to support this hypothesis.

Conclusions

The genotypic frequencies of the *DGAT1* K232A polymorphism evidence Hardy-Weinberg equilibrium, which suggests random mating between animals for this polymorphism. These findings imply the lack of indirect selection of this gene through milk fat phenotypic performance of Costa Rican Holstein cows. As far as we know, this is the first study to determine the *DGAT1* K232A polymorphism prevalence in Costa Rica. The inclusion of *DGAT1* K232A polymorphism as an additional criterion to select cows within dairy farms could mean a competitive advantage, by keeping animals with higher profit benefits for the productive system due to the current milk payment structure.

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Conflicts of interest

This experiment was approved by the Institutional Committee for Care and Usage of Animals (CICUA-050-16), the Scientific Council of the Animal Nutrition Research Center (CINA) and the Research Vice-Rectory of the University of Costa Rica (project number 739B7070).

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