Polymorphisms of pyruvate carboxylase gene (rs133566428) and its effects on economic traits in Holstein cows

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Abstract

A genetic selection strategy can be improved by identifying candidate genes and variants associated with economically important traits in dairy cattle. Specific genetic markers linked to traits such as milk production, fertility, and disease resistance can be pinpointed. The study was conducted at the Greater Diwaniyah Dairy Station in Diwaniyah Governorate during the 2023–2024 production season to investigate the effect of the Pyruvate Carboxylase (*PC*) gene variant rs133566428 on milk production, milk components, and reproductive traits in Holstein cows in Iraq. The study involved 50 cows aged three to five years, all maintained under identical feeding and management protocols. Each cow completed a lactation period of 305 days. Genotyping was performed to determine genotypes and allele frequencies. Sequence analysis of 816 bp amplicons identified a single nucleotide polymorphism (SNP), rs133566428. The results showed three genotypes of the *PC* gene: GG, GA, and AA. There were statistically significant differences ($P \leq 0.01$) among the genotype frequencies: GG 16.00%, GA 16.00%, and AA 68.00%, respectively. The results showed a significant effect ($P \leq 0.05$) of the GG and AA genotypes on total milk production of 4191.25 ± 414.89 kg and 4112.88 ± 296.92 kg, respectively, compared to the GA genotype, which produced 3276.25 ± 344.08 kg. The *PC* gene polymorphism had no significant effect on reproductive traits and milk components, except for fat percentage. It can be concluded that there is a statistically significant association between the *PC* gene and milk production, indicating its potential use for genetic improvement in dairy cattle; however, a larger sample size and a greater variety of cattle breeds are essential to validate the results.

Keywords: Cattle, milk, polymorphisms, pyruvate carboxylase, reproductive.

Introduction

Milk and dairy products are essential components of human nutrition, especially during childhood and increasing milk production has become a critical concern [29]. Lactose, fat, and protein are the primary nutritional components of milk; they serve as key indicators for assessing its nutritional value and are closely linked to the economic profitability of the dairy industry [23,27]. Therefore, identifying candidate genes associated with these traits can improve the accuracy of selecting animals for characteristics with moderate heritability, such as milk yield [6,18,16]. The moderate heritability associated with traditional breeding methods has limited their effectiveness in increasing milk production [18]. The efficiency of milk production can be improved by developing a better understanding of the genes that

encode proteins involved in the synthesis of fat, protein, and lactose in the mammary gland [10,26,1,20]. Among these, the pyruvate carboxylase (*PC*) gene emerges as a promising candidate influencing several functional traits in dairy cows [8].

The *PC* gene, located on cattle chromosome 29 (NCBI Reference Sequence NC_037356.1), encodes pyruvate carboxylase (PC), a rate-limiting enzyme essential for various metabolic pathways, including glucose production, lipogenesis, and amino acid metabolism [32]. The PC enzyme catalyzes the carboxylation of pyruvate to oxaloacetate, utilizing biotin and ATP as cofactors [28]. Pyruvate carboxylase participates in various metabolic processes such as gluconeogenesis, lipogenesis, insulin secretion, and the synthesis of the neurotransmitter glutamate [15,5,19,14]. Gluconeogenesis is essential for maintaining glucose homeostasis in the body and for lactose synthesis in the mammary glands [31]**.** Studies have highlighted the role of the *PC* gene in metabolic pathways related to milk synthesis, including lipid and pyruvate metabolism. SNPs rs109496284, rs110561408, rs137492467, rs42194999, rs42195000, rs42195007, and rs42197370, identified in the *PC* gene, were significantly associated with milk productive traits [17]. Recently, certain single nucleotide polymorphisms (SNPs) within the *PC* gene have been significantly associated with milk production traits in Chinese Holstein cows [8]. In dairy cattle, the *PC* gene is recognized as a key gene associated with milk production and reproduction; however, few studies have confirmed this association. This study aimed to investigate the effect of polymorphisms in the pyruvate carboxylase gene, specifically rs133566428, on functional traits in Holstein cows.

MATERIALS AND METHODS

The study was conducted at The Great Diwaniyah Cows Station, located in Diwaniyah Governorate, during the 2023/2024 productive season following the international recommendations [12], under approval number Agri, No. 021,10,24. Fifty Holstein cows, aged three to five years, were included in the study. All animals received the same feeding and maintenance regimen and completed a lactation period of 305 days. Phenotypic data were collected, including age, milking frequency, lactation number, calving information, and sire/dam identification. Two sires were randomly assigned to approximately 25 to 30 dams each within the studied herd. An industrial milk analyzer (EKOMILK) was used to measure the fat, protein, and solid content of a 10-milliliter milk sample.

Three to four milliliters of blood were collected from the jugular veins of 50 cows using anticoagulant tubes for genetic analysis. DNA was extracted from whole blood using Geneaid's gSYNC™ DNA extraction kit (New Taipei City, Taiwan). The concentration and purity of the extracted DNA were evaluated using a Nanodrop spectrophotometer (BioDrop Lite, Biodrop, UK), followed by analysis through 0.8% agarose gel electrophoresis [21]. Additionally, genotype distribution within the herd and allele frequencies were calculated. Polymerase chain reaction (PCR) was used to amplify the target fragment for molecular detection and polymorphism analysis of the *PC* gene. According to the sequencing protocol, samples of the PCR product (20 microliters per sample) from all cows were sent by the ASCo Learning Center to Macrogen Company in the United States to

ISSN 2072-3857

obtain the exact nucleotide sequences of the studied *PC* gene fragment. Primers targeting exon 7 on chromosome 29 of 816 bp fragment, were selected for molecular detection and identification of polymorphisms as reported by [8]. Forward = 5´- GTGAATGGAGCGAGTGAGGT -3´ and Reverse = 5´- CTCCGGTAGCTTTCCCAAA -3´.

PopGen32, version 1.31, was utilized to calculate genotype and allele frequencies [33]. The Statistical Analysis System SAS [25] program and the General Linear Model (GLM) procedure were used to assess the effect of the Pyruvate Carboxylase (*PC*) gene polymorphism (rs133566428) on various parameters. Duncan's multiple range test [9], following Analysis of Variance (ANOVA), and least squares means (LSMEANS) were employed to compare means significantly. The Chi-square test was used to compare percentages at significance levels of 0.05 and 0.01. The statistical model was:

$Yij = \mu + Gi + eij$

Where:

Yij = Dependent variable.

μ = Overall mean of traits.

Gi = Effect of Genotype/ Pyruvate carboxylase-*PC* gene (GG, GA and AA).

eij = Error term.

Chi-Square test was used to determine the significance of differences in genotype distribution percentages and to calculate the allele frequency of the Pyruvate Carboxylase (*PC*) gene according to Hardy-Weinberg equilibrium [11].

$$PA = \frac{2 * No.of\ Homozygous + 1 * No.of\ Heterozyg}{2 * Total\ number\ of\ sample}$$

$P + q = 1$

$qB = 1 - PA$

Age was not significantly affected by the analysis and was therefore excluded from further consideration.

RESULTS AND DISCUSSION

Genetic analysis

Genomic DNA was extracted to obtain the targeted 816 bp *PC* gene fragment using PCR technology. The amplified PC gene fragment was then analyzed by electrophoresis (Figure 1). The results obtained from Sanger sequencing technology, used to analyze the SNP in the studied *PC* gene fragment, revealed the presence of SNP rs133566428. Three genotypes were identified in the cow samples: GG, GA, and AA (Figure 2).

ISSN 2072-3857

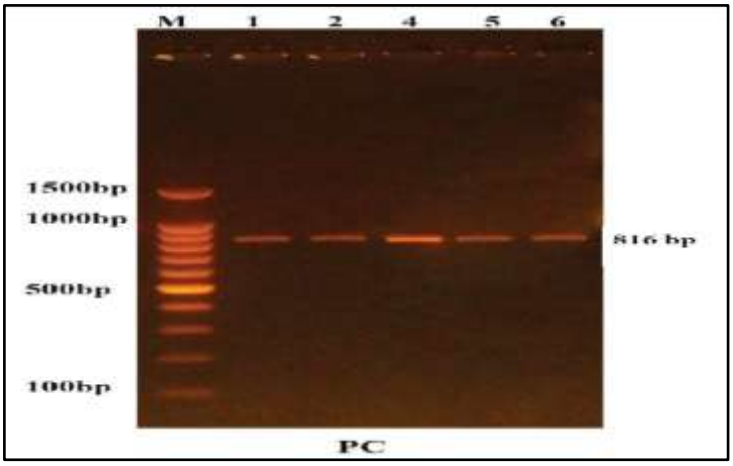


Figure 1. The extracted fragment (816 bp) of Pyruvate carboxylase-*PC* gene using PCR technique.

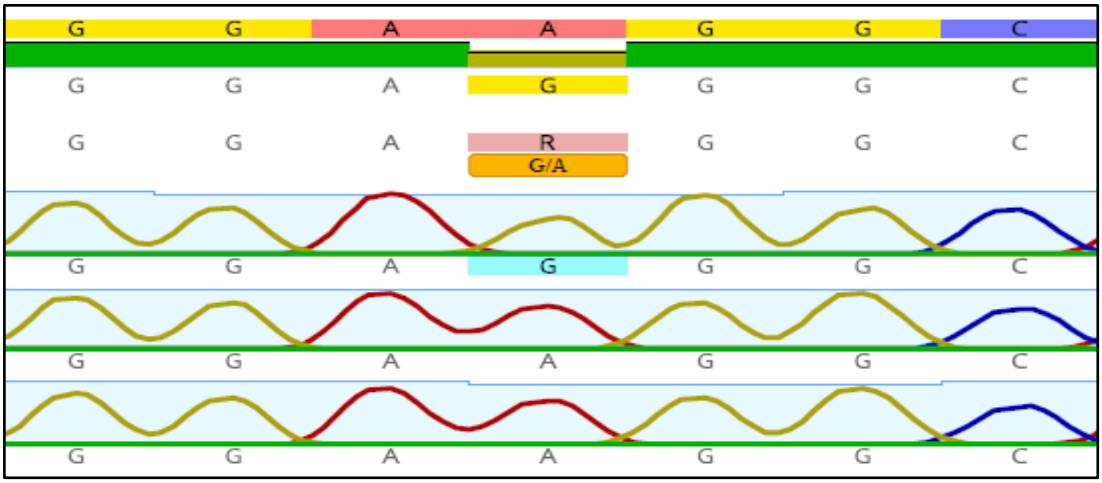


Figure 2. Sequence analysis of Pyruvate carboxylase-*PC* gene/ rs133566428.

The results in Table 1 show the number and percentage of genotypes, as well as the allelic frequency of the *PC* gene variant rs133566428. There were highly significant differences ($P \leq 0.01$) in genotype percentages, with GG and GA genotypes each representing 16.00% (n = 8), and the AA genotype representing 68.00% (n = 34). The frequency of the G allele was 0.24, while the A allele frequency was 0.76. These results indicate a predominance of the A allele in imported Holstein cows. Similarly, [8] identified six SNPs in the *PC* gene (rs42193753, rs42195007, rs110381742, rs109519857, rs133706500, and rs110386158), reporting highly significant differences ($P \leq 0.01$) in genotype ratios and allelic frequencies. However, few studies have confirmed variations in the *PC* gene in dairy cattle. This study provides genetic data along with a novel association that could enhance the effectiveness of marker-assisted selection for functional traits in future breeding programs. Incorporating this information into breeding programs can improve traits such as milk production.

ISSN 2072-3857

Table 1. Genotype distribution and allele frequency of Pyruvate carboxylase-*PC* gene/rs133566428 in Holstein cows

Genotype/rs133566428	**No**	**Percentage (%)**
GG	8	16.00
GA	8	16.00
AA	34	68.00
Total	50	100%
Chi-Square: χ2	---	50.160 **
Allele	Frequency	
G	0.24	
A	0.76	
** (P≤0.01).		

Association analysis

The results presented in Table 2 indicate a significant relationship ($P \leq 0.05$) between the genotypes of the *PC* gene, specifically rs133566428, and total milk production. Cows with the GG and AA genotypes produced 4191.25 ± 414.89 kg and 4112.88 ± 296.92 kg of milk, respectively, compared to cows with the GA genotype, which produced 3276.25 ± 344.08 kg. This finding may be explained by the conserved coding regions of DNA playing a vital role in biological functions [3]. The expression of the *PC* gene influences the metabolic processes involved in milk synthesis, and inhibiting its expression may enhance milk production traits [31]. Previous studies have demonstrated that the *PC* gene contains highly contiguous quantitative trait loci (QTLs: 2593, 2612, and 255006), which significantly affect milk and protein production [30,24]. [8] confirmed that pyruvate carboxylase has a significant effect on milk production traits, identifying six SNPs (rs42193753, rs42195007, rs110381742, rs109519857, rs133706500, and rs110386158) with notable genetic effects that can serve as markers for genomic selection in in Chinese Holstein cows. Advances in genomics have made it possible to identify genes linked to economically important traits [2,22]. It is possible that this mutation alters both the structure and function of the protein, thereby affecting the observed phenotype. TT genotypes of the *LEP* gene are associated with higher milk yields, elevated yield indices, and increased average daily milk production across three lactations [4]. [7] found that cows homozygous for the *SLICK1* allele produced 9% more milk than heterozygous cows. Polymorphisms in the pyruvate carboxylase gene at rs133566428 have not been previously studied in Holstein cows.

Table 2. Relationship between Pyruvate carboxylase-*PC* gene/rs133566428 polymorphism with total milk production, lactation period and dry period in Holstein cows (Least square means ± SE)

Genoptype/ rs133566428	Total milk production (kg)	Lactation period (day)	Dry period (day)
GG	4191.25 ± 414.89[a]	268.12 ± 21.54	114.87 ± 13.44
GA	3276.25 ± 344.08[b]	276.00 ± 15.54	104.37 ± 11.45
AA	4112.88 ± 296.92[a]	269.29 ± 11.28	111.88 ± 10.43
P-value	**0.03**	0.23	0.41

Different superscripts in the same column indicated significant differences ($P \leq 0.05$).

The results in Table 3 show a significant relationship ($P \leq 0.05$) between the genotypes of the *PC* gene, rs133566428, and the percentage of fat in milk. Cows with the recessive genotype AA and the homozygous genotype GG, which did not differ significantly from each other, recorded the highest fat percentages, reaching 4.31 ± 0.61% and 4.11 ± 0.19%, respectively. In contrast, cows with the heterozygous genotype GA recorded a fat percentage of 3.90 ± 0.28%, which did not differ significantly from the GG genotype. No significant differences were observed among the GG, GA, and AA genotypes in terms of protein percentage, glucose percentage, and solids non-fat percentage. The correlation between *PC* expression and fatty acid concentrations was previously reported by [32]. [8] also reported that certain SNPs identified in the *PC* gene were associated with milk components, particularly protein and fat production. However, the relationship between polymorphisms in the pyruvate carboxylasegene rs133566428 and milk composition in Holstein cows has not been previously studied.

Table 3. Relationship between Pyruvate carboxylase-*PC* gene/ rs133566428 polymorphism with milk composition in Holstein cows (Least square means ± SE)

Genoptype/ rs133566428	Fat (%)	Protein (%)	lactose (%)	SNF (%)
GG	4.11 ± 0.19[ab]	2.69 ± 0.07	4.34 ± 0.18	7.70 ± 0.20
GA	3.90 ± 0.28[b]	2.80 ± 0.14	4.48 ± 0.23	7.91± 0.18
AA	4.31 ± 0.61[a]	2.93 ± 0.11	4.40 ± 0.12	7.99 ± 0.17
P-value	**0.02**	0.35	0.43	0.16

SNF: solids-not-fat.

Regarding reproductive performance, the results presented in Table 4 indicate that there were no significant differences among the genotypes of the *PC* gene/rs133566428 with respect to reproductive efficiency indicators, including services per conception, calving interval, and days open. The strong metabolic correlation between glucose supply, milk production, and energy balance suggests that *PC* gene

polymorphism affects energy metabolism and may indirectly impact reproductive performance [13]. This relationship was investigated based on the premise that genes involved in metabolic processes can influence reproductive traits [31]. Despite this, an association between polymorphisms in the *PC* gene, specifically rs133566428, reproductive performance in Holstein cows has not been previously studied. While this study is the first to report a relationship between the PC/rs133566428 SNP and total milk production as well as fat percentage in Holstein cows, it has certain limitations. The results may be influenced by several factors, including environmental stressors, feed quality, and genetic variations within the sample population. Additionally, the findings are constrained by the small sample size. Further research is necessary to better understand how the rs133566428 variant in the *PC* gene affects milk composition and production.

Table 4. Relationship between Pyruvate carboxylase-*PC* gene/ rs133566428 polymorphism with reproductive performance in Holstein cows (Least square means ± SE)

Genoptype/ rs133566428	Service per conception-SPC	Calving interval-CI (day)	Days open-DO (day)
GG	102.62 ± 10.92	2.00 ± 0.32	382.87 ± 11.03
GA	100.00 ± 7.38	1.75 ± 0.31	380.37 ± 7.40
AA	100.18 ± 3.64	1.71 ± 0.13	381.29 ± 3.59
P-value	0.10	0.36	0.18

CONCLUSION

This study confirmed the presence of a single nucleotide polymorphism (SNP: rs133566428) in the *PC* gene and demonstrated that this gene has a significant genetic effect on milk production traits in Holstein cows. Therefore, SNPs with such genetic effects can be used as markers for genomic selection in dairy cattle. Although this conclusion is based on a small sample size (n=50), there is no evidence of a heterozygote effect, potential stratification, or functional validation. Future research should focus on increasing the sample size to over 200 cows to provide more robust data. Additionally, conducting allele-specific expression assays will help validate the findings by examining expression differences between alleles. Incorporating functional validation studies is also essential to understand the biological impact of rs133566428 on the traits of interest.

Conflict of interest

The authors declare that there is no conflict of interest

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Author contributions

Tahreer Mohammed Al-Thuwaini: Conceptualization, writing-original draft, supervision, methodology. Waleed Ahmed Mohammed: Data curation, investigation. Hameed Razzak Al-janabi: Data curation,

ISSN 2072-3857

investigation. In addition, all authors reviewed and approved the final manuscript.

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