Association of GH gene polymorphisms with some milk production traits in in Iraqi Holstein breed using PCR-RFLP technique

Noor Abdul Adhem Abdul Khudhur1 and Hayder Raheem Alnajm2*

1,2Department of Animal Production Techniques, Al-Musaib Technical College, Al-Furat Al-Awsat Technical University, Babylon, Iraq.

*Corresponding author's email: haider.raheem@atu.edu.iq, noor.adhem@atu.edu.iq

Abstract

In this study, a total of 50 Iraqi Holstein breed was used to research polymorphic and genotypes of the growth hormone (GH) gene using PCR-RFLP technique. The results showed that the frequencies of the C, and T alleles of the GH gene were 0.64, and 0.36, respectively. The genotype distributions for CC (0.12), CT (0.48), and TT (0.40), respectively. Also, the was a highly significant effect ($P< 0.01$) of the chi-square test on Hardy-Weinberg equilibrium for the GH gene. There was significant effect ($P< 0.05$) of TT genotype on length of the milk season, and total milk yield (kg) were (176.67 ±5.01), and 1810.00 ±142.17), respectively. In addition, the CC genotype of GH gene significant influence on fat (4.21 ±0.30%) compared with CT, and TT genotypes. The results showed that the GH gene polymorphic could be used as a candidate gene for selecting milk traits in Holstein breed in Iraq.

Keywords: GH gene, Iraqi Holstein, PCR-RFLP.

Introduction

The cattle raising is a good source of income for rural people, as it plays an important role in human culture and has a great impact on society [2, 7]. Holstein cattle are the dominant dairy breed worldwide, due to their impressive ability to produce large quantities of milk and their large body size [18]. In addition, Holstein cows have the ability to express their genetic potential for milk yields and composition traits. Therefore, The Holstein cows in Iraq have become the preferred choice for crossbreeding programs to enhance milk yields, and improve cow size for high carcass value4 [4.[

Milk production and components are phenotypic traits determined by the genotype and environment that show a great deal of variation in dairy cows [8]. Milk quantity and quality are determined by many numbers of genes with additive effects, that the candidate genes and genotypes associated with milk production and its composition such as GH, PIT-1 genes [3.[

The growth hormone gene (GH) plays an important role in influencing milk yield and its components [16]. The bovine growth hormone gene (GH) belongs to a multigene family that includes prolactin and placental lactogen is known as the prolactin/growth hormone (PRL/GH) family or the somatotropin family. The bovine GH gene (GenBank Acc. M57764) is located on the 19 chromosome and consists of five exons and four introns that encode several peptides comprising 217 amino acids [11,12]. The aim of the association between GH gene polymorphisms and milk production in Holstein cows in the Babylon region, Iraq.

Material and Methods

Ethical approval

The laboratory work was conducted at the laboratories of the College of Al-Musaib Technical, Al-Furat Al-Awsat Technical University, with ethical approval granted by the Animal Care Committee under approval number 7/37/5574 dated 20/10/2024.

Experimental materials

The study was carried out on 50 Holstein cows from the Al-Haydari cattle Station in Babylon city of the date from 1/8/ 2024 to 1/2/ 2025. Daily milk yield and growth records were collected fifty milliliters of milk were collected in sterile tubes. The fat, lactose, protein, and solids-not-fat (SNF) were measured using a Milko scan device (IndiFOSS, India). Milk samples were stored at −20 °C until DNA extraction.

DNA extraction and PCR amplification

Genomic DNA was extracted from milk following the method described by [10]. The quality and quantity of the extracted DNA were assessed using a 1% gel, with an electrophoresis voltage of 100 volts for 30 minutes. The polymerase chain reaction (PCR) amplification was performed in a final volume of 25 µl, consisting of [8 µl Master Mix (Syntol, Russia), 3 µl DNA template, 2 µl PCR buffer, 1 mM MgCl2, 1 mM dNTP, 1.0 U Taq polymerase, 2 µl RFLP primers (F: forward, R: reverse), 2 µl DNA sample, and 5 µl ddH$_2$ O]. Amplification of the GH gene was carried out using specific primer pairs (Table 1.(

Table 1. Information on the GH gene of the Holstein cows under study.

Gene/ Chr.	**Primers**	**Length of fragment (bp)**	**Restriction Enzymes**	**References**
GH **(19)**	F: 5'-CCCACGGGCAAGAATGAGGC-3' R: 5'-TGAGGAACTGCAGGGGCCCA-3'	329	*MspI*	[19]

The 35 cycles consisting of denaturation at 95 °C for 1min, annealing at 61 °C for 20 s and extension at 72 °C for 30 s A final extension step was performed at 72 °C for 10 mins. The PCR products were visualized as DNA bands under UV illumination after electrophoresis for 30 minutes at 80 V on a 2.5% agarose gel.

PCR-RFLP analysis

The polymerase chain reaction-restriction fragment length polymorphism (PCR-RLFP) method was applied for genotype analysis of GH gene using MspI restriction enzyme. Restriction digestion was performed in a total volume of 20 µl containing 10 µl of PCR product, 1 µl of MspI restriction enzyme for the GH gene, 4 µl of 10X buffer, and 5 µl of ddH_2O. The reaction mixtures were incubated at 37 °C for 15 hours using a thermal cycler. Following digestion, the PCR-RFLP products were subjected to electrophoresis on a 3% agarose gel and stained with ethidium bromide. The PCR-RFLP fragments were visualized under a UV transilluminator, the gel was documented using Gel Doc equipment. Then, the genotypes were determined according to band sizes.

Statistical analysis

Genotypic and allelic frequencies were estimated, and the Chi-square (χ^2) test was performed using the Pop Gene software [21]. The effect of GH gene polymorphisms on milk production and components was analyzed using SAS software [17]. The statistical model applied was:

$$Yijk = \mu + Bj + Gk + Eijk$$

Where: Yijk is the observation of ith animal of jth breed with kth genotype, Yijk is the length of the milk season, daily and total milk production, the milk yield/fat/ protein/ SNF/ lactose, µ the overall mean, Bj equal (Holstein breed), Gk is the 1-3 (genotypes for selected genes), and Eijk is the random error. Finally, Duncan test [6] was used to determine significant effects between the studied traits.

Rustles and Discussion

The GH gene fragment of 329 bp was successfully amplified from the genomic DNA of Holstein cows using the PCR-RFLP technique. Digestion of the 329 bp amplicons with the MspI restriction enzyme produced three genotypes: the CC genotype products one fragment of 329 bp, the CT genotype produced three fragments of 329, 224, and 105 bp, while the TT genotype generated two fragments of 224 and 105 bp(Figure 1). These results similar with in Holstein dairy cattle in Tunisia [1], in Friesian cattle in Egypt [14.[

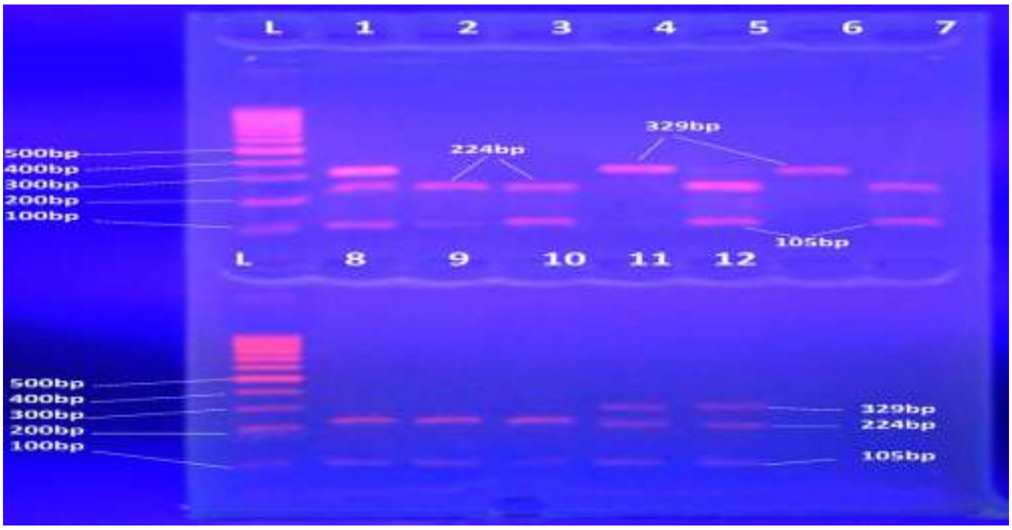

Figure 1. The PCR-RFLP of GH/ MspI enzyme restriction size of Holstein cows, CC; 329 bp, CT; 329, 224, and 105 bp, TT; 224 and 105 bp with marker ladder 100 bp.

The Table. 2 showed the allele and genotype frequencies of the GH gene in Holstein breed. The frequencies of the C and T alleles were 0.64 and 0.36, respectively. The observed genotype frequencies for CC, CT, and TT were 0.12, 0.48, and 0.40, respectively. The allele distribution in this study was still at Hardy–Weinberg equilibrium and statically significant (p<0.01). These results are similar to the highly significant chi-square results obtained in studies on local and Holstein cattle in Iraq [22] and on Holstein and Jersey breeds in Turkey [5 .[

Table 2. Genotypic and allelic frequencies in Holstein breed.

Gene	Genotype	Frequency	Alleles	Frequency	Chi-square
GH	CC	0.12	C	0.64	7.920 **
	CT	0.48	T	0.36	
	TT	0.40			

) **P < 0.01(

The Table 3 showed of the length of the milk season, daily milk yield, and total milk yield. The GH gene showed a significant effect on Length of the milk season (P < 0.05), that the TT genotype recorded the longest lactation period (176.67 ± 5.01 days) compared with CT (172.91 ± 5.33 days) and CC (170.00 ± 5.62 days). However, the significant effect (P < 0.05) of GH gene polymorphisms on total milk yield, with the TT genotype recorded higher total milk yield (1810.00 ± 142.17 kg) compared with CT (1581.75 ± 124.49 kg) and CC (1502.00 ± 144.28 kg). These results agreement with the Sahiwal cattle in India [15], the Holstein cows in Iran [13] reported that cows with the TT genotype significantly higher milk production traits than those with the TC and CC genotypes. Also, the cows carrying the T allele produced more milk compared to those carrying the C allele [20.[

Table 3. The GH gene genotypes and standard errors in terms of some milk traits in Holstein breed.

Genotypes	Number of cows.	Length of the milk season± S.E	Daily milk yield (kg/ day) ±S. E	Total milk yield (kg) ±S. E
CC	6	170.00 ±5.62c	11.60 ±0.43	1502.00 ±144.28c
CT	24	172.91 ±5.33b	11.16 ±0.48	1581.75 ±124.49b
TT	20	176.67 ±5.01a	11.17 ±0.91	1810.00 ±142.17a
P-value	50	*	NS	*

a, b, c: means showed different of the significant (*P<0.05, ** P<0.01, and NS: Non-significant) from one another, S.E: standard error .

The milk components traits are presented in Tables 4. The GH gene showed a significant effect on the fat % (P<0.05), that the CC genotype recorded the high (4.21 ±0.30%) compared with TT (3.99 ±0.24%) and CT (3.76 ±0.21%). These results similar with the Beijing Holstein cows in China [23]. However, the non-significant of the lactose, protein, and solids-not-fat % (table 4). The reason for the non-significance is due to the small sample size and the fact that the animals belong to the same breed. These results are in agreement with those of Friesian Holstein cattle in Indonesia, which found no significant association between GH gene polymorphisms and total protein percentage [9]. Our results also agreed with those of [22] in their study on local cattle and Holstein breeds in Iraq, as they observed a non-significant effect of protein %, lactose %, and solids-not-fat (SNF.% (

Table 4. The effect of GH genotypes on milk components traits in Holstein breed.

Genotypes	Number of cows.	Fat % ±S. E	Lactose% ± S. E	Protein% ± S. E	SNF% ± S. E
CC	6	4.21 ±0.30a	4.43 ±0.19	3.05 ±0.13	7.94 ±0.32
CT	24	3.76 ±0.21c	4.26 ±0.17	2.93 ±0.13	7.61 ±0.32
TT	20	3.99 ±0.24b	4.61 ±0.42	3.06 ±0.32	8.07 ±0.75
P-value	50	*	NS	NS	NS

a, b, c: means showed different of the significant (*P<0.05, ** P<0.01, and NS: Non-significant) from one another, SNF: Non-fat solids, S.E: standard error .

Conclusion

The GH gene investigated in this study was found to be polymorphic and had a significant effect on milk traits. Therefore, the GH gene can be proposed as a genetic marker for superior milk production traits in Holstein cows. Consequently, this study may serve as a reference for improving the economic traits of dairy cattle and for characterizing the status of this breed reared in Iraq.

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