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Detection of the effect of PIT-1 gene genotypes on some productive traits in Holstein cattle

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Abstract:

The research was conducted at the Taj Al-Nahrain cattle station in Al-Diwaniyah governorate in Iraq on a sample of 85 Holstein cows imported from Germany, which were selected during the third season of milk production, in order to determine the genotypes of the PIT-1 gene and its relationship to a number of production traits. The PCR product (451bp) of the PIT-1 gene was digested using the Hinf1 restriction enzyme, and the digestion results showed that the studied genetic segment of the POU1F1 gene appeared in three genotypes (AA, AB, and BB) with percentages of 55.3, 42.6, 2.1%, respectively, and there were highly significant differences (P≤0.01) between the distribution of these percentages, and the A allele (0.766) was superior to the B allele (0.234). The results of the analysis of variance showed that the effect of the genotype of the POU1F1 gene on the characteristics of milk production (daily milk production, total milk production, peak production) was not significant except in the characteristic of peak milk production in favor of the BB genotype at a rate of 538.50 kg on each of the genotypes AA and AB while the results did not show a significant effect between the genotypes (AA, AB, BB) in both the daily milk production rate and the total milk production. It was found that the effect of the POU1F1 gene on body dimension traits (body length, frontal body height, posterior body height) was significant (P≤0.05) in body length in favor of the BB genotype (164 cm) on each of the genotypes AA and AB, at 157,780. and 156,347 cm, respectively, while there were no significant differences between the genotypes in the anterior height and posterior height of the body.

I. INTRODUCTION

Holstein cows are characterized by their large size and high milk production compared to other breeds specialized in milk production (Al-Qudsi, 2010), but there are many factors that affect positively or negatively this production, including environmental and genetic factors, and the interaction between them, and that one the effects of the genetic factor are through mutations that occur in the sequence of nitrogenous bases constituting the candidate genes. The selection of such candidate genes that greatly affect productive traits such as growth and milk production is an important tool in improving the productive performance of cows and an alternative means of traditional selection that was followed previously, which requires additional time and effort. Among these genes is the POU1F1 gene or PIT-1which has been identified as one of the candidate genes and has been used to locate important quantitative traits (QTL) in selection and genetic improvement programs in farm animals (Sadeghi et al., 2014 and Woolard et al., 2000). POU1F1 gene is knowing the first pituitary transcription factor responsible for the development of the pituitary gland, where its expression is necessary for the

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differentiation of three types of pituitary cells (thyrotrophs, somatotrophs and lactotrophs) (Millan et al., 2016).The POU1F1 gene is associated with growth, reproduction, milk production and milk components (Işık et al., 2019), because it is responsible for the gene expression of many hormones such as Growth hormone (GH), Prolactin (PRL), Thyroid stimulating hormone (TSH-β) and Growth hormone receptor genes, and It was found that mutations that occur in the POU1F1 gene lead to different expression in the GH gene, PRL, TSH, and POU1F1 itself, thus affecting the level of growth hormones, prolactin and thyroid stimulating hormone, and this leads to changes in the body's ability to perform vital functions such as growth, development of the mammary gland, milk production and secretion (Renaville et al., 1997 and Sun et al., 2002).

II. MATERIALS AND METHODS

This study was conducted at Taj Al-Nahrain station in Al-Diwaniyah Governorate/Iraq, for the period from 10/15/2020 to 10/4/2021, in which 85 cows of the Holstein breed imported from Germany were used. They are black and white spotted cows, which were selected within the third season of milk production. The amount of milk production was calculated based on the data on milk production, which were collected from the station records for cows in the third season of production. Using a graduated cylinder in mm prepared for this purpose, the total milk production and the peak production were calculated through the following equations:

•The peak of production = the first rise in production, provided that it does not exceed 60 days.

• Total production = average daily production x length of production season

Also, the lengths and dimensions of the body (which were all in the third season of production) were taken using the tape measure inserted from (1-150 cm) as a tool for measuring both the length of the body and the height of the body at the front and the height of the body at the rear. The above-mentioned measurement is from the front of the chest to the end of the animal at the pin bones. As for the height of the body at the front, it was measured using the same measuring tape and vertically from the highest point in the shoulder to toward the ground. With regard to the height of the body at the back, it was measured by the same tape that was mentioned previously perpendicularly from the upper back end of the body to the ground.Blood samples were drawn from the udder vein of each cow with a volume of 3 ml per sample in test tubes containing EDTA K2 anticoagulant, and they were transferred to the laboratory for the purpose of extracting DNA from them for the purpose of conducting a molecular examination of the POU1F1 gene according to the instructions of the Kit supplied by Promega Company. To amplify the gene segment to be studied from the POU1F1 gene, a pair of primers were used as follow :(Woollard et al., 1994)

[5'-AAACCATCATCTCCCTTCTT-3'] (forward)

[5' -AATGTACAATGTGCCTTCTGAG-3'](reverse).

The primers used in this study were manufactured by Researcher. Co. LtD. Iraq. The polymerase chain reaction solution was carried out with a total volume of (25) µl consisting of 13 µL of Master Mix, 5 µL of DNA, 1.5 µL forward, 1.5 µL reverse, 4 distilled water. Touchdown amplification was performed with an initial step of 95 °C for 2 min, followed by 14

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cycles of 95 °C for 30 s, annealing temperatures starting at 58.7 °C for 30 s (decreasing by 0.5 °C/cycle), and 72 °C. C for 30 s to extend this step was followed by 19 cycles of 95 °C for 30 s, 51.7 °C for 30 s, 72 °C for 50 s, and a final extension at 72 °C for 5 min. After the completion of the polymerase chain reaction, the amplified segment of the POU1F1 gene was digested by using the HinfI enzyme. The enzymatic digestion process was carried out by adding 1 µl of HinfI enzyme to 20 µl of the PCR product for each sample, then they were mixed using a centrifuge for 30 seconds, then the reaction mixture was incubated at a temperature of 37 °C for a whole day. In the incubator, and during this period, the restriction enzyme identifies a specific site within the duplicated genetic piece to cut from it. Then, electrophoresis is carried out for the cut samples to detect the cut sites. The data were statistically analyzed using the statistical program (Statistical Analysis System -SAS, 2012) and using the General Linear Model (GLM) method to study the effect of polymorphism of the POU1F1 gene on body dimensions and milk production characteristics. The test of significant differences between the means was carried out using Duncan's polynomial test (Duncan, 1955), according to the following mathematical model:

$Yij = \mu + Gi + eij$

Since:

Yij: the observation value j of genotype i.

µ: the general mean of the trait studied.

Gi: effect of polymorphisms of gene i (AA, AB and BB.

eij: the experimental error that is normally and independently distributed with mean = zero and variance = $\delta^2 e$.

The Chi-Square-x^2 test was also used to compare the significance between the genotypes of the genotypes of the gene.

III. RESULTS AND DISCUSSION

The number, percentage of genotypes, and allelic frequency of the POU1F1 gene Table 1 shows the number of genotypes, their percentages, and the allelic frequency of the POU1F1 gene in the studied sample, as three genotypes appear for the studied segment of the POU1F1 gene after being digested by the Hinf I enzyme (AA, AB and BB) and the percentages of the genotypes were 55.3, 42.6 and 2.1, respectively, which means that there are highly significant differences ($P \leq 0.01$) between the percentages of the distribution of genotypes, as the individuals carrying the genotype AA were superior to the individuals carrying the two genotypes AB and BB. The sum of alleles in the genotypes was 47 alleles (26 alleles for AA genotype, 20 alleles for AB genotype, and 1 allele for genotype BB), and the allelic frequency of wild A and mutant B 0.766 and 0.234 respectively, which reflects the prevalence of the A allele on the allele. B in the studied sample of Holstein cows.

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Table 1. Number and percentage of genotypes and allelic frequency of POU1F1 gene.

Genotypes	No.	(%)
AA	26	AA 55.3
AB	20	42.6
BB	1	2.1
Total	47	100
Chi square (x^2)	-----	**15.891
Allels	**Frequency**	
A	0.766	
B	0.234	
**(P≤0.01)		

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The product of enzymatic digestion of the studied segment of the POU1F1 gene Figure 1 shows the product of enzymatic digestion by Hinf I of the POU1F1 gene studied segment (451 bp). The digestion product consisted of three genotypes AA, which appears in two bands of size (244 bp and 207 bp) homozygous, and AB, which appears in three bands of size (451 bp, 244 bp and 207 bp) heterozygous, and BB appears in one bundle (451 bp) because there is no cut in the two alleles, with the use of the molecular ladder (100-1000 bp).

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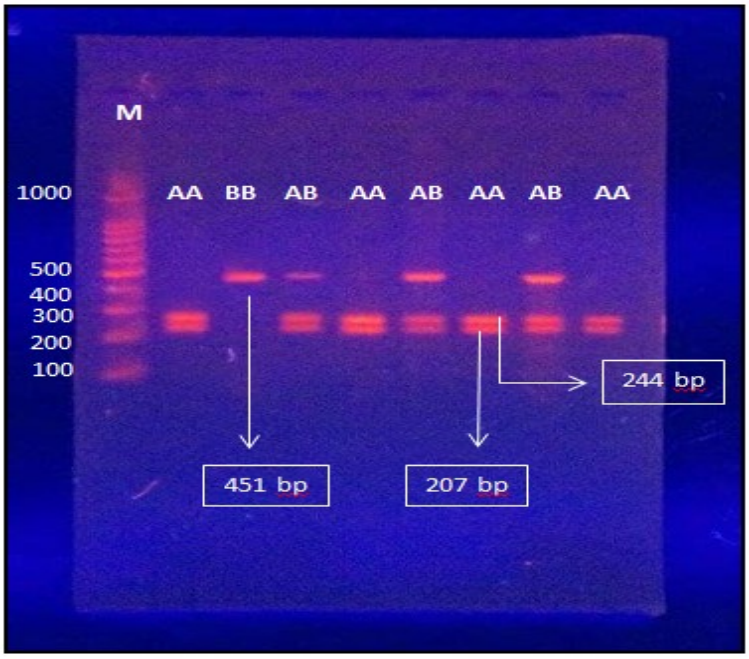

Figure 1. The product of the *Hinf I* enzymatic digestion of the POU1F1 gene stained with Ethidium Bromide dye and electrophoresed with agarose gel at a concentration of 2.5%.

The effect of the genotypes of the POU1F1 gene on the characteristics of milk production The results of the study in Table 2 showed that there were no significant differences between the genotypes (AA, AB, BB) of the POU1F1 gene in the rate of daily milk production and total milk production, as the daily milk production rate was 13.859, 14.440 and 14.00 kg for the three genotypes mentioned, respectively, and the total milk production was (4184.95, 4403.61 and 4271.40) kg for the genotypes (AA, AB, BB), respectively, while the results in the same table showed the significant effect of POU1F1 gene genotypes in the peak of milk production that the BB genotype was significantly ($P \leq 0.05$) superior, which amounted to 538.50 kg compared to 457.83 and 435.06 kg for the two genotypes (AA, AB), respectively. The results of this study were in agreement with Aytekin and Boztepe (2013), as it did not find any significant effect on milk production in his study of the genetic morphology of exon VI of the POU1F1 gene in Turkish cattle. Also, these results agreed with Trakovicka et al. (2015) in his study on Czech cows, as he indicated that there were no significant differences in genotypes in the second intron of the POU1F1 gene in milk production. In a similar study on Sarda goats in Italy, the genetic variation of the POU1F1 gene did not affect milk production (Daga et al., 2013). The results were similar to what was reached by Al-Khuzaei (2018) and Sherif (2019), who indicated in their study on Awassi sheep that there was no significant effect of the genotypes of the POU1F1 gene on milk production. While these results differed with Huang et al. (2008) and Chauhan et al. (2015) in their study on cows, as they obtained significant differences in the genetic morphology of the POU1F1 gene in milk production. Ozmen et al. (2014) also obtained a significant effect of POU1F1 gene on milk production in sheep. Zhou et al. (2016) also found significant gene differences in milk production in goats. The reason for the absence of a significant effect of the POU1F1 gene on daily and total milk production is that the mutations that occurred in the nitrogenous bases did not change any of the amino acids that make up the POU1F1

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protein, or it due to the difference in the studied genetic segment or the difference between the studied strains or the need for A larger number of animals for the purpose of ascertaining the real effect of this gene.

Table2. Effect of genotypes of POU1F1 gene on milk production characteristics

Genotypes		AA	AB	BB	Sig.
Trait(kg)	Number	26	20	1	level
Daily milk production		13.859±0.733 A	14.440±0.931 a	14.00±0.00 a	N.S
Total milk production		4184.95±223.74 A	4403.61±284.25 a	4271.40±0.00 a	N.S
Peak of milk production		457.83±20.70 B	435.06 ±20.85 b	538.50±0.00 a	*
Averages carrying different letters within the same line differ significantly between each other ,* (P≤0.05), N.S.: Not significant					

The effect of the genotypes of the POU1F1 gene on the characteristics of body dimensions Table 3 shows that the BB genotype was significantly (P≤ 0.05) superior in body length, which amounted to 164.0 cm compared to 157.780, 156.347 cm for the two genotypes (AB and AA), respectively. While there were no significant differences between the genotypes BB, AB,and AA in both the frontal height of the body and the rear height of the body.This result is similar to what was found by Kai et al. (2006) in his study on Nanyang cattle, where it was also found that the BB genotype was significantly (P < 0.05) superior in body length compared to the AB genotype at 12 months and 6 months of age. It is also agree with Al-Khuzaei (2018), which indicated that there was a significant effect (P≤0.05) of the genetic morphology of the POU1F1 gene on body length, while it did not find any significant effect in other body dimensions when studying it on Awassi sheep. Also agree with Zhang et al. (2019) and Zhu et al. (2019) in their studies on white cashmere goats in the presence of a significant effect of the genotypes of the POU1F1 gene on body dimensions. However, this result does not agree with Pan et al. (2008), who indicated that there was no statistically significant relationship between the POU1F1 gene polymorphism and body dimensions in his study on Nanyang cattle. It also does not agree with the findings of Jalil-Sarghale et al. (2014), as he did not find any significant differences in body dimensions when studying two breeds of Iranian local sheep. It also does not agree with Sharif (2019), who indicated that there was no significant effect of the POU1F1 gene polymorphism on the body dimensions of sheep at birth, at weaning and at the age of 6 months. The reason for the difference in results between studies in the effect of the genetic morphology of the POU1F1 gene on body dimensions may be due to the difference in the strains that are used to conduct research or the difference in the studied region of the gene.

Table 3. The effect of the genotypes of the POU1F1 gene on the characteristics of body dimensions.

genotypes		AA	AB	BB	Sig.
Trait (cm)	Number	26	20	1	level
Body length		157.780±0.977	156.347±0.868	164.000±0.00	*

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	b	b	a	
Frontal height of the body	150.038±0.975 a	150.325±1.358 a	153.000±0.00 a	N.S
Rear body height	150.288±1.021 a	150.200±1.468 a	154.000±0.00 a	N.S

Averages carrying different letters within the same line differ significantly between each other ,* (P≤0.05), N.S.: Not significant

In general, and because of the contribution of several genes, even if a very small amount, to the emergence of the measures of the studied productive trait, it can be concluded that the probability of identifying a particular gene in order to have the only effect in the occurrence of genetic variations as a result of a specific mutation in a specific site of that gene, whose effect is reflected on the associated trait (Supakorn, 2009). In order for the gene to affect the productive performance of the individual, the mutation must be present in one of two important sites in the gene: the coding regions of the gene, which work to modify the quality of the encoded protein so that it leads to a change in the amino acid as a result of the mutation and thus an incomplete stop of the translation process or for the case of alternative splicing.The other location is the regulatory regions of the gene, which affect the amount of transcription in the gene and the cell and the amount of the resulting protein without changing its quality. Therefore, these sites are influential in the external appearance of the economic trait (Dodds et al., 2007).

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