

Relationship of genotypes of *csn3* gene in milk production and proportions of its components in Holstein Friesian cows bred in Iraq

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Abstract

The study was conducted on 50 Holstein cows bred in Iraq at the Taj Al-Nahrain cow station in Al-Qadisiyah Governorate, of different ages and weights. The process of analyzing the milk components was carried out in the laboratories of the Animal Production Department in the College of Agriculture, Al-Muthanna University. The process of extracting the DNA and examining its purity inside the molecular genetics laboratory in the College of Agriculture University of Basra, and the period of conducting the study was from the beginning of drawing milk and blood samples from 02.12.2021 to 02.03.2022, followed by extraction of the DNA and sending it to China, in order to find out the genotypes of the milk protein gene CSN3 and its relationship to the productive characteristics of milk and its composition, when amplifying a piece. Two haplotypes were obtained from the CSN3 gene. The first pattern differs from the second by the shift of the nitrogenous base from C to T at sequence 69 and the change of the amino acid from ALA to threonine (THR). Isoleucine (ILE) to aspartate ASP, as two genetic mutations were detected that led to a change in the type of amino acid, and the number of cows that represented the first type was 30, and the second haplotype was 20. It was found that the patterns of the CSN3 gene had a highly significant effect ($P \leq 0.01$), as the second individual pattern, was superior to the first pattern in daily, weekly and monthly milk production and the production of the first stage of milk. The first is for the CSN3 gene on the second haplotype in protein and lipid ratios.

Keywords: *csn3 gene, milk production, Holstein Friesian cows.*

INTRODUCTION

Most of the research conducted in the past ten years on the discovery of candidate genes for production traits that are important in selection and breeding programs is that the most studied sites are CSN3, CSN2 and LGB, and it was found that there is a significant positive effect of the formation of these genes on the quantitative and qualitative traits of milk

(Getachew, 2010). Moreover, some other studies did not confirm these relationships, and for these reasons, it is necessary to continue work to obtain detailed studies to know the relationship between genetic formations of genes and milk production and its components in cattle.

On the other hand, genetic morphogenesis guides the relative differences between

individuals regarding the characteristics of milk production and its components. One study showed high genetic diversity in cattle raised in Iraq, and the genetic variation within the breed is greater than the genetic variation between strains (Faraj et al., 2020a). Markers for this gene have become part of "commercial genotype- ing plans" to design so-called Marker Assisted Selection markers in several species of farm animals (Schenkel et al., 2005) and (Nkrumah et al., 2005).

Milk protein genes have been studied extensively among ruminants, and different genetic variants have been identified and classified. These genetic variations influence milk composition and provide options for utilizing genetic diversity (Robinson et al., 1995; Stasio and Mariani, 2000). Some of these genetic variants lead to Functional changes in a protein, such as an amino acid substitution or deletion, which affects the biological properties of the protein in question. One of the studies indicated that the different genotypes for each gene are significantly related to the components of milk (Faraj et al., 2020b). Several studies indicated that milk protein genes are associated with quantitative and qualitative aspects of milk production (Asim et al., 2022; Ozdemir et al., 2018) and issues related to health and community consumption of products. In addition, milk protein variants were used to characterize the strains, and to investigate biodiversity, conducting evolution experiments on animal resources, milk and protein genes (Čítek et al., 2019). Casein genes are closely related to production and heritability, so they are considered a potential target among the marker-assisted selection criteria for milk production traits (Demirel and Bahattin, 2018). Therefore, it is necessary to study different genetic variants of milk proteins to evaluate their effect on milk production in milk cows of the original breeds and breeds of cows. Buffaloes However, no comprehensive study is available on the different variants of

milk protein genes and their possible association with milk production in these breeds.

Therefore, the current study was conducted to determine the genetic variants of different milk protein genes and their association with milk production traits in the Holstein-Friesian breed bred in Iraq.

Materials and Methods

The amount of milk and the analysis of milk components were calculated in the laboratories of the Department of Animal Production - College of Agriculture - University of Al-Muthanna, and the extraction of DNA was done in the laboratory of molecular genetics of the Department of Animal Production in the College of Agriculture, University of Basrah.

Study animals, milk analysis and DNA extraction:

Milk samples were collected from 50 cows (Holstein Friesian cows) from the morning milking period during the three-month continuous study period, and the proportions of its main components (non-fat solids, lactose, protein, and fat) were estimated. The Dutch EKO Milk Analyzer, originating in the Netherlands, analysed milk components in the laboratory of the Department of Animal Production, College of Agriculture, Al-Muthanna University.

DNA extraction:

Blood samples were drawn from the jugular vein of 50 cows (Holstein Friesian) (10 ml/head), and the extraction process was carried out in the molecular genetics laboratory of the College of Agriculture, University of Basra, using the DNA extraction kit produced by (Geneaid) of Korean origin. Chinese company Yang ling tianrun aoka biotechnology co.; Ltd.

A fragment (379 bp) in the P.1.1 promoter region of the CSN3 gene in cattle was amplified by using our designed primer F:

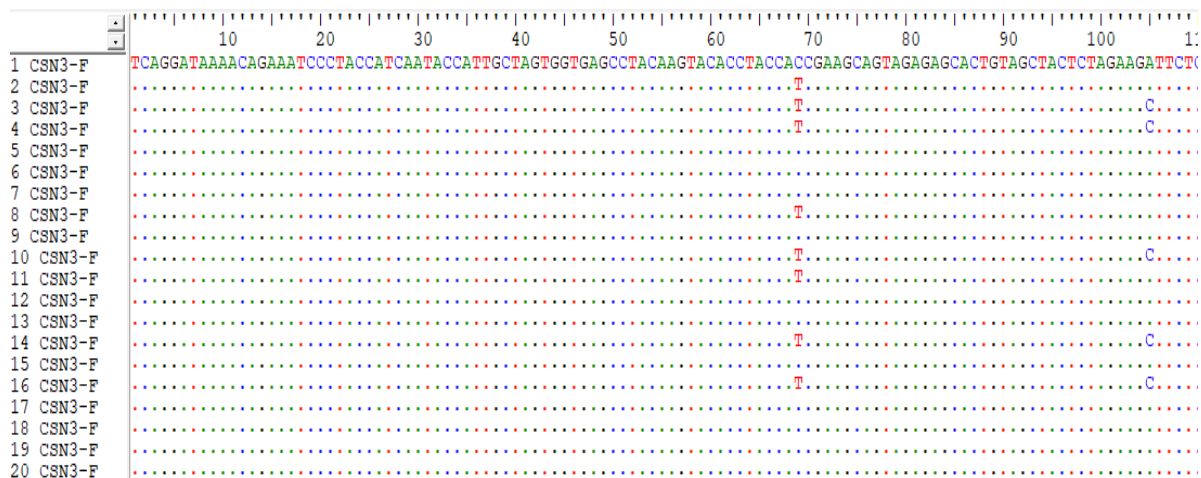
5'-CACGTCACCCACACCCACATTTATC-3' and R: 5'-TAATTAGCCCATTTCGCCTTCTCTGT-3'.

The PCR amplifications were conducted in a 50 µl volume containing 6 µl genomic DNA, 25 µl of Master Mix, 4 µl both primer, and 15 µl free water. The amplification conditions were as follows: initial denaturation at 94 C for 5 min followed by 30 cycles of denaturation at 94 C for 1 min, annealing at 55°C for 1 min, and extension at 72°C for 1 min, followed by the final extension at 72°C for 10 min. The PCR product was detected by 2% agarose gel electrophoresis, stained with Ethidium bromide and visualized by ultraviolet light. The PCR product was sent to Yang ling Tianrun Aoka Biotechnology Company, China, for sequencing.

Sequencing was performed for one strand of DNA (forward) as requested by the company to identify genetic mutations.

Statistical analysis:

Figure (1) The conformity DNA sequence of the studied cows with the reference DNA sequence of the csn3 gene, and it also shows all the substitution mutations according to their position in the PCR product, and the symbol “1 csn3-F” refers to the sequence that is attributed to NCBI



A completely random design (CRD) was used using the SPSS (2016) Version 24 statistical program, and the means were compared using the modified least significant difference test.

Results and discussion

SNP genotypes:

When amplifying a segment of the CSN3 gene with a size of 379 base pairs, two haplotypes were obtained when analyzing two (SNP1-SNP2) SNPs in the bovine csn3 gene. The first haplotype (Hap1) differed from the second haplotype (hap2) by the nitrogenous base with sequence 69 (Figure 1). It shifted from base C to base T (T>C) as the amino acid changed from ALA to threonine THR nine, as well as to the nitrogenous base of sequence 105 (Figure 1), as the nitrogenous base A changed to base C, which changed the amino acid from iso-leucine to ILE Aspartate ASP, as the number of individuals representing the first haplotype was 30 individuals with a frequency equal to 0.60 (Table 1), the number of cows representing the second haplotype was 20 cows with a frequency equal to 0.40.

Table (1) the number and frequencies of haplotypes of the csn3 gene in the cows under study

Gene	haplotype	Number	Repetition	chi-square value	Significant
Kappa casein Csn3	(hap1)	30	0.60	2.0	ns
	(hap2)	20	0.40		

Genotypes of CSN3 genes and their relation to milk production

Table No. (2) Shows that the haplotype of the kappa casein gene had a highly significant effect ($P \leq 0.01$) on milk, as the second haplotype was superior to the first in each of the daily milk production (23.15 ± 0.47 and

19.17 ± 0.15 kg for the two genotypes, respectively). Weekly (162.05 ± 3.29 and 134.22 ± 1.05 kg for the two genotypes, respectively), monthly (648.21 ± 13.19 and 536.88 ± 4.19 kg, respectively), and at the beginning of the milk phase (1944.62 ± 39.58 and 1610.63 ± 12.58 kg, respectively).

Table (2) Effect of multiple genotypes of the CSN3 gene on the quantities of milk production in kilograms of cows bred in Iraq

haplotypes	Number	daily milk production	weekly milk production	monthly production	Milk production for beginning stage of production
first	30	$0.15 \pm 19.17b$	$1.05 \pm 134.22b$	$4.19 \pm 536.88b$	$12.58 \pm 1610.63b$
second	20	$0.47 \pm 23.15a$	$3.29 \pm 162.05a$	$13.19 \pm 648.21a$	$39.58 \pm 1944.62a$

Means with different letters within one column for each gene are significantly different ($P \leq 0.01$).

The kappa casein gene (CSN3) is a candidate functional gene affecting milk formation and milk production traits in cattle, and polymorphisms in kappa casein have been extensively studied in cattle due to their association with milk production and composition (Grosclaude, 1988; Caroli et al., 2009). McSweeney and Fox, 2013). As found by Đedović et al. (2015) showed that CSN3 genotypes significantly affected milk production and milk fat in cattle and crossbreeds from Serbia.

Genotypes of the CSN3 gene and its relationship to the proportions of milk components

The first haplotype was superior to the second of the kappa casein gene (CSN3) in terms of fat (3.29 ± 0.04 and $3.86 \pm 0.06\%$ for the two haplotypes, respectively) and protein (3.09 ± 0.02 and $2.92 \pm 0.01\%$ for the two haplotypes, respectively) Table No. (3) . These results agreed with the findings of AL-hasnawi (2021), which showed that the percentage of fat and protein is significantly affected by the polymorphism of the kappa-casein gene, and the result also agreed with the percentage of lactose, which shows non-significant differences with the polymorphism of the kappa-casein gene. A group of researchers found that the genotype affects the high percentage of protein and fat in the milk (Kucerova et al., 2006).

Table (3) Effect of polymorphism of the CSN3 gene on the percentage of milk components in cows bred in Iraq

haplotypes	Number	fat %	protein %	lactose %	Non-greasy solids %
first	30	0.06±a3.86	0.02±a3.09	0.03±4.39	0.07±7.95
second	20	0.04±b3.29	0.01±b2.92	0.04±4.35	0.07±7.89

Means that different letters within one column for each gene are significantly different among themselves* ($P \leq 0.01$).

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