ISSN NO: 2771-8840
Date of Publication: 14-03-2023

Relationship of genotypes of csn2 gene in milk production and proportions of its components in Holstein Friesian cows bred in Iraq

Karrar thabit dragh,
Assist Prof.Dr. Raghdan Hashim Mohsin,
Assist Prof.Dr. Muntaha y.Yousief
Department of Animal Production, College of Agriculture, University of Basrah, Iraq.
agripg.karrar.dragh@uobasrah.edu.iq

Abstract
The study was conducted on 50 Holstein cows of different ages and weights raised in Iraq at the Taj Al-Nahrain cattle station in the Al-Qadisiyah governorate. The DNA was extracted, and its purity was examined in the Molecular Genetics Laboratory at the College of Agriculture, University of Basrah. The study was conducted from 12.03.2022 to 03.02.2022. Milk and blood samples were taken during this period, and the DNA was extracted and sent to China. In order to know the genotypes of the CSN2 milk protein gene and its relationship to the product characteristics and composition of milk. The study showed that when a piece of the CSN2 gene was amplified, three genotypes were obtained and that the second pattern differs from the first and third patterns by the nitrogenous base of sequence 88. The base shifted from A to C, and the third pattern differs from the first and second patterns by changing the nitrogenous base of the sequence 52, as it shifted from C to T without changing the amino acid sequence of the gene, and the distribution of the patterns among the study cows was 23 for the first pattern, 10 for the second pattern, and 17 for the third pattern. There were significant differences ($P \leq 0.01$) between the genotypes of the CSN2 gene in milk production, where the first individual genotype was superior to the second and third in daily, weekly and monthly milk production and the production of the first stage, as well as a significant effect ($P \leq 0.01$) of the genotypes of the CSN2 gene on the components of Milk (protein and fat), where the second and third individual of the CSN2 gene outperformed the first individual.

Key words: Holstein cows, cattle station, Milk

Introduction

All over the world, milk is an essential part of the components of human food, and the demand for dairy products is increasing, especially since milk is an essential source of protein, as it ranges from 3-3.5% of milk with an average of 3.11 (Caroli et al., 2009). The percentage of protein varies between strains (Marangoni et al., 2018). Proteins have a high biological value because they contain many essential amino acids. Milk protein consists of two proteins, casein and whey. Casein occupies 80% of milk proteins. It includes four forms - alpha-casein S1, alpha-casein S2, beta-casein, and kappa-casein - and is encoded by CSN1S1, CSN1S2, CSN2 and CSN3 genes, respectively. All of these genes are present on bovine chromosome 6. Milk production and its components is a quantitative trait controlled by several alpha, beta, and kappa casein genes related to casein proteins (Sebastiani et al. 2020).

One study showed that cattle raised in Iraq have high genetic diversity, with more significant genetic variation within breeds than between breeds (Faraj et al., 2020a). Studies have been conducted on identifying the genes that control the formation of milk and the regulation of gene expression in the tissues of cows because these genes are important regulators in the milk composition formed during the lactation process (Bionaz and Loor, 2007). One of the studies showed that the different individual patterns of Genes might be significantly associated with components of milk (Faraj et al., 2020b). The process of developing the mammary gland is a complex process that is subject to the influence of hormonal factors, including growth factors affecting the epithelium of the mammary gland during pregnancy and postpartum, as well as the influence of genetic aspects in it through the expression of milk protein genes as well as the production

ISSN NO: 2771-8840
Date of Publication: 14-03-2023

of milk fat, which are concentrated sources of energy along with Essential fatty acids (Robinson et al., 1995).

The study aimed to identify the genetic variation in the milk protein gene (CSN2) and its association with the characteristics of milk production and the proportion of its components in the Holstein-Friesen strain bred in Iraq.

Materials and Methods

The amount of milk was calculated, and its components were analyzed in laboratories of the Animal Production Department in the College of Agriculture, University of Al-Muthanna. DNA was extracted in the genetics laboratory of the Department of Animal Production, College of Agriculture, University of Basrah.

Study animals, milk analysis and DNA extraction:

Milk samples were collected from the morning paddock throughout the continuous study period for three months from 50 cows (Holstein Friesian), and the proportions of its main components (non-fat solids, lactose, protein and fat) were estimated. The components of milk were analyzed by the Dutch EKO Milk laboratory analysis device established in the laboratories of the Production Department of the College of Agriculture, Al-Muthanna University.

DNA extraction:

Blood samples were drawn from 50 cows (Holstein Friesian) (10 ml/cow) drawn from the jugular vein. The extraction process was carried out in the Molecular Genetics Laboratory of the College of Agriculture at the University of Basrah using the DNA extraction kit produced by (Geneaid) of Korean origin. The primers were manufactured and supplied by (Yang ling tianrun aoka biotechnology co.; Itd) of China.

A fragment (121bp) in the P.1.1 promoter region of the *CSN2* gene in cattle was amplified by using our designed primer F: 5´-CCTTCTTTCCAGGATGAACTCCAGG-3´and R: 5´-GAGTAAGAGGAGGGATGTTTTGTGGGAGGCTCT-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 95 ºC for 5 min followed by 30 cycles of denaturation at 95 C for 40 sec, annealing at 58ºC for 1 min, and extension at 72ºC for 1:30 min, followed by the final extension at 72ºC for 10 min. The PCR product was detected by 2% agarose gel electrophoresis, stained with Ethedium 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:

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:

A segment of the csn2 gene is amplified to the size of 121 bp. When analyzing three (SNP1-SNP3) SNPs in the bovine csn2 gene, three haplotypes were obtained. The second haplotype (Hap2) differed from the first and third haplotypes by the nitrogenous base of sequence 88 and shifted from base A to base (A>C) C. In contrast, the third haplotype (Hap3) differed from the first and second haplotypes by the nitrogenous base of sequence 52, as The nitrogenous base, C, was transformed into the base (C>T) T (Fig. 1). As the number of individuals representing the first haplotype was 23 with a frequency of 0.46 (Table 1), the number of cows representing the second haplotype was 10 with a frequency of 0.20. In contrast, the number of animals representing the third haplotype was 17, with a frequency of 0.34. The sequencing results showed no change in the amino acid sequence of the csn2 gene due to changes in SNPs identified in the CSN2 exon-7 region while comparing it to the reference sequence (GenBank: EF628290.1). At the same time, the three polymorphisms were identical to what was stated in GenBank, the SNP1 located in the intron VII and registered under accession number rs450367767 in NCBI and two SNP2 and SNP3 belonging to the eighth intron were previously registered under accession number rs135724333 and rs435192135, respectively.

ISSN NO: 2771-8840
Date of Publication: 14-03-2023

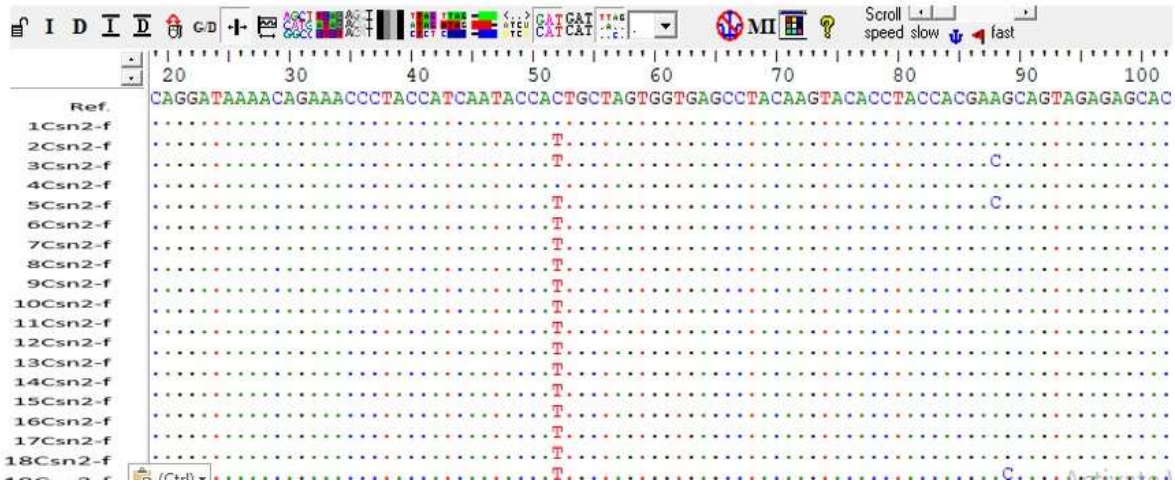

Figure (1) the conformity of the DNA sequence of the studied cows with the reference DNA sequence of the csn2 gene, and it shows all the substitution mutations according to their position in the PCR product, and the "ref" symbol refers to the sequence that is attributed to NCBI.

Table (1) The number and frequencies of haplotypes of the CSN2 gene in the cows under study

Gene	haplotype	Number	Repetition	chi-square value	Significant
beta casein Csn2	**(hap1)**	**23**	**0.46**	**5.0**	**ns**
	(hap2)	**10**	**0.20**		
	(hap3)	**17**	**0.34**		

Genotypes of the CSN2 gene and its relationship to milk production

Table (2) shows that there are significant differences (P≤0.01) between the haplotypes of the CSN2 beta-casein gene, as the first haplotype was superior to the second and third haplotypes in daily milk production (22.76 ± 0.46, 19.17 ± 0.27 and 19.00 ± 0.19 kg for the haplotypes). Individual, respectively), weekly (159.31 ± 3.23, 134.22 ± 1.88 and 133.01 ± 1.31 kg for haplotypes, respectively), monthly (637.25 ± 12.93, 536.88 ± 7.53 and 532.05 ± 5.25 kg for haplotypes, respectively) and production in the first stage (1911.76 ± 38.79, 1610.63 ± 22.58 and 1596.15 ± 15.76 kg for haplotypes, respectively) The result of these differences makes this gene a candidate gene for use as a marker in selection, and this is consistent with what was found by Marko et al. (2020) and Nilsen et al. (2009) and Oleński et al. (2012).

There are statistically significant differences in milk production between cows of different beta-casein genotypes for the genotype A1A1- vs. A2A2 and A1A2 (P < 0.01), indicating that the A1 allele was associated with decreased milk production in the tested cows, whereas Miluchová et al. (2023) No differences between the genotypes of the beta-casein gene for daily milk production in Slovak Holstein cows.

Table (2) Effect of polymorphisms of the CSN2 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	23	22.76a±0.46	159.31a±3.23	637.25a±12.93	1911.76a±38.79
second	10	19.17b±0.27	134.22b±1.88	536.88b±7.53	1610.63b±22.58
third	17	19.00b±0.19	133.01b±1.31	532.05b±5.25	1596.15b±15.76

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

Genotypes of the CSN2 gene and its relationship to milk component proportions

Table (3) shows that there is a significant effect (P≤0.01) of the haplotypes of the CSN2 gene in the fat and protein components of milk, the superiority of the second and third haplotypes over the first haplotype of the

ISSN NO: 2771-8840
Date of Publication: 14-03-2023

beta-casein gene (CSN2) in both percentages of fat (3.86 ± 0.11, 3.91 ± 0.08 and 3.33 ± 0.04% for haplotypes, respectively) and protein (3.09 ± 0.03, 3.11 ± 0.02 and 2.93 ± 0.01% for haplotypes, respectively) Table No. (3) while the results agreed with what was found by Albarella et al. (2020), Who got three genotypes that the milk of cows with the genotype A2A2 contains a higher percentage of protein and total solids when compared to the milk of cows A1A1 A group of researchers found that the genotype affected the high percentage of protein and fat in the milk (Kucerova et al., 2006).

Table (3) effect of polymorphism of the CSN2 gene on the percentage of milk components in cows bred in Iraq

haplotypes	Number	fat %	protein %	lactose %	Non-greasy solids %
first	23	0.04±b3.33	0.01±b2.93	0.04±4.35	0.06±7.90
second	10	0.11±a3.86	0.03±a3.09	0.05±4.39	0.117±7.95
third	17	0.08±a3.91	0.02±a3.11	0.04±4.39	0.09±7.94

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

References

1. Albarella, S., Selvaggi, M., D'Anza, E., Cosenza, G., Caira, S., Scaloni, A., Fontana, A., Peretti, V. & Ciotola, F. (2020). Influence of the casein composite genotype on milk quality and coagulation properties in the endangered Agerolese cattle breed. Animals 10(5): 892.
2. Bionaz, M. & Loor, J. J. (2007). Identification of reference genes for quantitative real-time PCR in the bovine mammary gland during the lactation cycle. Physiological genomics 29(3): 312-319.
3. Faraj, S. H., Ayied, A. Y. & Al-Rishdy, K. A .(2020a). Single Nucleotide Polymorphisms in the promoter of CYP19 gene in cattle bred in Iraq. Basrah Journal of Agricultural Sciences 33(1): 89-97.
4. Faraj, S. H., Ayied, A. Y. & Mahde, N. F. (2020b). ASSOCIATION OF ATP1B2 GENE POLYMORPHISM WITH MILK YIELD, MILK COMPOSITION AND HEAT RESISTANCE TRAITS OF CATTLE BRED IN IRAQ. Basrah Journal of Agricultural Sciences
5. Kucerova, J., Matejicek, A., Jandurová, O., Sorensen, P., Nemcova, E., Stipkova, M., Kott, T., Bouska, J. & Frelich, J. (2006). Milk protein genes CSN1S1, CSN2, CSN3, LGB and their relation to genetic values of milk production parameters in Czech Fleckvieh. Czech Journal of Animal Science 51(6): 241.
6. Marko, R., Uros, G., Branislav, V., Milan, M., Danijela, K., Vlado, T. & Zoran, S. (2020). Beta-casein gene polymorphism in Serbian Holstein-Friesian cows and its relationship with milk production traits. Acta Veterinaria 70(4): 497-510.
7. Miluchová, M., Gábor, M. & Candrák, J. (2023). The Effect of the Genotypes of the CSN2 Gene on Test-Day Milk Yields in the Slovak Holstein Cow. Agriculture 13(1): 154.
8. Nilsen, H., Olsen, H. G., Hayes, B., Sehested, E., Svendsen, M., Nome, T., Meuwissen, T. & Lien, S. (2009). Casein haplotypes and their association with milk production traits in Norwegian Red cattle. Genetics Selection Evolution 41: 1-12.
9. Oleński, K., Cieślińska, A., Suchocki, T., Szyda, J. & Kamiński, S. (2012). Polymorphism in coding and regulatory sequences of a beta-casein gene is associated with milk production traits in Holstein-Friesian cattle—animal Science Papers and Reports 30(1): 12.
10. Robinson, G. W., McKnight, R. A., Smith, G. H. & Hennighausen, L. (1995). Mammary epithelial cells undergo secretory differentiation in cycling virgins but require pregnancy to establish terminal differentiation. Development 121(7): 2079-2090.
11. Caroli, A., Chessa, S., & Erhardt, G. (2009). Milk protein genetic variation in cattle: Impact on animal breeding and human nutrition. Journal of dairy science, 92, 5335-5352.

ISSN NO: 2771-8840
Date of Publication: 14-03-2023

12. Marangoni, F.; Pellegrino, L.; Verduci, E.; Ghiselli, A.; Bernabei, R.; Calvani, R.; Cetin, I.; Giampietro, M.; Perticone, F.; Piretta, L.; et al. Cow's Milk Consumption and Health: A Health Professional's Guide. J. Am. Coll. Nutr. 2019, 38, 197–208.
13. Sebastiani, C., Arcangeli, C., Ciullo, M., Torricelli, M., Cinti, G., Fisichella, S., & Biagetti, M. (2020). Frequencies evaluation of β-casein gene polymorphisms in dairy cows reared in Central Italy. Animals, 10(2), 252.