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Effect of mutation site of k-casein gene on protein quantity, composition, and other milk constituents in Holstein cows

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Abstract

This study was conducted at the Taj Al-Nahrain Plant in Diwaniyah governorate on 50 cows of the Holstein, as well as the Ishaqi Dairy Laboratory in Baghdad and in the Laboratory of the department of Science / Babylon University for the period from 2017/10/1 / to 2018\3\1 to determine the genotypes of the k-casein gene and its relationship with quantitative and qualitative changes in the produced milk , especially the protein as well as milk production As well as the study of the percentages of the distribution of genotypes in the sample and the alleles frequencies obtained for the gene. The percentages of the distribution of AA, AB, and BB were significantly different from those of kappa casein (P <0.01) in the studied sample, the percentages were (38, 18 and 44%), respectively, with allelic frequency was 0.41 and 0.53 for A and B respectively and there were significant differences. (P <0.05) for protein and casein and . There were no significant differences between the other genotypes (pH, fat, density, freezing point, added water, and total solids) with the change in K-casein genotype, while for milk production, The effect was significant (P <0.05) during the first week of production only, while there were no significant differences in the following weeks There is also no significant difference between the production means or the total milk production. We can conclude by studying the genotypes of the k-casein gene that can be developed in the methods of genetic improvement in cows. The application of the study to larger sample and for many sites will give more accurate results for the application of methods of exclusion and substitution and determine the optimal method for managing cows.

Keywords: Holstein cows, kappa casein gene, milk production, milk quality.

INTRODUCTION

Casein is the most important part of cattle's milk. Casein genes and expression organizations have been the subject of many researches in the past (1). The knowledge of the fundamentals of the genes of milk proteins is very important in the progress and development of milk production. The identification of the regulatory factors of gene expression and the multiplicity of the genetic manifestations of the allele of the kappa casein gene provides a strong base for markers assisted selection (MAS) in dairy cattle. The accumulation of information and its availability about the genome over the past decades Has enabled more focused researches on the molecular mechanisms that control the manufacture of casein in the lactic gland (2). Caseins are the most important proteins in mammalian milk and has been classified as a combination of αs1- casein, β-casein, K-casein, αs2- casein, and y-casein which is derived from β-scasein and is located physiologically in a region of 200-250Kb (3 ,4 ,5). On chromosome 6, which is closely linked to each other and specifically at location 6q31-33 (6). Genetic variation in casine genes and its role in milk synthesis has been confirmed and used in livestock selection programs (7). The k-casein gene (k-casein gene (CSN3)) in cows is composed from five coded regions (exons) and four introns, Its length is about 13Kb of cow's genome (8,9). The registered allels (A, A1, B, C, E, F1, F2, G, H, I and J) (shape 1), for this gene are all located within the fourth exon within a region of 550bp length, (10 ,11). The genetic variants of k- casein were found in different frequencies in different groups of dairy cows. Most European cattle breeds have a very high frequency of allel A of k- casein (12 ,13). And this allel is the dominant in Holstein cows (14). The difference between the alleles of this gene depends on the change nitrogen bases except the allele A and A1(6). Chen et al. 2008 refered that The reading of the sequence of the nitrogen bases of this region will contribute to the identification of new variations in the form of individualities (haplotypes) and other researchers have proposed a genetic survey of this gene in the domestic cows to identify the genetic variation and development in the composition of this gene (10 ,11). The current study aimed at determining the genotypes of the k-casein gene in the cow's milk sample through the use of PCR-SSCP technique and its relation to quantitative and qualitative changes in the produced milk , especially the protein as well as the daily and total milk production, in a sample of Holstein cattle.

MATERIALS AND METHODS

This study was conducted at the Taj Al Nahrain Plant in Diwaniyah Governorate on a group of Holstein cattle (50 cows) during the period from 2017/10/1 to 1/3/2018. The genetic analysis of the blood samples were conducted in the laboratories of department of Science / Babylon for the purpose of separating the genetic material DNA and determining the genotypes of the k-casein gene for the exon 4 region and its relation to the quantity and quality of the milk, as well as the extraction of the proportions of the distribution of its genotypes in the herd and the repeated alleles obtained. The PCR-SSCP technique was used to amplify the required particle to complete the molecular detection procedure and to identify the polymorphism of the k-casein gene according to the size of the piece and the type of primers used. The primers (Exon 4 amplification area) were selected for molecular detection and

polymorphism of the gene resulting from the presence of mutations of the k-casein gene (15). The studied gene pieces were identified through the use of Electronic browsers specialized in the vertebrates genome (NCBI National Center for Biotechnology Information), Ensembl Genome Browser and UCSC (University of California Santa Cruz).

Primers:

Forword=5" CAGAGCCACTGTGGAGAACA 3"

Reverse=5" TCAGCTCTTGCTTGGCAGTA 3"

SSCP technique, PCR products were denaturation using SSCP dye (EDTA, formamid and bromophynol blue) 1/1 V:V in water bath for 5 min at 95C then its child in ice for 2 min.;

SSCP electrophoresis, the products were electrophoresis as a following About 10 µl of the samples into wells of an 8% acrylamide/bis gel (37.5:1), containing 7% glycerol, and 1x TBE buffer. And for recipe a 20 x 20 x 0.1 cm gel format. 8 ml of 40% acrylamide/bis (37.5:1) mixed with 8 ml of 5x TBE , 2,8 ml 100% glycerol, then 40 µl TEMED and 400 µl of 10% ammonium per sulfate were added with 20.8 ml of dH_2O After gel was casting sample were loaded and Run under the following conditions.

RESULTS AND DISCUSSION

Extraction of kappa casein gene:

The k-casein gene was extracted using PCR technology. Five microliters of each sample were loaded in 0.8% agarose gel and the voltage was controlled at 70 V and 40 amperes for 90 min. The loading product was visualized to ensure that the extraction process was completed successfully and obtained the desired gene size 935 base pairs (bp) of k-casein gene, a known size and concentration DNA fragments were used (Marker bp 100-1500) (Figure 1).

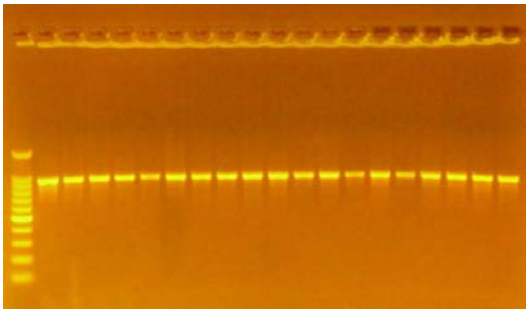

Figure 1: relaying PCR product for exon 4 region

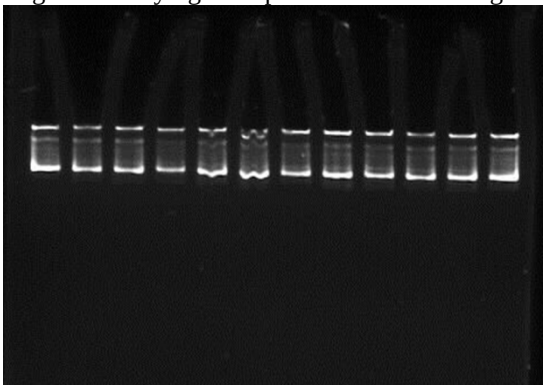

Figure 2: relaying SSCP product of exon 4

Genetic polymorphisms of exon 4 region

1- SSCP technique was conducted to identify the genotypes of the K-casein gene of the experimental animals in exon 4 region as it shown in Figure (2), a three genotypes were found (two, three and four bands).

2. In order to identify the location and type of changing bases of the resulting genotypes, representative samples were sent for nitrogen bases sequencing technique process for each genotype in order to identify the genetic difference between the resulting forms (two , three and four bands), as follows:

In case there were two bands (22 samples) and after conducting the sequencing technique, it showed the presence of BB allele as follows:

TACCAYCGAAGCAGTAGAGAGCACTGTAGCTACT
CTAGAAGMTTCTCCAGAAGTTATTGAGAGCCCAC
CTGAGATCAACACAGTCCAAGTTACTTCAACTGCR
GTCTAAAWACTCTAAGGAGACATCAAAGAAGACA
ACGCAGGTAAAT

This change causes the amino acids to be as follows

RCEKDERFFS DKIAKYIPIQ YVLSRYPSYG
LNYYQQKPVA LINNQFLPYP YYAKPAAVRS
PAQILQWQVL SNTVPAKSCQ AQPTTMARHP
HPHLSFMAIP PKKNQDKTEI PTINTIASGE
PTSTPTTEAV ESTVATLEDS PEVIESPPEI
NTVQVTSTAV

---------- ---------X YVLSRYPSYG
LNYYQQKPVA LINNQFLPXP YYAKPAAVRS
PAQILQWQVL SNTVPAKSCQ AQPTTMARHP
HPHLSFMAIP PKKNQDKTEI PTINTIASGE
PTSTPTIEAV ESTVATLEAS PEVIESPPEI
NTVQVTSTAV

In case there were three bands (19 samples) and after conducting the sequencing technique, it showed the presence of AA allele as follows:

TACCATCGAAGCAGTAGAGAGCACTGTAGCTACTC
TAGAAGCTTCTCCAGAAGTTATTGAGAGCCCACCT
GAGATCAACACAGTCCAAGTTACTTCAACTGCGGT
CTAAATACTCTAAGGAGACATCAAAGAAGACAAC
GCAGGTAAAT

This change causes the amino acids to be as follows

RCEKDERFFS DKIAKYIPIQ YVLSRYPSYG
LNYYQQKPVA LINNQFLPYP YYAKPAAVRS
PAQILQWQVL SNTVPAKSCQ AQPTTMARHP
HPHLSFMAIP PKKNQDKTEI PTINTIASGE
PTSTPTTEAV ESTVATLEDS PEVIESPPEI
NTVQVTSTAV

---------- ---------X YVLSRYPSYG
LNYYQQKPVA LINNQFLPYP YYAKPAAVRS
PAQILQWQVL SNTVPAKSCQ AQPTTMARHP
HPHLSFMAIP PKKNQDKTEI PTINTIASGE
PTSTPTTEAV ESTVATLEDS PEVIESPPEI
NTVQVTSTAV

And In case there were four bands (9 samples) and after conducting the sequencing technique, it showed the presence of AB allele as follows:

TACCACCGAAGCAGTAGAGAGCACTGTAGCTACT
CTAGAAGATTCTCCAGAAGTTATTGAGAGCCCACC

TGAGATCAACACAGTCCAAGTTACTTCAACTGCAG TCTAAAAACTCTAAGGAGACATCAAAGAAGACAA CGCAGGTAAAT

This change causes the amino acids to be as follows

RCEKDERFFS DKIAKYIPIQ YVLSRYPSYG LNYYQQKPVA LINNQFLPYP YYAKPAAVRS PAQILQWQVL SNTVPAKSCQ AQPTTMARHP HPHLSFMAIP PKKNQDKTEI PTINTIASGE PTSTPTTEAV ESTVATLEDS PEVIESPPEI NTVQVTSTAV

---------- ---------X YVLSRYPSYG LNYYQQKPVA LINNQFLPYP YYAKPAAVRS PAQILQWQVL SNTVPAKSCQ AQPTTMARHP HPHLSFMAIP PKKNQDKTEI PTINTIASGE PTSTPTXEAV ESTVATLEXS PEVIESPPEI NTVQVTSTXV

The number and percentage of the genotypes and alleles frequencies for K-casein gene:

The percentages of the distribution of the genotypes were 44, 18 and 38%, There was a significant difference in distribution ratios when compared to mandalas (1,2,1)This is due to indirect selection based on milk production. The allelic distribution was 0.47% and 0.53 A, B Respectively. The distribution of alleles was in the direction of the B as it shown in table (1)

Table 1: The number and percentage of the genotypes and alleles frequencies for K-casein gene

Genotype	Number	Percentage %
AA	19	38
AB	9	18
BB	22	44
total	50	100%
Kai square (χ^2)	-----	20.840**
allele	Frequency	
A	0.47	
B	0.53	
.(P<0.01) **		

The relationship of the polymorphism in the region of the Exon IV with milk components and the quantity of production:

The results of the current study, as shown in Table (2), showed that differences in the exon IV had significantly affected P <0.05) on protein and casein in milk, but did not differ significantly for other components. These results correlate with the fact confirmed by the direct effect of changes in the exon region on the quality of the produced protein (7). The variation in the bases was associated with the difference in the type of amino acid. The variation in the genotype (AA, AB, BB) was associated with The variation of total protein content and the ratio of casein in milk as all alleles of this gene fall within the area of Exon IV (10 ,11). The genotype BB was higher than that of AA but similar to the effect of AB in total protein and casein within the total protein. The total protein content of the three genotypes (AA, AB, BB 3.183, 3.132 and 2.75,respectively). The ratio of casein protein was 2.546, 2.506 and 2380 for the three genotypes respectively. this results consistent with (16) and (17)and (18) who showed that the cows with genotype BB recorded the highest protein content, the ability to produce more cheese, And milk coagulation faster than the genotype AA, this is due to the decrease in the ratio of casein and increase colloidal colloids (19). In terms of fat percentage there were differences in the calculation but not significant for the genotype AA, as well as for water and the proportion of salts, This may be due to that the kappa casein gene is responsible only for the encoding of the major gene.

Table (3) shows the relationship of the polymorphism in the area of the fourth exon with milk production. The table showed significant differences (P <0.05) in the first week only and no significant differences were observed in the following weeks. There is no significant difference between the production means or total production of the milk .

AA genotype was higher than AB genotype in milk production and was similar to BB genotype in effect in the first week. The daily milk production was 10.184, 8.812 and 11.375 for the three genotypes (AA, AB, BB) respectively in the following weeks, there were no significant differences between the three genotypes. That this result was consistent with a number of studies that recorded the superiority of AA genotype in milk production (19,12,20, 21,22) which also confirmed The role played by allele A in this but differed with other studies that reported that hybrid genotype tends to increase milk production (16, 23,21) which are opposite The current study results, the high temperature and low level of nutrition may be due to the absence of the possibility of genetic systems to express the production potential, which in turn explains the absence of significant differences between the Three genotypes in the following weeks.

Table 2: The relationship of the polymorphism in the region of the Exon 4 with milk components

Sold	Frez	Water	Dencity	Fat	Czen	Proten	Ph	SSCP
8.296a ±0.159	56.010a ±2.361	4.466a ±1.024	27.265a ±0.999	4.33a ±0.446	2.546a ±0.054	3.183a ±0.068	7.039a ±0.034	**2(BB)**
8.210a ±0.117	54.068a ±0.678	3.379a ±1.025	26.112a ±0.867	4.266a ±0.472	2.506ab ±0.022	3.132ab ±0.028	6.989a ±0.032	**3(AB)**
7.803a ±0.217	51.512a ±1.22	7.340a ±2.190	24.150a ±0.965	5.003a ±0.312	2.380b ±0.062	2.975b ±0.078	6.932a ±0.021	**4(AA)**
NS	NS	NS	NS	NS	*	*	NS	**F**

Table 3: The relationship of the polymorphism in the region of the Exon 4 with milk production

Total	Mean	W5	W4	W3	W2	W1	SSCP
53.375a ±2.916	10.675a ±0.583	10.375a ±0.660	10.815a ±0.517	10.315a ±0.465	10.736 ±0.606	10.184ab ±0.621	**2(BB)**
48.166a ±2.029	9.633a ±0.405	9.800a ±0.427	9.937a ±0.558	9.781a ±0.693	10.062 ±0.722	8.812b ±0.568	**3(AB)**
52.375a ±5.4900	10.475a ±1.098	11.750a ±1.108	11.375a ±0.323	9.625a ±0.962	9.875 ±1.241	11.375a ±0.998	**4(AA)**
NS	NS	NS	NS	NS	NS	*	**F**

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