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Detection of β-Lactoglobulin Gene Polymorphism In Exon IV And Intron IV Region.

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ABSTRACT

The study was conducted at the Taj Al-Nahrain Plant in Diwaniyah Governorate on 50 cows of imported Holstein cattle. The analysis of the milk components and its qualitative characteristics were conducted in the laboratory of Al-Ishaqi in Baghdad. Molecular genetics analyzes were also carried out in the Advanced Scientific Bureau (ASCO) laboratory for Biotechnologies and Molecular Genetics Analysis. in order to identify the polymorphism of exon IV and intron IV region of β-Lactoglobulin gene to determine the genotypes frequencies in the sample and allelic frequency as well as identifying the relationship of this polymorphism with milk yield and components in the sample. Two point mutations were detected (g.5305C>T) in exon IV and (g.5490C>T) in intron IV, and three genotypes were found for both mutations, the wild genotype CC and the hybrid genotype CT, while the mutated genotype was TT.

Keywords: β-Lactoglobulin, gene polymorphism, exon IV, intron IV.

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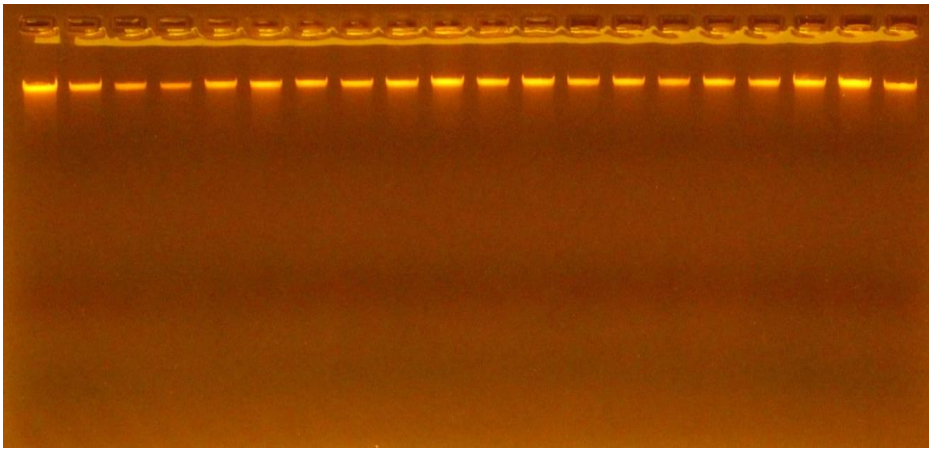
INTRODUCTION

Cows are the main producers of milk in the world, contributing 90% of the world's production (FAO, 1998). As the world's population worldwide saves 13% of their daily protein requirement through milk and its derivatives (Mir et al., 2014).Researches and attempts to improve the productive traits of milk began more than a hundred years ago using traditional methods that adopted morphological changes in traits. These methods are useless and time consuming. Genetic improvement methods have developed in recent years through the discovery of molecular techniques and their use in genetic improvement programs. and became possible to predict the animal production early without depending on age or sex or waiting for the trait to express itself through studying the genotype and also the most important polymorphisms at the molecular level (Otaviano et al., 2005). The productive traits of milk are controlled by a combination of genetic factors and therefore the differences between these factors can be exploited and used as genetic markers of productive milk traits dairy cattle (Ng-Kwai-Hang et al, 1990). Milk proteins play an important role in food industry since the properties of each protein in milk proteins determine the function of this protein in food (Darewicz et al., 2006), The reason why milk proteins attract so much attention is that they contain the nine essential amino acids needed by humans (Gigli, 2016). The quantitative traits selection efficiency in dairy cattle depends on the identification of the candidate genes responsible for these traits, as well as identifying the most important changes responsible for polymorphism in these genes (Abu Khaizaran, 2013). Among these genes affecting economically important traits in cattle is the β-LG gene, which has been extensively studied (Tsiaras et al., 2005). This gene is located on chromosome 11 in cattle and encodes (β-LG) protein , which is an important protein in the whey, and affects milk yield and milk protein quality (Karimi et al., 2009).This gene is consisting of 7 exons and 6 introns, a protein (β-LG) is a highly acid stable protein and is present in the normal pH of bovine milk (Karimi, 2009). It has a molecular weight of approximately 18000KDa and consists of 162 amino acids. The amino acid sequence has been fully recorded as well as the genetic variation of the amino acid sequence (Creamer et al., 1983; Rachagani et al., 2006).

Objective of the research: identify betalactoglobulin gene polymorphism in a sample of Holstein cows and the effect of genotypes of this gene on milk yield and composition.

MATERIALS AND METHODS

Blood samples were derived from 50 cowsc and the blood samples were put in EDTA tubes (5ml) and stored in -18^{o}C.The conventional phenol–chloroform method was adopted to extract genomic DNA from blood samplesand a (Promega) kit was used in the process. Theelectrophoresis process was used to make sure that the DNA extraction process was successful where the DNA samples were loaded in 2% agaros and stained with ethidium bromide, then the samples were visualized by UV device as shown in shape (1).The milk indices (including weekly yield and total milk yield) were recorded for biological statistics.



Shape 1: DNA samples electrophoresis

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PCR analysis

A primer was taken from a study made by (Badola et al, 2003) used in this study as shown in table (1).

Table 1: The primer used in the study

Primer
Forward primer 5'-CGAGAACAAAGTCCTTGTGCT-3'
Reverse primer 5'-CCGGTAACAAAGGCTGTTAGA-3'

Badola et al (2003)

The PCR reaction was carried out in a final volume of 25 µl mixture containing 12.5 master mix,1 µl of primer F, 1 µl of primer R, ,5 µlDNA template and 5.5 µlddH2O. The PCR condition was shown as follows:intial denaturation at 94C for 5 min; 1 cycle, denaturation at 94C for 30 s: 35 cycles; annealing at 60C for 30 s: 35 cycles, extension at 72C for 5.5 min.

Standard Sequencing technique:

PCR product were send for Sanger sequencing using ABI3730XL, automated DNA sequencer, by Macrogen Corporation – Korea. The results were received by email then analyzed using genious software.

Milk components analysis:

50 milk samples were taken from the studied cows and were being analyzed in Al-Ishaqi laboratory to determine the percentages of milk components, four milk components were analyzed (total protein, β-LG protein, fat and solid non fatty (SNF)) using a device called (Milkana).

Statistical analysis

GLM procedure was used to explore the relationship of β-lactoglobulin gene polymorphism with milk yield and components, the following model was adopted:

$$Y_{ijk} = \mu + G_i + P_j + e_{ijk}$$

Y_{ijk} : observation value 1 of the genotype i and the parity
µ: general mean of the trait
G_i: effect of the β-lactoglobulin gene polymorphism for both mutations (g.5305C>T) and (g.5490C>T).
P_j: effect of productive cycle sequence (second and third).
e_{ijk}:Random error which is distributed naturally at an average of zero and a variation of σ2e

The following equation was applied to calculate the allelic frequency according to the Hardy-Weinberg equation:

Frequency of first allele:

$$P_A = \frac{2 * \text{No. of Homozygous} + 1 * \text{No. of Heterozygous}}{2 * \text{Total number of sample}}$$

Frequency of second allele:

$$q_B = 1 - P$$

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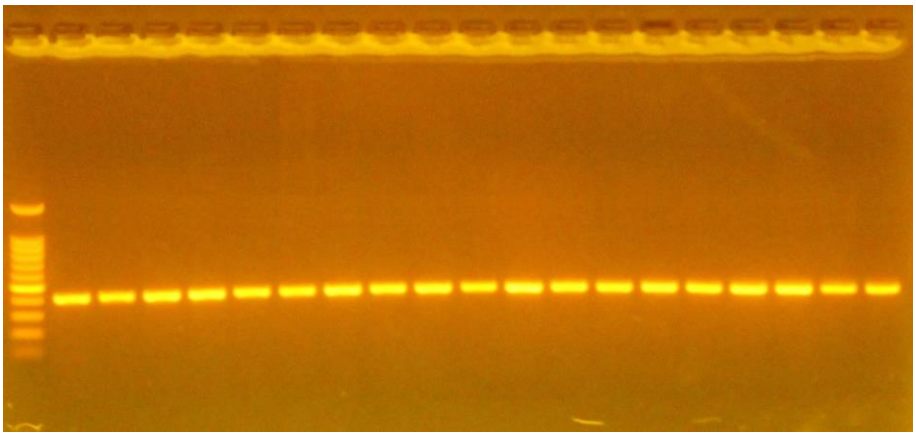
RESULTS AND DISCUSSION

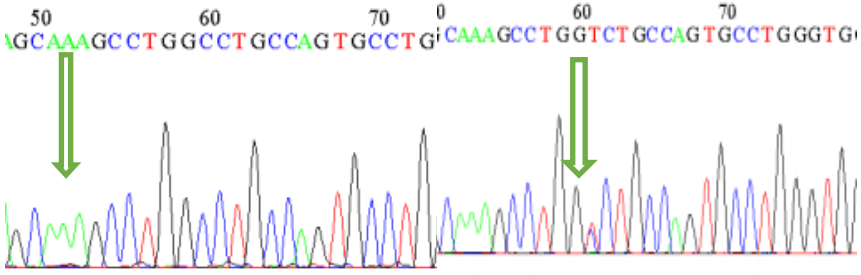
The size of PCR products amplified with the primer was 398 bp (104 bases of exon IV and 294 bases of intron IV). As shown in shape (2).



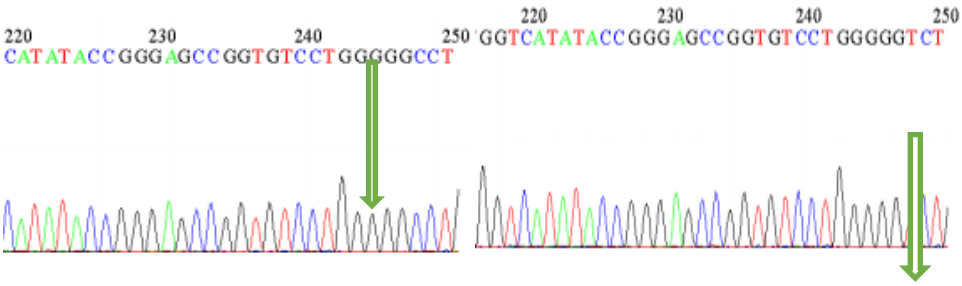
Shape 2: amplified PCR products

DNA sequencing technique:

DNA sequencing technique was applied to identify the genotypes. Two mutations were found leading produced a change in the nitrogen base of cytosine (C) to thymine (T). The first was in exon IV (g.5305C> T) (shape 3) represented a non-synonymous mutation producing amino acid change of alanine to valine, These results were similar to that reported by Getachew (2010), Piatkowska et al (2011), Yang et al (2011), Hristov et al (2013) Mir et al (2014) and Zaglool et al (2016). The second mutation was located in the intron IV (g.5490C> T) (shape 4). This substitution did not result in any change in the amino acid chain of the resulting protein. Each mutation resulted in three genotypes, wild genotype CC and hybrid CT and TT.



Shape 3: mutation (g.5305C> T) loci in exon IV



Shape 4: mutation (g.5490C> T) loci in intron IV

As for the first mutation (g.5305C> T) the genotypes frequencies of the three genotypes (CC, CT and TT) were 40.82, 40.82 and 18.36 respectively with a significant differences for both genotypes (CC and CT) than genotype TT. While the allelic frequency for the both alleles (C and T) was 0.61 and 0.39 respectively and the

wild allele showed a significant superiority on allele T. and also there were a significant differences between the distributed percentages (P<0.01) as it shown in table (2).

Table 2: the number and frequencies of genotypes and alleles for mutation (g.5305C> T)

Genotype	Number	Percentage %
CC	20	40.82
CT	20	40.82
TT	9	18.36
Total	49	100%
Kai square (χ^2)	-----	6.588**
Allele	Frequency	
C	0.61	
T	0.39	
.(P<0.01) **		

While for the second mutation (g.5490C> T), the genotypes frequencies of the genotypes (CC, CT and TT) were 36.73, 44.90 and 18.37 respectively and there was a significant difference for the hybrid genotype CT compared with the other genotypes. The alleles frequencies for C and T were 0.59 and 0.41 respectively with the superiority of wild allele C, and also there were a significant differences between the distributed percentages (P<0.05) as it seen in table (3).

Table 3: The number and frequencies of genotypes and alleles for mutation (g.5490C> T)

Genotype	Number	Percentage %
CC	18	40.82
CT	22	40.82
TT	9	18.36
Total	49	100%
Kai square (χ^2)	-----	3.815*
Allele	Frequency	
C	0.59	
T	0.41	
.(P<0.05) *		

The relationship of β-lactoglobulin gene polymorphism with milk components:

As for the relationship of β-lactoglobulingene polymorphism with milk components, the first mutation (g.5305C> T) didn't show any significant differences in all of the genotypes on milk components (Table 4). While the second mutation (g.5490C> T) revealed a significant difference for individuals with hybrid genotype CT than other individuals with different genotypes in fat percentage, and there weren't any significant differences for the genotypes in any other components as it shown in table (4).

Table 4: Statistical analysis of the mean values of milk components of (g.5305C> T and g.5490C> T) genotypes

Genotype	Components Mean ± SE			
g.5305C> T	Total protein (gm)	β-LG protein (gm)	Fat %	SNF
CC	3.07 ± 0.04a	0.35 ± 0.005a	4.79 ± 0.36a	8.04 ± 0.14a
CT	3.16 ± 0.06a	0.36 ± 0.007a	3.93 ± 0.48a	8.36 ± 0.18a
TT	3.14 ± 0.08a	0.35 ± 0.009a	4.58 ± 0.55a	8.08 ± 0.15a
Significant difference	N.S	N.S	N.S	N.S

g.5490C> T	Total protein (gm)	β-LG protein (gm)	Fat %	SNF
CC	3.08 ± 0.04a	0.35 ± 0.005a	4.83 ± 0.38a	8.05 ± 0.15a
CT	3.15 ± 0.06a	0.36 ± 0.007a	3.94 ± 0.45b	8.34 ± 0.17a
TT	3.14 ± 0.08a	0.35 ± 0.009a	4.58 ± 0.55a	8.08 ± 0.15a
Significant difference	N.S	N.S	*	N.S

(* = P<0.05) N.S= no significant difference

The relationship of β-lactoglobulin gene polymorphism with milkyield:

The first mutation (g.5305C> T) showed a significant difference for the individuals with the wild genotype CC in both of the weekly mean milk yield and total milk yield with (10.65±0.53 and 53.26±2.67 Kg) respectively than other individuals with other genotypes as it shown table (5), and this result corresponds with the result in Zaglool et al (2016) study, it also referred to the superiority of the individuals with wild genotype in total milk yield with 11461 ± 494 Kg. As for the second mutation (g.5490C> T) the wild genotype CC also revealed a significant difference in both of weekly milk yield and total milk yield with (10.65±0.58 and 53.29±2.91) respectively as it seen in table (5).

Table 5: relationship of β-lactoglobulin gene polymorphism with weekly mean milk yield and total milk yield

Genotype g.5305C> T	Mean ± SE	
	Weekly mean milk yield (Kg)	Total milk yield (Kg)
CC	10.65 ± 0.53a	53.26 ± 2.67a
CT	10.05 ± 0.51a	50.29 ± 2.58a
TT	9.21 ± 0.65b	46.08 ± 3.29b
Significant difference	*	*
g.5490C> T	Weekly mean milk yield (Kg)	Total milk yield (Kg)
CC	10.65 ± 0.58a	53.29 ± 2.91a
CT	10.10 ± 0.47ab	50.50 ± 2.38ab
TT	9.21 ± 0.65b	46.08 ± 3.29b
Significant difference	*	*

* = P<0.05

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