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ASSOCIATION BETWEEN BTN1A1 GENE POLYMORPHISM AND MILK PRODUCTION AND ITS CONTENTS IN HOLSTEIN COWS

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Abstract

This study was conducted in Al-Salam station for Dairy cattle/private sector, for the period from 1-11-2016 to 1-11-2017, to determine the association between *BTN1A1* gene polymorphism and productive performance traits in 50 Holstein cows. The results of *BTN1A1* gene analysis showed a highly significant Different (P<0.01) between genotypes of *BTN1A1* gene's genotypes AA, AB the percentage were 72.00, 28.00% respectively. Results showed that total milk production and lactation period in Holstein cows was highly significant (P<0.01) affected by *BTN1A1* gene polymorphism and for cows with AA genotype. The period from birth to the peak of lactation trait, the results showed that there was a highly significant different (P<0.01) for this trait affected with *BTN1A1* gene polymorphism and for cows with AB genotype, while the length of peak lactation was highly significant affected (P<0.01) with *BTN1A1* gene polymorphism and for AA genotype, the fat and lactose percentage was significantly affected (P<0.05) by *BTN1A1* gene's polymorphism, the highest percentage reached in the cow's milk which had genotype AB, while protein and non-fat solids and milk density were not significantly affected with the *BTN1A1* gene polymorphism. It was possible to conclude from this study the possibility of *BTN1A1* gen's polymorphism in the development of genetic improvement strategies and breeding programs that achieved the best productive performance in dairy cows.

***Keywords*:** Holstein cattle, productive performance, *BTN1A1* gene polymorphism

Introduction

Due to increasing interest in animal's products for their highly nutritional and biological value, milk is also consider one of that important animal's products and good source for human nutrition (Mourad *et al*., 2014), the aim of dairy industry has been to identify an efficient and economical way of increasing milk production and its constituents without increasing the size of dairy herd, improving productivity and quality of milk and contents (Ratwan *et al*., 2017).

In all the world , breeding strategies for dairy animals must concentrate not only for increasing milk yield but also on milk contents and its quality, such as fat percent and fat yield in milk, it has economical aspect associated with it as in organized sectors and at dairy cooperative (Kumar *et al*., 2016).

Since the improvement of cattle linkage maps, therefore many researchers have managed to identify quantitative trait were controlled by a number of genetic loci known as quantitative sites QTL (Quantitative trail loci) which relied on statistical methods and focused on the selection of individuals with a better phenotypic structure, which achieved significant gains in the field of genetic improvement, but scientific acceleration and the availability of large information on the work of the genome has enabled to set a selection programs more accurate and less time-consuming and cost (Ashwell *et al*., 2004), At the beginning, investigations focused on identifying QTL affecting milk production traits, however traditional selection ways have been effectual in development milk production in dairies without the DNA marker information it is possible to predict the phenotype variation of the traits by identified these loci and associated genetic markers with it to be improved early and to build the selection programs on them, These markers are functional mutations in the genes affecting traits (Singh *et al*., 2014).

Butyrophilin (*BTN1A1*) is a QTL candidate gene having role in milk production and composition (fat) in dairy animals, and its protein was identified as a main component of the milk fat globule and important in secreting and stabilizing of milk fat droplets (Franke *et al*., 1981), the butyrophilins (*BTN*) belong to the immunoglobulin family of transmembran proteins(Yardibi etal. 2013), the bovine butyrophilin gene (*BTN*) was mapped to the long arm of chromosome 23 consists of 8 exon and 7 intron and 893 bp long gene fragment (Brunner *et al*., 1996; Taylor *et al*., 1996), milk fat is a major contributor to energy density of whole milk and affects the physical and manufacturing properties of various dairy products (Ratwan *et al*., 2017).

The genetic variations in bovine *BTN1A1* gene has been exploited as a marker for QTL controlling milk yield and fat percentage, and affects economically important trait in dairy animal because it is specifically expressed in lactating mammary tissue and gene product *BTN1A1* may function in secretion of milk lipid (Zegeye, 2003) and disease resistance (Jonchere *et al*., 2010; Moyes *et al*., 2011).

The objective of this study was knowledge the association of *BTN1A1* polymorphism with many productive traits in Holstein cows for selection purpose.

Materials and Methods

This study was conducted in Al-Salam station for Dairy cattle /private sector (Al-Latifia district 25 km southern Baghdad), from 1-11-2016 to 1-11-2017, on 50 Holstein cows and their, for DNA extraction and *BTN1A1* gene analysis in were carried out in Al-Takadom scientific lab to determine the association between polymorphisms of *BTN1A1* gene and it's rate and allelic frequency with milk production and lactation season length and period from birth to the peak of lactation and length of peak lactation as well as milk content for the lactation season 2016- 2017.

Blood collected by a medical syringe from the jugular vein in a 15 ml sterile polypropylene tubes containing 0.5 ml of EDTA (0.5 M) as an anticoagulant by the phenol chloroform extraction by the veterinarian at the station, The blood samples were then transferred by a cool box then

stored in freezing at -20 ºC temperature till transferred to the lab to extracting DNA, the DNA samples were checked for their quality, purity and concentration, the quality of the genomic DNA was checked by using agarose gel electrophoresis, DNA samples of good quality, purity and concentration were used for further analysis, the polymerase chain reaction (PCR) technique for *BTN1A1* typing is based upon the extensive polymorphism that is present an 893 region of exon 8 of the butyrophilin gene was amplified by using primers (Sadr *et al*., 2008):

F: TCCCGAGAATGGGTTCTG

R: ACTGCCTGAGTTCACCTCA

After the polymerase reaction was completed, the polymorphism of *BTN1A1* gene were identified in blood samples from the cows after proceed the cutting to the required piece of gene (893 bp) by restriction enzyme *HeaIII* from Haemophilus aegyptius bacteria. The digestion with *HaeIII* revealed, *HaeIII* restriction site were found in the A allele as 371, 231, 185, 83 and 32 bp fragment. In B allele, 371 were replaced with 338, 185, 83 and 32 bp. This restriction enzyme was obtained by American Promega Company, the concentration of enzyme was 2500 U, 10u-l µ.

The data was analyzed by used Statistical Analysis System (SAS, 2012) to study the polymorphism of *BTN1A1* gene according the mathematical model, significant differences was compared by used least square means method.

$$Yijk = \mu + Gi + OJ + eijk$$

Yijkl: observed value K which belong to phenotype i and month of birth j , µ: general mean , Gi: effect of *BTN1A1* polymorphism (AA,AB), Oj: effect of month of birth (April, may ,June), eijk: Random error which distributed normally with mean= 0 and variation σ2e. Chi-square- $\chi 2$ test were used to compare between the percentages of polymorphisms.

Results and Discussion

BTN1A1 gene was extracted with polymerase chain reaction (PCR) technique by used PCR kit and primers and total DNA samples, in a final volume of 5 µl, the restriction fragments were resolved on 1.2% agarose gel electrophoresis at 100 volt for 70 minutes in 1×TBE buffer and documented through photography using a gel documentation system to ensure from DNA extraction succeed and got 893bp of required piece as the figure no.1. 1000 bp DNA ladder was used to estimate the size of the fragments.

The polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP) technique for *BTN1A1* and restriction enzyme *HeaIII* to identified *BTN1A1*polymorphism according to the method that mentioned in material and methods, the restriction fragments were resolved on 3% agarose gel electrophoresis at 100 volt for 70 minutes in 1×TBE buffer and documented through photography using a gel documentation system to ensure from DNA extraction succeed and got 893 bp of required piece, the analysis showed tow allelic: allelic AA (371, 231, 185, 83 and 32)bp, allelic AB (371, 338 , 185, 83 and 32)bp, as the figure no.2.

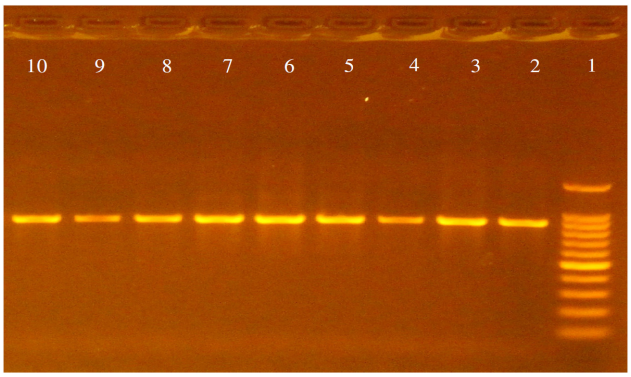

Fig 1: *BTN1A1* gene extracted by Polymerase chain reaction method, column no.1 represented DNA Ladder 1000bp; column no.2-10 represented *BTN1A1* gene (893pb exon8) piece amplified with Polymerase chain reaction method

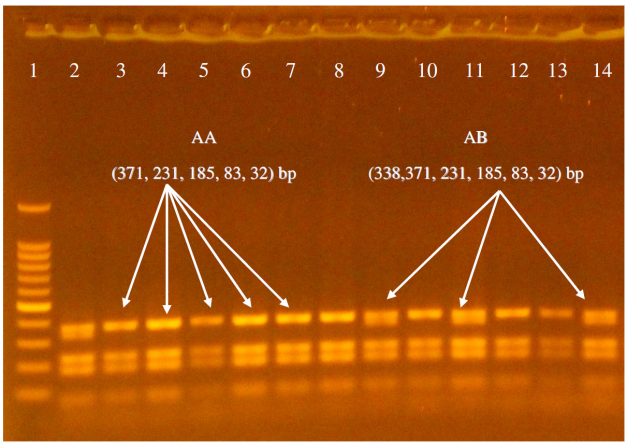

Fig 2: *BTN1A1* gen tow genotypes identified as the size of bands, column no.1 represented DNA Ladder 1000bp,column no.3,4,5,6,7,8,10,12,13 represented AA allelic (371, 231, 185, 83, 32)bp, column no. 2,9,11,14 represented AB allelic (338, 371, 185, 83, 32)bp

Table (1) showed the number and percentage of *BTN1A1* gene polymorphism, there were a highly significant different (*P<0.01*) between distribution ratio of *BTN1A1* gene polymorphism which reached to 72.00, 28,00 % for AA,AB respectively, there was a common for genotype AA if compared with AB genotype, these results are proof that *BTN1A1* gene primer we used in this study was really exist in the genome of the Holstein cattle, the results of the previous studies indicated that there are highly significant differences (*P<0.01*) between distribution ratio of *BTN1A1* gene polymorphism (Ratwan *et al*., 2017; Yardibi *et al*., 2013; Sadr *et al*., 2008; Muszyńska *et al*., 2010).

The prevalence of allelic AA and Scarcity of allelic AB in this study maybe due to the adapted of first allelic to the environmental conditions that cows adapted in their original country and their association with the higher production, which made them within the selection programs, or may be due to the adapted cows to the environmental condition in central Iraq that Holstein cattle lived from high temperatures for most months of the year and scarcity of rainfall and deficiency in nutrition or because animals depended roughages on the feed therefore perhaps natural selection play a role against the AB allelic.

Association of *BTN1A1* gene polymorphism with milk production and lactation length

The results of this study showed that there were a highly variation ($P<0.01$) in total milk production and length of lactation season with different *BTN1A1* genotypes, as the cows with AA genotype achieved maximum total milk production mean (1992.24 ± 48.63 Kg) and (195.35 ± 4.61 day) respectively compared with cows with AB genotype (1486.07 ± 35.29 Kg) and (158.74 ± 1.82 day) respectively (Table 2), this may be due to the cows with AA genotype were excellent more than AB genotype's cows to the commonness of this allele and perhaps this supports survival theory for the better or may be to the association between *BTN1A1* gene and milk production (Heid and Keenan, 2005), in other previous study founded a significant different in milk production (Muszyńska *et al*., 2010; Yang *et al*., 2015; Ashwell *et al*., 2004; Komisarek and Dorynek, 2003; Rychtářová *et al*., 2014).

Association of *BTN1A1* gene polymorphism with period from birth to the peak of milk production and length of peak production

As reported in table (3) that the period from birth to peak of milk production was highly significantly (*P<0.01*) affected with genotype of *BTN1A1* polymorphism as it reached (47.72 ± 0.80 day) for cows with AB genotype, also the length of peak production affected highly significantly (*P<0.01*) for cows with AA genotype and reached (59.21± 1.85 day).

This may be attributed to the role of *BTN1A1* gene and its association with immunoglobulin's to increase the immunity of the body against pathogens, including the bacterial causes of mastitis, which affects the mammary gland in cows and therefore reflected negatively on the production of milk during the milking season and thus will be affected by the duration of birth to reach the peak production and the peak production in these cows (Ratwan *et al*., 2017), and may be due to the cows with AA genotypes gave a maximum milk production than cows with AB genotype so these cows will be more persistency because younger cows are more persistent than bigger cows (Rehak *et al*., 2012).

Association of *BTN1A1* gene polymorphism with milk content

It is cleared from the table (4) that fat and lactose percentage was significantly ($P<0.05$) affected by polymorphism of *BTN1A1* gene allelic, it was reached (3.87 ± 0.41%) and (4.83 ± 0.09%) respectively in cows with AB genotype, while it was at minimum rat in genotypes 3.66 ± 0.27 & 4.38 ± 0.11 % respectively, the increase of fat percentage in AA genotype came together with decrease in total milk production in same cows, increasing of milk produced is usually accompanied by a decrease in fat content (Bushara, 2013), and may be due to that BTN1A1 responsible for secretion of milk lipid droplets during lactation and considered to be involved in the milk fat secretion (Ogg *et al*., 2004), in other previous study founded a significant different in milk fat percentage (Muszyńska *et al*., 2010; Yang *et al*., 2015; Ashwell *et al*., 2004; Komisarek and Dorynek, 2003; Rychtářová et al., 2014).

Table 1: Number and percentage for *BTN1A1* gene polymorphism

Polymorphism	Number	Percentage %
AA	36	72.00
AB	14	28.00
Total	50	100 %
Chi-square- χ^2 value	----	63.13**
Allele	Frequency	
A	0.86	
B	0.14	
(*P<0.01*) **		

Table 2: Association between *BTN1A1* gene polymorphism with milk production and lactation season length

Polymorphism	Cows number	Mean ± Standard error	
		Total milk production (Kg)	Lactation season length (Day)
AA	36	1992.24 ± 48.63 a	195.35 ± 4.61 a
AB	14	1486.07 ± 35.29 b	158.74 ± 1.82 b
Total	50	**	**
Significantly			
The means with different letters within the same column are significantly between them (*P<0.01*) **			

Table 3: Association between *BTN1A1* gene polymorphism with period from birth to the peak of milk production and length of peak production

Polymorphism	Cows number	Mean ± Standard error	
		Period from birth to the peak of milk production (day)	Length of peak production (day)
AA	36	35.96 ± 1.66 b	59.21 ± 1.85 a
AB	14	47.72 ± 0.80 a	45.53 ± 0.78 b
Total	50	**	**
Significantly			
The means with different letters within the same column are significantly between them (*P<0.01*) **			

Table 4: Association between *BTN1A1* gene polymorphism with milk content

Polymorphism	Cows number (Samples)	Mean ± Standard error				
		Fat (%)	Lactose (%)	Protein (%)	Non-fat solids (%)	Milk density g/cm^3
AA	36 (108)	3.66 ± 0.27 b	4.38 ± 0.11 b	3.26 ± 0.07	8.41 ± 0.12	1.030 ± 0.02
AB	14 (42)	3.87 ± 0.41 a	4.83 ± 0.09 a	3.12 ± 0.05	8.52 ± 0.14	1.034 ± 0.02
Total	50 (150)	*	*	N.S	N.S	N.S
Significantly						
The means with different letters within the same column are significantly between them ($P<0.05$) *, N.S= no significant						

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