



ASSOCIATION BETWEEN *BTN1A1* GENE POLYMORPHISM AND SOME REPRODUCTIVE EFFICIENCY INDICATOR AND HEAT TOLERANCE 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 reproductive efficiency indicator and heat tolerance 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 services per conception and days open was significantly ($P<0.05$) affected by polymorphism of *BTN1A1* gene and for cows with AA genotype, there was also a significant difference ($P<0.05$) between the genotypes of *BTN1A1* gene for IgG concentration in calves blood who belong to mother's with AA genotypes compared with AB genotype, for the heat coefficient tolerance trait the results showed a significant different ($P<0.05$) with *BTN1A1* polymorphism and for cows with AB genotype in third month of lactation, while there are no significant differences in other months of lactation with different genotypes of *BTN1A1* gene.

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.

Key words: Holstein cattle, reproductive efficiency, heat tolerance, *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, 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 its contents (Ratwan *et al.*, 2017).

In Iraq there has been a deterioration in the animals production sector in general and cattle in particular and the decline in the number of farm animals compared with the population increase in recent years and the infection of animals with infectious diseases lead to decrease in reproductive performance and this leads to increase in veterinary costs and therefore high production costs such as milk production, veterinary costs, premature discard of animals, milk rejection due to antibiotic contamination,

among others (Oltenacu and Broom 2010), because of that 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.*, 2017),

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 trait 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 *et al.*, 2013)., members of the butyrophilin (*BTN*) gene family are attracting increasing attention because they may play multifunctional roles in diverse physiologies, including lactation, selection and regulation of T-cells in the immune system, and modulation of autoimmune disease (Jeong *et al.*, 2009).

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 50 offspring, for DNA extraction and DRB3 gene analysis in were carried out in Al-Takadom scientific lab to determine the association between polymorphisms of DRB3 gene and it's rate and allelic frequency with some reproductive traits (services per conception and days open), immunoglobulin's concentration (IgG) and heat Tolerance coefficient 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 freezer at -20°C temperature till transferred to the lab to extracting DNA, for the calves blood also collected by medical syringe from the jugular vein in a 10 ml tubes, 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 *HaeIII* from Haemophilus aegyptius bacteria. The digestion with *HaeIII* revealed, *HaeIII* restriction site were found in the AA allele as 371, 231, 185, 83 and 32 bp fragments. In AB 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-1 μ.

For the heat tolerance coefficient (HTC) it was calculated according to (16) equation:

$$HTC = 100 - 10 (ART-38.33)$$

As the:

18: fixed.

ART: Average of Rectal Temperature at morning and afternoon.

38.33: normal of rectal temperature (centigrade)

The data was analyzed by used Statistical Analysis System (2012) to study the polymorphism of DRB3 gene according the mathematical model, significant differences was compared by used least square means method.

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

Y_{ijkl} : observed value K which belong to phenotype i and month of birth j, μ : general mean, G_i : effect of *BTN1A1* polymorphism (AA, AB), O_j : effect of month of birth (April, may, June), e_{ijk} : Random error which distributed normally with mean= 0 and variation σ^2_e .

Chi-square- χ^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 μ l, the restriction fragments were resolved on 1.2% agarose gel electrophoresis at 100 volt for 70 minutes in 1 \times 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 fig. 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 \times TBE

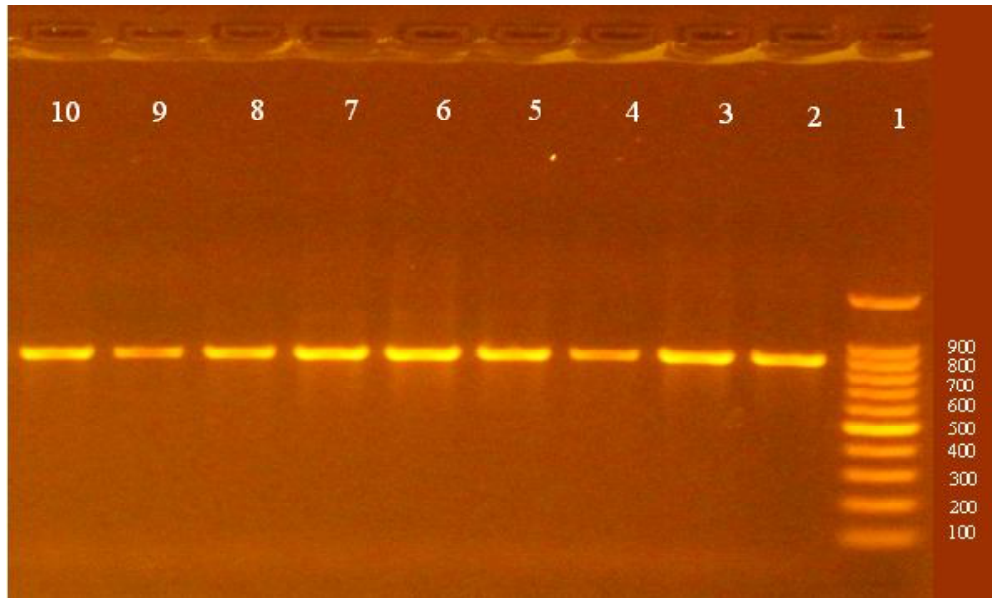


Fig. 1: *BTN1A1* gene extracted by Polymerase chain reaction method, column no.1 represented DNA Ladder 1000bp, column no.2-10 represented *BTN1A1* gene (893pb exon8) piece amplified with Polymerase chain reaction method

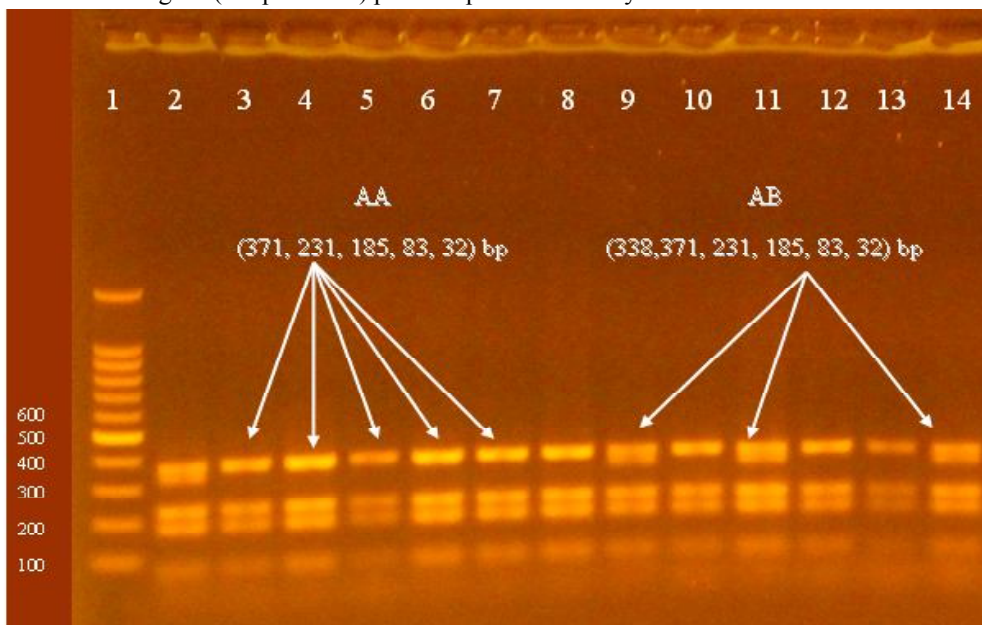


Fig. 2: *BTN1A1* gen tow genotypes identified as the size of bands, column no.1 represented DNA Ladder 1000bp,column no.3,4,5,6,7,8,10,12,13 represented AAallelic (371, 231, 185, 83, 32)bp, column no. 2,9,11,14 represented AB allelic (338, 371, 185, 83, 32)bp

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, homozygotes, allelic AB (371, 338 , 185, 83 and 32) bp heterozygotes with mutant in one sequence (C → T) , as the fig. 2.

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; Brunner *et al.*, 1996); 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 that highly allelic frequency indicate that allele have been supported by selection for dairy production (Sadr *et al.*, 2008, Taylor *et al.*, 1996; Husaini *et al.*, 1990; Komisarek and Dorynek, 2003).

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	
$(P<0.01)$ **		

Association of *BTN1A1* gene polymorphism with services per conception and days open

Table 2 showed the association between *BTN1A1* polymorphism and services per conception and days open, there was a highly different significant ($P<0.01$) in both traits when the genotypes was different, cows with AA genotype needed minimal services per conception (1.55 ± 0.10) and thus less days open (81.59 ± 3.28 day), while the rates of these two traits were 1.86 ± 0.12 & $96.36 \pm$

6.06 day for cows with AB genotype, the reason may be due to the association between *BTN1A1* allelic and immunity throw controlling the phagocytes which play a role in the elimination of pathogenic bacteria affecting the reproductive system and uterus and then the lack of infections in the reproductive system after birth and the lack of the occurrence of polycystic ovary and retained placenta, which in turn will lead to a small number of services for fertilization and then will cows require less days from birth to pollination fertilization (Vandre *et al.*, 2014), these two traits were the most important indicators of reproductive efficiency in dairy cows because they reflect the number of births during lactation season and the physiological and health status of the cow and herd management and attention to the fertilization process and thus the number of births during productive life, which determines the economic return of cattle breeding projects and the possibility of applying the selection programs.

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

Polymorphism	Cows No.	Mean \pm Standard error	
		Services per conception (day)	Days open (day)
AA	36	1.55 ± 0.10 a	81.59 ± 3.28 a
AB	14	1.86 ± 0.12 b	96.36 ± 6.02 b
Total	50	**	**
Significance			
The means with different letters within the same column are significantly between them			
$(P<0.01)$ **			

Association of *BTN1A1* gene polymorphism with Immunoglobulin's concentration

Table 3 showed that there was a different significance ($P<0.05$) between the genotypes derived from the analysis of *BTN1A1* gene for cows for IgG concentration in calves blood, as the concentration of IgG in this study reached 45.74 ± 1.65 g/L calves blood derived from cows with AA genotype, while the concentration of IgG Decreased in blood of calves Belongs to cow with AB genotype reached 39.07 ± 0.83 g/L calves blood, this results may be due to that AA allelic are the most common and then animals that possess these genotypes are more immune and resistant to pathological and bacterial infections compared to AB genotype and this may lead to increase in the number of immunoglobulin's in calves, or may be to the association between immunoglobulin's and *BTN1A1* genes allelic because it was a member of immunoglobulin's superfamily because most family members structurally comprise two exoplasmic Ig folds (Ogg *et al.*, 2004), and suggesting that the structural

Table 3: Association between *BTN1A1* gene polymorphism and Immunoglobulin's concentration

Polymorphism	Cows number (samples)	Mean ± Standard error
		Immunoglobulin's Concentration (g / L blood) calves
AA	36(72)	45.74 ± 1.65 a
AB	14(28)	39.07 ± 0.83 b
Total	50(100)	*
Significance		
The means with different letters within the same column are significantly between them (P<0.05) *		

Table 4: Association between *BTN1A1* gene polymorphism and heat tolerance coefficient

Polymorphism	Cows number (samples)	Mean ± Standard error for heat tolerance coefficient		
		1 st month of lactation season	2 nd month of lactation season	3 rd month of lactation season
AA	36(72)	101.96 ± 0.77	102.18 ± 0.59	101.64 ± 0.35 b
AB	14(28)	102.68 ± 1.52	102.42 ± 0.83	104.57 ± 0.91 a
Total	50(100)	N.S	N.S	*
Significance				
The means with different letters within the same column are significantly between them (P<0.05) *, NS= No significant				

domains of *BTN* proteins may have both universal and tissue-specific functions. *BTN1A1* is structurally related to proteins of the immune system, which may suggest its possible immunologic function (Vernet *et al.*, 1993), a milk protein and common dietary antigen, and a similar Ig fold in myelin oligodendrocyte glycoprotein may modulate autoimmune responses (Guggenmos *et al.*, 2004).

Association of *BTN1A1* gene polymorphism with Heat tolerance coefficient

Observed from table 4 a different significant (P<0.05) between *BTN1A1* gene genotypes and heat tolerance coefficient, It was found that there was a significant difference between the genotypes of the *BTN1A1* allelic in the heat tolerance coefficient and for cows with AB genotype in the third months of the lactation season, the heat tolerance coefficient was 104.57 ± 0.91 and reached 101.64 ± 0.35 for cows with AA genotype, while there was no effected of *BTN1A1* gene genotypes of the heat tolerance coefficient of cows during the first and second months of the lactation season, this may be due to the fact that disease is a stressful factor for living organisms, or maybe due to the association between *BTN1A1* allelic and immunoglobulin's because of the relationship of butyrophilin to proteins of the immune system and its

possible immunologic function (Taylor *et al.*, 1996).

Conclusion

We can conclude from this study that cows with AA genotype had better services per conception and days open, and gave the best levels of immunoglobulin's in their calves blood when compared it with AB genotype, for the heat tolerance coefficient we found that difference in *BTN1A1* gene genotype was significant during the third month of the lactation season.

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