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RELATIONSHIP OF THE DQA1 GEN POLYMORPHISM WITH PRODUCTIVE PERFORMANCE IN HOLSTEIN CATTLE

H.K. Al-Waith[1], N.N. AL-Anbari[1] and T. R. Mohamed[2]

[1]Department of Animal Production, College of Agriculture, University of Baghdad, Iraq.
[2]Department of Animal Production, College of Agriculture, University of Al-Anbar, Iraq.

Abstract

The present 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 relationship between genotype for the DQA1 gene and productive performance traits in Holstein cattle. Results showed that total milk production in Holstein cows was highly significant ($P<0.01$) affected by genotypes of DQA1 gene and for cows with C genotype (1699.65 ± 65.78 kg), while the lactation period was affected significantly ($P<0.05$) with different genotypes of DQA1 gene. There was no significant different in period from birth to the peak of lactation, while the length of peak production was significantly affected ($P<0.05$) with different genotypes of DQA1 gene and the highest average for C & B genotype 50.26 ± 1.78, 48.14 ± 1.73 day respectively, the fat percentage as well as milk density was significantly affected ($P<0.05$) by different genotypes of the DQA1 gene's genotypes, the highest percentage reached in the cow's milk which had genotype B 5.82 ± 1.31 % fat and best milk density in A & C genotypes 31.82 ± 0.67 g/cm^3 and 30.39 ± 0.69 g/cm^3 respectively, while lactose, protein and non-fat solids were not significantly affected with the different of DQA1 gene genotypes.

It was possible to conclude from this study the possibility of DQA1 gen's genotypes in the development of genetic improvement strategies and breeding programs that achieved the best productive performance in dairy cows.

Key words: Holstein cattle, productive performance, DQA1 gene polymorphism

Introduction

The livestock production sector is an important in the economies of countries including Iraq because of its role in food security, which contributing about 40% of the value of agricultural products [Singh, *et al.* (2014)], 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 [Vandre, *et al.* (2014)], 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 The costs associated to these types of problems, are mainly represented by the decrease in milk production, veterinary costs, premature discard of animals, milk rejection due to antibiotic contamination, among others [Rothschild, *et al.* (2000)], scientific acceleration and the availability of the large information about the genome work has made it possible to put a selection program more specific and less time and coast, and for economic characteristics were controlled by a number of genetic loci known as quantitative sites (QTL-Quantitative trail loci) 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 and resistance to infectious diseases [Vandre, *et al.* (2014)], there were more evidences about major histocompatibility complex polymorphisms such as DQA1 gene in many pervious study [Park, *et al.* (2004); Takeshima, *et al.* (2007)]. DQA1 gene was one of the major histocompatibility complex class II gene which belong to Immunoglobulin super family and it's consider a glycoproteins and this gene was one of the most of major histocompatibility complex gene polymorphism was located on the short arm of chromosome 23 in cattle

[Kulaj, *et al.* (2015)], DQA1 gene allelic frequently showed more unify to the most suitable alleles in families which belong to accident, and major histocompatibility complex allelic founded related together in different loci, there were more active technique to found the polymorphism in animals, so this study aimed the association of DQA1 polymorphism with many productive traits in Holstein cows for selection purpose.

Materials & methods

This study was conducted in Al-Salam station for Dairy cattle / private sector (Al-Latifia district 25 km southern Baghdad), for the period from 1-11-2016 to 1-11-2017, on 34 Holstein cows and their offspring, for DNA extraction and DQA1 gene analysis in were carried out in Al-Takadom scientific lab to determine the association between polymorphisms of DQA1 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 freezer at -20^{0}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 DQA1 typing is based upon the extensive polymorphism that is present in Exon 2 of the BoLA-DQA1 gene under consideration depending on the size of the pieces and type of primers used, the 373bp fragment consisting [Kulaj, *et al.* (2015)] present in the genomic DNA of cattle was amplified by employing the corresponding primer pairs (forward and reverse), The details of the primer sequences are as follows:

F: TCAATTTCTTCTTTCACTTTGCT

R: GGTTTGAAGGGGTAGATTAATAAA

After the polymerase reaction was completed, the polymorphism of DQA1 gene were identified in blood samples from the cows by used sequence technique through the program Blast software from NCBI (National Center For Biotechnology Information) and information program. And the genotype's of DQA1 identified by the different between the sequences in nitrogen bases for the studied cows and compared it with the wild sequence of the gene in NCBI.

The data was analyzied by used Statistical Analysis System [SAS. (2012)] to study the polymorphism of DQA1 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

Gi: effect of DQA1 polymorphism (A, B, C)

Oj: effect of month of birth (April, may, June)

e_{ijk}: Random error which distributed normally with mean= 0 and variation $\sigma^2 e$.

Chi-square- χ^2 test were used to compare between the percentages of polymorphisms.

Results and Discussions

DQA1 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, and resolved on 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 373 bp of required piece as the figure no.1. 1000 bp DNA ladder was used to estimate the size of the fragments.

The sequencing method used to identified DQA1 polymorphism according to the method that mentioned in material and methods, by the different between the

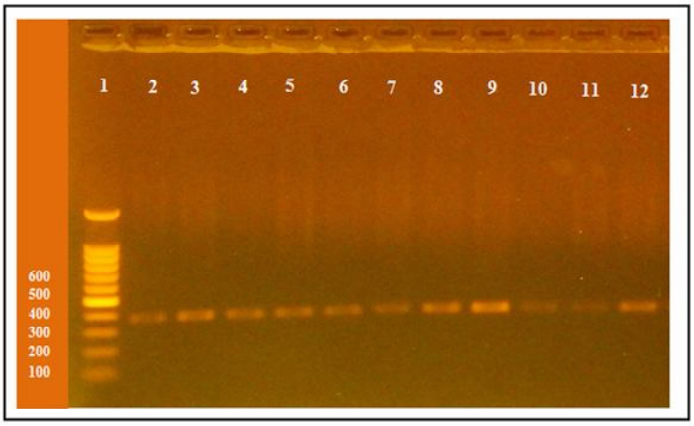

Fig.1: DQA1 gene extracted by Polymerase chain reaction method, column no.1 represented DNA Ladder 1000bp, column no.2-12 represented DQA1 gene piece amplified with Polymerase chain reaction method exon 2

sequences in nitrogen bases for the studied cows and compared it with the wild sequence of the gene in NCBI, the result of sequencing showed three allelic as fig. 2.

Association of DQA1 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 with different DQA1 genotypes, as the cows with C genotype achieved maximum total milk production mean (1699.65 ± 65.78 Kg) then the cows with A genotype (1586.19 ± 63.83 Kg) while the cows with B genotype came in minimum total milk production mean 1506.62 ± 82.05 Kg (table 1), this may be due to the cows with C or A genotype were excellent more than B genotypes cows to the commonness of these two allelic and perhaps this supports survival theory for the better or may be to the association between DQA1 gene and the hormones which responsible on milk production, or on the growth and development of the mammary gland and the ducts system

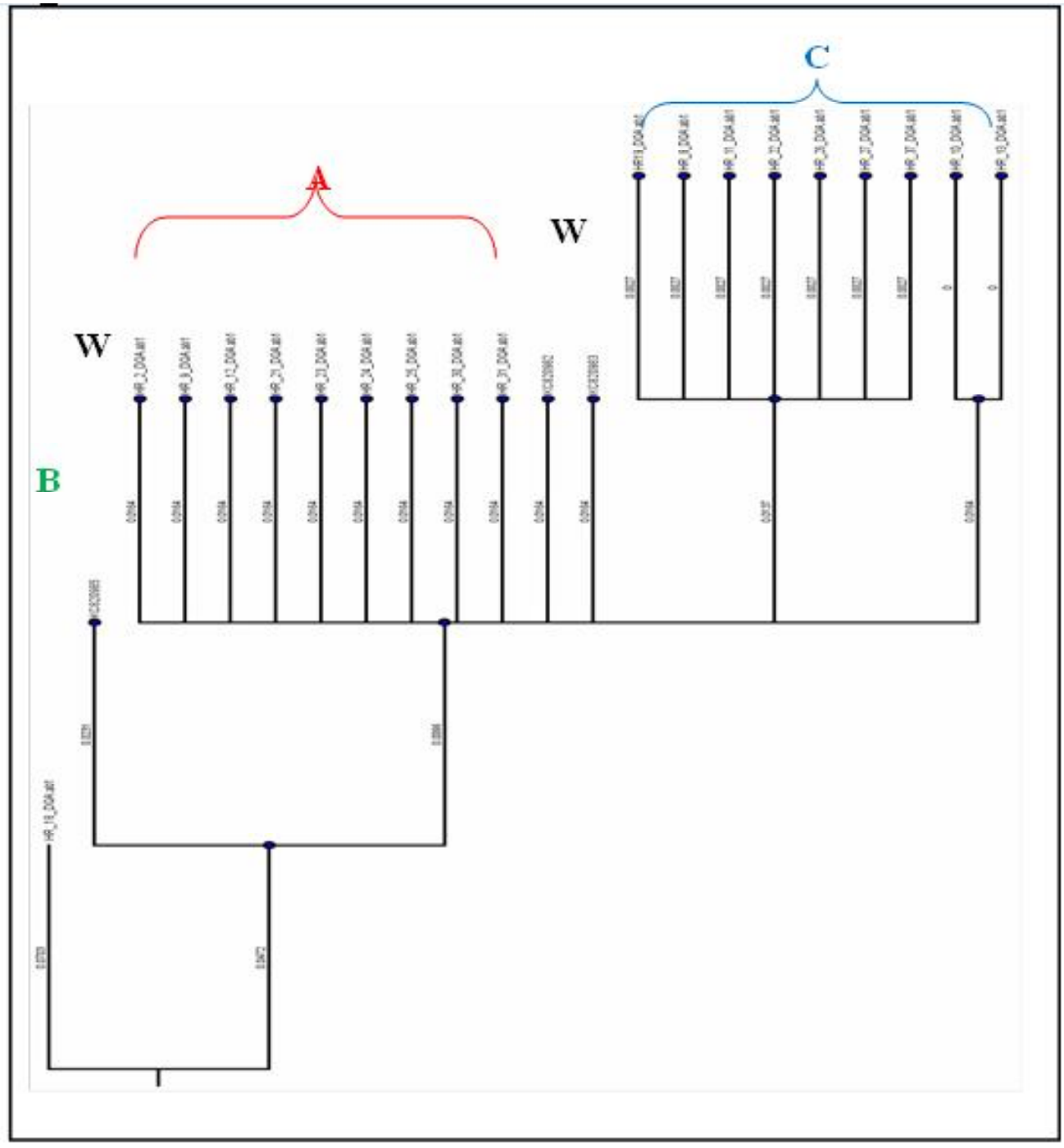

Fig. 2: Tree shape for three allelic of DQA1 gene

in the udder that leading to improvement and increase milk production [Fitzpatrick, *et al.* (1992)] , and the results showed a different significant (P<0.05) between genotypes of DQA1 gene in lactation season length trait, as it reached the maximum rate in cows with C genotype 175.88 ± 3.85 day, and it was in minimum rate in cows with A genotype 161.59 ± 3.74 day, while compromise in cows with B genotype 168.22 ± 10.67 day (table 1).

Association of DQA1 gene polymorphism with period from birth to the peak of lactation and length of lactation period

As reported in table (2) that the period from birth to peak of milk production was not significantly affected with

Table 1: Association between DQA1 gene polymorphism with milk production and lactation season length

Polymorphism	Cows number	Mean ± Standard error	
		Total milk production (Kg)	Lactation season length (Day)
A	16	1586.19 ± 63.83 b	161.59 ± 3.74 b
B	2	1506.62 ± 82.05 c	168.22 ± 10.67 ab
C	16	1699.65 ± 65.78 a	175.88 ± 3.85 a
Total	34	**	*
Significantly			
The means with different letters within the same column are significantly between them (P<0.01) **, * (P<0.05)			

Table 2: Association between DQA1 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)
A	16	44.08 ± 1.75	48.14 ± 1.73 a
B	2	41.64 ± 5.00	41.98 ± 4.94 b
C	16	40.92 ± 1.81	50.26 ± 1.78 a
Total	34	N.S	*
Significantly			
The means with different letters within the same column are significantly between them (P<0.01) **, * (P<0.05)			

genotype of DQA1 allelic although there are some mathematical differences in A genotype as it reached 44.08 ± 1.75 day , while it was in both B & C genotype was 41.46 ± 5.00 and 40.92 ± 1.81 day respectively, the variation in the length of the peak of production was significantly (P<005) in cows with C genotype then A genotype maximum rats and reached 50.26 ± 1.78, 48.14 ± 1.73 respectively, while it was less from that rats in cows with B genotype (41.98 ± 4.94 day).

This may be attributed to the role of major histocompatibility complex gene 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 [Chakraborty, *et al.* (2015)], and may be due to the cows with C and A genotypes gave a maximum milk production than cows with B genotype so these cows will be more persistency because younger cows are more persistent than bigger cows [Rehak, *et al.* (2012)].

Association of DQA1 gene polymorphism with milk content

It is cleared from the table (3) that fat percentage was significantly (P<0.05) affected by polymorphism of DQA1 gene allelic, it was reached the maximum rate (5.82 ± 1.31 %) in cows with B genotype, while it was at minimum rat in both genotypes A&C 2.64 ± 0.46 & 3.51 ± 0.47 % respectively, the increase of fat percentage in C 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, or may be to the association between major histocompatibility complex genes and milk fat percentage [Hines, *et al.* (1986)].

There was a significant different (P<0.05) in milk density with different of DQA1 genotypes, as it reached a maximum rate 31.82 ± 0.67 g/cm^3 for A

Table 3: Association between DQA1 gene polymorphism with milk content

Polymorphism	Cows number	Mean ± Standard error				
		Fat (%))	Lactose (%)	Protein (%)	Non-fat solids (%)	Milk densityg/cm^3
A	16 (48)	2.64 ± 0.46 b	4.69 ± 0.06	3.11 ± 0.04	8.64 ± 0.10	31.82 ± 0.67 a
B	2 (6)	5.82 ± 1.31 a	4.59 ± 0.16	3.06 ± 0.11	8.51 ± 0.29	27.83 ± 1.92 b
C	16 (48)	3.51 ± 0.47 b	4.64 ± 0.06	3.07 ± 0.04	8.54 ± 0.11	30.39 ± 0.69 a
Total	34 (102)	*	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						

genotype and a minimum rate 27.83 ± 1.92 g/cm^3 for B genotype, this may be due to that cows with A genotype have a lower fat percentage than cows with B genotype, since there is an inverse relationship between fat percentage and the specific gravity of the milk, that is increasing the fat percentage in the milk leads to a reduction in the specific gravity of the milk, and then increase milk density [Bushara, (2013)].

There was no significant different found with the different of DQA1 gen's genotype for lactose, protein, non-fat solids and milk density in cow's milk with A, B & C genotype respectively (table3).

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