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ASSOCIATION BETWEEN DQA1 GENE POLYMORPHISM AND REPRODUCTIVE, IMMUNITY PERFORMANCE AND HEAT TOLERANCE IN HOLSTEIN CATTLE

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Abstract

This investigated 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 DQA1 gene's genotypes with some reproductive traits, immunity and heat tolerance coefficient in Holstein cows. The results DQA1 gene analysis showed a highly significant Different (P<0.01) between genotypes of DQA1 gene's genotypes A, B& C, the percentage were 47.06, 5.88 and 47.06% respectively. The results of the current study showed that services per conception and days open was significantly affected (P<0.05) by different genotypes of the DQA1 gene for the cows with C genotype. There was also a significant difference (P<0.05) between the genotypes of DQA1 gene for IgG concentration in calves blood who belong to mother's with A& C genotypes compared with B genotype. The concentration of IgG in this study 44.73 ± 2.68&43.55 ± 1.82 g/L. blood which belong to cows with A &C genotypes respectively and it was higher than claves which belong to cows with C genotype (37.09 ± 1.77 g / L. blood). For the heat tolerance coefficient it was found that there was a significant (P<0.05) difference between the genotypes of the DQA1 gene in this trait and for the cows with B &C genotype in the second and third months of the lactation season, while there was no effect of DQA1 gene genotypes of the heat tolerance coefficient during the first month of the lactation season.

It was possible to conclude from this study the possibility of DQA1 gene's genotypes in the development of genetic improvement strategies and in breeding programs in dairy cows.

Key words : Holstein cows, reproductive, immunity performance, heat tolerance, 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 (FAO, 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 (The Ministry of Planning, 2008) 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 (Oltenacu and Broom, 2010). Therefore, the researchers resort to perform the traditional genetic improvement of the agricultural animals in general and cattle especially, 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, 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 disease (Singh *et al*., 2014), DQA1 gene is one of the Major Histo compatibility complex type –A genes (MHC-Class II) which belonged to Immunoglobulin super family and these genes were a glycoproteins and it's one of the major histo compatibility complex genes had more polymorphism, it was located on the short arm of chromosome 23 in cattle (Vandre *et al*., 2014).

Aim of this study was to determine the polymorphism of DQA1 gene and elicitation Distribution rate of that polymorphism and allelic frequency and it association with some reproductive traits and immunity and heat tolerance coefficient.

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 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 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^{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. Heat tolerance coefficient was calculated (HTC) according to Rhoad (1944) equation:

HTC = 100 – 10 (ART-38.33)

As the:

RT: average of rectal temperature at mornig and afternooon.

38.33: normal of rectal temperature (centergate)

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 ,

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

O_j : 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 Discussion

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 fig. 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 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.

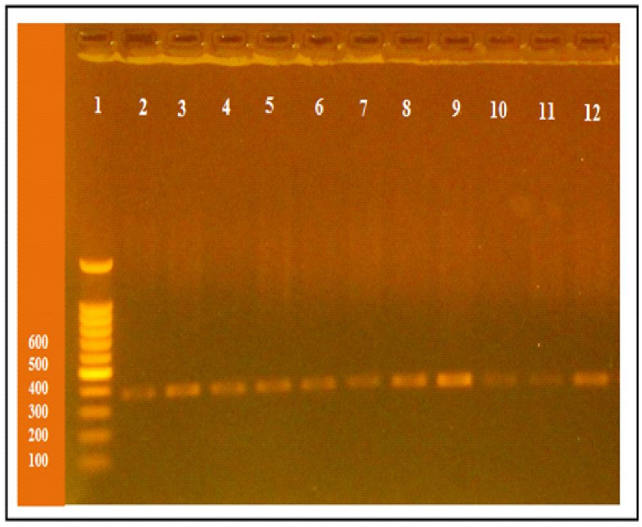

Fig. 1 : DQA1 gene extracted by Polymerase chain reaction method, column no.1 represented DNA Ladder 1000bp, column no. 2-12 represented DQA1 gene piece amplified with Polymerase chain reaction method exon 2.

Table 1: Number and percentage for DQA1 gene polymorphism.

Polymorphism	Number	Percentage %
A	16	47.06
B	2	5.88
C	16	47.06
Total	34	100 %
Chi-square- χ^2 value	—	11.913 **

(P<0.01) **

Table 2 : Association between DQA1 gene polymorphism and services per conception and days open.

Polymorphism	Cows number	Mean ± Standard error	
		Services per conception	Days open
A	16	0.78 ± 0.16a	88.65 ± 6.10a
B	2	1.75 ± 0.46ab	84.49 ± 6.29ab
C	16	1.59 ± 0.16b	77.17 ± 17.41b
Total	34	*	*
Significantly			

*The means with different letters within the same column are significantly between them (P<0.05).

Table 1 showed the number and percentage of DQA1 gene polymorphism, there were a highly significant different(P<0.01) between distribution ratio of DQA1 gene polymorphism which reached to 47.06, 5.88 & 47.96% for A, B & C allelic respectively, there was a common for genotype A then C if compared with B genotype, these results are proof that DQA1 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) in DQA1 gene polymorphism (Park *et al*., 2004; Takeshima and Aida, 2006, 2007; Sharif *et al*., 1998). The prevalence of allelic A and C and Scarcity of allelic B in this study maybe due to the adapted of first and third allelic to the environmental conditions 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 B allelic.

Table 3 : Association between DQA1 gene polymorphism and Immunoglobulin's concentration.

Polymorphism	Cows number (samples)	Mean ± Standard error
		Immunoglobulin's concentration (g/L blood) calves
A	16 (32)	44.73 ± 2.68a
B	2 (4)	37.09 ± 1.77 b
C	14 (32)	43.55 ± 1.82 a
Total	34 (68)	*
Significantly		

*The means with different letters within the same column are significantly between them(P<0.05).

Association of DQA1 gene polymorphism with services per conception and days open

Table 2 shows the association between DQA1 polymorphism and services per conception and days open, there was a different significant (P<0.05) in both traits when the genotypes was different, cows with C genotype needed minimal services per conception (1.59 ± 0.16) and thus less days open (77.17 ± 17.41 day), while the rates of these two traits were 1.75 ± 0.46 & 84.49 ± 6.29 day for cows with B genotype and 1.785 ± 0.16&88.65 ± 6.10 day in cows with A genotype, the reason may be due to the association between major histo compatibility complex 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,

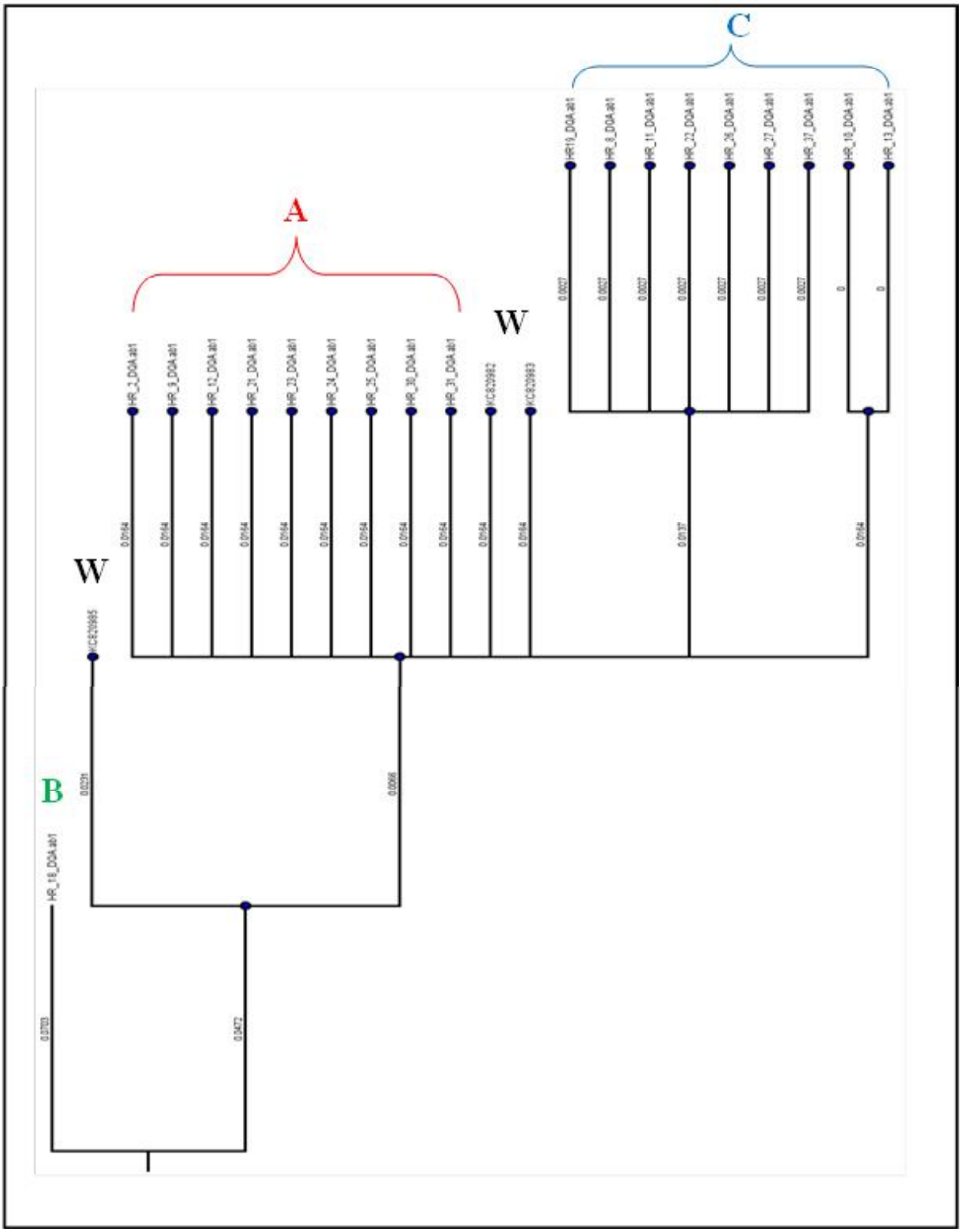

Fig. 2 : Tree shape for three allelic of DQA1 gene.

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 4 : Association between DQA1 gene polymorphism and heat tolerance coefficient.

Polymorphism	Cows number (samples)	Mean ± Standard error for heat tolerance coefficient		
		1st month of lactation season	2nd month of lactation season	3rd month of lactation season
A	16(32)	92.75 ± 0.96	92.52 ± 0.48 b	93.15±0.52 b
B	2(4)	93.04 ± 1.12	95.47 ± 0.39 a	95.61 ± 0.42 a
C	16(32)	92.82 ± 0.67	94.52 ± 0.56 a	95.72 ± 0.38 a
Total	34(68)	N.S	*	*
Significantly				

*The means with different letters within the same column are significantly between them(P<0.05), NS = No significant.

Association of DQA1 gene polymorphism with Immunoglobulin's concentration

Table 3 shows that there was a different significance (P<0.05) between the genotypes derived from the analysis of DQA1 gene for cows for IgG concentration in calves blood, as the concentration of IgG in this study reached 44.73 ±2.68 & 43.55 ± 1.82 g/L calves blood derived from cows with A, C genotype respectively, while the concentration of IgG Decreased in blood of claves Belongs to cow with B genotype reached 37.09 ± 1.77 g/L claves blood, this results may be due to that A and C allelic are the most common and then animals that possess these genotypes are more immune and resistant to pathological and bacterial infections compared to B 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 major histocompatibility complex genes, whereas class II release T cells type CD4+ then becomes a helper T cell, which in turn stimulates the effectiveness of phagocytes and B cells to provide a response to inflammation and antibodies (Behl *et al*., 2012).

Association of DQA1 gene polymorphism with Heat tolerance coefficient

Observed from table 4 a different significant (P<0.05) between DQA1 gene genotypes and heat tolerance coefficient. It was found that there was a significant difference between the genotypes of the DQA1allelic in the heat tolerance coefficient and for cows with B and C genotype in the second and third months of the lactation season, the heat tolerance coefficient was 95.47 ± 0.39 and 95.61 ± 0.42 for the cows carrying the genotypeB and 94.52 ± 0.56 and 95.72± 0.38 and reached 92.52 ± 0.48 and 93.15 ±0.52 for cows with A genotype, while there was no effected of DQA1 gene genotypes of the heat tolerance coefficient of cows during the first month of the lactation season, this may be due to the fact that disease is a stressful factor for living organisms, and since the cluster functions of the first and second type of major histocompatibility complex was the speed, specialization and effectiveness, heat shock proteins facilitate molecular processes and serve as targets for the immune system in healthy individuals, they consider themselves the dominant antigens on a wide range of pathogens, play a role in the immune response and act as an early warning of the body's immune system (Tkáèová and Angelovièová, 2012).

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