



E-ISSN: 2320-7078

P-ISSN: 2349-6800

JEZS 2018; 6(2): 2363-2366

© 2018 JEZS

Received: 25-01-2018

Accepted: 26-02-2018

Al-Waith KH

Department of Animal
Production/College of
Agriculture/University of
Al-Anbar, Iraq

AL-Anbari NN

Department of Animal
Production/College of
Agriculture/University of
Baghdad, Iraq

Mohamed TR

Department of Animal
Production/College of
Agriculture/University of
Al-Anbar, Iraq

Relationship of the DRB3 gen polymorphism with productive performance in Holstein cows

Al-Waith KH, AL-Anbari NN and Mohamed TR

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 DRB3 and productive performance traits in Holstein cows. Results showed that total milk production in Holstein cows was highly significant ($P < 0.01$) affected by genotypes of gene DRB3 and for cows with B (1628.36 ± 60.05 kg) and A genotype (1601.83 ± 60.25 Kg) compared with those which had C genotype (1510.77 ± 81.89 Kg), while the lactation period was not affected with different genotypes of DRB3 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 DRB3 gene and the highest average for B & A genotype 50.27 ± 1.73 , 49.91 ± 1.74 day respectively, the fat percentage was significantly affected ($P < 0.05$) by different genotypes of the DRB3 gene, the highest percentage reached in the cow's milk which had genotype C $3.97 \pm 0.22\%$, lactose, protein, non-fat solids and milk density were not significantly affected with the different of DRB3 gene genotypes.

It was possible to conclude from this study the possibility of DRB3 gen's genotypes in the development of genetic improvement strategies and breeding programs that achieved the best productive performance in dairy cows.

Keywords: holstein cows, productive performance, DRB3 gene

1. 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 [1], 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 [2], 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 [3], 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 [4], there were more evidences about major histocompatibility complex polymorphisms such as DRB3 gene in many pervious study [5]. DRB3 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 [6], DRB3 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 [7], so this study aimed the association of DRB3 polymorphism with many productive traits in Holstein cows for selection purpose.

Correspondence

Al-Waith KH

Department of Animal
Production/College of
Agriculture/University of
Al-Anbar, Iraq

2. 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 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 °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 submarine agarose gel electrophoresis, DNA samples of good quality, purity and concentration were used for further analysis.

The polymerase chain reaction (PCR) technique for BoLA-DRB3 typing is based upon the extensive polymorphism that is present in exon 2 of the BoLA- DRB3 gene under consideration depending on the size of the pieces and type of primers used, The 284 bp fragment consisting^[8] of the 267 bp exon 2 region of the DRB3 gene and the flanking intron of 17 bp present in the genomic DNA of cattle was amplified by employing the corresponding primer pairs (forward and reverse) as described by Van Eijk *et al.*,^[9] The details of the primer sequences are as follows:

F: ATCCTCTCTCTGCAGCACATTTCC

R: TCGCCGCTGCACAGTGAAACTCTC

After the polymerase reaction was completed, the polymorphism of DRB3 gene were identified in blood samples from the cows after proceed the cutting to the required piece of gene (284 bp) by restriction enzyme *HeaIII* from *Haemophilus aegyptius* bacteria The digestion with *HaeIII* revealed four restriction sites, which resulted in three pieces (167,65,52 bp) for *HeaIIIa* and two pieces (219,65 bp) for *HeaIIIb* and (167,117) for *HeaIIIc*. This restriction enzyme was obtained by American Promega Company, the concentration of enzyme was 2500 U, 10u-1 μ .

The data was analyzed by used Statistical Analysis System^[10] 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 DRB3 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 σ^2e .

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

3. Results and Discussions

DRB3 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 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 284 bp of required piece as the figure no.1. 1000 bp DNA ladder was used to estimate the size of the fragments.

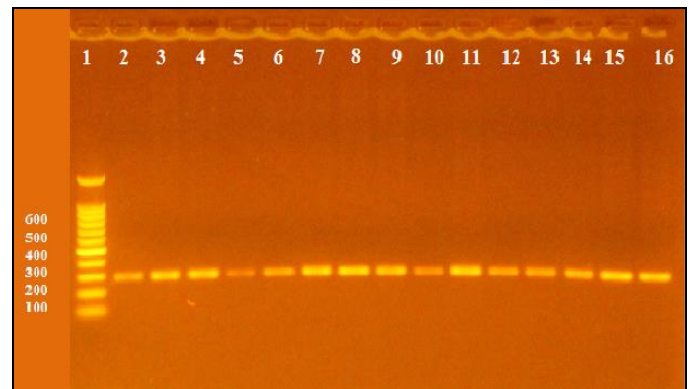


Fig 1: DRB3 gene extracted by Polymerase chain reaction method, column no.1 represented DNA Ladder 1000bp, column no.2-16 represented DRB3 gene piece amplified with Polymerase chain reaction method

The polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP) technique for BoLA-DRB3 and restriction enzyme *HeaIII* to identified DRB3 polymorphism according to the method that mentioned in material and methods, the restriction fragments were resolved on 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 284 bp of required piece, the analysis showed three allelic: allelic A (219,167,52)bp, allelic B (167,52)bp, allelic C (219,52)bp as the figure no.2.

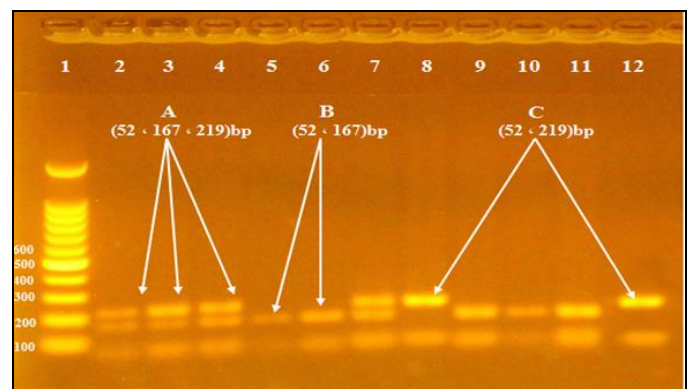


Fig 2: DRB3 gen three genotypes identified as the size of bands, column no.1 represented DNA Ladder 1000bp, column no.2,3,4,7 represented A allelic (219,167,52)bp, column no. 5,6,9,10,11 represented B allelic (167,52)bp, column no. 8,12 represented C allelic (219,52)bp with 17 bp not shown when compare it with gene sequence in NCBI (wild gene)

Association of DRB3 gene 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 DRB3 genotypes, as the cows with B genotype achieved maximum total milk production mean (1628.36 ± 60.05 Kg) then the cows with A genotype (1601.83 ± 60.25 Kg) while the cows with C genotype came in minimum total milk production mean (1510.77 ± 81.89 Kg (Table 1), this may be due to the cows with B or A genotype were excellent more than C genotype's cows to the commonness of these two

allelic and perhaps this supports survival theory for the better or may be to the association between DRB3 gene and the hormones which responsible on milk production, in other previous study founded a significant different in milk

production [11-16], and the results showed that there were no different significant between genotypes of DRB3 gene in lactation season length trait.

Table 1: Association between DRB3 gene polymorphism with milk production and lactation season length

Polymorphism	Cows number	Mean ± Standard error	
		Total milk production (Kg)	Lactation season length (Day)
A	23	1601.83 ± 60.25 a	169.43 ± 3.43 a
B	21	1628.36 ± 60.50 a	168.67 ± 3.40 a
C	6	1510.77 ± 81.89 b	162.35 ± 6.68 a
Total	50	**	N.S
Significantly			
The means with different letters within the same column are significantly between them ($P < 0.01$) **, N.S= no significant			

Association of DRB3 gene 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 genotype of DRB3 gene although there are some mathematical differences in A,C genotype, the rate of each of them for this period 43.04 ± 1.52 , 43.73 ± 2.99 day, while the rate was in the group of cows with B genotype for this gene 41.99 ± 1.52 day, the variation in the length of the peak of production was significantly ($P < 0.05$) cows with B genotype then A genotype were achieved maximum rats 50.27 ± 1.73 , 49.91 ± 1.74

respectively, while it was less from that rats in cows with C genotype (42.41 ± 3.40 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 [15].

Table 2: Association between DRB3 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	23	43.04 ± 1.52 a	49.91 ± 1.74 a
B	21	41.99 ± 1.52 a	50.27 ± 1.73 a
C	6	43.73 ± 2.99 a	42.41 ± 3.40 b
Total	50	N.S	*
Significantly			
The means with different letters within the same column are significantly between them ($P < 0.05$) *, N.S= no significant			

Association of DRB3 gene with milk content

It is cleared from the table (3) that fat percentage was significantly ($P < 0.05$) affected by polymorphism of DRB3 gene, it was reached the maximum rate ($3.97 \pm 0.22\%$) in cows with C genotype, while it was at minimum rat in both genotypes A&B 3.51 ± 0.21 & $3.52 \pm 0.09\%$ 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.

There was no significant different found with the different of DRB3 gen's genotype for lactose, protein, non-fat solids and milk density in cow's milk with A, B & C genotype respectively (table3), and in previous study founded different significant in milk content with the different of DRB3 genotype [7, 12, 14, 17].

Table 3: Association between DRB3 gene polymorphism with milk content

Polymorphism	Cows number (Samples)	Mean ± Standard error				
		Fat (%)	Lactose (%)	Protein (%)	Non-fat solids (%)	Milk density Cm ³ /ml
A	23 (69)	3.51 ± 0.21 b	4.56 ± 0.56 a	3.02 ± 0.04 a	8.41 ± 0.11 a	29.37 ± 0.72 a
B	21 (43)	3.52 ± 0.09 b	4.60 ± 0.06 a	3.05 ± 0.03 a	8.46 ± 0.11 a	30.02 ± 0.71 a
C	6 (18)	3.97 ± 0.22 a	4.62 ± 0.12 a	3.08 ± 0.07 a	8.50 ± 0.21 a	30.20 ± 1.41 a
Total	50 (150)	*	N.S	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						

4. Conclusion

It can be concluded from this experiment that the cows with B & A genotypes had the best rate of total milk production and Length of peak production, while the cows with C genotype gave best of fat percentage.

5. Acknowledgement

The authors express their deep gratitude to Dr. Hassan S.A. Jawad\ Department of Animal Production\ Faculty of

Agriculture, for his valuable assistance in publishing this research.

6. References

- Food and Agriculture Organization (FAO). Statistical yearbook. Asia and the Pacific Food and Agriculture, 2014.
- The ministry of Planning. National survey of animal resources. Central Statistical Organization, 2008.

3. Oltenacu PA, Broom DM. The Impact of genetic selection for increased milk yield on the welfare of dairy cows. *Animal Welfare*. 2010; 19(5):39-49.
4. Singh U, Rajib D, Alyethodi RR, Alex R, Kumar S, Chakraborty S *et al.*, Molecular markers and their applications in cattle genetic research: A review. *Biomarkers and Genomic Medicine*. 2014, 6:49-58.
5. Vandre RK, Sharma AK, Gowane GR, Rajoriya R, Rajoriya S, Sinha RK *et al.*, Polymorphism and disease resistance possessions of MHC Class II BoLA genes. *International Journal of Biomedical and Life Science*. 2014, 5(2):362-372.
6. Rothschild MF, Skow L, Lamont SJ. The major histocompatibility complex and its role in disease resistance and immune responsiveness. In: *Breeding for disease resistance in farm animals*. (R.F.E. Axford, S.C. Bishop, F. W. Nicholas and J.B Owen, Eds.) CAB International, Wallingord, 2000, 243-252.
7. Russell GC, Smith JA, Oliver RA. Structure of the BoLA-DRB3 gene and promoter, *European Journal of Immunogenetics*. 2004; 31(3):145-151.
8. Paswan C, Bhushan B, Patra BN, Kumar P, Sharma A, Dandapat S. Characterization of MHC DRB3.2 Alleles of Crossbred Cattle by Polymerase Chain Reaction-Restriction Fragment Length Polymorphism. *Genetic Marker Laboratory, Animal Genetics. Division, Indian Veterinary Research*, 2005, 1226-1230.
9. Van-Eijk MJ, Stewart-Haynes JA, Lewin HA. Extensive polymorphism of the BoLA-DRB3 gene distinguished by PCR-RFLP. *Animal Genetics*. 1992; 23:483-496.
10. SAS. *Statistical Analysis System, User's Guide*. Statistical. Version 9.1th ed. SAS. Inst. Inc. Cary. N.C. USA, 2012.
11. Duangjinda M, Buayai D, Pattarajinda V, Phasuk Y, Katawatin S, Vongpralub *et al.*, Detection of bovine leukocyte antigen DRB3 alleles as candidate markers for clinical mastitis resistance in Holstein \times Zebu. *Journal of Animal Science*. 2007; 87(2):469-476.
12. Wojdak-Maksymie K, Kmiec M, Kowalewska-Luczak I, Warlinsk I. DRB3 polymorphism and somatic cell count in milk Jersey cows. *Journal of Animal and Veterinary Advance*. 2010; 9(9):1295-1300.
13. Gowane GR, Vandre RK, Nagre M, Sharma AK. Major histocompatibility complex (MHC) of bovines: an insight into infectious disease resistance. *Livestock Research International*. 2013; 1(2):46-57.
14. Juan-Zambrano A, Julián-Echeverri Z, López-Herrera A. Association of gene BoLA DRB3.2 with production traits in a dairy herd of Antioquia, Colombia. *Rev. MVZ Córdoba*. 2014; 19(2):4116-4129.
15. Chakraborty D, Singh A, Tantia MS, Verma A, Chkravarty AK. Polymorphism of BOLA-DRB3.2 locus in Sahiwal cattle. *Animal Science Reporter*. 2015; 9:33-40.
16. Sermiyagin AA, Kovalyuk NV, Ermilov AN, Yanchukov IN, Satuck VF, Dotsev AV *et al.*, Association of BoLA-DRB3 genotypes with breeding values for milk production traits in Russian dairy cattle population. *Agricultural Biology*. 2016; 51(6):775-781
17. Nascimento CS, Machado MA, Martínez ML, Barbosa MV, Martins MF, Campos AL. Association of the bovine major histocompatibility complex (BoLA) BoLA-DRB3 gen with fat and protein production and somatic cell score in Brazilian Gyr Dairy cattle (*Bos indicus*). *Genetics and Molecular Biology*. 2006; 29(4):641-647.