Plant Archives Vol. 18 No. 2, 2018 pp. 2311-2316 **e-ISSN:2581-6063 (online), ISSN:0972-5210**

GENETIC POLYMORPHISMS OF LEPTIN GENE ASSOCIATED WITH PRODUCTION TRAITS IN HOLSTEIN PRIMIPAROUS COWS

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Abstract

This study was conducted on 50 Holstein cows at Al Salam Station for dairy cows / Private Sector (Latifia township, 25 km south of Baghdad), fifty samples collected of Holstein primiparous cows from a period of lactation / 2016 – 2017, in addition the Laboratory of Scientific Progress of Biotechnology and Molecular Genetics Analysis. Determination of the genotypes at the position LEP/Sau3AI of Leptin gene and the relationship with some of production traits in Holstein cows. Genotype differed for the target leptin gene encoding region due to different genetic bundles resulting from enzymatic digestion, which were genotyped in both AA and AB and their distribution ratios were 80.00 and 20.00 % respectively, and that the differences between these percentages were highly significant ($P < 0.01$), and allele frequency were 0.90 and 0.10 for both A and B respectively. The results of the present study showed that the total milk production and the lactation period for the Holstein cows were significantly affected ($P < 0.01$) by the Genotype of the leptin gene and for the cows with heterozygous Genotype AB. There was a significant variation ($P < 0.01$) in the period from birth to the peak of production, at 44.95 ± 0.85 and 33.78 ± 1.73 days for the two genotypes AA and AB respectively, as for the length of the production of peak reached the two genotypes in the same order 46.78 ± 0.94 and 60.46 ± 1.92 days ($P < 0.01$). The percentage of fat was significantly affected ($P < 0.05$) by genotypes of the Leptin gene, reaching the highest percentage of cow milk with the Genotype of AA ($3.93 \pm 0.62\%$) and the lowest of the genotype AB ($3.69 \pm 0.30\%$), while the percentage of lactose, protein and non-fat solids and specific gravity of milk had not significantly affected by the genotypes of leptin gene. It could be concluded by studying the genotypes of leptin gene that could be adopted in the development of genetic improvement strategies in milk cows to maximize the economic return of their breeding projects by selecting and crossing the genotypes that achieved the best economic characteristics.

Key words : Leptin gene, production traits, Holstein cows.

Introduction

Selective breeding has long been used by farmers to improve the quality of livestock. The improvement of productive and reproductive performance in agricultural animals, including cows under traditional breeding conditions, has many difficulties which have led to a decline in their productive performance, therefore, livestock in agriculture-dependent countries need special attention to improving and sustaining their productive traits because the economy and the large population depend heavily on livestock, it is therefore necessary to adopt some strategies for obtaining more adaptive animals and their productivity, which has led researchers and educators to find alternative means of traditional selection over the past decades that require considerable time and effort (Saleem *et al*., 2015). Molecular genetics techniques allow the direct determination of genotypes for animals by molecular markers, and through advanced techniques of molecular genetics, differences can be investigated in the structure of the primary or primary gene and directing animal breeders approach the selection decision in a new and improved method, molecular markers of polymorphism at the genes level, along with the traditional method of selection, play a crucial role in animal selection to improve the production of milk and meat (Elibox and Umaharan, 2008; Deb *et al*., 2012; Ebegbulem and Ozung, 2013). Leptin is a hormone that is produced mostly by white lipid tissue in the body, it has many roles, including energy balance, tissue growth, body composition, reproduction, immunity and leptin helps to reduce the intake of feed by

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animals through the feed-back mechanism, by informing the central nervous system about body energy and fat reserves, and plays a major role in regulating the productive performance of animals (Agarwal *et al*., 2009; Priyadarshini *et al*., 2015; Jamre *et al*., 2016; Mota *et al*., 2017). Given Iraq's the scarcity of ongoing studies, the aim of the research is determination of the polymorphisms of leptin gene in a sample of Holstein primiparous by the RFLP technique and extract the distribution ratios of those polymorphisms and allele frequency to them, and study the relationship of these polymorphisms of leptin gene with productive performance of cows.

Materials and methods

This study was conducted at the Al-Salam Station for dairy cows, Private Sector (Latifia township, 25 km south of Baghdad), on a sample of 50 Holstein primiparous cows (imported from Germany) from a period of lactation / 2016 – 2017. Samples of milk produced from dairy cows were taken in the morning for the analysis of milk components in the research and development division of the Abu Ghraib dairy factories using an electrical device called Ultrasonic Milk Analyzer (Master LM2), to estimate some milk components, as well as the monthly analysis of the milk components. Which is played by the station by agreement with the milk marketing lab of Jawhara company in Latifiyah. The genetic analysis of the blood samples in the Laboratory of Scientific Progress in Al-Harithiya for the period from 1/11/2016 to 1/11/ 2017 with the aim of extracting the genetic material and determining the genotypes of the leptin gene and its relation to production performance, as well as extraction ratios for distribution of genotypes in herd and allele frequencies obtained.

Polymerase chain reaction (PCR) technique was used to amplify the required fragment to complete the molecular detection and polymorphism of the Leptin gene and according to the size of the fragment and the type of primers used, the primers were selected as shown below (Intron 2, Gen Bank Accession No. U50365) for the purpose of conducting molecular detection and knowledge of polymorphism of the gene resulting from the presence of mutations of the LEP gene (Liefers *et al*., 2002). The gene fragment studied and their location have been confirmed and verified by electronic browsers for vertebrate genome: National Center for Biotechnology Information (NCBI), Ensembl Genome Browser and University of California Santa Cruz (UCSC).

Forward = 5′-TGG AGT GGC TTG TTA TTT TCT TCT- 3′

Reverse = 5′-GTC CCC GCT TCT GGC TAC CTA ACT- 3′

The data were analyzed statistically using the Statistical Analysis Program - SAS software (SAS, 2012) to study the effect of the genetic polymorphism of the Leptin gene according to the mathematical model below and significant differences between the means were compared with the application of the least square mean method.

$Yijk = ì + Gi + Oj + eijk$

Yijk: view value (k) of genotype (i) and the month of birth (j), μ: the general average of the traits, Gi: effect of genetic polymorphism of Leptin (AA and AB), Oj: effect of month of birth (April, May and June), eijk: random error which is distributed naturally at an average of zero and variance of σ^2e. The Chi-square- χ^2 test was used to compare the percentage distribution of genotypes.

Results and Discussion

DNA extraction and Determination of the genotypes of Lepin gene

Leptin gene fragment was extracted by PCR technique and used the PCR kit, primers, total DNA samples and Adjust your thermal cycles, the samples were then migrated from each sample model and imaging the output of the migration to make sure the extraction process is successful and obtained the required fragment size 422 bp for leptin gene, the size of the DNA fragments were used as a marker 1500-100 bp DNA ladder (fig. 1).

The genotypes of the experiment cows were determined by the studied fragment of leptin gene (422 bp) using a technique PCR-RFLP and Sau3AI restriction enzyme, after that, the samples were migrated from each model and imaging the output of the migration to determine the distribution of the genotypes of the study cows according to the number and size of the formed bands, the size of the DNA fragments were used as a marker 1500-100 bp DNA ladder (fig. 2).

The Sau3AI cutting was performed after identification of the sensitive position within the specific sequence for restriction position LEP/Sau3AI or LEP/Arg2059Cys of the studied fragment of leptin gene, so that the cutting process consisted of two or four bands of each model that can be compared with the ladder bands, because this enzyme has two restriction sites in this studied fragment, the first site (GATC) shows all samples at 32 bp, and this restriction site does not contain any SNP, therefore, the studied fragment of leptin gene (422 bp) will be two fragments of size 32 bp and 390 bp for all samples after enzyme cutting at the first site, the second

site of the enzyme work is the SNP container (GACC) and depends on presence of the SNP (replace base C with base T), in some samples, it is shown at 85 bp from the first restriction site, it cut the fragment 390 bp into two fragments of 85 bp 305 bp, note that the SNP is the result of replacing base C with base T, the letters A and B were used instead of the original bases for distinguishing them from the studied fragment of leptin receptor gene, because of the similarity of the changing bases in both genes, genotypes have been identified within intron 2 for the studied fragment of bovine leptin gene in this way as follows :

1. If the cutting happened in both bands at the first site only, will be two bands of each tape, the first band is the size of 32 bp and the second band of size 390 bp show as two bands, so as to obtain interference each two bands of the same size of both the two tapes with one bands, this means the genotype of this model is homozygous and represents the original genotype (AA) or wild genotype (without SNP).
2. If the cutting happened in both tapes in the first site and in one of the two tapes in the second site, it will consist of four bands (32 bp, 85 bp, 305 bp and 390 bp), as well as the cutting in the first site in both tapes, which gives two fragments size of 32 bp (not shown with gel) and 390 bp, there is a cut in the second site in one of the tapes without the other of the fragment 390 bp, consisting of two bands the size of 85 bp and 305 bp of one of the tapes because of the cutting happened in second site, this means that the genotype of this model is heterozygous and represents the heterozygous genotype AB (fig. 3).

Show the SNP itself, recorded in NCBI and polymorphism of single nucleotide (SNP) and numbered (rs29004501), which were analyzed as being within Intron 2 in the bovine LEP gene located on chromosome 4 (fig. 3), this SNP results from the replacement of cystosine nucleotide (C) with thymine (T), at the base pair is 85 bp from the first restriction site of the studied fragment of leptin gene (422 bp), resulting in change of the amino acid Arginine to Cysteine at the 2059 residue of the protein sequence (SNP/Arg2059Cys) (Liefers *et al*., 2002, 2004).

Distribution of genotypes and allele frequency of leptin gene

The number and percentage of genotypes and the allele frequency of leptin gene in the studied cattle sample (table 1). Two genotypes (AA and AB) of German imported Holstein cattle were bred in Iraq. There were significant differences (P <0.01) between the ratio of the two genotypes, with 80.00 and 20.00% for the genotypes

Table 1 : Number and percentages of genotypes and allele frequency of leptin gene.

Genotype	Number	Percentages (%)
AA	40	80.00
AB	10	20.00
Total	50	%100
(χ^2) Chi-Square value	—	** 13.250
Allele	Frequency	
A	0.90	
B	0.10	

** (P<0.01)

Table 2 : Relationship of leptin gene polymorphism in total milk production and lactation period.

Genotype	Number of cows (Total number 50)	Mean ± SE	
		Total milk production (kg)	Lactation period (day)
AA	40	32.52 ± 1508.11b	1.74 ± 162.55b
AB	10	66.21 ± 1976.55a	3.55 ± 190.73a

Means having with the different letters in same column differed significantly.
(P<0.01). **

AA and AB respectively, there was clear prevalence of homozygous genotype (AA) and low heterozygous genotype (AB) in the studied cow sample. These results were relative to genotypes close with a number of previous studies of the same position LEP/Sau3AI or Arg2059Cys / LEP and the same studied fragment of the leptin gene and the breed of Holstein cows. However, all studies show that homozygous genotype (AA) is more prevalent with low heterozygous genotype ratio (AB) and the scarcity of recessive genotype (BB). Moussavi *et al*. (2006) showed that there were significant difference between the ratio of the two genotypes, which were 89.00 and 11.00% for the genotypes AA and AB respectively, when studying on the same position and the fragment of the leptin gene and the Iranian Holstein breed. Javanmard *et al*. (2010) recorded lower ratios of the two genotypes, the same position and fragment of the leptin gene and of the Iranian Holstein breed as well, with 90.00 and 10.00% of the genotypes AA and AB, respectively. The results showed in table 1 that the allele A return of leptin gene in the sample of Holstein cows was 0.90 while the allele B frequency was 0.10 and this result reflected the prevalence of the allele A in the Holstein cattle of German imported. The A allele is not cut by the enzyme, but the B allele is cut off. The results of previous studies on the same position Sau3AI / LEP and the studied fragment of leptin gene and of the Iranian Holstein breed showed

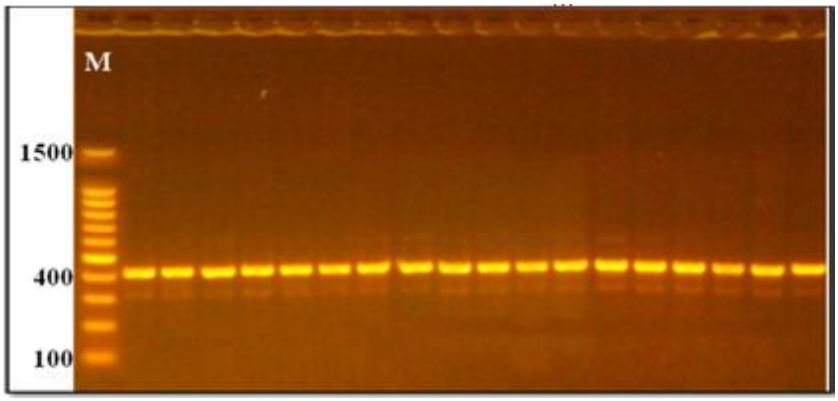

Fig. 1 : Extraction fragment (422 bp) of the leptin gene by PCR technique.

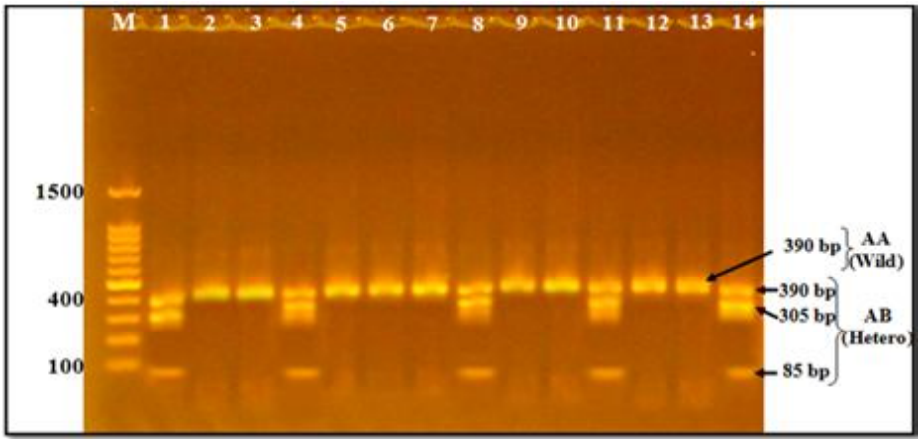

Fig. 2 : The digestion products of the studied fragment of leptin gene using restriction enzyme (Sau3AI).

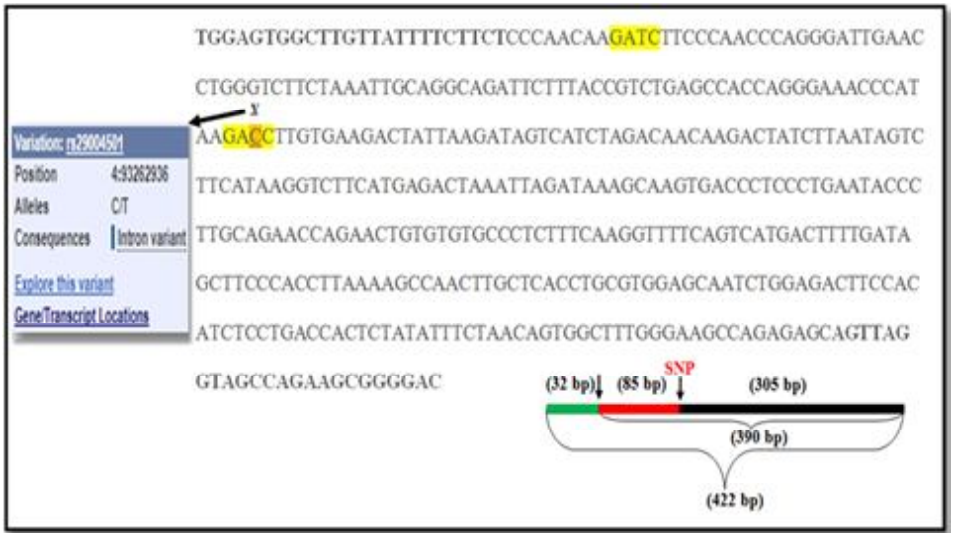

Fig. 3 : The studied fragment of the bovine leptin gene in intron 2.

that the allele frequency was 0.947 and 0.053 for both A and B, respectively (Moussavi *et al*., 2006).

Relationship of leptin gene polymorphism with milk production and lactation period

The results showed in table 2 that there were significant differences ($P < 0.05$) in total milk production between the genotypes of leptin gene, the cows with heterozygous genotype (AB) achieved the maximum milk production rate was 1976.55 ± 66.21 kg, while the production rate below in the genotype AA of 1508.11 ± 32.52 kg. The studies confirmed that there is a significant correlation between the SNP in the position Sau3AI / LEP and leptin genes with milk production traits Liefers *et al*. (2002; Anton *et al*., 2012). Liefers *et al*. (2002)

Table 3 : Relationship of leptin gene polymorphism with the period from birth to the peak of production and the length of the peak of production.

Genotype	Number of cows (Total number 50)	Mean ± SE	
		The period from birth to the peak of production (day)	The length of the peak of production (day)
AA	40	a 0.85 ± 44.95	b0.94 ± 46.78
AB	10	b 1.73 ± 33.78	a 1.92 ± 60.46
Significance level		**	**

**Means having with the different letters in same column differed significantly.(P<0.01).

Table 4 : Relationship of leptin gene polymorphism with milk composition.

Genotype	Mean ± SE					
	Number of cows (Total number 50) Number of samples (Total number 150)	Fat (%)	Lactose (%)	Protein (%)	Non-fat solids (%)	Specific gravity of milk
AA	40(120 sample)	0.62 ± 3.93a	0.04 ± 4.61a	0.02 ± 3.05a	0.07 ± 8.47a	0.02± 1.030a
AB	10(30 sample)	0.30 ± 3.69b	0.08 ± 4.49a	0.05 ± 2.99a	0.16 ± 8.34a	0.04± 1.029a
Significance level *		NS	NS	NS	NS	

*(P<0.05) Means having with the different letters in same column differed significantlyNS: Not significant.

reported that the Holstein primiparous with genotype AB produced milk more (1.32 kg/day) compared with the genotype AA. They suggested that allele B can give higher yield of milk production without effect negatively on energy balance and fertility.

The results in table 2 showed significant differences (P <0.05) between genotypes of gene leptin in the lactation period. The mean lactation period was 162.55 ± 1.74 and 190.73 ± 3.55 for the two genotypes AA and AB, respectively. Therefore, the relationship of the genotype AB of leptin gene with milk production and the lactation period suggests that this marker may be useful for selecting animals on the basis of difference at the DNA level, direct markers that were identified and associated with milk production and the lactation period in breeding programs of Holstein cows can lead to faster genetic progress for milk produce.

Relationship of leptin gene polymorphism with the period from birth to the peak of production and the length of the peak of production

The results of current study (table 3) should that significant differences (P<0.05) for the period from birth to peak of production and the length of the peak of production with difference of leptin genotypes, as cows achieved with genotype AA the best rate in the longest period from birth to the peak of production (44.95±0.85 day), while those registered with genotype AB lowest rates (33.78 ± 1.73 days). As cows achieved with genotype AB the best rate in the length of the peak (60.46 ± 1.92 days), while those registered with genotype AB lowest rates (46.78 ± 0.94 days). Seangjun *et al*. (2009) reported dairy cows that have higher initial production and higher production yield and more ability to continue produce milk at near peak levels throughout the lactation are expected to have higher milk yields per lactation.

Relationship of leptin gene polymorphism with milk composition

The results showed in table 4 that there were significant differences (P <0.05) in fat percentage between the genotypes of leptin gene, the cows with the homozygous genotype (AA) recorded the highest fat percentage (3.93 ± 0.62%), while the cows with the heterozygous genotype (AB) a fat percentage (3.69 ± 0.30%). The results in table 4 showed non-significant differences in the percentage of lactose, protein, non-fat solids, and specific gravity of milk for leptin gene. The cows with the homozygous genotype (AA) recorded percentages of milk content of 4.61 ± 0.04, 3.05 ± 0.02, 8.47 ± 0.07 and 1.030 ± 0.02 lactose, protein, non-fat solids and specific gravity of milk respectively, and cows with heterozygous genotype (AB) recorded content of milk 4.49 ± 0.08, 2.99 ± 0.05, 8.34 ± 0.16, 1.029 ± 0.04 lactose, protein, non-fatty solids and specific gravity of milk respectively. Studies have shown that the allele A of

the studied position (LEP/Sau3AI), plays a role in determining the level of production of milk, protein, fat and age at the first birth, the cows with the genotype AA were characterized by higher milk production, protein and fat and lower age at birth compared to cows with AB and BB (Trakovicka *et al*., 2013).

Conclusion

The polymorphisms in the studied fragment of leptin gene in population of Holstein cows were detected. Genotyping was carried by the multiplex PCR-RFLP analysis. Our finding results from association analyses suggested for milk performance traits can be said that the B allele had potential positive effect with AB genotype. Analyses of LEP/Sau3AI genotype effect on production shows potential positively effect of B allele occurrence on milk production performance, the selection of animals based on the genotype AB (LEP/Sau3AI) could result in production traits improvement in dairy cattle through participation of cows with these allele in genotype in future selection breeding program. It is generally accepted that the leptin has an important role in various economic traits of farm animals (growth, in feed intake, reproduction and milk production), therefore can be considered as a strong candidate gene for economically important production traits in Holstein cattle. The results of the present study confirmed mainly the significant impact of polymorphisms in the bovine leptin (LEP) gene on milk production. Since our study was biased by small sample size, the analysis of bigger population is recommended, that would increase of the reliability of the obtained results.

Acknowledgement

The authors express their deep gratitude to Dr. Adnan Nassif Jassim and Dr. Hassan S.A. Jawad\ Department of Animal Production\ Faculty of Agriculture, for their valuable assistance in publishing this research.

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