



THE RELATIONSHIP BETWEEN LEPTIN RECEPTOR GENE POLYMORPHISMS AND SOME PRODUCTIVE AND REPRODUCTIVE TRAITS IN HOLSTEIN PRIMIPAROUS COWS

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Abstract

This investigate was conducted 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 of the LEPR/Thr944Met position of Leptin Receptor gene and the relationship with some of productive and reproductive traits in Holstein cows. The percentages of CC and CT distribution of LEPR were significantly different ($P < 0.01$) in the studied sample, were 84.00 and 16.00%, respectively with allele frequency 0.92 and 0.08 for both C and T respectively. The results of the present study showed that the total milk production of the Holstein cows was significantly affected ($P < 0.05$) by genotype of the LEPR gene and for the cows with the Genotype of CT, while the lactation period was not affected by the genotype of the leptin receptor gene, while the period from birth to peak of production was not significantly affected by the genotype of the LEPR gene, the difference was significant in the length of the peak of production and for the cows with the genotype of CT. The percentage of lipid and non-fat solids were significantly affected ($P < 0.05$) by the genotype of the leptin receptor gene, where the milk of cows with the heterozygous genotype were higher than those homozygous genotype CC, while the percentage of lactose, protein and specific gravity of milk were not significantly affected different genotype of leptin receptor gene. The services per conception was not affected by the genotype of the LEPR gene, whereas cows with homozygous genotype CC have achieved days open (85.76 ± 3.78 days) were significantly better ($P < 0.05$) than those with heterozygous genotype CT (95.91 ± 6.00 days). It could be concluded by studying the genotypes of leptin receptor 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 receptor gene, productive and reproductive 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 reproductive 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 (Bollongino *et al.*, 2012 and Saleem *et al.*, 2015). The regulatory roles and biological function of leptin when it binds to its receptors on the cell surface, and this causes the intracellular domain to complex signaling pathways within cells and binds the different cloning factors to the DNA sequence of the target genes, leptin plays a role in regulating the expression of target genes (Agarwal *et al.*, 2009). In 1995, one year after the discovery of leptin gene and the leptin receptor Gene (LEPR) or Obese Receptor Gene (Ob-R), bovine leptin receptor gene maps were located on the BTA 3q33 chromosome, consisting of 20 exons divided into 1.75 Mb within the gene, the

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leptin receptor is a glycoprotein with a single transmembrane-spanning region, and there were six forms of leptin receptors in different tissues (Liefersm *et al.*, 2004). Polymorphism of the leptin receptor gene studied in beef and dairy breeds had been associated with studied reproductive and reproductive traits (Schenkel *et al.*, 2006; Clempson *et al.*, 2011; Hill *et al.*, 2016). Due to the scarcity of studies in this regard in Iraq, the research aims at determining the polymorphism of the leptin receptor gene in a sample of Holstein primiparous through the Restriction Fragment Length Polymorphism- RFLP technique, the extraction of polymorphism distribution ratios and their initial replication and the study of the relationship between polymorphism of LEPR in the 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 to the aim of extracting the genetic material and determining the genotypes of the leptin receptor gene and its relation to production performance, as well as extraction ratios for distribution of genotypes in herd and allele frequency obtained.

Polymerase chain reaction (PCR) technique was

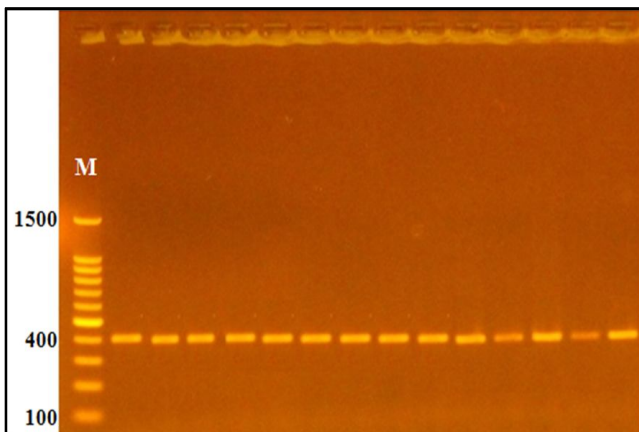


Fig. 1: Extraction fragment (400 bp) of the leptin receptor gene by PCR technique.

used to amplify the required fragment to complete the molecular detection and polymorphism of the leptin receptor gene and according to the size of the fragment and the type of primers used, the primers were selected as shown below (Exon 20, Gen Bank Accession No. AJ580801) for the purpose of conducting molecular detection and knowledge of polymorphism of the gene resulting from the presence of mutations of the LEPR gene (Komisarek and Dorynek 2006). The gene fragment studied and their location have been confirmed and

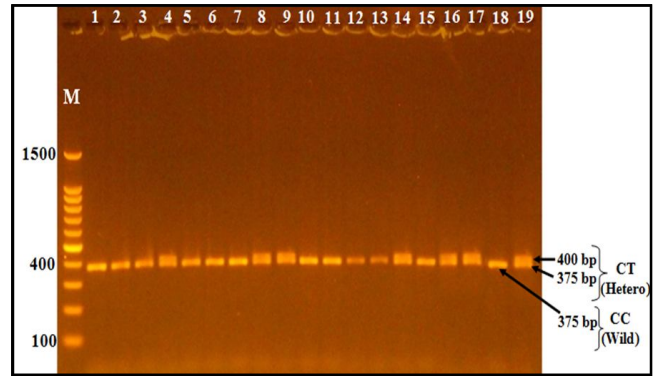


Fig. 2: The digestion products of the studied leptin receptor gene fragment using *TaqI* restriction enzyme.

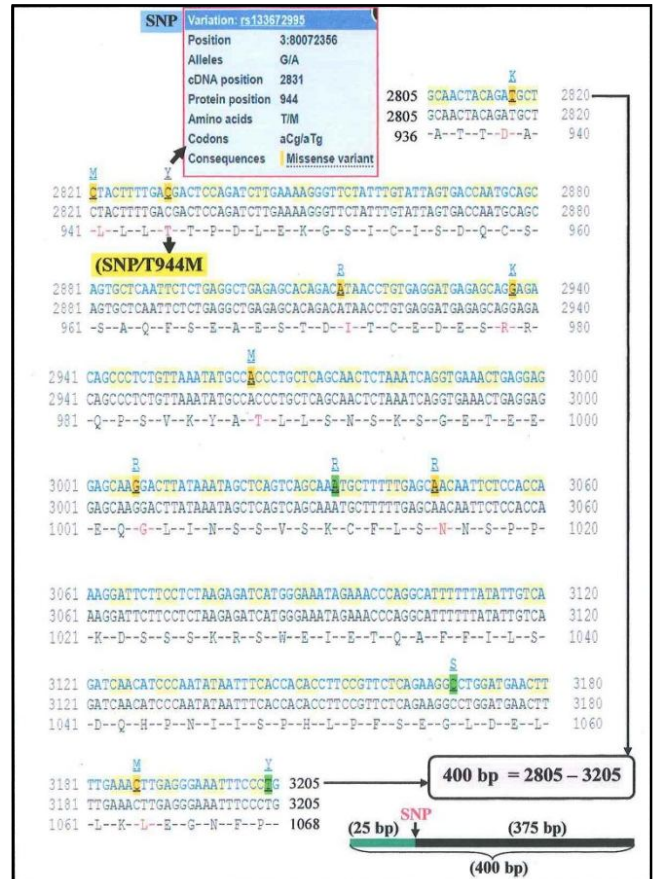


Fig. 3: Leptin receptor gene fragment (400 bp) and SNP (rs: 133672995) studied according to Ensemble Genome Browser.

verified by electronic browsers for vertebrate genome: National Center for Biotechnology Information (NCBI), Ensemble Genome Browser and University of California Santa Cruz (UCSC).

Forward =

5′- GCAACTACAGATGCTCTACTTTTGT* -3′

Reverse =

5′- CAGGGAAATTTCCCTCAAGTTTCAA -3′

*: The forward primer included a purposeful mismatch (marked with an asterisk) that incorporated a *TaqI* restriction site to the sequence, for the purpose of completion the sequence of the restriction site of enzyme *TaqI* (TCGA).

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 receptor gene according to the mathematical model below. Significant differences between the averages were compared with the application of the least square mean method.

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

Y_{ijk} : view value (k) of genotype (i) and the month of birth (j), μ : the general average of the traits, G_i : effect of genetic polymorphism of leptin receptor gene (TT and TC), O_j : effect of month of birth (April, May and June), e_{ijk} : random error which is distributed naturally at an average of zero and variance of σ^2_e . The Chi-square- χ^2 test was used to compare the percentage distribution of genotypes.

Results and Discussion

Table 1: Relationship of leptin receptor gene polymorphism in total milk production and lactation period.

Geno type	Number of cows (Total number 50)	Mean ± SE	
		Total milk production (kg)	Lactation period (day)
CC	42	b 42.94 ± 1588.26	a 2.43 ± 167.46
CT	8	a 72.11 ± 1680.82	a 5.79 ± 172.51
Significance level		*	NS
*Means having with the different letters in same column differed significantly (P<0.05)NS: Not significant.			

Table 3: Relationship of leptin receptor gene polymorphism with milk composition.

Geno type	Number of cows (Total number 50) Number of samples (Total number 150)	Mean ± SE				
		Fat (%)	Lactose (%)	Protein (%)	Non-fat solids (%)	Specific gravity of milk
CC	42(126 sample)	0.70 ± 3.99 a	0.04 ± 4.56 a	0.02 ± 3.03 a	0.07 ± 8.40 b	0.02 ± 1.029 a
CT	8(24 sample)	0.29 ± 3.68 b	0.09 ± 4.72 a	0.06 ± 3.13 a	0.18 ± 8.84 a	0.05 ± 1.031 a
Significance level		*	NS	NS	*	*
Means having with the different letters in same column differed significantly (P<0.05)NS: Not significant.						

DNA extraction and Determination of the genotypes of Lepin receptor gene

Leptin receptor 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 400 bp for leptin receptoe 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 receptor gene (400 bp) using a technique PCR-RFLP and *TaqI* 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 genotype CC (Wild): show in columns 1, 2, 3, 5, 6, 7, 10, 11, 12, 13, 15 and 18. The genotype CT (Heterozygous): show in columns 4, 8, 9, 14, 16, 17 and 19.

The *TaqI* restriction enzyme was performed after identification of the sensitive position within the specific sequence (LEPR/Thr944Met), of the studied fragment of leptin receptor gene, so that the cutting process consisted of two or three bands of each model, note that

Table 2: Relationship of leptin receptor gene polymorphism with the period from birth to the peak of production and the length of the peak of production.

Geno type	Number of cows (Total number 50)	Mean ± SE	
		The period from birth to the peak production (day)	The length of the peak of production (day)
CC	42	a 1.08 ± 42.69	b 1.24 ± 47.99
CT	8	a 2.58 ± 42.67	a 2.58 ± 53.50
Significance level		NS	*
*Means having with the different letters in same column differed significantly (P<0.05)NS: Not significant.			

this enzyme has restriction site (TCGA) in this studied fragment, the site shows in sequence at the base pair 25 bp (Fig. 3), the cut is studied fragment (400 bp) to two fragments 25 bp and 375 bp in the absence of the SNP, this restriction site contains SNP (TCGA) resulting from the replacement of base C with the base T (TTGA), the genotypes within exon 20 were identified for the studied fragment of the bovine leptin receptor gene in this way as follows:

- 1- If cutting happened in both the two tapes at the base pair sequence (25 bp) of studied fragment will be two bands of each tape, the first band size is 25 bp (not shown gel) and the second band size is 375 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 (CC) or wild genotype (without SNP).
- 2- If the cutting happened in one of the two tapes at the base pair sequence (25 bp) of studied fragment will be three bands of each tape of size 25 bp (not shown gel), 375 bp and 400 bp, the two bands (25 bp and 375 bp) consist of one of the tape because of the enzyme cutting and the third bands (400) is for the other tape (without cutting because of the presence of the SNP). This means that the genotype of this model is heterozygous and represents the heterozygous genotype (CT), which the presence of SNP in one of the two tapes resulting from the replacement of base C with base T, and this SNP is the one that stops or inhibits the action of the restriction enzyme (*TaqI*) within the specific sequence of restriction site in the studied fragment of the leptin receptor gene.

Show the missense mutation itself, recorded in NCBI (T944M) and polymorphism of single nucleotide (SNP) and numbered (rs133672995), which were analyzed within exon 20 in the bovine LEPR gene on chromosome 3, the mutation resulted in the replacement of cytosine nucleotide (C) with thymine (T), at the base pair of 25 bp of the studied dose of 400 bp of the leptin receptor gene,

Table 4: Relationship of leptin receptor gene polymorphism with the services per conception and days open.

Geno type	Number of cows (Total number 50)	Mean \pm SE	
		The services per conception (insemination)	Days open (day)
CC	42	a 0.09 \pm 1.72	b 3.78 \pm 85.76
CT	8	a 0.23 \pm 1.86	a6.00 \pm 95.91
Significance level		NS	*
*Means having with the different letters in same column differed significantly (P<0.05)NS: Not significant.			

resulting in change of the amino acid Threonine to Methionine at the 944 residue of the protein sequence (SNP/Thr944Met) (Matteis *et al.*, 2012). Note that this site according to the sequencing of the FASTA of the leptin receptor gene and according to the browsers of the genome of vertebrates (Fig. 3), whereas previous studies had indicated this mutation on site 945 (T945M) of the leptin sequence (Liefers *et al.*, 2002; Liefersm *et al.*, 2004).

Relationship of leptin receptor gene polymorphism with milk production and lactation period

The results showed in table 1 that there were significant differences (P<0.05) in total milk production between the genotypes of the leptin receptor gene, the cows with the heterozygous genotype CT achieved the maximum milk production rate of 1680.82 \pm 72.11 kg, while below was found in the homozygous genotype CC, which were 1588.26 \pm 42.94 kg. The studies confirmed that there is a significant relationship between the polymorphism at position T944M/LEPR of leptin receptor gene with milk production traits and composition^[12,13]. Trakovic *et al.*, (2015) showed that Pinzgau cows with genotype CC gave the highest milk production compared with the genotype CT. Suchocki *et al.*, (2010) and Clempson *et al.*, (2011) showed a low association between polymorphism of position T944M/LEPR with milk production in Holstein cows, but it found a significant relationship between other polymorphism of leptin receptor gene with lactogenesis and energy storage and fertility performance (Giblin *et al.*, 2010). While non-significant differences were observed between the SNP relationship at position LEPR/T944M of leptin receptor gene with the milk-related traits, note that the effect of this SNP was significant on circulating leptin concentration and may effect on hormone signaling pathways (Liefersm *et al.*, 2004); Liefers *et al.*, 2005; Matteis *et al.*, 2012). Non-significant differences were observed between the genotypes of the leptin receptor gene in the lactation period (Table 1), the mean lactation period was 167.46 \pm 2.43 and 5.79 \pm 172.51 for the genotypes CC and CT respectively. Note that relationship of leptin receptor gene polymorphism at position T944M/LEPR with the lactation period was not previously studied in cows.

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

The results in table 2 showed non-significant differences to the period from birth to peak of production between genotypes of leptin receptor gene, this period was 42.69 \pm 1.08 and 42.67 \pm 2.58 days for the cows

that the heterozygous genotype CT and the homozygous genotype CC respectively, the results of table 2 showed significant differences ($P < 0.05$) for the length of the peak of production between the genotypes of the leptin receptor gene, the cows with genotype CC recorded the shortest of the production of peak 47.99 ± 1.24 days, while the cows with the heterozygous genotype CT recorded the longest of the production of peak 53.50 ± 2.58 days. It has been attributed to the fact that the cows with the heterozygous genotype CT gave the highest production so it was the longest the peak of production compared to cows with genotype CC in this study. Note that relationship of leptin receptor gene polymorphism at position T944M/LEPR with the length of the peak of production was not previously studied in cows.

Relationship of leptin receptor gene polymorphism with milk composition

The results showed in table 3 that there was significant difference ($P < 0.05$) in fat percentage between genotypes of leptin receptor gene, the cows with the homozygous genotype (CC) recorded the highest fat percentage (3.99 ± 0.70 %) and the lowest percentage of non-fatty solids was $0.40 \pm 0.07\%$, while the cows with the heterozygous genotype (CT) recorded the lowest fat percentage (3.68 ± 0.29 %) and the highest percentage of non-fatty solids amounted to $8.84 \pm 0.18\%$. The results in table 3 showed non-significant differences in the percentage of lactose, protein specific gravity of milk between the genotypes of studies site of leptin receptor gene, the cows with genotype CC recorded percentage of lactose, protein and specific gravity of milk $4.56 \pm 0.04\%$ and 3.03 ± 0.02 and 1.029 ± 0.02 respectively, and cows with genotype CT recorded percentage of lactose, protein, and specific gravity of milk $4.72 \pm 0.09\%$, $3.13 \pm 0.06\%$ and 1.031 ± 0.05 respectively. Studies have reported relationship of leptin receptor gene polymorphism at position LEPR/T944M with milk components in some breeds. Trakovicka *et al.*, (2015) showed that Pinzgau cows with genotype CC had the highest production of fat and protein compared with genotype CT. While Suchocki *et al.* (2010) found that heterozygous genotype cows for the same studied site were associated with increased fat content in Holstein-Friesian cows compared to the Jersey breed. It was observed that the Jersey cows with the genotype TT gave the lowest values for the percentage of fat and protein compared to genotype of cows CT and CC (Komisarek and Dorynek 2006).

Relationship of leptin receptor gene polymorphism with the services per conception and days open

Non-significant differences were observed between

the genotypes of leptin receptor gene and the services per conception (Table 4), the cows with homozygous genotype CC recorded the services per conception was 1.72 ± 0.09 , while the cows with the heterozygous genotype CT recorded the services per conception of 1.86 ± 0.23 service. Studies showed significant relationship between SNP at position LEPR / T944M of leptin receptor gene with reproductive traits (Komisarek 2010; Clempson *et al.*, 2011), non-significant differences were observed between the polymorphism relationship of the studies position T944M / LEPR with the number of services per conception (Clempson *et al.*, 2011).

The results in table 4 showed significant differences ($P < 0.05$) for days open between the genotypes of leptin receptor gene, the cows with the homozygous genotype CC were shorter for days open was 85.76 ± 3.78 days, while cows with the heterozygous genotype CT recorded the longest for days open of 95.91 ± 6.00 days. Studies showed that there were non-significant differences between the polymorphism relationship of the studies position T944M / LEPR with days open and insemination interval and age at first calving (Trakovicka *et al.*, 2013) [18]. The polymorphism relationship of the studies position T944M/LEPR with some reproductive traits represented in calving interval, the studies showed significant differences, the cows with the heterozygous genotype CT were shorter compared to the cows with the homozygous genotype CC (Trakovicka *et al.*, 2013), Suchocki *et al.*, (2010) and Clempson *et al.*, (2011) found a weak relationship (non-significant) between the polymorphism relationship of the studies position T944M/LEPR with the number of days to first service in Holstein's cows.

Conclusion

This is the first study that evaluated the effect of SNP/T944M of the LEPR gene on dairy traits in Iraq. In this study, the T944M polymorphisms in the leptin receptor gene in Holstein primiparous cows were detected. The PCR-RFLP method was for this identification efficient and successful. Animals with heterozygous genotype (CT) compared with homozygous genotype (CC) had higher milk in lactation period. Based on these results it can be said that the C allele had potential positive effect on parameters evaluated and participation of cows with T allele in genotype in future genomic selection program can increase milk production, because the allele substitution effects of the SNP of receptor gene were evident. The results of this study confirm the role that LEPR gene play in influencing productive trait, and due to relatively small size of the considered animal sample,

the detected effects need to be further confirmed in independent dairy populations.

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