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ASSOCIATION OF TRANSFERRIN GENE POLYMORPHISM A14037G AND C14081T SNPS WITH THE PRODUCTIVE PERFORMANCE OF HOLSTEIN-FRISIAN COWS

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ABSTRACT
transferrin (*Tf*) protein plays a crucial role in immunity against microbial pathogens like *Escherichia coli*, *Streptococcus dysgalactiae*, *Streptococcus uberis*, *Staphylococcus aureus*, and *Streptococcus agalactiae* those considered the main causes of mastitis in bovine. The effectiveness of transferrin protein lies in the prevention of microbial cells from getting iron. The study of polymorphisms and detection of the single nucleotide polymorphisms (SNPs) of *Tf* gene could support the description of the inheritance differences of mastitis resistance and milk production traits. In this study A14037G SNP has been detected and new SNP was observed (C14081T) of *Tf* gene (entron 8) in Holstein-Frisian cows in Iraq. DNA was collected from 165 cows and the sequencing technique was used to investigate the genetic variation of two SNPs, A14037G and C14081T respectively. The percentages of genotype distribution for the transferring gene in sample of cows were 23.64, 58.18 and 18.18 % for the genotypes AA, AG and GG respectively and the differences between these percentages were significant (P< 0.01). Allele's frequencies for alleles (A) and (G) were 0.53 and 0.47 respectively according to the analysis of transferrin gene (*Tf*). High correlation was found between polymorphism and three genotypes, namely, AACC, AGCT and GGTT by two alleles AC (A), as a wild and GT as a mutant allele respectively were observed. Homogenotype in A14037G SNP (AG) AG was superior to other genotypes (AA and GG) in all traits except for fat percentage.

KEYWORDS: *Tf gene- entron 8, A14037G, C14081T, Cows.*

INTRODUCTION

The Transferrin *(Tf)* gene located on bovine chromosome 1q41-q46 (Chowdhary *et al*., 1998), where it consists of 17 exons and spans about 39 kb of genomic DNA. Many polymorphisms have been found in the bovine *Tf* gene (Ashton *et al*., 1964, Zhang *et al*., 2008, Sanz *et al*., 2010). Transferrin is an iron-binding β-globulin plasma protein synthesized by the liver with a molecular weight about 80 kDa (Fletcher and Huehns, 1968). It has two separate iron-binding sites, and each of them is capable of binding one atom of ferric iron (Fletcher and Huehns, 1968). In human plasma, transferrin is normally at 2.0-3.2 mg/mL and is typically one-third saturated with iron (Macgillivray *et al*., 1982). Transferrin may contribute to innate host defense against bacterial and fungal pathogens by limiting microbial access to iron (Chaneton *et al*., 2008). Transferrin also inhibits bacterial adhesion (Ardehali *et al*., 2003) and has an iron-independent antifungal effect (Bond *et al*., 2005). In addition, in animals with diagnosed mastitis, the transferrin concentration in milk is higher than that in healthy animals (Kmiec, 1998). These results suggest a possible relationship between the *Tf* gene and mastitis in dairy cattle. It is useful to study the genetic variations of candidate genes and their associations with milk production and somatic cell count (SCC) (Khatib *et al*., 2007 and Huang *et al*., 2010), which have a high genetic positive correlation with mastitis (with an estimated average coefficient of 0.7) (Pösö and Mäntysaari, 1996 and Heringstad *et al*., 2000). The objective to this study was to identify the polymorphism transferrin gene (*Tf*) A14037G, C14081T SNPs and their association with the productive performance (some milk and growth parameters) of 165 Holstein-Frisian cows and their progeny.

MATERIALS & METHODS

Animals

This study was conducted in Dairy Cattle Farm and the Biotechnology Laboratory, College of Agriculture, Department of Animals Production, Abu-Ghraib (West of Baghdad) and also at the Laboratory dealing with the analysis of molecular genetic during the period from 1/1/2014 to 31/1/2015.

Genomic DNA extraction

Genomic DNA was isolated from bovine blood samples by the method described by American PROMEG company kit procedure. DNA concentration was estimated spectrophotometric and then adjusted to 50 ng/ µL. All DNA samples were stored at -20°C.

PCR amplification and sequencing

To amplify the bovine *Tf* gene (NW_001493777) including exon 8 and intron 8 we used the primer was designed by **Ju, Z.H** at el .2011, (F: 5'-GGTCTGA CTGCCCTCTCTC -3' and R: 5' – GTTCAAACACACC TCTAATG -3').

The PCR mixture with a final volume of 25 µL consisted of12.5 µL Profi taq PCR premix kit 2.5,1µL $P_{F,}$ 1µL P_R, 21µL Template DNA ,8 µL H2O . PCR was performed as

follows: 94°C for 5 min, followed by 30 cycles of 94°C for 30 s, annealing 65 C° for 30 s, and 72°C for 30 s, and a final extension at 72°C for 8 min. (Ju, Z.H *et al*., 2011). PCR products were evaluated by electrophoresis on 2.5% agarose gels after staining with ethidium bromide (Figure 1).

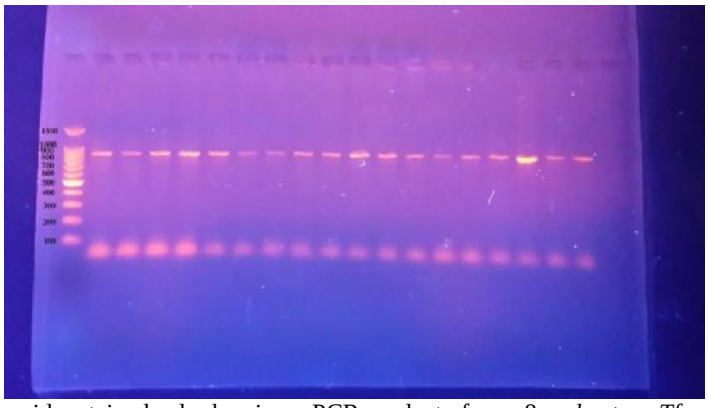

FIGURE 1. Ethidium bromide -stained gels showing PCR product of exon8 *and entron Tf* gene (882bp) evaluated by electrophoresis on 2.5% agarose gels.

Directly sequenced with Macrogene USA CO. Sequence data were analyzed using peaks file by Visual comparison of color peaks after made aliment to the base data in NCBI (Figure 2).

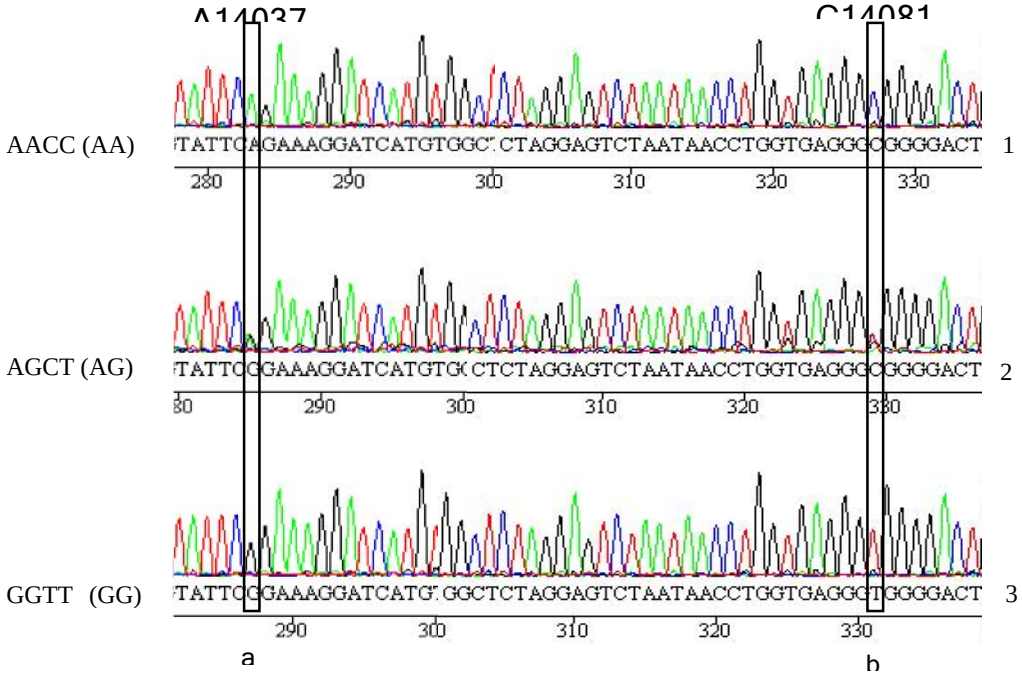

FIGURE 2. Column (a) refers to SNP A14037G, column (b) refers to novel SNP C14081T, row (1) refers to a haplotype AACC (AA), row (2) refers to a haplotype AGCT (AG), and row (3) refers to a haplotype GGTT (GG).

TAAGTATTC***A***GAAAGGATCATGTGGCTCTAGGAGTCTAATAACCTGGTGAGGG***C***GG

Wild Allele AC (A)

TAAGTATTC***G***GAAAGGATCATGTGGCTCTAGGAGTCTAATAACCTGGTGAGGG***T***GG

Mutant Allele GT (G)

FIGURE 3. Show both of the wild and mutant Allele in intron 8 *Tf* gene according to two SNPs A14037G and C14081T .

Novel SNP

According to sequencing results new SNP was detected C14081T distance between novel SNP (C>T) and SNP A14037G detected by Ju *et al.* (2011) was submitted to the National Centre for Biotechnology Information under accession Nos. ss250608651)and studied in resent study of Holstein Frisian cow was 43 pb with complete correlation as ahaplotype.

Genotyping tests

According to sequencing results, SNPs A14037G and C14081T were identified in *Tf* gene (entron 8) as a haplotype block in Holstein cattle which have been bred in Iraq , AA/CC, AG/CT and GG/TT (Figure 2), that finding common SNP g.- A14037G and novel SNP C14081T was found in Holstein Frisian cows and had a relationship with studied traits.

Statistical analysis

The Statistical Analysis System- SAS (2012) program was used to investigate the effect of polymorphism transferrin gene-*Tf*: (SNPs A14037G, C14081T-exon 8- haplotype combinations) in some milk parameters and growth of progeny. Chi-square test was used to assess the significant difference between percentage (distribution of polymorphism and allele frequency of *Tf* gene according to Hardy and Weinberg low).

RESULTS & DISCUSSION

Genetic polymorphisms of *Tf* gene in Holstein cattle

Two SNPS A14037G and C14081T were revealed from 165 samples by direct sequencing and comparisons with the reference sequence (NW_001493777) (Figure 2). A14037G were submitted to the National Centre for Biotechnology Information under accession Nos. ss250608651by Ju *et al*. (2011) C14081T as a new SNP using peaks file by Visual comparison of color peaks after made aliment to the base data in NCBI (the peak has two color as a hetero genotype but that has only one as a homo genotype).

Distribution of *Tf* in the cow studied sample genotypes

Table (1) revealed the percentages of genotype distribution of transferrin gene in sample of studied cows were 23.64, 58.18 and 18.18 % for the genotypes AA, AG and GG respectively and allele frequency of A and G alleles (Figure 3) was 0.53 and 0.47 respectively. The difference between these percentages was highly significant. The linkage between two SNPs (A14037G and novel SNP recorded in this study C14081T) was estimated (complete stratification, r = 1).

TABLE 1: Number and percentage of Genotype *to Tf* gene (A14037G) in Holstein-Frisian cows

Genotype	No.	Percentage (%)
AA	39	23.64
AG	96	58.18
GG	30	18.18
Total	165	100%
Chi-square $-\chi^2$	---	73.032 **
Allele frequency		
A	0.53	
G	0.47	

** (P<0.01)

Milk production and lactation period

The effect of the genotypes of the transferrin gene in the total milk production and the lactation period was significant (P≤ 0.05), while the cows with AG genotype gave the highest total milk production (2149.03 ± 295.05 kg/season) with a lactation period of 170.92 ± 18.59 days but non-significant effect in others (AA and GG) (Table 2). The current results are similar with those obtained by Ju *et al.* (2011) which detected this SNP and its relationship with the milk production in several types of Chines cows. This may be due to the site of SNP (9 bp downstream of the 5' intron splicing site GT) and it may affect directly on splicing of intron 8 (Ju *et al*., 2011) and to the important role of intron 8 in regulating gene expression and splicing (Nott *et al*. 2003). This result was agreed with Jacek *et al*. (2009) who found that transferrin gene had an effect on health state and milk production in ewes.

TABLE 2: Effect of Polymorphism *Tf* gene in Holstein Frisian cows on total milk production and lactation period

Polymorphism (Genotype)	No.	Mean ± SE	
		Total milk production (kg)	Lactation period (day)
AA	39	1553.59 ± 154.09 b	158.07 ± 14.97 ab
AG	96	1978.44 ± 138.28 a	169.21 ± 17.65 a
GG	30	1404.63 ± 98.37 b	146.39 ± 14.79 b
Level of sig.	---	*	*

Means with the different superscripts within the same column differ significantly (P≤ 0.05).

Milk constituents

Table (3) shows non-significant differences between different genotypes (GG, GA and AA) of lactose (%) and protein (%) but the homogenotypes (AA and GG) have significant effect on fat and SNF percentages as compared with the heterogenotype AG, this result agreed with the results obtained by Bailey *et al*. (2005) about the negative relationship between milk production and fat percentage. This result .may due to the high correlation with another SNPS in exon 8 and exon 12 (Ju *et al*., 2011) as haplotype

which have a significant association (P≤ 0.0006) with high fat production in Holstein-Friesian breed (Sanz, *et al*. 2010). This result was in line with Steppa *et al*. (2009) who observed that contents of protein, fat and dry matter in milk of ewes having different transferrin genotypes, and they concluded that it is useful to use the *Tf* gene as a maker to improve milk production in sheep.

TABLE 3: Effect of polymorphism *Tf* gene in Holstein-Frisian cows on milk contents

Polymorphism (Genotype)	No.	Mean ± SE			
		Fat (%)	Lactose (%)	Protein (%)	SNF (%)
AA	39	3.37 ± 0.06 a	4.31 ± 0.11 a	3.05 ± 0.07 a	8.85 ± 0.12 a
AG	96	3.06 ± 0.08 b	4.25 ± 0.09 a	2.97 ± 0.12 a	7.32 ± 0.18 b
GG	30	3.44 ± 0.08 a	4.26 ± 0.12 a	3.04 ± 0.10 a	8.47 ± 0.13 a
Level of sig.	---	*	NS		

Means with the different superscripts within the same column differ significantly (P≤ 0.05).

Growth traits

The significant differences (P≤ 0.05) were noticed in growth traits (Table 4). the heterogenotype AG has a superior effect than the other genotypes (AA and GG) in birth, weaning and gain between birth and weaning weight .This result is accordance with Ju et al (2011) who found that haplotype contained this SNP with other SNPS in *Tf* gene have a high resistance to pathogens caused mastitis leading to support health and improve the growth. The results of the current study support that transferrin gene was posted in 4 QTL (birth weight, adjusted weaning weight, adjusted yearling weight, and adjusted fat) called bovine QTL Viewer (http:// genomes.sapac. edu.au/ bovineqtl/ (A. Sanz1, *at al.*, 2010) and to the important role of intron 8 in regulating gene expression (Nott *et al*. 2003).

TABLE 4: Effect of Polymorphism *Tf* gene in Holstein-Frisian cows on growth traits

Polymorphism (Genotype)	No.	Mean ± SE		
		Birth weight (kg)	Weaning weight (kg)	Gain: birth to weaning (kg)
AA	39	29.72 ± 1.07 b	74.81 ± 2.68 b	45.09 ± 1.06 ab
AG	96	33.48 ± 1.64 a	80.25 ± 1.92 a	46.77 ± 1.74 a
GG	30	29.52 ± 1.33 b	73.79 ± 1.74 b	44.27 ± 0.95 b
Level of sig.	---	*	*	*

Means with the different superscripts within the same column differ significantly (P≤ 0.05).

CONCLUSION

A14037G SNP has been detected and a new SNP was observed (C14081T) of *Tf* gene (entron 8) in Holstein-Frisian cows in Iraq. The percentages of genotype distribution for the transferring gene in sample of cows were 23.64, 58.18 and 18.18% for the genotypes AA, AG and GG respectively. Homogenotype in A14037G SNP (AG) AG was superior to other genotypes (AA and GG) in all traits except for fat percentage.

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