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RELATIONSHIP OF STAT5A GENE (EXON 7) POLYMORPHISM WITH MILK PRODUCTION TRAITS IN HOLSTEIN COWS IN IRAQ

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Abstract

This study was conducted inTaj Al-Nahrain National Cattle Milk Production Station in Diwaniyah Governorate in Iraq. The genetic analysis of blood samples were carried out in the Scientific Advancement Laboratory for Biotechnology and Molecular Genetics. Milk samples were taken from 50 Holstein cows. The chemical analysis of their components were carried out in the R&D Division of Abe Ghraib Factories for the period from 1/7/2018 to 1/4/2019, in order to separate DNA and determine the genetic polymorphism of STAT5A gene and its relationship with milk production traits (daily milk, total milk yield and lactation period) and its components (protein, fat and lactose) as well as study the distribution genotypes percentage and alleles frequencies for the studied gene. Two mutations were identified for the seventh expression region, the first at C12483T and the second at C12471T with two genotypes (CC and CT). The distribution of CC and CT genotypes within the studied region of the 7th exon of STAT5A was significantly ($P < 0.01$), they were 70% and 30%, respectively while the allelic frequency was 0.85 and 0.15 of C and T alleles respectively. Results showed that amino acid in both studied mutations of the seventh exon did not change, which was considered silent mutation. There were no significant differences between the genotypes in the daily and total milk yield and lactation period of seventh exon region of STAT5A gene, while the fat and protein percentage of CC genotype cows was significant ($P<0.05$) at July. No significant differences were showed within lactose levels and within three months of the study in the percentage of milk components in the cows carriers CT. There were no significant differences between the two genotypes within the month of measurement in milk components.

Key words: STAT5A gene, milk production, Holstein cows.

Introduction

Due to the economic importance of milk cows, which is a major source of milk in the world and it's involved in the fields of manufacturing dairy products. It covers 90% of the world's milk as a human food source (Seijan *et al*., 2016). Over the past decade, there has been a growing scientific interest in using genetic information (Veer kamp, 2007) as a tool for selection. The use of molecular genetics techniques in animal breeding and improvement is a tool for improving farm animals and for a wide range of traits and early life as well as enhancing the reliability of prediction of phenotypic of individual (Selvaggi *et al*., 2009). Therefore Genetic markers were there adopted in selection programs as being more accurate than phenotypic and biochemical markers (Williams, 2005). Signal transducers and activators of transcriptions are transcription factors family including STAT1, 2, 3, 4, 5A, 5B and 6. The DNA-binding activity of STAT5 was first identified in the mammary gland. STAT5 exists in two closely related forms, A and B, encoded by two separate genes (Darnell, 1997).

Many Transcription Factor-TF sites approximately Promoter have an important role in the initiation of transcription: C / EBP, Nf1, GR and STAT5A (Hennighausen and Robinson, 1997 and Chughtai *et al*., 2002). The STAT5A gene plays an important role in many physiological processes, as this gene has a multiply of traits (He *et al*., 2012), including the viability of embryos, milk production and some growth traits in cattle (Brym *et al*., 2004). The functions of the STAT5A gene lie in the mammary gland factors (MGF), which are involved in the development of the mammary gland and prolactine hormone (PRL) singles as well as transcription factor to milk genes (Wakao *et al*., 1994). Therefore, the aim of this study was to determine the genotypes and their frequencies for the 7th expression region of STAT5A gene

and its relation to milk production traits and its components in Holstein cows.

Materials and Methods

This study carried out in Taj Al-Nahrain National Cattle Milk Production Station in Diwaniya Governorate in Iraq on a sample of 50 Holstein cows for the period from 1/7/2018 to 1/4/2019. Milk samples were taken and chemical components were analyzed in the research and development division of Abu Ghraib factories by the MASTER LM2 (Ultrasonic milk analyzer).

Total milk yield was calculated for 50 cows of Holstein breed (50 records) for one production season based on daily milk, which Milk production was recorded monthly at two time per day (morning and evening) for cows. Then it multiplied by days, month and measurement number. The total periodic measurements of milk production according to the total milk production and adjusted to 305 days per cow by automatic milking. Milk composition (fat, protein and lactose) to 50 cows (150 records) was estimated in three phases (mid-July, mid-October and mid-December).

The genetic analysis (laboratory part) of blood samples was carried out in the laboratory of scientific progress specialized in biotechnology and molecular genetics analyzes in order to separate DNA and determination of genotypes of STAT5A gene for the seventh exon region and its relation to milk production traits represented by Daily and total milk production and its components (protein, fat and lactose). Five ml of blood was taken from the udder vein of each animal in test tubes containing an EDTA anticoagulant and transported in a refrigerated box to the laboratory for freezing at-18°C until the time of use. The DNA was extracted from the blood samples from the studied cows using a measurement kit (Promega). The primer was selected for the purpose of molecular detection and to identify genetic polymorphism of the exon 7 expression region of STAT5A gene as shown in table 1.

All PCR reaction materials were prepared under sterile conditions in the sterilization cabinet. The PCR reaction mixture was (25µl) in table 2. The molecular detection program was followed using PCR technique as shown in table 3.

After amplification of the desired fragment (215 bp), the PCR product was electrophoresis by 1% agarose gel stained with ethidium bromide in TAE buffer. 5 µl of PCR product has been to keep and loaded in the gel and electrophoresis at 80 voltage for 90 minutes. After migration, the packages were photographed using a Gel Imaging System. After the identification of the seventh fragment of the expression region of PCR, genetic polymorphism of DNA sequencing were identified. The studied samples were sent to South Korea for the purpose of determining the genetic polymorphism of the studied fragments.

Table 1: Primer sequences of position exon7 to STAT5A gene.

Gene's name	Size (bp)	Primer sequence	Position	References
STAT5A	215	F:5'-CTGCAGGGCTGTTCTGAGAG-3'	Exon 7	Flisikowski *et al*., 2003
		R:5'-TGGTACCAGGACTGTAGCACAT-3'		

Table 2: PCR reaction mixture.

Chemical material	Master mix (µl)	Primer (µl)	DNA Template (µl)	Distilled water (µl)	Final size (µl)
Size	5	R-1; F-1	5	13	25

Statistical analysis

Data were analyzed statistically using SAS, (2012) to study genetic polymorphism of exon 7 to STAT5A gene in the studied traits. The significant differences between the averages were compared using Duncan taste (1955) by applying the Least square means (LSM) using the following model:

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

As:

Yijk: observation value y of the genotype i and the parity j.

µ: over all mean.

Gi: Effect of Genotypes (CC and TC).

Pj: Effect of parity (first, second and third).

e_{ijkl}: The random error, which is normally distributed with an average of zero and a variation of σ2e.

Chi-square- $\chi 2$ was also used to compare the percentages of the distribution of genotypes of the gene mutation studied in exon 7.

The law below has been applied to calculate allele frequency according to the Hardy Weinberg's equilibrium.

Frequency the first allele:

$$P_C = \frac{2 \times \text{No. of Homozygous} + 1 \times \text{No. of Heterozygous}}{2 \times \text{Total number of sample}}$$

Since: P + q = 1

The second allele is repeated according to the formula: $q_T = 1 - P_C$.

Results and Discussion

The studied fragments of STAT5A gene were amplified using PCR. The gene fragment (215 bp) and its

Table 3: Program of PCR technique.

Stages	Temperature	Time	No. of cycles
initial denaturation	95	5 Min	1
denaturation	95	30 Sec	30
annealing	64	30 Sec	
extension	72	30 Sec	
final extension	72	7 Min	1
	4	-	
(Selvaggi *et al.*, 2009)			

genotypes were identified using DNA sequencing technique. The two mutations (C12483T and C12471T) were identified within exon 7. There were 11bp between the two mutations were occurred due to replace of C allele to T allele (transition mutation). The wild C allele and mutant T allele as well as two genotypes (CC, CT) were observed as shown in fig. 2 and 3. The C12483T mutation was consistent with what Paramitasari *et al*., (2015) found in the Indonesian cattle according to the AvaI cut-of position within the sequence (C | CCGAG) which found no genetic polymorphism since all animals had a monomorphism (CC). While there was a genetic polymorphism of a number of breeds such as Italian brown cows (Flisikowski *et al*., 2003) and Italian cattle (Dario *et al*., 2009a). The CC frequency was lower compared to other genotypes and TT genotypes was lower in Turkish Holstein cattle (KIYICI *et al*., 2018), Podolica cattle (Dario *et al*., 2009b) and jersey cows (Dario and Selvaggi, 2011). All previous studies indicated C12483T mutations as silent mutation which carries both codes CCC and CCT that giving the same amino acid (proline). The mutation C12471T also a silent mutation

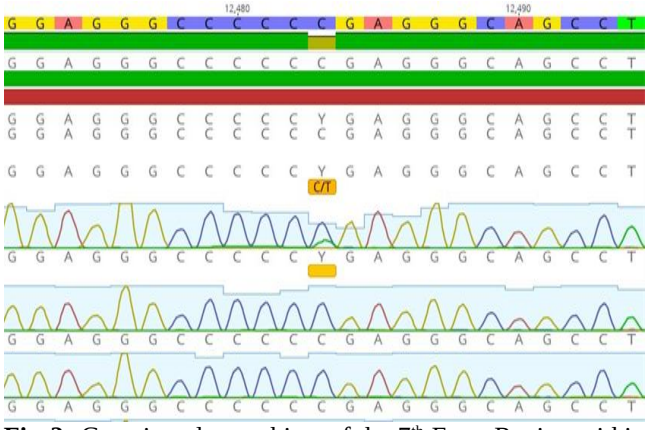

Fig. 2: Genetic polymorphism of the 7th Exon Region within Site 12483.

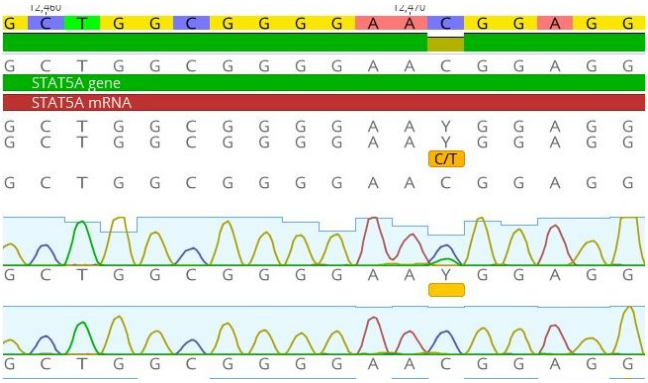

Fig. 3: Genetic polymorphism of the 7th Exon Region within Site 12471.

that carries both codes AAC and AAT which coding to same amino acid (Asparagine). The correlation coefficient between the two mutations was one, that's mean they change in same time by same way table 4.

Distribution of genotypes and alleles frequencies of the studied region of STAT5A gene

There were significant differences of genotypes distribution of CC and CT genotypes (70% and 30%, respectively), while the allele distribution of allele C (wild) and T (mutant) was 0.85 and 0.15 for both mutations from the studied region (Table 5). The allele frequency of C allele were high compared to the T allele. Result of present study was consistent with a number of previous studies, as most of them indicated a high percentages of the genotype CC on the rest of the genotypes (CT and TT). Selvaggi *et al*., (2009) showed in their study of Italian brown cattle, that CC genotype frequency was 68.67% which higher than to other genotypes (CT 29.18% and TT 2.15%). Also Sadeghi *et al*., (2009), Kmiec *et al*., (2010) and Kiyici *et al*., (2018) recorded superiority of CC genotype on other genotypes in their studies.

Relationship of genetic polymorphism within exon 7region of the STAT5A gene with daily milk, total milk production and lactation period

The present study showed that no-significantly differences to Exon 7 in daily milk, total milk production and lactation period traits (Table 6). The site of mutation C12483T in this study was consistent with a number of previous studies which not recorded effects on milk production, at example Kiyici *et al*., (2018) reported that there were no significant differences in the genotypes CC, CT and TT of (7836.2, 7735.0 and 7992.6 kg respectively) for total milk production in Holstein cows, also what Kmiec *et al*., (2010) which found no Significant differences of CC and CT genotypes in total milk production when studied on Polish

Table 4: Genotypes of both mutations and their effect on amino acids of STAT5A gene expression region.

Mutation Position	Genotype	Mutation type	Type of histidine before the mutation	Type of histidine after the mutation
C 12483 T	CCandCT	Silent (replacement)	Proline (P)	Proline (P)
C 12471 T	CCandCT	Silent (replacement)	Asparagine (Asn)	Asparagine (Asn)

Table 5: Number and percentages of genotypes and alleles frequencies for exon7 of STAT5A in Holstein cows in Iraq.

Allele frequency	Alleles	%	No.	Genotype	(Exon 7)
0.15	T	70	35	CC: Wild	C12483T
		30.00	15	CT: Hetro	
0.85	C	70.00	35	CC: Wild	C12471T
		30.00	15	CT: Hetro	
51.56**	value of χ^2	100	50	Total	
(**P<0.01)					

cows. The results of this study differed with other studies, which confirmed the difference in milk production with different genotypes. Selvaggi *et al*., (2009) in a study of Italian brown cows found that individuals with CC genotype were superior to total milk yield (5418.68 kg) compared to CT (5149.54 kg) which was consistent with Cosier *et al*., (2010). Which recorded the superiority of the CC genotype in milk production compared to the rest of the genotypes (Dario and Selvaggi, 2011).

Current result may be happened due to the change of the cows environment which Imported from Germany, especially high temperatures as well as low level of nutrition, that inhibited animal to express its genetic ability, which explains the absence of significant differences between the two genotypes despite the existence of an arithmetic difference in milk production. Otherwise, there were no studies on the relationship of mutation site 12471 within exon 7 with milk production.

Relationship of genetic polymorphism of region of the seventh exon of STAT5A gene with milk components (fat, protein and lactose)

There was no significant effect of STAT5A gene with fat percentage (Table 8) within month of measurement, which came in accordance with the aforementioned traits of this study and may be for the same reasons mentioned above. While there was a significant effects (P<0.05) of month on fat percentage within individuals of CC genotype cows. The fat percentage in July was higher (3.46%) compared to December (2.72%). This may be due to lower milk production in summer, which is inversely proportional to the fat percentage in milk, whereas in winter the fat percentage decreased due to higher milk production in the cold season, in otherwise there was no effect of months on individuals carrying CT genotype. This finding is consistent with what Sadeghi *et al*., (2009) who study Holstein bulls in Iran, Kmiec *et al*., (2010) in the Polish Friesian Holstein cows and Cosier *et al*., (2010) and kiyici *et al*., (2018) in the Turkish Holstein cows who indicated no- Significant differences of genotypes in fat percentage. The result of this study is contrary to Selvaggi *et al.*, (2009), that found a high Significant of CC genotype in Exon 7 region in fat percentage, its amount was 201.32 kg in favor of animals carrying CC genotype of CT carriers (192.63 kg) in Italian brown cows but found no significant difference in fat percentage.

Table 9, showed that there is no significant difference in protein percentage between genotypes. While there were significant

Table 6: Relationship of Genotypes of exon 7 region in Daily, Total Milk Production and lactation period in Holstein Cows.

Genotype	Cows No.	LSM ± SE		
		Daily milk production (kg)	Total milk production (kg)	lactation length (day)
CC	35	0.48±12.30	174.52±4429.89	362.51 ± 2.42
CT	15	11.96± 0.71	257.65± 4305.78	361.93±4.06
Significant		N.S	N.S	N.S

Table 8: Relation of Polymorphism of the exon 7 region in milk Fat percentage in Holstein Cows.

Genotype	Animal No.	LSM ± SE			
	50	Fat % (July)	Fat % (October)	Fat % (December)	Significant level
CC	35	0.16±3.46 Aa	0.20±3.35 Aa	0.23 ±2.27 Ab	*
CT	15	3.05 ±0.24 Aa	3.30±0.30Aa	2.82 ±0.34Aa	N.S
Significant level		N.S	N.S	N.S	

Different letters within one column (uppercase) and within a row (lowercase) are significantly different between them. * (P<0.05) NS (no significant).

Table 9: Relation of polymorphisms of the exon 7 region in milk protein percentage in Holstein cows.

Genotype	Animal No.	LSM ± SE			
	50	Protein % (July)	Protein % (October)	Protein % (December)	Significant level
CC	35	2.79±0.05Aa	2.65±0.05 Aa	2.53±0.06 Ab	*
CT	15	2.73±0.08 Aa	2.63±0.08 Aa	2.56±0.09 Ab	N.S
Significant level		N.S	N.S	N.S	

Different letters within one column (uppercase) and within a row (lowercase) are significantly different between them. * (P<0.05) NS (no significant).

Table 10: Relation of polymorphism of the exon 7 in lactose percentage for milk in Holstein cows.

Genotype	Animal No.	LSM ± SE			
	50	Protein % (July)	Protein % (October)	Protein % (December)	Significant level
CC	35	30.98±0.08 Aa	3.92±0.08 Aa	3.88±0.10 Aa	N.S
CT	15	4.01±0.13 Aa	3.97 ±0.12 Aa	3.99±0.15 Aa	N.S
Significant level		N.S	N.S	N.S	

Different letters within one column (uppercase) and within a row (lowercase) are significantly different between them. NS (no significant).

differences (P<0.05) for individuals carrying the CC genotype between the months, proteins percentage in July was higher (2.79%) than December (2.53%). This significant difference was not observed between months of individuals with CT genotype. The results of this study were consistent with those found by Kmiec *et al*., (2010) and kiyici *et al*., (2018) were no significant differences observed between CC and TT genotype in protein percentage. The results were contrary to Selvaggi *et al*., (2009), which indicated a highly significant effect of CC genotype in the seventh expression region in protein percentage when studied this trait in Italian Brown Cattle breed, he confirmed that the cows carrying the genotype CC recorded the highest quantity (183.99 kg) and protein percentage (3.40%) compared to TT genotypes for both traits, contrary to what pointed out by Sadeghi *et al*., (2009) in Holstein bulls that CT carrying individuals outperformed CC in content of protein In Iran. Cosier *et al*., (2010) also found a significant effect of CC genotype in the 7th expression region on milk protein content.

There were no significant differences between the two genotypes within the measurement month and between the months in percentage of lactose (Table 10). The results of the study are in line with what Kiyici *et al*., (2018) found that there were no significant differences between the genotypes (CC, TT and CT) in Holstein cows in Turkey. We find that from previous studies of the STAT5A gene, does not determine the relationship of the mutation T12471C with milk production and its components. Therefore, we need to clarify the role for SNP in exon 7 of the gene in productive and reproductive traits in cattle because there is a strong correlative correlation between the two mutations studied for the exon 7 in this study, it may be interesting to study changes in the STAT5B gene because they are genetically related, *i.e*. because of their genetic convergence (Selvaggi *et al*., 2009).

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