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Study of the allelic frequency of HSPA6 gene and its genotypes and their effect on some characteristics of milk production in Holstein cows

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Conclusion

The study was conducted at the Taj Al-Nahrain cattle station in Al-Diwaniyah Governorate, which is 200 km from the center of Dhi Qar Governorate. For the period from 10/15/2021 to 10/3/2022, 49 Holstein cows imported from Germany were used in this experiment, all in the third production cycle. And the laboratory part of the study was conducted in the laboratory of the Marsh Research Center \ Dhi Qar University for the purpose of separating the genetic material from cow blood samples and conducting genetic analysis on it and extracting the possible genotypes HSPA6 heat shock protein gene. The animals in the station were fed according to the season and the year in terms of the availability of fodder and the percentage of costs involved in raising the cows, as well as on the general state of the productivity of the cows, but in general they are provided with green fodder, which includes jet, barley, corn stalks and a percentage of each of the dry fodder (hay and hay).) and concentrate (barley, bran and cake) according to what is available from them, as well as limestone and table salt. Data on milk production were collected from the station records, where the milk production was recorded weekly for the morning and evening fenugreek by the station, and the milk components were analyzed in the Abu Dairy Company Gharib / Research and Development Division by Milk Analyzer. 3 ml of blood was collected from the udder vein of each cow by 5 ml medical syringes, suitable for the size of the animal’s vein, and placed in collection tubes containing an anticoagulant substance (K2 EDTA) and transported in a cooler box for safekeeping Freezing at -18°C until extraction time.

From the results obtained from this study, we can conclude that the mutation detected in the studied segment of the 6HSPA gene was a type of missense mutation and worked to change the code of the amino acid valine to the code of the amino acid leucine. Three genotypes were shown: GG, GT, and TT. It was found that there was a clear commonality of cows carrying the GG genotype with a rate of 88%, followed by the GT genotype with 8%, while the TT genotype got the lowest percentage and amounted to 4%, and with allelic recurrence Its value is 0.92 for the G allele and 0.08 for the T . allele

Groups of cows carrying the genotypes (GG, GT and TT) did not differ significantly among themselves in the characteristics of milk production and its components, but an arithmetic superiority between the three genotypes was observed and referred to in all the studied traits.

Key words: HSPA6 gene, milk production, Holstein cows

I. INTRODUCTION

Summer in Iraq is considered a critical season for dairy cows in terms of their production and reproduction, due to a decrease in milk production and a decrease in the fertility of dairy cows. Subtropical climate A group of purebred milk cows are reared under heat stress from high temperatures and intense vertical solar radiation at midday in summer, resulting in a significant reduction in milk production and their reproductive efficiency (El-Dessouky et al. 1977, Ali et al. 1983b).

Heat stress is one of the well-defined phenomena that negatively affects agricultural animals in general and dairy cows in particular, and this effect is one of the main determinants of cow production and physiological performance (Bohmanova et al., 2007). The reduced production and fertility associated with heat stress may be due to the negative effects of high temperature on embryonic development or hormonal balance (Ealy. et al., 1993). Exposure to sunlight and other climatic factors (relative humidity, air movement, air temperature) directly leads to an increase in body temperature because animals cannot get rid of excess heat in the usual way, which negatively affects cows production and efficiency (Prawechenski, 1982). Another harmful effect of heat stress is its effect on feed intake, milk production, growth rate and reproduction (Gaughan et al., 2012). Heat stress is not only harmful to animal production and reproduction but also seriously damages the health of breeding animals (Banerjee et al., 2014). The importance of selecting animals that are more resistant and adaptive to climate change and different stress conditions, due to their high genetic capacity to protect cells, can reduce the effects of heat stress, and one of the most important molecular mechanisms possessed by the body is the heat shock protein HSPA6. (Guerriero et al., 2004).

The HSPA6 protein also known as HSP70B (70 kDa) is involved in the maintenance of cellular proteins (Heldens et al., 2010) HSP70 family plays an important role in cell heat tolerance, Beckham et al., 2004). It is also a characteristic of the HSP70 family. Stimulated after severe cellular stress (Noonan et al. 2007) HSPA6 was identified as having higher expression in cattle and goats under heat stress (Mohanarao et al. 2014) This may be due to severe stress conditions that cause HSPA6 to evolve into a gene that maintains basic biological functions (Hyder et al. 2017).Therefore, HSPA6 can be used as a candidate gene for breeding heat-tolerant flocks (Hyder et al. 2011).

Because of the importance of this type of genetic studies, the current study aimed to detect the genetic manifestations of the HSPA6 gene in Holstein cows and to show its allelic recurrence.

And linking the genetic phenotypes of the above gene with some productive and physiological traits in Holstein cows in the third season of production.

II. MATERIALS AND RESEARCH METHODS

The study was conducted at the Taj Al-Nahrain cattle station in Al-Diwaniyah Governorate, which is 200 km from the center of Dhi Qar Governorate. For the period from 2/10/2021 to 1/4/2022, 49 Holstein cows imported from Germany were used in this experiment, all in the third production cycle. As for the laboratory part of the study, it was conducted in laboratories conducted in the laboratory of the Marshes Research Center \ Dhi Qar University for the purpose of separating the genetic material from cow blood samples and conducting genetic analysis on it and extracting the possible genetic structures HSPA6 heat shock protein gene and identifying genetic mutations in the studied piece and the following are the most important The results obtained

From the results of the nucleotide sequencing study on the gene segment of 475 base pairs, the presence of the mutation G>T.113 was detected in the studied segment and located at site 473 in the complete gene registered under accession number XM-027527626.1 and after making an alignment of the sequence of samples that this obtained The change was found to be a missense mutation, and it caused the change of the base G to T at the site specified above. It appeared in three structures: wild GG, hybrid GT and TT mutant. Since the mutation site occurred in the valine amino acid code at site 44 of the complete peptide chain of HSPA6 protein. The code was changed from GTG to TTG and thus the acid changed to Lucine. This mutation was registered under accession number XP-027383427 on the NCBI website.

The initiators of the HSPA6 gene were prepared by the Korean company Macrogene in the form of lyophilized powder of two initiators separated from each other, each of them placed in a special tube with a label showing the sequence of nitrogen bases. Stock solution was taken and 10 microliters were taken from it and 90 microliters of dd Water was added again so that the concentration of the initiator became 10 Picamol which is the concentration required to perform the PCR reaction.

Table (1): Prefixes used in the experiment

Primers	start	stop	GC %	Length (base)	Tm (C)
Forward **GAAACCACAACCATGTCCGC**	**236**	**256**	**55**	**20 b**	**60 C**
Reverse **AGTCGTTGAAGTAGGCAGGC**	**711**	**691**	**55**	**20 b**	**60 C**

Table (2): Program for PCR technology for HSPA6 . gene

number of courses	time (minutes)	Temperatures	stages	gene
1	**5**	**95C°**	**Initial Denaturation**	
	0.30	**95C°**	**Denaturation**	

35	**0.45**	**54 C$^{\circ}$**	**Annealing**	**HSPA6**
	0.45	**72C$^{\circ}$**	**Extension**	
1	**10**	**72C$^{\circ}$**	**Final Extension**	

Acarose gel was prepared with the same previous steps in which the DNA material was removed from the samples, but the concentration of agarose prepared for the transfer of samples of the PCR product was 1.5%, where 0.45 g of agarose was added to 30 ml of 1X TAE solution, 5 µl of the PCR product was loaded with 2 One microliter of Diamond™ Nucleic Acid Dye dye was diluted to each hole on the agarose gel and a marked hole was used to load 5 microliter of DNA Ladder with the dye and set the migration program using 70 V and 85 mA for 45 minutes. Photos using a UV Gel Documentation device

After confirming the size of the specialized PCR product for the studied gene by comparing it with the standard DNA strip DNA Ladder, 20 microliters were taken for each sample of the PCR product and sent to the Korean company Macrogene, where the samples were purified and then the base sequence analysis process using the sanger sequencing technique. The base sequence results were received and analyzed using BLAST tools The NCBI website with the use of some bioinformatics programs.

The data of the experiment were statistically analyzed using the ready-made statistical program SAS (2012) (Statistical Analysis System), as the complete random design (CRD) was used to study the effect of multiple genetic phenotypes for the resulting mutation on the studied traits in Holstein cows, and the significant differences between the averages were compared using Duncan's test Polynomial (Duncan, 1955) by applying the least squares mean (LSM) method.

$$Yij = \mu + Gi + Eij$$

whereas :

Yij: the observation value j of genotype i.

µ: the general average of the adjective.

Gi: effect of polymorphism of 6HSPA gene (GG, GT, TT).

Eij is a random error that is normally distributed with a mean of zero and a variance of 2δ

Chi-square-x2 test was used to compare the percentages of the distribution of genotypes for the studied gene.

III. RESULTS AND DISCUSSION

From the results of the study of the nucleotide sequence of the studied segment of the HSPA6 gene and its relationship with the productive traits:

From the results of the nucleotide sequencing study on the 475 base pair gene segment, the presence of the G>T.113 mutation was detected in the encoded region (exon 3) of the HSPA6 gene in the studied cattle sample.

Mutation G>T.113

It appeared in three combinations: wild GG, hybrid GT and mutant TT, and it was found that this mutation is a type of missense mutation (Missense Mutation), which led to a change in the resulting amino acid code to be Lucine instead of Valine.

Table (3) shows that there are highly significant differences ($p \leq 0.01$) in the percentages of genetic phenotypes of the 6HSPA gene for genetic heterogeneity (G>T.113), and also found that there is a clear commonality of pure individuals carrying the genotype GG, which obtained the highest percentage of 85.71%, followed by the GT genotype with a percentage of 10.20%, while the TT genotype had the lowest percentage and reached 4.09%, and with an allelic frequency of 0.91 for the G allele and 0.09 for the T allele. From 0.02 to 0.21.

Mutation	G>T.113			the total
genotype	GG	GT	TT	--
the number	42	5	2	٤٩
The ratio Centennial(%)	٨٥,٧١	١٠,٢٠	٤,٠٩	%١٠٠
allele	G		T	--

Frequency	٠,٩١	٠,٠٩	١,٠٠
Ka i-square value X^2	٢٥,٣٦**		**** p≤0.01**

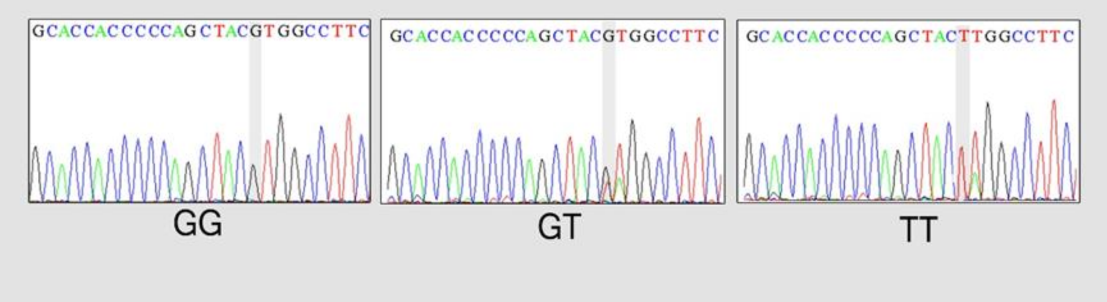

Figure (1): Genotypes resulting from the mutation at site 113G>T. HSPA6 . gene

It is evident from Table (4) that there is no significant effect of the mutation G>T.113 on the heat tolerance coefficient and all the studied milk production traits, noting the arithmetic superiority in the trait of the heat tolerance coefficient of the individuals carrying the TT genotype, which amounted to 2.64 compared to the individuals carrying the GG genotypes, GT (2.48 and 2.51, respectively), and this result was in contradiction to the result of the study of Liu et al. (2010) for the same gene, which indicated that there is a significant effect of these genotypes on the trait of heat tolerance coefficient.

From the same table below, it was also noted that the arithmetic superiority of the individuals carrying the GT genotype in the average daily and total milk production traits reached 15 and 5400 kg, respectively, compared to individuals with the genotype GG and TT. In the same context of simple arithmetic superiority, the GG genotype outperformed in the trait of average persistence in production, which amounted to 1.41% compared to the two genotypes GT and TT (1.39, 1.340%, respectively), while it was noticed that the TT genotype had an arithmetic superiority in both the trait of peak production and length The milk season averaged 538.5 kg and 304 days compared to the other two genotypes. The insignificance of the effect of this mutation on the heat tolerance coefficient can be explained by the fact that this genetic mutation is a point mutation, which is often attributed to adaptation to environmental conditions different from the homeland of the Holstein breed The original (Ahmed, 2020). Also, the reason for the absence of significant differences between the three genotypes in the characteristics of milk production (noting the presence of simple arithmetic differences) can be attributed to the large discrepancy in the number of observations between the genotypes.

Table (4) The effect of the genotypes of the G>T.113 mutation on the characteristics of milk production

season long (day)	perseverance (%)	Summit production (kg)	total milk production kg/season) (	daily milk production (kg/day)	Thermal Endurance Coefficient	the number	Mutation T / G
302.47 0.316±	1.413 0.018±	445.5 ±15.93	4782.24 207.3±	13.32 0.574±	2.48 0.03±	42	GG
302.60 0.871±	1.394 0.053±	509.4 52.8±	5400 440.88±	15.00 1.224±	2.51 0.10±	5	GT
304.5 0.5±	1.340 0.10±	538.5 172.5±	5220 1260±	14.50 3.50±	2.64 0.29±	2	TT

N.S	**N.S**	**N.S**	**N.S**	**N.S**	**N.S**	**49**	**morale level**

NS: insignificant

It can be concluded from all of the above that the mutation G>T.113 in the studied segment of the 6HSPA gene changed the code for the amino acid valine to the code for the amino acid leucine.

This mutation showed three genotypes, GG, GT and TT, which did not differ from each other significantly in the characteristics of milk production.

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