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drdof the genotype of HSP70B gene in some characteristics of the components of milk

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Abstract

The study was conducted at Taj Al-Nahrain station in Al- Diwaniyah Governorate, which Is approximately 200 km from the center of Dhi Qar Governorate, for the period from 10/2/2021 to 1/4/2022.And the laboratory part of the study was conducted In the Molecular Genetics Laboratory of the Marshes Research Center at Dhi Qar University. The study includeincludeed 49 Holstein cows Imported in their third productive season, Blood samples were drawn from them for the purpose of separating the genetic material (DNA) from It and studying the heat shock protein HSPA6 gene and knowing Its relationship to milk production characteristics In Holstein cows ,The results showed the nucleotide sequence on the 475bp gene segment extracted from the HSP70B gene the presence of the point mutation G>T.113 was detected In the third exon of the above gene In the cattle sample under study.

And the mutation discovered In the studied piece of the HSP70B gene was a missense mutation and It worked to change the code for the amino acid valine to private code With the amino acid lucine .Three

genotypes were shown: GG, GT, and TT.

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

Key words: genetic morphology, components of milk, HSP70B gene, Holstein cows.

I. INTRODUCTION

Cows are the main source of milk production among farm animals, as the livestock sector contributes to the agricultural economy effectively, as the contribution rate reached 90% of the total production In the world (FAO, 2009 and Sejian , 2016),Friesian cows, which are one of the most widespread breeds In the world One of the main sources of milk characteristics of economic importance, Including the characteristic of milk production, depend largely on the environment In which the cow Is raised ,From this aspect, we note that the change In the productive capacity of cows Is a combination of environmental changes on the one hand, and the genetic capacity of cows on the other hand, because milk production Is a quantitative trait controlled by a large number of genes, and the selection process Is one of the methods used to Improve this trait .Climate is one of the environmental factors that affect the agricultural production sector In general and the animal production sector In particular. It has been recently pointed out that the effects of climate are an element of threat denouncing the danger towards this sector as a result of climate warming (2009, FAO)

Iraq is located within the equatorial region, where temperatures reach between 46-47 degrees Celsius during the summer, according to what Is mentioned in local climatic reports, as climate change has significantly led to changes in temperature and precipitation and exacerbated global warming In the atmosphere, which led to Extensive climatic changes in the tropics and subtropics, and consequently, increased heat stress that affects all ecosystem ,Including farm animals In the region, as It ultimately affects the survival of different species, ecosystems and the sustainability of animal production systems around the world, especially In tropical and temperate countries, these high temperatures are usually associated with high humidity and the availability of poor quality forage in most parts of the country (International Panel on Climate Change at the World Food Organization) (IPCC, 2007).Organisms differ In their ability to withstand high temperatures In different species, resulting

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from differences in their genetic structures when referring to cows, we find that they are affected by high temperatures above 20 degrees Celsius, and when they reach the point of heat shock, It leads to a decrease In their productive performance to a large extent. (Liu and co., 2010).
The HSP70B protein (70 kDa) Is involved In the maintenance of cellular proteins (Heldens et al., 2010), and the 70HSP family plays an Important role in the cell's heat tolerance (Beckham et al., 2004).It is also characteristic of this family and Is stimulated after severe cellular stress (Noonan et al. 2007)It is also characteristic of this family and is stimulated after severe cellular stress (Noonan et al. 2007).HSP70B was Identified as having higher expression in cattle and goats under heat stress (Mohanarao et al. 2014) and this may be due to severe stress conditions causing HSP70B to evolve Into a gene that maintains basic biological functions (Hyder et al. 2017) Therefore, it can be used as a candIdate gene for breeding heat-resistant flocks (Hyder et al. 2011).

I. MATERIALS AND METHODS

This study was conducted at Taj Al- Nahrain station in Al- Diwaniyah Governorate, which Is approximately 200 km from the center of Dhi Qar Governorate, for the period from 10/2/2121 to 1/4/2022 throughout the experiment period, the feeding was uniform and at 6 kg of concentrated feed for each cow it was divided Into two morning and evening meals, and the feeding depended mainly on concentrated fodder consisting of wheat bran ,crushed barley, on the Calcine and salt In addition to hay and dried alfalfa who was presenting When green fodder Is scarce ,As for water, it was available In front of the animals around the clock, and 49 dairy cows were selected at the beginning of their productive seasons ,the cows were mechanically milked twice a day, in the morning and In the evening, and the daily production was recorded throughout the trial period for each cow In kg to know the daily milk production and to calculate the total milk production ,then the number of breathing times was measured by estimating the number of flank movements per minute and for each cow twice a week at 7 am and 3 pm, with an average of 8 readings per month for each cow. The rectal temperature of each cow was also measured twice a week at 7 am and 3 pm, with an average of 8 readings per month For each cow with a medical thermometer and a lily ,Blood samples were collected regularly from the jugular vein in the neck area every 30 days at seven o'clock in the morning before the morning meal, through a 10-ml wine syringe placed in clean, sterile wine plastic tubes and left to precipitate for an hour at laboratory temperature ,After that, the tubes were placed In a cooled cork at a temperature (4°C) for two hours, during which time they were transferred to the laboratory and placed in a centrifuge for twenty minutes at a speed of 3000 rpm for the purpose of separating the blood serum from the rest of the components ,the serum was used directly In conducting chemical and hormonal tests .The temperature and humidity were recorded twice a day at two o'clock in the afternoon and with sunrise throughout the experiment, and the measurement process was done through a thermometer (Hygro) of German origin that measures temperature and humidity at the same time.

The Initiators of the HSPA6 gene were prepared by the Korean company Macrogene In the form of a lyophilized powder of two separated primers, each of which is placed In a special tube with a label showing the sequence of nitrogen bases. The primers were prepared by adding 300 µL of distilled water to be the Initiator at a concentration of 100 bicamol this is considered stock solution and 10 microliters were taken from It and again add 90 microliters of dd Water At a concentration of 10 bicamol This is the concentration required to perform the PCR reaction The following table shows the dilution of the starters and the added quantities of dd Water:

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 (3 – 4): Materials used In PCR and their quantities

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Final size	Dd wather	Prefixes		template DNA Concentration (100 ng)	Master Mix	Chemical
		Reverse	forward			
35	9-12	1.5	1.5	2-5	18	Volume (microliter)

Table 3-5: Program for PCR technology for HSPA6 gene

Number of courses	Time (minutes)	Temperatures	Stages	Gene
1	5	95C°	Initial Denaturation	HSPA6
35	0.30	95C°	Denaturation	
	0.45	54 C°	Annealing	
	0.45	72C°	Extension	
1	10	72C°	Final Extension	

Acarose gel was prepared with the same previous steps that removed the DNA of the samples, but the concentration of agarose prepared for the migration of PCR product samples was 1.5% Where 0.45 g of agarose was added to 30 ml of TAE solution 1x focus,5 µL of PCR product was loaded with 2 µL of Dilute Diamond™ Nucleic Acid Dye Into each hole on the agarose gel A marked hole was used to load 5 microliters of DNA Ladder with the dye and set the migration program using 70 volts and 85 mA for 45 minutes and after the migration process of the PCR product Images were taken using a UV Gel Documentation device.

After confirming the volume of the specialized PCR product for the studied gene by comparing It with the standard DNA strand, 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 sanger sequencing technology and the base sequencing results were received and analyzed using BLAST tools from the NCBI International Gene Bank website with the use of some bioinformatics programs(Bioinformatics).

The data of the experiment were statistically analyzed using the ready-made statistical program SAS (2012) (Statistical Analysis System),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 means were compared using Duncan's polynomial test (Duncan, 1955) by applying the least squares mean (LSM) method.

Mathematical model:

$$Yij = \mu + Gi +$$

whereas :

Yij:View value j belonging to genetic makeup i.

µ: The general average of the adjective

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

Eij: Random error that Is normally distributed with a mean of zero and a variance of 2δ

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

I. RESULTS AND DISCUSSIONS

DNA extraction was carried out using the Promega Kit and according to the method Indicated in the separation of materials and methods, and clear packages of total DNA were obtained After that, the

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product was migrated from each sample and the migration product was photographed to detect the presence or absence of DNA as shown In Figure (1).

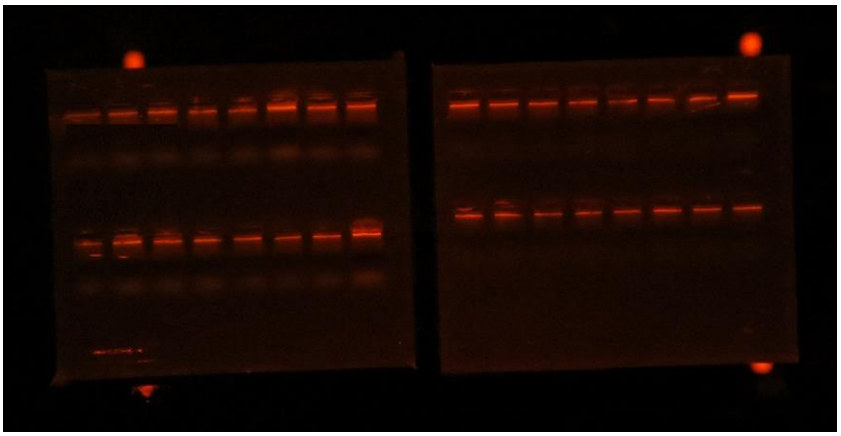

Figure (1): electric deportations of DNA genetic material after extraction

The segment to be studied was extracted from the HSPA6 gene with a size of 475 bp on exon 3 of the gene using special primers, and the segment was multiplied by Polymerase Chain Reaction technique and adjusting the program of the PCR Thermo cycler device, according to what was mentioned in the chapter on materials and working methods, and using a piece of known size (Ladder), placing the gel In the designated basin Inside the electrophoresis device filled with the bound solution, and the voltage has been adjusted ,And a photograph of the product of the electrophoresis to ensure the success of the extraction process for the target genetic segment, as shown in the figure(2) .

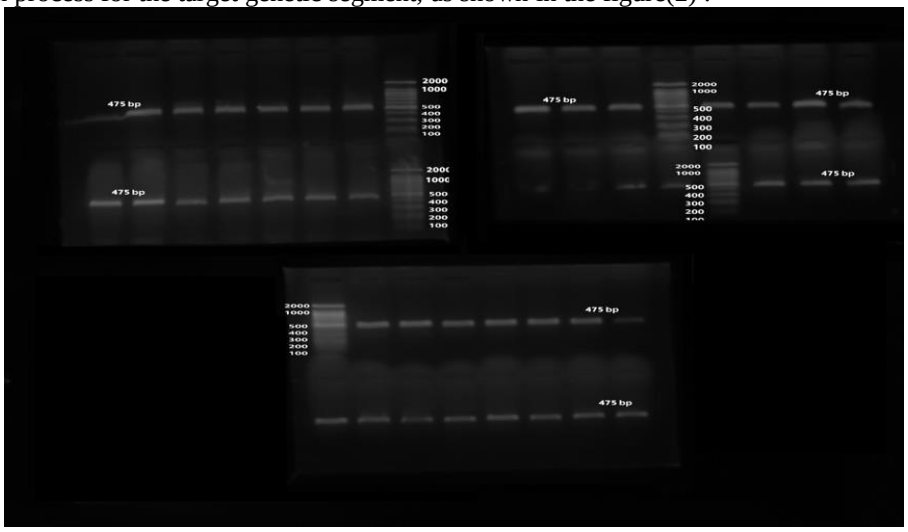

Figure 2: Electrophoresis of the PCR product of a segment of the 6HSPA gene with a size of 475 bp in Holstein cows.

It is clear from Table (3) that there are highly significant differences (0.01P>) In the percentages of genetic phenotypes of the 6HSPA gene for genetic heterogeneity (G>T.113),also, it was found that there Is a clear prevalence of pure Individuals carrying the GG genotype, which obtained the highest percentage of 85.71%,it was followed by the GT genotype with a percentage of 10.20%, while the TT genotype had the lowest percentage and amounted to 4.09%,with a night frequency of 0.91 for the G allele and 0.09 for the T allele, a result that differed from what was found by Baena et al. (2018) of the nightly frequency of several mutations ranging from 0.02 to 0.21.

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Table No. (3) Frequency of genotypes and frequency of alleles for the mutation G>T.113

Chi-square value X^2	Repetition Frequency	Allele	percentage(%)	number	Genetic makeup	Mutation
**25.36	0.91	G	85.71	42	GG	G>T.113
			10.20	5	GT	
	0.09	T	4.09	2	TT	
** p≤0.01	1.00	--	100%	49	--	total

The results of the statistical analysis of the characteristics of the milk components in Table (4) were similar to what was found in the characteristics of milk production in terms of the absence of a significant effect of the mutation G>T.113 on the characteristics of the milk components with the presence of simple mathematical differences In the characteristics of milk components between the wild, hybrid and mutant genotypes, where the cows with the GT genotype achieved the highest average for the characteristic of the percentage of fat In milk, which amounted to 3.466%, while the lowest percentage was (3.405%) for the TT genotype While the characteristic of non-fatty solids of milk components was the highest for Individuals with the genotype GG (7.283) and the lowest for individuals with the genotype TT (7.165%) the highest for Individuals with a genotype GG (7.283) and the lowest for individuals with a genotype TT (7.165%) .

Regarding milk density and lactose content In milk, the Individuals with the GT genotype showed the highest mean of the two traits (5 26.0 g/cm3 and 4.05%, respectively), while the lowest value for milk density was recorded for the individuals with the TT genotype, which amounted to 25.32 g/cm3 While the lowest value for the average percentage of lactose was recorded for Individuals with the GG genotype (3.97%), and the cows with the GG genotype were also mathematically superior to the average protein percentage and reached 2.63%, In contrast to the lowest in the cows with the TT genotype (2.54%). And when comparing with the very few sources available to us on the effect of this gene on the milk characteristics of cows, Liu et al. (2010) indicated in his study on a sample of Holstein cows for gene1. 70HSP Indicated that the presence of four SNPs In the UTR and different genotypes, including FF, DD and AB, gave higher milk fat content, protein content and higher milk yield, respectively.

Similar to genes In the 70HSP family, HSPA6 may also function under severe heat stress conditions, which was indicated by Mohanarao et al., (2013) when they observed a 14.4-fold increase in expression of this gene when goats were exposed to high temperatures ,the polymorphism within the heat shock protein genes contributes to some susceptibility to disease and stress tolerance in European dairy cattle .Some mutations can change the translation pattern, protein folding and/or function, and It depends on the mechanism of the codon using the translation rate by Introducing pauses caused by non-optimal (rare) codons by introducing changes in the mRNA structure that affect the Folding and Co-translation (Bartosziwesky et al., 2016).Although SNPs In some genes do not change the amino acid sequence, they may be regulatory and play an Important role in the coding region of HSP genes, affecting the peptide-binding mechanism or affinity of HSP proteins (Baena et al., 2018).According to Curi (2004), mutations can cause protein dysfunction and consequently phenotypic variation. Therefore, this mutation In our current study could be Important for the formation, function, and expression of HSP proteins for future studies to develop MAS (Markers Assist Selection) strategies for heat stress In breed breeding programs holstein .

In general, It can be concluded that It is impossible to Identify a particular gene so that It has the only effect In its effect on a trait, due to the contribution of several genes, albeit In a small amount, to the emergence of measures of economic trait (Supakorn, 2009) . accordingly, we can attribute the

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disappearance of significance in the current study to the different superior interactions between the quantitative trait sites or because of the experimental design and statistical analysis followed (Curi et al., 2006) .

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

Protein ratio%	Freezing point of milk (C0)	Lactose ratio%	density g/cm3	Non - greasy solids%	Fat percentage %	number	Mutation G/ T
2.634 0.017±	-0.66 0.012±	3.975 0.027±	26.04 0.271±	7.283 0.066±	3.455 0.026±	42	GG
2.628 0.04±	-0.676 0.037±	4.056 0.039±	26.048 0.653±	7.250 0.077±	3.466 0.104±	5	GT
2.545 0.125±	-0.623 0.099±	4.02 0.38±	25.32 1.32±	7.165 0.475±	3.405 0.115±	2	TT
N.S	N.S	N.S	N.S	N.S	N.S	49	Morale level

NS: non incorporeal

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 (Lucine).

This mutation showed three genotypes, GG, GT they did not differ from each other spiritually In the characteristics of the components of milk.

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