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RELATIONSHIP OF THE GENETIC POLYMORPHISM OF TWO POINT MUTATIONS WITHIN HSP70 GENE WITH THE TRAITS OF MILK AND THE THERMAL TOLERANCE OF THE HOLSTEIN COWS IN IRAQ

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Abstract

The current study was conducted at Taj Al-Nahrain Cows Station, located in Al-Qadisiya Governorate, Diwaniya District, which is 180 km from the center of Baghdad for the period (19/3/2018 to 20/3/2019). HSP70 gene was analyzed Nitrogen base reading were conducted and the relationship between the two point mutations with the traits of milk (daily milk lection period, peak production, persistence on production) and milk components (fat ratio, non-fat solids, density, protein ratio, lactose content) and the Heat Tolerance Coefficient represented (summer Heat Tolerance Coefficient, autumn Heat Tolerance Coefficient, winter Heat Tolerance Coefficient and Heat Tolerance Coefficient General). The results show the presence of point mutations (T825C and A826G) The first mutation T825C appeared in three genotypes TT = 3 TC = 50 CC = 6 As for the second mutation A826G appeared in two genotypes AA = 54 AG = 5 the first mutation (T825C) had no significant effect on milk production Characteristics , milk components, but its effect was significant in the Heat Tolerance Coefficient for summer and general , as individuals carrying TC genotypes outperformed individuals carrying pure genotypes TT - CC. The second mutation (A856G) did not have a significant effect on the traits of milk production and the Heat Tolerance Coefficient, but it had a significant effect on the milk components, as the individuals carrying the hybrid formulation (AG) overtook the individuals carrying the wild composition (AA) in the ratio of fat, non-fat solids, and protein. And the ratio of lactose.

***Keywords*:** Holstein cows, milk production, HSP70 gene.

Introduction

Heat Stress is one of the clear phenomena that weigh down and its negative effects on domestic animals in general and dairy cattle in particular, which results from the abnormal rise in temperatures, as this rise is one of the main determinants of productive and physiological performance (Bohmanova *et al.*, 2007) and due to the intensive election of an increase milk production, which clearly improved production and deteriorated the traits of the animal's heat tolerance due to the inverse relationship between them, so dairy cattle became more sensitive to heat stress (Miglior *et al.*, 1995) . The animals that are most resistant and acclimatized to climatic changes and different stress conditions, because they have some molecular mechanisms that are able to protect cells from the effects of heat stress, among the most important of these molecular mechanisms that the body possesses are the family of heat shock proteins (HSPs), especially the heat shock proteins HSP70, which have a very large role in protecting and maintaining cells when exposed to different stress conditions (Guerriero *et al.*, 2004). Because the HSP70 heat shock protein gene is one of the most important mechanisms for stress management in the body, its genetic diversity is directly and closely related to the ability of animals to tolerance heat stress conditions in cows (Bhat *et al.*, 2016). The research aims to uncover the relationship between the genetic manifestations of some mutations in the HSP70 gene in the sample of the Holstein cows, extract the distribution ratios of those manifestations and night allergies to them and link them with the characteristics of milk production and its components and the Heat Tolerance Coefficient.

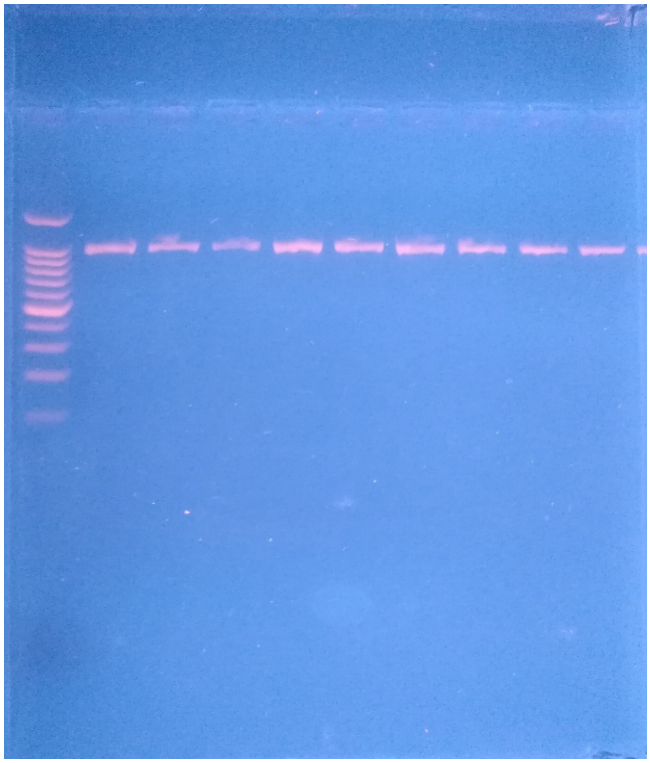
Materials and Methods

Experimented animal seventy five cows from Holstein cows imported from Germany were randomly chosen from among the 700 cows in the station. Molecular genetic analyzes were performed in the biotechnology laboratory of the Department of Animal Production / College of Agricultural Engineering Sciences / University of Baghdad for the period from 1/3/2019 to 1/9/2019 for the purpose of separating the genetic material (DNA), conducting the electrophoresis and analyzing the PCR chain reaction of the HSP70 thermal shock gene. The data on milk production were collected from the station's records my weekly milk production is recorded for the morning and evening arenas from the station, Ten ml of milk was drawn from each cow with plastic tubes for three periods. The milk components were analyzed at the Alban Abu Gharib Company / Research and Development division of the device (Milk Analyzer). Heat Tolerance Coefficient (HTC) was calculated according to the following equation (Benezra 1954) HCT = RR/23 + TR/38.3) (RR) Cow respiratory rate (TR) Cow rectal temperature (23) Cow natural respiration rate (38.33) represents the ideal rectal temperature of the cows . Blood samples were drawn from the udder vein 2.5 ml using test tubes of a volume of 5 ml container on anticoagulant Di - amine Tetra acidic acid Ethylene (EDTA). After the withdrawal the blood samples were frozen at (-18)Cº for DNA extracting Kit FAVORGEN Taiwanese origin .

Table 1 : Primers designed with a length of 922 Nitrogenous bases starting from the site (433-1355) of the HSP70 gene in Holstein cows

(Seq- Primer)	Primer Name	Amplicon size (pb)	name gene	Annealing temp Cº
F- GCCTGGAGAGAGCTGATAA	Primer–F	922 pb (433…1355)	HSP70	65º
R- CCTTCTTGTGCTTCCTCTTG	Primer-R			

After that, the studied segment of the HSP70 gene was revealed using the technology of polymerase chain reaction (PCR). After the reaction was completed, the polymerization reaction result is transmitted to ensure the presence of PCR products using the same method of preparing the carouse gel in the DNA transfer as the known molecular weight DNA is loaded DNA Ladder Marker (100 -1500) Nitrogenous base in the first hole of the gel mold and then load the PCR output by 5 micro liters in drilling the gel mold on (70 volts for a period of 90 minutes) to see the packages with the UV Light Transillminator and photograph these packages with a special camera as The packages appear as an orange pigment, with an ethyl bromide color .



Picture 1 : Depicts the electrophoresis of the studied piece of the HSP70 gene

After the electrophoresis, 20 microliter PCR output was sent to Macrogen Corporation-Korea. After obtaining the results by e-mail, Genious Software was used to analyze the results on the global website of the Genebank www.ncbi.nlm.nih.go. A file was used, Nucleotides are followed to determine the presence or absence of mutation and curves file to determine the phenotypic multiplication of the two HSP70 gene mutations. The data were statistically analyzed using the SAS (Statistical Analysis System) (2010) to study the relationship of HSP70 genotypes in milk production traits and components, and Heat Tolerance Coefficient. Significant differences among means test were performed using Duncan multiple rang test (Duncan, 1955).

$$Yijm = \mu + G_i + eij$$

Yij = viewing value of genotype

i = the general average of the studied traits μ

G_i = the effect of simple polymorphism HSP70 gene on the studied traits .

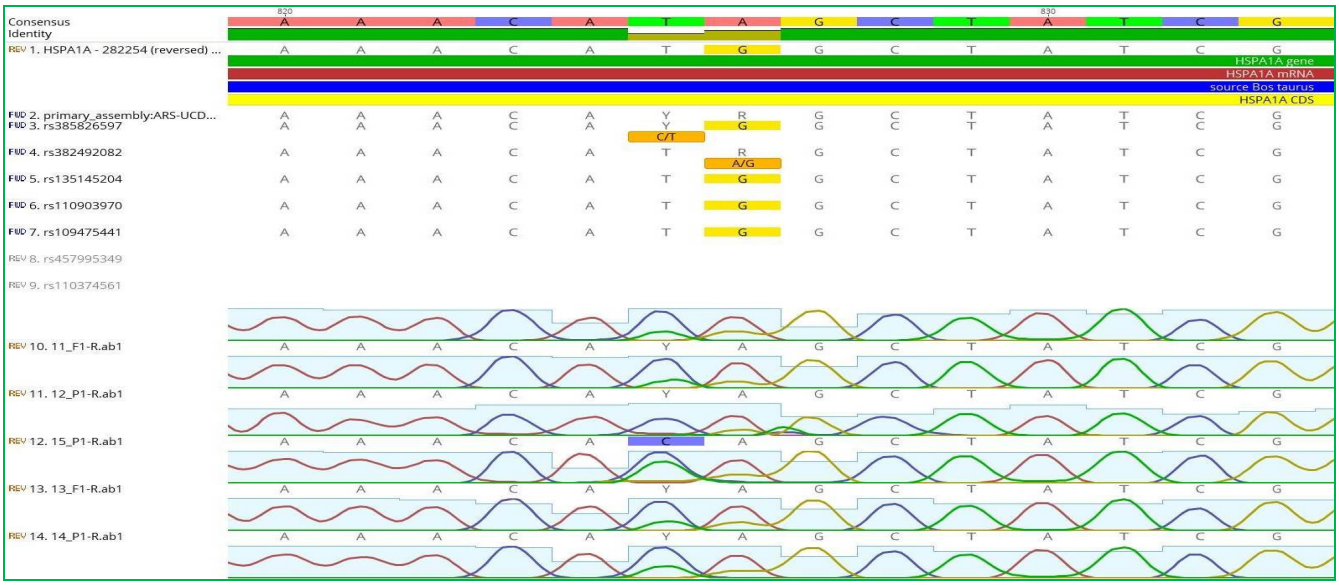
eij random error, which is assumed to be random and natural distribution with an average of zero and the variance of $\sigma^2 e$.

Results and Discussion

In the present research, the point mutations in the studied sample of a segment of the HSP70 gene consisting of 922 bp were detected. When analyzing the sequence of Nitrogenous bases, two first point mutations (rs385826597) (T825C) were detected with three wild genotypes TT and hybrid TC and the CC mutant led to a change in the amino acid from Alanine to Isoleucine and the second rs382492082 (A826G) with two genotypes wild AA and hybrid GA resulted in the change of the amino acid from Threonine to Aspartic acid. Table (2) shows the number of genotypes, percentages, and nocturnal repetitions of the two HSP70 genes, as it is observed in the first mutation (T825C) that the percentage of individuals carrying TC (84.8%) significantly exceeded significantly in both wild pure genotypes TT (5.1%) the CC mutant (10.1%) also showed that the recurrence of the wild allele T was 0.74, while the mutant allele C was 0.53 and no significant effect was shown for the nocturnal repetition. As for the second mutation (A826G), two genotypes are observed resulting from this mutation, while the third genotype did not show the small sample size or the receding allele G, as the percentage of individuals carrying the pure genotype AA (91.5%) was superior to the hybrid genotype AG (8.5%) a significant increase in the frequency of the wild allele A was observed, as it appeared by 0.96 compared to the allele G that showed 0.04. In a study by Basirico *et al.* (2011) to study some mutations of the HSP70 gene, heterogeneity G / T was found with one genotype = 446 GG and CD deletion mutation in three genotypes: CC = 298 CD = 128 D = 20 and allele frequency C = 81.2 and D = 18.8

Table 2: Number and percentages of genotypes and frequency of two point mutations in the HSP70 gene in Holstein cows

Allele Frequency	Percentages	Number	Genetic Composition	Genetic Variation
T=0.47 C=0.53 (N.S)	5.1	3	TT	T/C
	84.8	**50**	TC	
	10.1	6	CC	
	The value of the Chi square for variance C/T χ^2=27.20**			
A=0.96 G=0.04	91.5	**54**	AA	A/G
	8.5	**5**	AG	
(p≤0.01)	The value of the Chi square for variance A/G 8.59** χ^2=			



Picture 2: Location of the T/C and A/G point mutations in the HSP70 gene in Holstein cows

The results of the statistical analysis, table (3) showed that there was no significant effect of genetic variation (T825C) and genetic variance (A856G) on milk traits represented (daily milk production, total milk production, production peak, persistence on production and length of production season), and the reason can be attributed to the lack of the presence of a significant difference, noting the presence of mathematical differences to the large variation in the number of observations between genotypes.

Table 3 : Effect of genotypes on milk production for the first part

Length of production season/ day	persistence on production %	production peak kg/month	Total milk production kg/season	Daily milk production kg/day	Number 59	Traits T/C
280±36.05	1.46±0.17	420±91.65	4499±996.18	1.99 ± 15.75	3	TT
294.60±5.33	1.47±0.08	352.20±14.41	4064.96±150.23	13.67±0.40	50	TC
310±6.32	1.23±0.25	355±44.77	4178.70±469.38	13.41±1.38	6	CC
Genotype A/G						
296.11±5.01	1.44±0.08	357.22±13.69	4152.32±144.12	13.91±0.38	54	AA
288±20.34	1.56±0.15	342.0±68.80	3518.34±578.01	12.04±1.62	5	AG

It is clear from table (4) that there was no significant effect of the first mutation (T825C) on the milk components (fat ratio, non-fat solids, density, protein ratio, lactose ratio) , while the second mutation (A826G) had a significant effect as the individuals carrying the composition excelled genetic hybrid AG on pure genotypes AA in the fat ratio (4.19-3.06%), non-fat solids (8.11 - 7.41)%, protein ratio (2.91 - 2.68)%, and lactose ratio (4.40, 4.06) % , in the Li *et al* (2011) study on the HSP70 gene in Chinese Holstein cows found that individuals with AA-AB genotypes did not significantly differ in milk fat rate (3.90, 3.75) % , protein milk (3.14 and 3.21), while the C/ D mutation, which was distributed in three genotypes CC - (CD - DD), affected a significant effect on the fat percentage (4.11, 4.10, and 3.85) and did not affect the protein ratio (3.13, 3.06, 3.06) %

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Table 4: Effect of genotypes on milk components

Lactose ratio %	Protein ratio %	Density %	Non-fat solids %	Fat ratio %	Number 59	Traits T/C
4.17±0.14	2.76±0.08	27.83±0.21	7.75±0.25	2.43±0.25	3	TT
4.10±0.05	2.70±0.03	26.84±0.39	7.48±0.09	3.18±0.15	50	TC
4±0.22	2.63±0.14	27.92±1.68	7.31±0.43	3.27±0.48	6	CC
genotype A/G						
4.06±0.04 **b**	2.68±0.03 **b**	26.91±0.39	7.41±0.08 **b**	3.06±0.14 **b**	54	AA
4.40±0.18 **a**	2.91±0.11 **a**	27.92±1.01	8.11±0.30 **a**	4.19±0.56 **a**	5	AG

Averages with different letters within one column differ significantly with each other at a significant level (P≤0.05)

Results represented in table 5 showed the presence of the effect of the genotype of the mutation (T825C) on the Heat Tolerance Coefficient, as a significant difference was observed between the individuals carrying the genotypes in favor of the hybrid individuals (TC) in the characteristic of the Heat Tolerance Coefficient in summer and the general Heat Tolerance Coefficient on both pure genotypes. Wild (TT) and mutant (CC) (2.70, 2.59, 2.58) (2.29, 2.24, 2.24) respectively,

while there was no significant effect of the second boom (A826G) on the Heat Tolerance Coefficient for summer, autumn and winter and the general Heat Tolerance Coefficient, the low Heat Tolerance Coefficient is more resistant to conditions of heat stress and since heat tolerance is one of the quantifiable traits that are heritable trait. Breeders should focus on this trait and elect cows with a low Heat Tolerance Coefficient to enhance animal efficiency in conditions of heat stress in Iraq .

Table 5 : Shows the effect of genotypes on the Heat Tolerance Coefficient

General Heat Tolerance Coefficient	**Heat Tolerance Coefficient for Winter**	**Heat Tolerance Coefficient for Autumn**	**Heat Tolerance Coefficient for Summer**	**Number** 59	**Traits T/C**
2.24±0.03 **b**	1.94±0.02	2.20±0.08	2.58±0.02 **b**	3	TT
2.29±0.01 **a**	1.93±0.01	2.26±0.01	2.70±0.02 **a**	50	TC
2.24±0.02 **b**	1.90±0.02	2.25±0.05	2.59±0.07 **b**	6	CC
genotype A/G					
2.29±0.01	1.93±0.01	2.26±0.01	2.68±0.02	54	AA
2.29±0.04	1.90±0.03	2.27±0.08	2.72±0.07	5	AG

Different letters within the same column indicate a significant difference at the level ($P \leq 0.05$).

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