Diyala Agricultural Sciences Journal, 2024, Vol. (16) No 1: 88-105
ISSN: 2073-9524
eISSN: 2310-8746

Study of HSP70 Gene and Relationship to Milk Productivity and Fat Ratio Holstein Cows

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Article history:

Received: 14 November 2023
Accepted: 20 April 2024
Published: 30 June 2024

Keywords: *Holstein cows Breeding value, Dominance effect, Mutations.*

Abstract

The study was conducted on 75 Holstein cows imported from Germany to determine the chi-square value, the breeding value, the additive and dominance effect, and the effect of the allele of the HSP70 gene on the variation of total milk production and fat ratio. The HSP70 gene was divided into three bands, each with a length of 922, 935 and 932 bp. Molecular analysis of the gene shows the presence of 12-point mutations distributed in three primers (T825C, G856A, G937A, A1135G, G1185C, G1753A, C1867T, A2017G, C2548T, G2572T, G2688C and T2734C). Mutation T825C changed the amino acid Ata to Lie, and mutation G856A changed the amino acid Aca. to Thr. The result showed that the chi-square value was significant in the mutations (C1867T, A2017G and G2688C) reached (4.25, 3.90 and 4.16). The higher milk production in the CT genotype of the C1867T mutation was 5442.7 kg/season, the higher breeding value, dominance deviation, additive and dominance variance (652.3, 1460, 726856 and 235183) respectively for total milk production. The higher ratio of fat in the CT genotype of the C2548T mutation was (5.04%), and the higher breeding value, dominance deviation, additive and dominance variance (1.30, 1.70, 0.576 and 0.0793), respectively for the ratio of fat.

https://dx.doi.org/10.52951/dasj.24160108

Introduction

Stress heat is one of the well-defined phenomena that weigh heavily and negatively on the shoulders of agricultural animals in general and milk cows in particular, which results from an abnormal temperature rise, as this rise is one of the main determinants. of productive and physiological performance (Bohmanova *et al*., 2007), The HSP70 heat shock protein gene is the most critical mechanism for stress tolerance in the body, and the genetic diversity of the HSP70 gene is related to the ability of animals to withstand heat stress conditions (Bhat *et al.*, 2016). The HSP70 gene is located on chromosome 23 in cows, as well as, the length of the gene is (3126) bp and contains only one exon with a length of (1926) bp. The HSP70 gene analyses and terminates cellular proteins that have been greatly affected by stress and that cannot be repaired (Mayer and Bukau, 2006). The HSP70.1 protein is encoded by the HSP70.1 gene, also known as HSP70A1A, which is located at position 1323 in the bovine genome (NCBI, 2017). Raza *et al*. (2021) found that the HSP70 gene is located on chromosome 23 of cows, zebu cows, and goats. It is nonnucleus and contains one exon, and that HSP70 encodes 641 amino acids. In goats, the HSP70 protein contains seven variable amino acid residues. In sheep and cows, only one amino acid was detected, and HSP70 contains 1926 nitrogenous bases. Younis (2020) showed that the gene expression pattern of HSP70 and HSP90 can be used as a reference point in breeding in order to identify, manipulate, and crossbreed, to improve genetic potential. The intensive selection increases milk production but deterioration in the thermal tolerance of the animal due to the presence of an inverse relationship between them, so the milk cows became more sensitive to heat stress (Miglior *et al.*, 1995). Estimating breeding value using genetic markers will increase the genetic yield as a result of reducing the generation ranges, increasing genetic accuracy, and lower cost (Al-Zaidi, 2022). The study genetic variation and genotypes that play a role in raising heat tolerance in cows and thus obtaining animals that can withstand high temperatures better (Sejian and Scientist, 2017), The opportunity to select animals with a good ability to withstand stressful temperatures, by using genes that are related to raising heat tolerance, and thus heat-tolerant animals are selected in high-production breeds that must retain and inherit this high productivity ability when exposed to conditions of heat stress (Das *et al*. 2016).

This study aims to identify the mutations of the HSP70 gene in a sample of the genetic material of Holstein cows, extract the distribution ratios of phenotypes, allelic frequencies and genetic markers, and link them with total milk production and fat percentage.

Materials and Methods

Ethics Approval

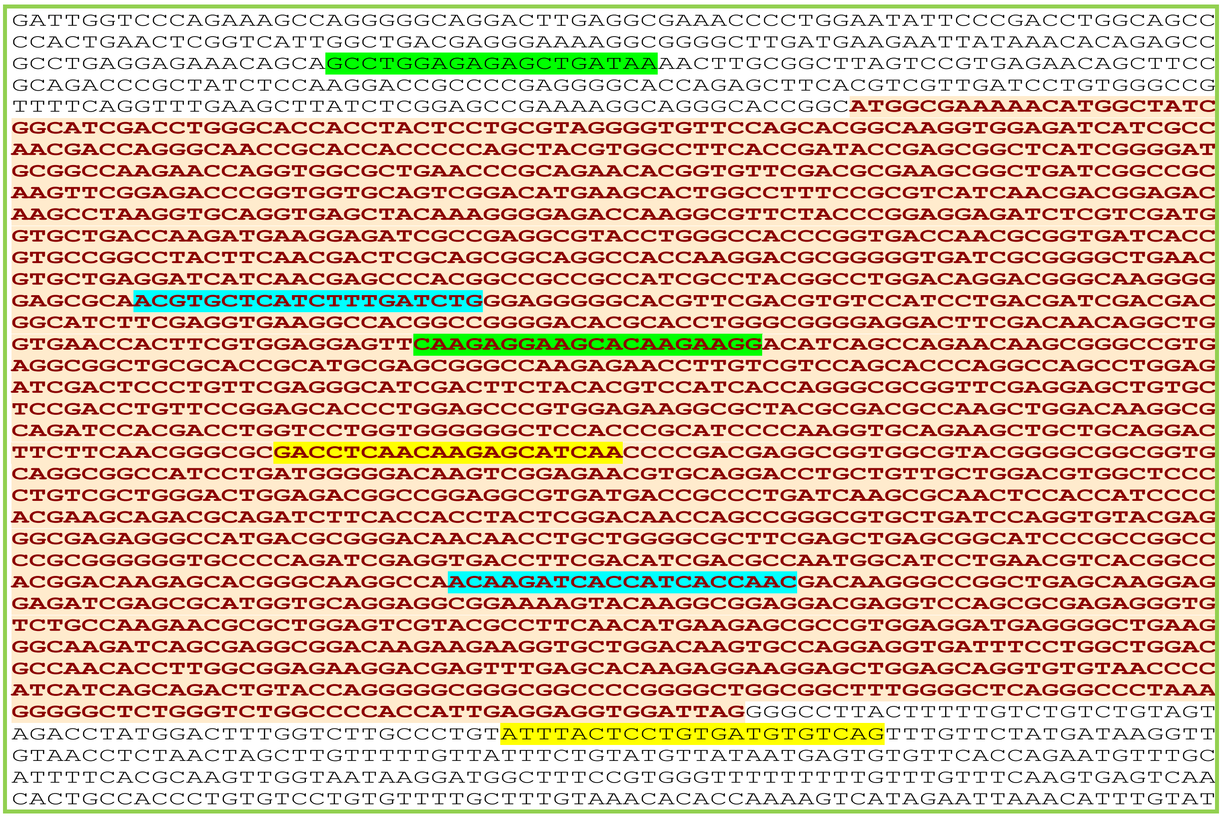
The Scientific Ethical Committee of the Department of Animal Production, College of Agriculture, University of Diyala, approved this study. All applicable national and international guidelines for the care and use of animals were followed.

Design of Experiment

This study was conducted at the Taj Al-Nahrain cow station in Al-Qadisiyah Governorate, Al-Diwaniyah District, The cows used in the experiment were 75 Holstein cows imported from Germany. Molecular genetic analyzes. were conducted in the Biotechnology Laboratory of the Department of Animal Production, College of Agricultural Engineering Sciences, University of Baghdad. Blood was drawn from the udder vein, the blood was collected in anticoagulant tubes, and the samples were frozen at a temperature of -20. to extract (DNA) FAVORGEN Taiwanese and conducting electrophoresis and PCR analysis of the Heat shock gene HSP70 Taiwanese, The gene was designed into three pieces by the Scientific Advancement Company, Baghdad Table 1.

Table 1. Show the cutting primers used in the study

First piece is 922 bp	Start	Stop	Length bp	Tm	GC%
Forward GCCTGGAGAGAGCTGATAA.	433	452	19	60	52.6
Reverse CCTTCTTGTGCTTCCTCTTG	1335	1355	20	61	50
Second piece is 935 bp					
Forward ACGTGCTCATCTTTGATCTG.	1181	1201	20	60	45
Reverse GTTGGTGATGGTGATCTTGT	2096	2116	20	60	45
Tthird piece is 932 bp					
Forward GACCTCAACAAGAGCATCAA	1672	1692	20	60	45
Reverse CTGACACATCACAGGAGTAAAT.	2582	2604	22	60	40.9



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GATTGGTCCCAGAAAGCCAGGGGGCAGGACTTGAGGCGAAACCCCTGGAATATTCCCGACCTGGCAGCC
CCACTGAACTCGGTCATTGGCTGACGAGGGAAAAGGCGGGGCTTGATGAAGAATTATAAACACAGAGCC
GCCTGAGGAGAAACAGCAGCCTGGAGAGAGCTGATAAAACTTGCGGCTTAGTCCGTGAGAACAGCTTCC
GCAGACCCGCTATCTCCAAGGACCGCCCCGAGGGGCACCAGAGCTTCACGTCGTTGATCCTGTGGGCCG
TTTTCAGGTTTGAAGCTTATCTCGGAGCCGAAAAGGCAGGGCACCGGCATGGCGAAAAACATGGCTATC
GGCATCGACCTGGGCACCACCTACTCCTGCGTAGGGGTGTTCCAGCACGGCAAGGTGGAGATCATCGCC
AACGACCAGGGCAACCGCACCACCCCCAGCTACGTGGCCTTCACCGATACCGAGCGGCTCATCGGGGAT
GCGGCCAAGAACCAGGTGGCGCTGAACCCGCAGAACACGGTGTTCGACGCGAAGCGGCTGATCGGCCGC
AAGTTCGGAGACCCGGTGGTGCAGTCGGACATGAAGCACTGGCCTTTCCGCGTCATCAACGACGGAGAC
AAGCCTAAGGTGCAGGTGAGCTACAAAGGGGAGACCAAGGCGTTCTACCCGGAGGAGATCTCGTCGATG
GTGCTGACCAAGATGAAGGAGATCGCCGAGGCGTACCTGGGCCACCCGGTGACCAACGCGGTGATCACC
GTGCCGGCCTACTTCAACGACTCGCAGCGGCAGGCCACCAAGGACGCGGGGGTGATCGCGGGGCTGAAC
GTGCTGAGGATCATCAACGAGCCCACGGCCGCCGCCATCGCCTACGGCCTGGACAGGACGGGCAAGGGG
GAGCGCAACGTGCTCATCTTTGATCTGGGAGGGGGCACGTTCGACGTGTCCATCCTGACGATCGACGAC
GGCATCTTCGAGGTGAAGGCCACGGCCGGGGACACGCACCTGGGCGGGGAGGACTTCGACAACAGGCTG
GTGAACCACTTCGTGGAGGAGTTCAAGAGGAAGCACAAGAAGGACATCAGCCAGAACAAGCGGGCCGTG
AGGCGGCTGCGCACCGCATGCGAGCGGGCCAAGAGAACCTTGTCGTCCAGCACCCAGGCCAGCCTGGAG
ATCGACTCCCTGTTCGAGGGCATCGACTTCTACACGTCCATCACCAGGGCGCGGTTCGAGGAGCTGTGC
TCCGACCTGTTCCGGAGCACCCTGGAGCCCGTGGAGAAGGCGCTACGCGACGCCAAGCTGGACAAGGCG
CAGATCCACGACCTGGTCCTGGTGGGGGGCTCCACCCGCATCCCCAAGGTGCAGAAGCTGCTGCAGGAC
TTCTTCAACGGGCGCGACCTCAACAAGAGCATCAACCCCGACGAGGCGGTGGCGTACGGGGCGGCGGTG
CAGGCGGCCATCCTGATGGGGGACAAGTCGGAGAACGTGCAGGACCTGCTGTTGCTGGACGTGGCTCCC
CTGTCGCTGGGACTGGAGACGGCCGGAGGCGTGATGACCGCCCTGATCAAGCGCAACTCCACCATCCCC
ACGAAGCAGACGCAGATCTTCACCACCTACTCGGACAACCAGCCGGGCGTGCTGATCCAGGTGTACGAG
GGCGAGAGGGCCATGACGCGGGACAACAACCTGCTGGGGCGCTTCGAGCTGAGCGGCATCCCGCCGGCC
CCGCGGGGGGTGCCCCAGATCGAGGTGACCTTCGACATCGACGCCAATGGCATCCTGAACGTCACGGCC
ACGGACAAGAGCACGGGCAAGGCCAACAAGATCACCATCACCAACGACAAGGGCCGGCTGAGCAAGGAG
GAGATCGAGCGCATGGTGCAGGAGGCGGAAAAGTACAAGGCGGAGGACGAGGTCCAGCGCGAGAGGGTG
TCTGCCAAGAACGCGCTGGAGTCGTACGCCTTCAACATGAAGAGCGCCGTGGAGGATGAGGGGCTGAAG
GGCAAGATCAGCGAGGCGGACAAGAAGAAGGTGCTGGACAAGTGCCAGGAGGTGATTTCCTGGCTGGAC
GCCAACACCTTGGCGGAGAAGGACGAGTTTGAGCACAAGAGGAAGGAGCTGGAGCAGGTGTGTAACCCC
ATCATCAGCAGACTGTACCAGGGGGCGGGCGGCCCCGGGGCTGGCGGCTTTGGGGCTCAGGGCCCTAAA
GGGGGCTCTGGGTCTGGCCCCACCATTGAGGAGGTGGATTAGGGGCCTTACTTTTTGTCTGTCTGTAGT
AGACCTATGGACTTTGGTCTTGCCCTGTATTTACTCCTGTGATGTGTCAGTTTGTTCTATGATAAGGTT
GTAACCTCTAACTAGCTTGTTTTTGTTATTTCTGTATGTTATAATGAGTGTGTTCACCAGAATGTTTGC
ATTTTCACGCAAGTTGGTAATAAGGATGGCTTTCCGTGGGTTTTTTTTTGTTTGTTTCAAGTGAGTCAA
CACTGCCACCCTGTGTCCTGTGTTTTGCTTTGTAAACACACCAAAAGTCATAGAATTAAACATTTGTAT
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Figure 1. Distribution of primers designed to study heat shock gene HSP70 in Holstein cows

After that, the studied segment of the HSP70 gene was detected using the polymerase chain reaction (PCR) table 2 technology, and after the completion of the reaction, the product of the polymerase reaction was carried over to ensure the presence of PCR products, using the same method of preparing the agarose gel in the transfer of the DNA, as the DNA known molecular. weight (DNA Ladder Marker Promega USA (100-1500) bp is loaded. 1500 bp nitrogenous bases in the first hole of the gel template, then load the PCR output by 5 microliters into the hole of the gel template at (70 volts for 90 minutes) so that the bands are viewed.by a UV Light Transilluminator and these packages are photographed .with a special camera. The bands are stained with ethidium bromide orange.

Table 2. Reaction conditions for the three studied fragments of the HSP70 gene using PCR technology, according to temperature and number of cycles

Stages	Temperature	Time	Number of cycles
Initial Denaturation	95	5 min	5
Denaturation	95	30 sec	30
Annealing	65	30 sec	
Extension	72	2 min	
Final Extension	72	10 min	1
Hold	4	-	-

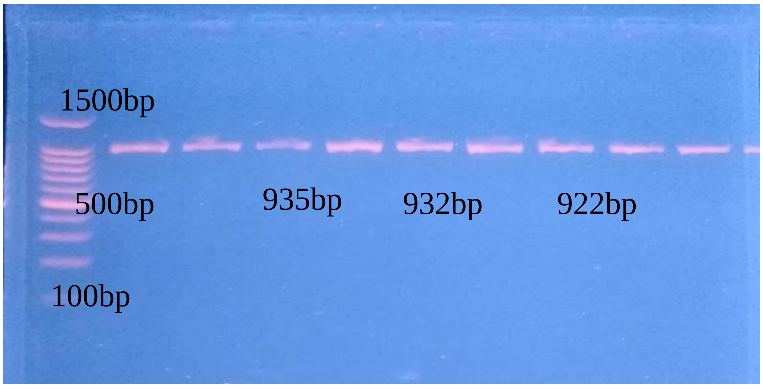

Figure 2. Electrophoresis of the three pieces of the HSP70 gene

After electrophoresis, a 20-microliter PCR product was sent to Macrogen, Korea. After obtaining the results by e-mail, Genious Software was used to analyze. the results on the global website of the Gen bank https://www.ncbi.nlm.nih.gov/. The nucleotide sequence file was used to determine the presence or absence of the mutation and the profile of the curves to determine the polymorphism of the mutations of the HSP70 gene.

To calculate the allelic frequency according to the Hardy-Weinberg rule (Falconer and Mackay, 1996).

First allele frequencies:

2 x No. of Homozygous + No. of heterozygous

PA= 2x total number of samples

$p + q = 1$

So the second allele

$qB = 1 – PA$

PA=Allele frequency A qB=Allele frequency B

The chi-square test (Ch-square - $\chi 2$) was used to compare the percentages of the distribution of genotypes for each mutation also used to compare the rates of the distribution of alleles.

The laws for each computed value were applied (Falconer. and Mackay, 1996) as follows:

1. Mean effect of A allele: $q[a+d(q-p)] = \alpha A= \alpha 1$

   Mean Effect of B Allele: $\alpha 2=\alpha B=-p\ [a+d(q-p)]$

   The replacement mean is the difference between the two values. $\alpha = \alpha 1-\alpha 2$

2. The breeding values were as follows:

   $\alpha 1$, $AB= \alpha 1+\alpha 2$, $BB= 2\alpha 2$ $AA= 2$

3. Dominance deviations:

   $AA= -2q^2d$, $AB= 2pqd$, $AA= -2p^2d$

4. The different contrasts were. extracted as follows:

   α^2, $VD= 4p^2q^2d^2$, $VG=VA+VD$ $VA=2pq$

p and q: frequency of alleles A and B, respectively, a: genotype value for genotype AA.

d: genotype value for genotype Aa.

VG = genetic variance, VA = additive variance, VD = dominance variance

Results and Discussion

In the current study, the largest part of the heat shock gene HSP70 (2171bp) was covered by dividing the gene into three bands (922, 932 and 935 bp) with 12 point mutations, as shown in Figure 3. These genetic mutations are caused by mutations Point, which is often attributed to adaptation to different environmental conditions from the original.home of the Holstein breed.

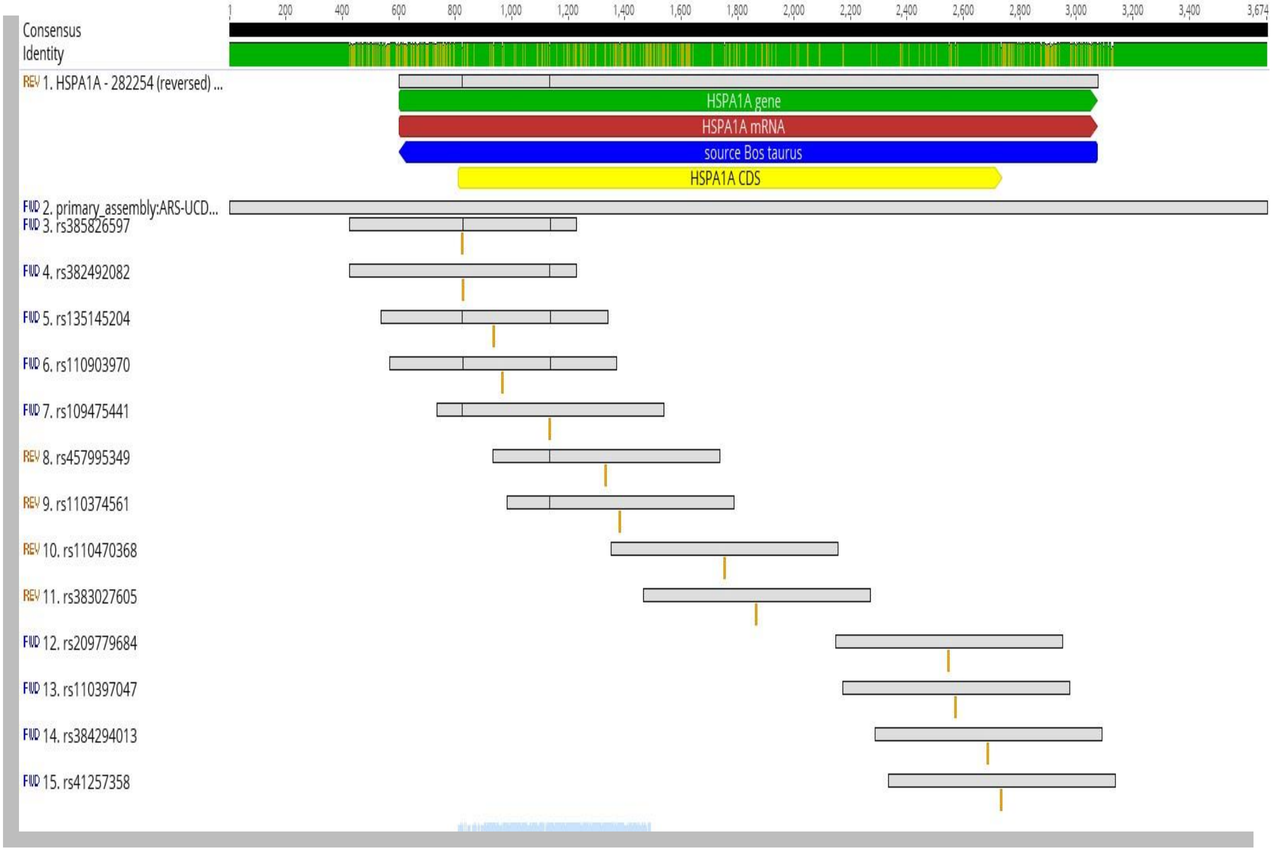

Figure 3. Location of point mutations of the HSP70 gene in Holstein cows

Table 3 Through searching on the Internet and scientific research, it was found that the mutations that were studied in this research on the HSP70 gene in Holstein cows are all registered with an identification number in the NCBI gene bank and in (ensemble), the T825C mutation changed the amino acid Ata. to the amino acid .Lie and the G856A mutation changed the amino acid Aca. to the amino acid Thr. The G2688C mutation changed the amino acid Gly. to the stop codon, while the other mutations are all silent and did not change the amino acid. The number of cows in the first piece was 59, in the second 69, and the third 53. Significant chi-square value ($P<0.05$) in the mutations (C1867T, A2017G and G2688C) reached (4.25, 3.90 and 4.16), respectively. As for the rest of the mutations, there was no significant change.in the chi-square value. This result is close to the study of Oner *et al*. (2017) on the HSP70 gene in Holstein-Friesian cows in Turkey and agrees with the findings of Kerekoppa *et al*. (2015); and Said and Bayu (2018).

The difference in the distribution of genotypes for mutations occurring in the studied Holstein cows is due to several factors, including the difference in sample size, the difference in the breed, and its suitability for environmental conditions. Hardy-Weinberg disequilibrium due to selection, genetic drift, and small sample size.

The results of Table 4 showed that the total milk production varied between the genotypes of each mutation due to the exposure of cows to heat stress, where the temperature in the summer reached 50 degrees Celsius, as the highest production was in the C1867T mutation of the CT hybrid genotype (5442.7) kg season^{-1} and less milk production in the mutation G937A for the recessive genotype AA (1869) kg season^{-1}. The results of the current study were consistent with Deb *et al*. (2013) in their study of the HSP70 gene in traits of total milk production in cows Low production compared to researcher Efimova *et al*.(2020) when he studied Holstein cows. The hybrid genotype GC of the G2688C mutation recorded the highest breeding value of 652.3, while the dominance deviation was the highest value of 1460 in the recessive genotype TT of the C2548T mutation. We note that the additive variance had the highest value in the G937A mutation, which amounted to 726856, so it is possible to select the GA hybrid genotype for the high breeding value resulting from the additive variance. This variance is important in genetic improvement because it is transmitted from one generation to the next (Jiang *et al*., 2017) and the highest dominance variance amounted to 235183. In the mutation, C2548T, it is possible to benefit from the genetic mutations revealed in this study in improving the breeding programs of Holstein cows in Iraq and making faster genetic progress in order to increase milk production.Holstein cows in Iraq and making faster

Table 5 Shows that the ratio of fat in milk ranged from 1.47 to 5.07%, which is within the normal rates for the ratio of fat. The highest breeding value amounted to 1.30 in the hybrid genotype GC of the mutation G2688C, and the highest dominance deviation reached 1.70 in the recessive genotype TT of the mutation C2548T This result is consistent with Mariana *et a* (2020). As for the additive variance, its highest value was 0.576 in the C2548T mutation. Estimating breeding value using genetic mutations will increase the genetic yield as a result of reducing the generation ranges, increasing genetic accuracy and lower cost, and the accuracy of use.

Genetic mutations in estimating breeding value and improving animal performance under field conditions. From the results of Tables 2 and 3, it is shown that the molecular side of the research is linked with quantitative calculations of variance, thus reaching the best selection efficiency. This is the real gain for this type of scientific research aimed at genetic and productivity improvement. Accordingly, quantitative estimates are calculated for some genetic mutations that significantly affected traits of total milk production.

Table 3. The number and percentages of genotypes and allelic frequency of the HSP70 gene

Primer	Mutation site	Reference sequence (rs)	Amino acids	Genotype	NO.	Percentage	Observed	Expected	Chi-square value	Allelic frequency
P1	T825C	385826597	Ata/Lle	TT	3	5.10	0.051	0.220	0.369	T=0.47 C=0.53
				TC	50	84.74	0.847	0.498		
				CC	6	10.16	0.101	0.280		
	G856A	382492082	Aca/Thr	GG	1	1.70	0.017	0.002	0.120	G=0.05 A=0.95
				GA	4	6.77	0.067	0.095		
				AA	54	91.52	0.915	0.902		
	G937A	135145204	Val/Val	GG	15	25.42	0.254	0.384	0.295	G=0.62 A=0.38
				GA	43	72.88	0.728	0.471		
				AA	1	1.70	0.017	0.144		
	A1135G	109475441	Lys/Lys	AG	36	61.01	0.610	0.420	0.120	A=0.30 G=0.70
				GG	23	38.98	0.389	0.580		
	G1185C	110374561	Gly/Gly	GG	48	81.35	0.813	0.828	0.0034	G=0.91 C=0.09
				GC	11	18.64	0.186	0.163		
P2	G1753A	110470368	Leu/Leu	GG	43	62.31	0.623	0.608	0.007	G=0.78 A=0.22
				GA	22	31.90	0.319	0.343		
				AA	4	5.79	0.057	0.048		
	C1867T	383027605	Asp/Asp	CC	65	94.20	0.942	0.940	4.250	C=0.97 T=0.03
				CT	4	5.80	0.058	0.058		
	A2017G	382780148	Gly/Gly	AA	64	92.75	0.927	0.921	3.908	A=0.96 G=0.04
				AG	5	7.25	0.072	0.076		
P3	C2548T	209779684	Ser/Ser	CC	39	73.85	0.738	0.562	0.934	C=0.75 T=0.25
				CT	1	1.80	0.018	0.375		
				TT	13	24.50	0.245	0.062		
	G2572T	110397047	Ala/Ala	GG	26	49.05	0.490	0.396	0.196	G=0.63 T=0.37
				GT	14	26.41	0.264	0.466		
				TT	13	24.50	0.245	0.136		
	G2688C	384294013	Gly/….	GG	51	96.22	0.962	0.960	4.166	G=0.98 C=0.02
				GC	2	3.77	0.037	0.039		
	T2734C	41257358	Asp/Asp	TT	27	50.99	0.509	0.396	0.223	T=0.63 C=0.37
				TC	13	24.50	0.245	0.466		
				CC	13	24.50	0.245	0.136		

Table 4. Breeding values, dominance deviation, and mean alleles for the genotypes of the HSP70 gene for total milk production

Primer	Mutation site	Genotype	NO.	Total milk production	Breeding value	Dominance deviation	Additive variance	Dominance deviation	Genetic variation	Average effect allele	Average allele exchange
P1	T825C	TT	3	4499	152.16	-153.9	10267	18606.8	28873.8	T=76.08 C=67.47	T=8.61 C=-8.61
		TC	50	4064	143.55	-136.9					
		CC	6	4178.7	134.94	-121.4					
	G856A	GG	1	3060	-1182	-146.1	36753	59.18	36812.18	G=-591 A=-31.1	G=-560 A=560
		GA	4	3529.4	-622	-7.69					
		AA	54	4159.9	-62.2	-0.405					
	G937A	GG	15	4677.4	944	194.4	726856	100776	827632	G=472 A=75.6	G=396.4 A=-396.4
		GA	43	3948	547.6	318					
		AA	1	1869	151.2	837					
	A1135G	AG	36	4372.3	-16	112.14	11296	12575	23871	A=-11.2 G=-4.8	A=-6.4 G=6.4
		GG	23	3834	-9.6	261.6					
	G1185C	GG	48	4117.3	15.2	-0.76	1169	58.52	1228.52	G=7.6 C=76.9	G=-69.3 C=69.3
		GC	11	4025.3	84.5	-7.69					
P2	G1753A	GG	43	4175.7	-139.4	-37.63	34487	17938	52425	G=-69.7 A=-247.2	G=177.5 A=--177.5
		GA	22	4320.5	-316.9	-134.5					
		AA	4	5248.5	-494.4	-476.6					
	C1867T	CC	65	4212.8	-71.6	1.10	82833	6682	89515	A=-35.8 G=-1157.2	A=1121.4 G=-1121.4
		CT	4	5442.7	-1193	35.8					
	A2017G	AA	64	4231.6	2.32	1.16	64.5	776.6	841.1	A=1.16 G=27.8	A=-26.6 G=26.6
		AG	5	4956	28.9	27.87					
P3	C2548T	CC	39	4128	-326	161	159.4	235183	235342.4	C=-163 T=-489	C=-326 T=326
		CT	1	5430	-632	487					
		TT	13	4133.6	-978	1460					
	G2572T	GG	26	4005.2	-120.4	103.3	12234	31107	43341	G=-60.23 T=-102.56	G=60.33 T=-60.33
		GT	14	4449	-162.7	177.15					
		TT	13	4133.6	-205.1	300.9					
	G2688C	GG	51	4179	26.8	-0.266	16664	170.32	16834.3	G=13.4 C=638.9	G=-625.5 C=625.5
		GC	2	3514	652.3	-23.05					
	T2734C	TT	27	3908.4	-224.2	101.7	42801	30126	72927	T=-112.1 C=-190.8	T=-92.3 C=92.3
		TC	13	4491.4	-302.9	169.6					
		CC	13	4326.5	-381.6	296.2					

Table 5. Breeding values, dominance deviation, and mean alleles for the genotypes of the HSP70 gene for Ratio fat

Primer	Mutation site	Genotype	NO.	Ratio fat	Breeding value	Dominance deviation	Additive variance	Dominance deviation	Genetic variation	Average effect allele	Average allele exchange
P1	T825C	TT	3	4.17	0.20	0.101	0.01778	0.0080	0.0257	T=0.10 C=0.08	T=0.012 C=-0.012
		TC	50	3.79	0.188	0.141					
		CC	6	3.77	0.176	0.169					
	G856A	GG	1	4.27	1.248	0.776	0.0410	0.0016	0.0426	G=0.624 A=0.032	G=0.592 A=-0.592
		GA	4	4.70	0.656	0.0408					
		AA	54	3.73	0.064	0.043					
	G937A	GG	15	4	-0.122	-0.170	0.01248	0.0793	0.0917	G=-0.061 A=-0.102	G=0.041 A=-0.041
		GA	43	3.72	-0.163	-0.282					
		AA	1	4.63	-0.204	-0.078					
	A1135G	AG	36	3.61	0.124	-0.0798	0.00642	0.00636	0.01278	A=0.086 G=0.037	A=0.049 G=-0.049
		GG	23	4	0.0744	-0.0342					
	G1185C	GG	48	3.87	0.05	-0.0024	0.0131	0.00061	0.0137	G=0.0025 C=0.357	G=-0.232 C=0.232
		GC	11	3.56	0.282	-0.024					
P2	G1753A	GG	43	2.60	0.106	-0.0211	0.0202	0.00560	0.0258	G=0.053 A=0.189	G=-0.136 A=0.136
		GA	22	2.26	0.242	-0.075					
		AA	4	2.36	0.378	-0.267					
	C1867T	CC	65	2.48	-0.0011	0.000018	0.000021	0.0000003	0.0000213	A=-0.00058 G=-0.0188	A=0.013 G=-0.013
		CT	4	2.50	0.013	0.00048					
	A2017G	AA	64	2.56	0.082	-0.0018	0.0843	0.00159	0.08589	A=0.041 G=1.006	A=-0.963 G=0.965
		AG	5	1.47	1.047	-0.039					
P3	C2548T	CC	39	3.07	-0.62	0.188	0.576	0.3177	0.893	C=-0.31 T=-0.93	C=0.62 T=-0.62
		CT	1	5.07	-1.24	0.57					
		TT	13	4.04	-1.86	1.70					
	G2572T	GG	26	3.03	-0.354	-0.095	0.1072	0.0262	0.1334	G=-0.177 T=-0.302	G=-0.178 T=0.178
		GT	14	3.26	-0.479	-0.163					
		TT	13	4.19	-0.604	-0.277					
	G2688C	GG	51	3.42	0.052	-0.00053	0.0672	0.00068	0.0678	G=0.026 C=1.28	G=1.25 C=-1.25
		GC	2	2.08	1.30	-0.026					
	T2734C	TT	27	3.15	-0.228	-0.097	0.0447	0.0277	0.0724	T=-0.144 C=-0.195	T=0.081 C=-0.081
		TC	13	3.20	-0.309	-0.167					
		CC	13	3.98	-0.39	-0.285					

Cconclusions

We conclude that Holstein cows imported from Germany, which were studied, were subjected to heat stress in Iraq, which led to a decrease in milk production and the emergence of a number of genetic mutations when studying the HSP70 gene, as it is considered the most important gene expressing heat stress, We conclude that the C187T mutation has high additive variation for milk production The C2548T mutation has a high additive variation in fat percentage, and this variation is important in genetic improvement because it is transmitted from one generation to another.

Conflict of interest

Regarding the publication of this manuscript, the authors declare that there are no conflicts of interest.

Acknowledgments

We would like to express our deep thanks to the Taj Al-Nahrain cows breeding station in Al-Diwaniyah, Al-Qadisiyah Governorate for helping us to complete this work.

References

Al-Zaidi, O. R. W. (2022). Relationship of genetic polymorphism to the heat shock gene (HSP 70) and some genetic factors in some reproductive and productive traits of local female goats. *Master's thesis*, College of Agriculture, University of Diyala.

Bhat, S., Kumar, P., Kashyap, N., Deshmukh, B., Dige, M. S., Bhushan, B., and Singh, G. (2016). Effect of heat shock protein 70 polymorphism on thermotolerance in Tharparkar cattle. *Veterinary world*, *9*(2), 113-117. https://doi.org/10.14202%2Fvetworld.2016.113-117

Bohmanova, J., Misztal, I., and Cole, J. B. (2007). Temperature-humidity indices as indicators of milk production losses due to heat stress. *Journal of dairy science*, *90*(4), 1947-1956. https://doi.org/10.3168/jds.2006-513

Das, R., Sailo, L., Verma, N., Bharti, P., Saikia, J., and Kumar, R. (2016). Impact of heat stress on health and performance of dairy animals: A review. *Veterinary world*, *9*(3), 260-268. https://doi.org/10.14202%2Fvetworld.2016.260-268

Deb, R., Sajjanar, B., Singh, U., Kumar, S., Brahmane, M. P., Singh, R., and Sharma, A. (2013). Promoter variants at AP2 box region of Hsp70. 1 affect thermal stress response and milk production traits in Frieswal cross bred cattle. *Gene*, *532*(2), 230-235. https://doi.org/10.1016/j.gene.2013.09.037

Efimova, I. O., Zagidullin, L. R., Khisamov, R. R., Akhmetov, T. M., Shaidullin, R. R., Tyulkin, S. V., and Gilmanov, K. K. (2020). Assessment on milk productivity and milk quality in cattle with different genotypes by HSP70. 1 gene. In *IOP Conference Series: Earth and Environmental Science* (Vol. 604, No. 1, p. 012016). IOP Publishing. https://doi.org/10.1088/1755-1315/604/1/012016

Falconer, D.S. and Mackay, T. F. C. (1996). Introduction. to quantitative genetic. *4th edition, Longman Group Ltd.*

Jiang, J., Shen, B., O'Connell, J. R., VanRaden, P. M., Cole, J. B., and Ma, L. (2017). Dissection of additive, dominance, and imprinting effects for production and reproduction traits in Holstein cattle. *BMC genomics*, *18*(425), 1-13. https://doi.org/10.1186/s12864-017-3821-4

Kerekoppa, R. P., Rao, A., Basavaraju, M., Geetha, G. R., Krishnamurthy, L., RAO, T. V. N., and Mukund, K. (2015). Molecular characterization of the HSPA1A gene by single-strandconformation polymorphism and sequence analysis in Holstein-Friesiancrossbred and Deoni cattle raised in India. *Turkish Journal of Veterinary and Animal Sciences*, *39*(2), 128-133.https://doi.org/10.3906/vet-1212-3

Mariana, E., Sumantri, C., Astuti, D. A., Anggraeni, A., and Gunawan, A. (2020). Association of HSP70 gene with milk yield and milk quality of Friesian Holstein in Indonesia. In *IOP Conference Series: Earth and Environmental Science*, *425*(1), 1-6. https://doi.org/10.1088/1755-1315/425/1/012045

Mayer, M. P., and Bukau, B. (2005). Hsp70 chaperones: cellular functions and molecular mechanism. *Cellular and molecular life sciences*, *62*(6), 670-684. https://doi.org/10.1007/s00018-004-4464-6

Miglior, F., Jansen, G., and Schaeffer, L. R. (1995). Inclusion of time-region-age-parity effect in the Canadian genetic evaluation for production traits. *Interbull Bulletin*, (11).

NCBI. National Center for Biotechnology Information. (2017). https://www.ncbi.nlm.nih.gov/gene/281825

Oner, Y., Keskin, A., Üstüner, H., Soysal, D., and Karakaş, V. (2017). Genetic diversity of the 3' and 5' untranslated regions of the HSP70. 1 gene between native Turkish and Holstein Friesian cattle breeds. *South African Journal of Animal Science*, *47*(4), 424-439.

Raza, S. H. A., Hassanin, A. A., Dhshan, A. I., Abdelnour, S. A., Khan, R., Mei, C., and Zan, L. (2021). In silico genomic and proteomic analyses of three heat shock proteins (HSP70, HSP90-α, and HSP90-β) in even-toed ungulates. *Electronic Journal of Biotechnology*, *53*, 61-70. https://doi.org/10.1016/j.ejbt.2021.07.002

SAID, S., and PUTRA, W. P. B. (2018). Novel single nucleotide polymorphisms (SNPs) in the 5'UTR of bovine heat shock protein 70 (bHsp70) gene and its association with service per conception (S/C) of Pasundan cattle. *Biodiversitas Journal of Biological Diversity*, *19*(5), 1622-1625. https://doi.org/10.13057/biodiv/d190504

Sejian, V., Bagath, M., Krishnan, G., Vaswani, S., Archana, P. R., Pragna, P., and Bhatta, R. (2017). Impact of Adverse Environmental Stress on Productive and Reproductive Performance in Osmanabadi Goats. *Sustainable Goat Production in Adverse Environments: Volume I: Welfare, Health and Breeding*, 407-428. https://doi.org/10.1007/978-3-319-71855-2_23

Younis, F. (2020). Expression pattern of heat shock protein genes in sheep. *Mansoura Veterinary Medical Journal*, *21*(1), 1-5. https://doi.org/10.21608/mvmj.2020.21.001