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Studying the effect of the genotypes of the GH gene on blood parameters and some structural characteristics of Iraqi buffalo milk

[1]Mazen Majed Malbas Al-Hasnawi , [2]Meitham K, Ali AL-Galiby

[1,2]Department Of Animal Production , College Of Agriculture And Marches University Of Thi-Qar , 64001, Iraq

[1]Email:mazen.m@utq.edu.iq

[2]Email:Maitham@utq.edu.iq

Summary

The samples of this study were collected from the private fields of buffalo breeders in the Al-Tar district of Dhi Qar Governorate / Karma Bani Said district, where 50 samples of milking female buffaloes were used in the experiment, with ages ranging from (2-3 years), this study was conducted in the laboratories of the College Agriculture and Marshes Department of Animal Production for the period from 15/11/2021 to 30/5/2022 with the aim of diagnosing the genetic morphology of the growth hormone gene in Iraqi buffaloes and its relationship to blood parameters and some characteristics of milk composition. Where the blood parameters measurements included the number of red blood cells, the number of white blood cells, the percentage of hemoglobin in the blood and the volume of the aggregated cells. As for the measurement of milk components, it included protein percentage, lactose sugar percentage, fat percentage, percentage of non-fat solids, milk density, freezing point, ash percentage and water percentage. Then DNA was extracted from animal blood samples in the Molecular Genetics Laboratory of For the Marshes Research Center / Dhi Qar University, the primer of the growth hormone gene was amplified and then the genotypes in the growth hormone gene were determined using (PCR) technique by electrophoresis with a relay device, and then the sequence of bases was analyzed using the sanger sequencing technique. And then study the relationship between the genetic structures and the traits mentioned above.

The results of the polymerase chain reaction (PCR) analysis of the growth hormone gene showed the appearance of a bundle with a size of 1196 base pairs, and the appearance of two alleles, G and T, where their frequency was (0.64 and 0.36), respectively, and by three genotypes: GG, TT and GT, where the frequency of these structures reached genetic (0.60, 0.32 and 0.08), respectively. Whereas, the value of chi-square was 17.1, which indicates the mismatch of the observed and expected animals, which indicates the imbalance of the population, which was observed in the study from the genetic frequencies of the unbalanced population. A single silent mutation was also found at position 118 of the 1196-base-pair-long studied region in the non-coding region 2Intron and at site 611 of the complete gene.

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The appearance of a single silent mutation at position 118 of the 1196-base-pair-long studied region in the 2Intron noncoding region and at site 611 of the complete gene. The frequency of the G allele was 0.64, while the frequency of the T allele was 0.36, and the frequency of the genotypes GG, TT and GT was (0.60, 0.32 and 0.08), respectively. Whereas, the value of chi-square was 17.1, which indicates the mismatch of the observed and expected animals, which indicates the imbalance of the population, which was observed in the study from the genetic frequencies of the unbalanced population. Through the obtained results, it was found that the red blood cells, the hemoglobin percentage and the volume of the compacted cells were not affected by the genotypes, as the highest value of the red blood cells was recorded by the GT genotype, which was (7.11 ± 0.69) * 106 cells/ml. Whereas, the GG genotype recorded the highest value for both hemoglobin and the volume of packed blood cells (13.022 ± 2.076) g/dL and (40.07 ± 6.23)%, respectively. As for white blood cells, they were significantly affected ($P < 0.05$) by genotypes, where the GT genotype was superior to the two genotypes GG and TT, and its value was (a 9.225 ± 0.317) * 103 cells/ml.

Also, the composition of milk was not affected by the different genotypes of the growth hormone gene, where the highest value of the degree of freezing and milk density was recorded by the GT genotype, as its value was (0.430 ± 0.025) m5 and (27.400 ± 2.540) g/cm3, respectively. While the GG genotype recorded the highest value for lactose, protein and non-fat solids (5.180 ± 1.302, 3.398 ± 0.141 and 18.138 ± 2.322)%, respectively. As for the percentage of fat, ash and water, the highest value was recorded in the TT genotype (7.608 ± 2.027, 2.596 ± 0.284 and 81.868 ± 1.531) %, respectively.

Key words: blood parameters, milk composition, growth hormone gene

I. INTRODUCTION

Livestock is one of the important sources to supplement the national economy and meet the citizen's need for animal products in Iraq. Buffalo is one of the sources of livestock that constitute an important source of red meat and milk, as buffalo milk is characterized by a high percentage of fat compared to milk of other species in addition to other products, and buffalo is characterized by its tolerance To difficult environmental conditions and rapid adaptation to the environment (Al-Ghaliby, 2020).

Recent studies have resorted to the use of molecular genetics methods and modern techniques in detecting the genetic formations of productive genes and choosing the best ones, comparing the genetic structures with the structures of global breeds with high productivity, identifying genetic mutations and linking them to phenotypic and productive traits, which leads to raising the productivity of local animals and improving their performance, especially growth and milk production. (Jaayid and Drag, 2013 ; Unal et al., 2014 and Rushdi et al., 2017). The available information on the mechanism of work of the endocrine glands regulating the onset of puberty in buffalo calves is scarce, especially related to the concentrations of GH and other growth hormones in the blood plasma. However, some studies have indicated the importance of the endocrine glands in the secretion of these hormones in the growth of buffalo calves (Mondal and Prakash , 2004).Growth hormone plays an important role in growth, reproduction, and lactation primarily through induction of cell proliferation, protein and lipid synthesis, and metabolism (Akers, 2006 ; Katoh et al., 2008 and Davis et al., 2021), by binding to its GHR receptor, which is expressed In many tissues, especially the liver, muscle, and adipose tissue (Herrington and Carte, 2001).

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Due to the lack of available studies on the genes and their genetic structures related to the physiological and productive characteristics of the Iraqi local buffalo and their effects on the ability to produce and grow, this study aimed to reveal the growth hormone gene in the Iraqi local buffalo, the number of alleles that make up the gene, the number of its genotypes and its relationship to some physiological traits, production and milk components.

II. MATERIALS AND WORKING METHODS

study samples

Blood samples of 2.5 ml were collected for 50 animals of female buffaloes aged between (2 - 3) years through the jugular vein from the neck area and randomly from animals affiliated to buffalo breeders in Dhi Qar Governorate / Karma Bani Saeed District / Al Tar district. A sample of samples taken in test tubes containing an anticoagulant substance (Ethylene diamine tetraacetic acid (EDTA)), where a blood parameters test, which includes agglutinated cell volume, red blood cell count, white blood cell count and hemoglobin blood within less than 24 hours of sampling, was tested. Then the remaining blood placed in the test tube containing the anticoagulant was stored by freezing for the purpose of DNA extraction. Also, 50 milk samples with a volume of 40 ml were collected for each sample in a 50 ml plastic cup, then tests were conducted on them to detect the proportions of milk components, which include protein percentage, lactose sugar, fat percentage, milk density, freezing point, non-fat solids, ash and water.

DNA extraction (Geneaid Kit)

The DNA extraction process was carried out in the Molecular Genetics Laboratory of the Marsh Research Center / Dhi Qar University and a DNA extraction kit produced by the Korean company Geneaid was used using a refrigerated centrifuge as well as a water bath and a pipette of 1 ml inserted from (100-1000) microliters and (10- 200) microliters and (1-10) microliters according to the method of work mentioned in the kit, and after DNA extraction and transfer of samples on agarose gel by means of an electrophoresis device at a voltage of 70 volts and 85 milliamps for half an hour, the agarose gel was examined after the expiry time Migration using UV Gel Documentation device and images of the migration were taken using the camera installed and designated for this purpose. Then the primers of the growth hormone gene were prepared by Alpha DNA company in the form of a lyophilized powder of initiators separated from each other as shown in Table (1) to identify alleles, genotypes and mutations of the growth hormone gene.

Gene	Primers	Size of fragment
Growth hormone	5'- GCTGCTGACACCTTCAAAGA- 3'	1196 bp

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	5'– TGACCCTCAGGTACGTCTCC– 3'	

Table (2) The PCR program for the GH gene using the Touchdown method

	Stages	Temperature	Time	Number of cycle
initial program	initial metamorphosis	95C°	5	1
	Mutant	95C°	0.30	20
	Adhesion	63–53 C°	0.45	
	Elongation	72C°	1.10	
	Final elongation	72C°	10	1
	initial metamorphosis	95C°	5	1
	Mutant	95C°	0.30	

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Specialized Amplification Program	**Adhesion**	53 C°	0.45	20
	Elongation	72C°	1.10	
	Final elongation	72C°	10	1

Nitrogenous base sequence analysis

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 International Gene Bank website with the use of some bioinformatics programmes.

Calculation of blood parameters

Red Blood Cells (RBC)

The number of red blood cells was calculated according to the method (Schalm et al., 1975), using a hemocytometer slide and a special pipette for drawing blood. Blood was drawn up to the mark 1.1 with a Heims solution after mixing the contents of the solution for 10 seconds by shaking, the first drop of blood was removed and placed The second drop was at the edge of the contact between the slide and the slideCover, and then left for two minutes for the purpose of cell stability. The red blood cells were counted by light microscopy in five medium squares, each square containing 16 small squares, then the total number of red blood cells was extracted according to the following equation:

Red blood cell count/cm^3 = number of red blood cells in five medium squares * 200 (dilution correction factor) * (volume correction factor)

White Blood Cells (WBC)

Hemocytometer slide was used according to the method of (John and Lewis, 1984), blood was drawn using micropipet, 380 microns were taken from a solution consisting of (1.5 ml glacial acetic acid, 1.0 ml concentration of methyl violet dye, and distilled water supplemented to 100 ml and 20 microns from the blood sample) and agitated. Well after being placed in a glass tube and left for 5 minutes, then 20 microns of the mixture was placed on the counting slide after getting rid of the first drops and left the slide for two minutes for the purpose of stabilizing the pellets.

WBC/cm^3 = WBC inside squared * 20 (dilution correction factor) * (volume correction factor)

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Packed Cell Volume(PCV%)

Using the Microhematocrit method, the volume of the compacted cells was calculated, where a sample of blood was drawn into the capillary tube from one end to a height of at least 15 mm by capillary action, and then the second end of the tube was closed with wax and then placed in a centrifuge (Hematocrit) (3000 revolutions/min) for 5 minutes, and then read with a ruler (Dacie and Lewis, 1974).

Hemoglobin Concentration (Hb)

The hemoglobin concentration was measured using a Sahli apparatus, which is a glass tube containing divisions dedicated to calculating the hemoglobin as a ratio and concentration (g / 100 cm^3) by calibrating the hemoglobin of 0.1 N hydrochloric acid (Schalm et al., 1975).

Measurement of milk components

After taking 40 ml milk samples from all animals and placing them in a 50 ml plastic cup, the ratios of milk components (fats, proteins, lactose sugar, non-fat solids, milk density, freezing point, ash and water) using MilkEko device manufactured from by the German Gerber Company.

statistical analysis

The data were statistically analyzed and significant differences between the means were tested using the Revised Least Significant Differences (RLSD) using the ready-made statistical program SPSS (V.26) (2021).

$Yij= \mu+ Gi. +Eij$

Yij = the value of any observation j within the i genotype for any studied trait.

μ = the general average of any studied trait.

Gi = effect of genotype i (i = 3) on any studied trait.

Eij = the effect of the experimental error inherent in observation j within the genotype i with a mean of zero and variance σe

III. RESULTS AND DISCUSSION

DNA analysis product

The result of polymerase chain reaction (PCR) analysis of the growth hormone gene migrated on an agarose gel showed a package of 1196 base pair size using the DNA Ladder marker.

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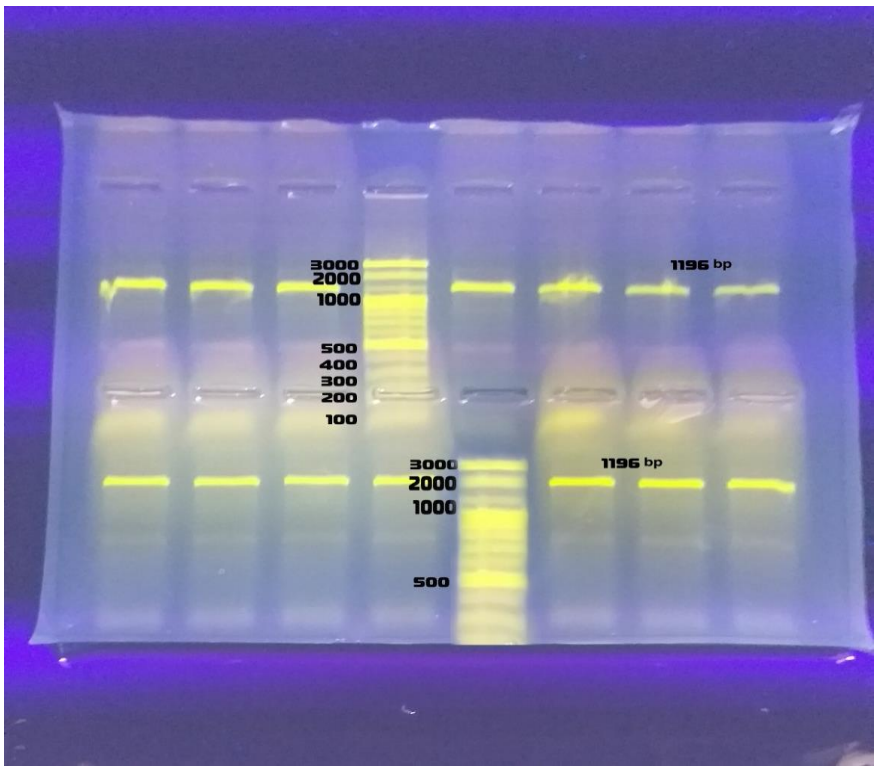

Figure (1) A picture of a GH gene amplification product with a size of 1196 base pairs relayed by akarose with a concentration of 1.5% at a voltage of 70 volts and 85 mA for 45 minutes

Allele frequency, genotype frequency, and chi-square value.

It was shown in Table (3) that the frequency of the G allele amounted to (0.64) while the frequency of the T allele amounted to (0.36). As for the frequency of the genotypes, it reached the highest frequency of the GG genotype, whose value was 0.60, then came the TT genotype, where its frequency was 0.32 and finally the structure The GT genotype had a frequency of 0.08. Whereas, the value of chi-square was 17.1, which indicates the imbalance of the population, i.e. the mismatch of the numbers of observed and expected animals that were observed in the study from the genetic frequencies of the unbalanced population. The results of the current study agreed with what was found by (Othman et al., 2012) where three genotypes (LL, LV, VV) were shown and the LL genotype was the most common with a frequency of 0.871, and the least common was the VV genotype with a frequency of 0.005 in the Egyptian water buffalo (Konca et al., 2016) confirmed that the LL genotype was the most common genotype with a frequency of (0.755) and the VV genotype was the least common with a frequency of (0.017) in the Anatolian water buffalo. In terms of the presence of the two alleles, they are L and V, but with two genotypes: LL and LV, and with frequency (94 and 6), respectively, while most of the buffaloes carried the genotype LL. It also agreed with the study (Saed et al. 2017) that was conducted on the buffaloes of Khuzestan, where it showed the presence of two alleles (L and V), but also with two genetic structures, LL and LV, where most of the buffaloes were identical to the zygote LL. While it did not agree with the results obtained by T. K. Biswas et al. (2003) in the Sahiwal

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buffalo and (Andreas et al. 2010) in the Andalusian buffalo, where all the tested buffaloes had one allele (L) and only one genotype is LL.

Table (3) shows the frequency of the genotypes, the frequency of the G and T alleles for the GH gene and the chi-square value

Repeat genotypes and allele repeat value of the GH gene relative to locus 92.G>T				
Genetic structure	Genetic structure frequency	Allel	frequency	chi square X^2
GG	0.60	G	0.64	17.1
GT	0.08			
TT	0.32	T	0.36	

Genetic morphology of the GH gene in Iraqi local buffalo

When aligning the sequence of samples under study to detect changes or mutations that occur and form genetic structures, a single change (mutation) was found at site No. 118 of the studied region, the length of which is 1196 base pairs of the GH gene, specifically in the region of intron No. (2) 118.G>T. No. (3), which is a silent mutation, and the location of this mutation is (611) for the complete growth hormone gene, which is registered with accession number KC107770.1 in the NCBI Gene Bank. The study did not observe any mutation in the encoded regions among all samples in the current study. These results did not agree with what was reached by (Othman et al., 2012) in his study on the Egyptian buffalo, where there was one SNP (A/C) at site 203, also the results of the current study did not agree with Hussain (2015) who found one very low mutation at the exon The fifth in water buffalo, and it did not agree with the results of the study of(El-Komy et al. , 2021), where two error mutations were discovered in exon5 in Egyptian water buffalo.

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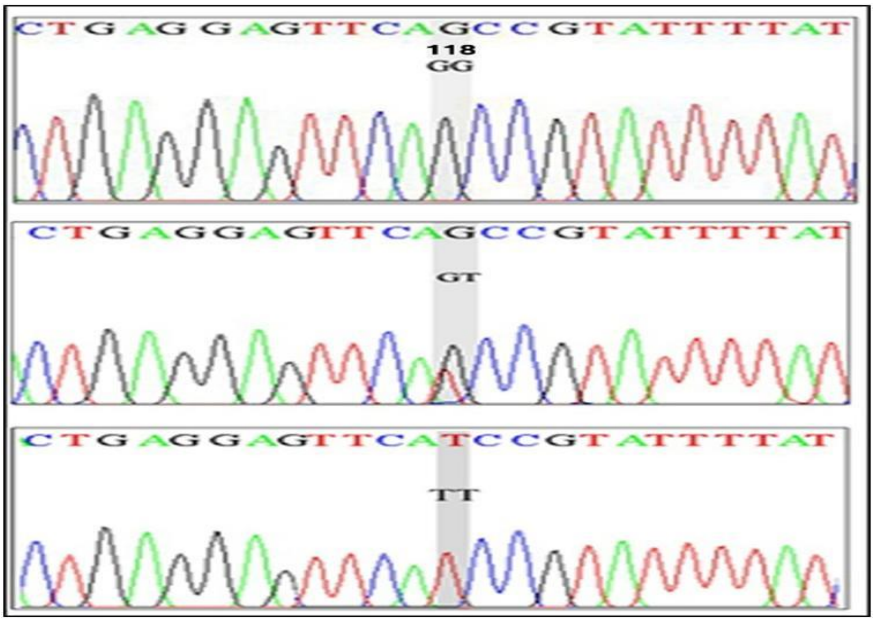

Figure (2) Mutation in the G>T118 site of the growth hormone gene of Iraqi buffalo

Relationship of the genotypes of the growth hormone gene with blood parameters in Iraqi buffalo

The results of Table No. (4) showed that there were no significant differences in the number of red blood cells, hemoglobin concentration, and the volume of stacked blood cells for the different genotypes of the growth hormone gene, where the highest value of the number of red blood cells was for the genotype TT (0.69±7.11) * 10^6 cells/ Then came the genotypes GT and GG, where their values were (0.54 ± 6.910 and 6.53 ± 0.78) * 10^6 cells/ml, respectively. As for the hemoglobin concentration, the GG genotype recorded the highest value, which amounted to (2.076 ± 13.022) g/dL, while the value of the genotype TT and GT were (2.076 ± 13.022 and 1.347 ± 11.500) g/dL, respectively, while the volume of the compacted blood cells recorded the highest value of the genotype GG, which reached (6.231 ± 40.07)%, in the following The two genotypes TT and GT recorded values of (5.183 ± 37.75 and 4.041 ± 35.50)%, respectively. These results did not agree with what was found by (Makhlouf et al., 2017) in his study on the ranks of red blood cells, as it was found that their value for the AA and AB genotypes is (4.79 ± 0.30 and 5.10 ± 0.07) * 106 cells/ml, respectively. While the results of the current study agreed with the results indicated by (Makhlouf et al., 2017) in his study on rabbits regarding the values of hemoglobin concentration and the volume of packed blood cells for genotypes AA and AB, where hemoglobin values were (13.35 ± 0.64 and 14.02 ± 0.13) g/ deciliter and respectively, and the values of the volume of packed blood cells are (40.13 ± 1.35 and 40.45 ± 0.27) %, respectively. Since there is a direct relationship between the number of red blood cells, hemoglobin and the volume of compacted blood cells, in the event of an increase in the number of red blood cells accompanied by an increase in the concentration of hemoglobin and the volume of compacted cells, this depends on the physiological condition and the high and low metabolic rates of the animal and the extent of its need for oxygen (Ali, 2011) and this What explains the results of the current study, as all the values of blood indicators are within the normal range, and this shows the extent of the vital physiological relationship of hemoglobin to the transport of gases to and from body tissues naturally (Njidda et al., 2006). As for the average number of white blood cells, a significant increase was recorded in favor of the GT genotype,

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which amounted to (0.317 ± 9.225) * 103 cells/ml compared to the average white blood cells of the TT genotype, which recorded an average of (0.465 ± 8.435) * 103 cells/ml. Whereas, the average white blood cell GG genotype did not differ significantly from the average of the two previous genotypes, and the recorded value was (8.766 ± 0.412) * 103 cells/ml. The results of the current study approximated the results between them (Makhloof et al., 2017) in the polymorphism analysis study. The genetic differences of rabbit growth hormone to evaluate its effect on the live body weight, blood and biochemical parameters, where the white blood cell values were (7.92 ± 0.29 and 8.20 ± 0.06) * 103 cells/ml for genotypes AA and AB, respectively. To develop the immune system of animals (Ullrey et al., 1965).

Table (4) The genotype of the growth hormone gene and the calculation of the number of red blood cells, the concentration of hemoglobin and the volume of the compacted blood cells, preparing the white blood cells (± standard deviation).

genetic makeup	Red blood cell count * 10^3 cells/ml	hemoglobin concentration g /dl	aggregated blood volume %	White blood cell counts * 10^3 cells/ml
GG	6.53 ± 0.78	13.02 ± 2.07	40.07 ± 6.23	8.76 ± 0.41 ab
GT	6.91 ± 0. 54	11.50 ± 1.347	35.50 ±4.04	9.22 ± 0.31 a
TT	7.11 ± 0.69	12.25 ±1.72	37.75 ± 5.18	8.43 ± 0.46 b
Significant	NS	NS	NS	0.05

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The relationship of the genotypes of the growth hormone gene with the components of buffalo milk.

The current study did not show significant differences for milk components with different three genotypes of the growth hormone gene Table (5), where the GT genotype recorded the highest value for the degree of freezing, which was (0.025 ± (-0.430)) m5, followed by the two genotypes GG and TT, which recorded values were (0.018 ± (- 0.435) and 0.042 ± (- 0.448)) m°, respectively. As for lactose, the highest recorded value of the genotype GG was (1.302 ± 5.180)%, while the values of the genotypes GT and TT were (0.207 ± 4.810 and 0.623 ± 4.647)%, respectively. As for the percentage of fat, the TT genotype recorded the highest value, which amounted to (2.027 ± 7.608)%, followed by the two genotypes GG and GT, which recorded values of (1.798 ± 7.096 and 2.027 ± 7.608)%, respectively. While the GT genotype recorded the highest value for milk density, which reached (2.540 ± 27.400) g/dl, while the genotypes GG and TT recorded values of (2.157 ± 26.833 and 5.538 ± 25.013) g/dl, respectively. While the GG genotype recorded the highest value for milk protein, which amounted to (0.141 ± 3.398)%, followed by the two genotypes GT and TT, which gave values of (0.121 ± 3.355 and 0.378 ± 3.278)%, respectively. As for the percentage of milk ash, the values recorded for the genotypes (TT, GT, and GG) were (2.59620,284 ± 0.346 ± 2.5200, 2.4620 ± 0.205)%, respectively. While the recorded results for the genotypes (GG, TT, GT) which included the percentage of non-fat solids were (18.148 ± 2.322, 18.141 ± 1.531, 16.730 ± 0.958)%, respectively. As for the percentage of water, the values recorded for the genotypes of the growth hormone gene (GT, TT, GG) were (83.270 ± 0.958, 81.868 ± 1.531, 81.862 ± 2,322) %, respectively. The results of this study agreed with the results obtained by (Yogesh and Ankit, 2021) in the study of dairy cows, where the measurements related to milk were not significantly affected by the difference in the genotypes of the growth hormone gene at a significant level (P>0.1) in all genetic models. It also did not agree with what Sadeghi et al. (2010) found in a study of Iranian Holstein cows about the association of the LL genotype of the hormone gene with the percentage of fat and protein compared to the VL genotype . While Dario et al. (2008) confirmed that the LV genotype gave a percentage of fat and a percentage of protein. higher than LL and LV genotypes in Jersey cows.

Table (5) Genetic structure of the growth hormone gene and measure of the degree of freezing M5, lactose percentage, fat percentage, milk density g/dL, protein percentage, ash percentage %, non-fat solids %, water percentage (± standard deviation).

	The genotypes of the growth hormone gene			Significant
	GG	GT	TT	

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Milk ingredients				
Freezing point C°	(− 0.435) ± 0.01	(− 0. 430) ±0.02	(− 0. 448) ±0. 04	NS
lactose % sugar	5.81 ± 1.30	4.81± 0.20	4.64 ± 0.62	NS
fat %	7.09 ± 1.79	6.04 ± 1.25	7.60± 2.02	NS
Milk density g/dL	26.83 ± 2.15	27.40± 2.54	25.01 ± 5.53	NS
protein %	3.39± 0.14	3.35± 0.12	3.27± 0.37	NS
Ash %	2.46 ± 0.20	2.52 ± 0.34	2.59 ±0.28	NS
non−greasy % solid	18.14 ± 2.32	16.73± 0.95	18.14 ±1.53	NS
Water%	81.86 ± 2.32	83.27 ± 0.95	81.86 ±1.53	NS

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