GENETIC DIVERSITY AND PRODUCTIVE PERFORMANCE IN LOCAL AND IMPORTED IRAQI COWS USING MICROSATELLITE MARKERS

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ABSTRACT.
The study was conducted us to identify the genetic features of the microsatellite markers CSSM66 and ETH10 and ILSTS34 and their relationship to genetic diversity and productive performance this study was carried out using 100 cows of five breeds presented in Iraq ; Restaki (n=10) , Janoubi (n=10) , Sharabi (n=10) , (local), Holstein (n=50) and male Brahman (n=20) (imported).The results showed the presence of a very high diversity and a highly significant difference in a sample of studied cows in the allele distribution ratios for the three markers, the results showed a significant effect (P≤0.05) superiority of the genotype LY within the ETH10 marker in the least number of inseminatons required for fertilization of Restaki cows, namely 1.16 insemination, and the genotype GP and AB within ETH10 and CSSM66 markers, respectively, recorded the highest total average milk yield with a significant value (P≤0.05) for Holstein cows, reached 1.44 . The CC genotype within markers CSSM66 had the highest mean total weight with an average of 502.75 among Brahman bulls. Therefore, microsatellites markers contributed to the mapping of quantitative traits in the studied cows

Keywords: restaki cows , janoubi cows , CSSM66 , milk yield.
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دراسة التنوع الوراثي والاداء الانتاجي في الابقار العراقية المحلية والمستوردة باستعمال واسمات المايكروستلايت

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المستخلص
أجريت الدراسة بهدف الكشف وتحديد المظاهر الوراثية لواسمات التتابعات الجزيئية الدقيقة (CSSM66 (Microsatellite و ETH10 و ILSTS34 وعلاقتها بالتنوع الوراثي والاداء الانتاجي باستخدام 100 بقرة من خمسة سلالاتَ موجوده في العراق , رستاكي (عدد 10), جنوبي (عدد 10) , شرابي(عدد 10) (محلية) وابقار الهولشتاين (عدد 50) و البراهما (عدد 20). اوضحت النتائج وجود تنوع عالي جدا وبفارق عالي المعنوية في عينة لأبقار المدروسة في نسب توزيع الاليلات للواسمات الثلاث , بينت النتائج عن وجود تأثير معنوي (P≤0.05) تفوق المظهر الوراثي LY ضمن الواسم ETH10 في اقل عدد التلقيحات اللازمة للأخصاب لابقار الرستاكي إذ بلغ 1.16 تلقيحة , و سجل المظهر الوراثي GP وAB ضمن الواسمين ETH10 وCSSM66 على التوالي اعلى متوسط كلي في المثابرة على انتاج الحليب وبفرق معنوي (P≤0.05) لدى ابقار الهولشتاين اذ بلغ 1.44 لكل منها , و سجل المظهر الوراثي CC ضمن الواسم CSSM66 اعلى معدل وزن كلي بمتوسط 502.75 لدى ذكور البراهما . لذا ساهمت واسمات المايكروستلات في رسم خريطة الصفات الكميه في الابقار المدروسة.

الكلمات المفتاحية: ابقارالرستاكي. ابقار الجنوبي. CSSM66. المثابرة على انتاج الحليب.
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INTRODUCTION

The livestock sector contributes to the livelihoods of one billion of the world's poorest people and employs nearly 1.1 billion people (21). Cows are the main producer of milk in the world, as they account for about 90% of the total production (1,5). There are more than 750 genetically different types of livestock around the world, as well as countless unspecified breeds, this wealth of genetic diversity has helped livestock breed in most human-inhabited environments, the abundance of genetic diversity in livestock populations has provided the opportunity for selective breeding of livestock at present, the selection of livestock adapted to environmental conditions has gone beyond choosing according to desirable traits (4, 17, 18, 19, 33). Most of the important economic features in animal production are quantitative in nature and complex in etiology such as milk production and other economic quantitative traits where these traits are controlled by many genomic regions,the QTL is very important for mapping genes underlying a particular trait change and mapping of these loci can be achieved by analyzing their association with known molecular markers such as microsatellites it is possible to predict the phenotypic variance of the traits to be improved (2,14,26). The current genetic linkage map of cattle consists of more than 1500 genetic markers, the majority of these markers are polymorphic microslate markers with known sites and maps that are very important for genome scans, and through the use of ETH10 and CSSM66 markers to perform scans on quantitative traits (QTL) important on chromosomes, which have an impact on the health and milk production characteristics of cows (6). Its ETH10 site is in exon 1 of the signal transducer and transcription activator gene (STAT6) located on bovine chromosome 5 (10). STAT proteins are involved in the influx of cell signaling after cytokines or hormones that bind their receptors play an essential role in mammary gland development and differentiation, this is reflected in the differential expression of genes during mammary gland development, and across different stages of lactation (6,11). Reproductive performance is one of the main factors affecting the efficiency of milk production (16) . The D14S31-CSSM66 locus on chromosome 14 is the region close to the quantitative trait site of bovine chromosome 14 (Bos taurus autosome 14) and genes related to economically important (6,12,27). Markers, including CSSM66, contributed QTL mapping in beef cattle is mainly based on growth traits, carcass and meat quality (32). The role of microsatellites molecular markers is essential for determining the genetic makeup of an individual and is very important for genetic improvement of agricultural and livestock animals, its application being useful to improve breeding programs for desired traits,for the purpose of obtaining better productivity and high-quality products, these markers provide more accurate genetic information and better knowledge of animal genetic resources (30). Given the importance of microsatellites markers for mapping quantitative traits and studying genetic diversity, this study aims to study genetic diversity in a sample of local and imported cows, as well as compare diversity and phenotypes with the productive performance of cows using three microsatellites markers

MATERIALS AND METHODS

The Genetic Resources Conservation Directorate, Directorate of Animal Resource, and the Ministry of Agriculture was consulted to deteet the locations of the animals. They were of different ages and different breeds. From these samples, 30 cows were selected according to the scientific and formal specifications, and 50 cows of the Holstein breed, white and black spotted, and 20 Brahman bulls (Figure 1).

Figure 1. Model of the Iraqi local cows used in the study

Blood sampling

Blood samples (3 ml) were collected his jugular vein puncture using K2EDTA tubes from all animals and stored under - 18^{o} c until assay.

DNA Isolation

DNA was isolated from the blood according to the instructions of the diagnostic kit (kit) supplied by the Korean company Geneaid in the laboratory of the Faculty of Agricultural Engineering Sciences.

PCR and **Gel Electrophoresis**

Primers were selected for three of the genetic markers for micro-sequences Table (1) which includes primer sequences a replicon ,size , degree of annealing and remittance Table (2) the materials used in molecular detection using an enzyme reaction sequential PCR of the studied genes using the PCR GreenMasterMix KIT diagnostic kit with a volume of 25 microliters, and placed in the PCR device according to the reaction conditions for each duplicated gene segment. After completion of the PCR, the electrophoresis of the PCR product was carried out using the agarose gel preparation method to migrate the DNA genetic material. The electrophoresis process began after the PCR reaction and DNA amplification, as 7 lμ of the PCR product is taken and placed in the pits with the use of a ladder with a size of 25 and 100 nitrogen bases (DNA Marker 100 -25bp). The PCR product was passed on an agarose gel at a concentration of 2.5 mm in TAE (1X) solution and the voltage was fixed at 90 volts and 60 amperes for an hour and a half, then the gel was immersed in a solution containing ethidium bromide dye for 20 minutes.

Milk component assay

The samples were analyzed to estimate the basic components of milk using the Milk Composition Analyzer (Mlikoscope Julie Z7) which is located in the laboratory of the Ruminant Station, Animal Wealth Department, Agricultural Research Department

Statistical analysis

The data were statistically analyzed using the program) (24)to study the effect of phenotypes of the genetic markers ETH10 (the first mathematical model) and CSSM66 (the second mathematical model) on different traits, and the significant differences between the means were compared using the (11)polynomial test By applying the Least square means method.

$Y_{ijk} = \mu + E_i + P_j + e_{ijk}$

Yijk: k watch value

μ: overall mean

Ei: influence of genotypic effects of the ETH10 marker

Pj: influence of birth sequence (2, 3, 4 and morer).

eijkl: the naturally distributed random error with a mean of zero and a variance of 2eσ

$Y_{ijk} = \mu + S_i + P_j + e_{ijk}$

Si: influence of genetic phenotypes of the CSSM66 marker

Table 1. Sequences of primers supplied by Macrogen

Marker	Chr.	Primesr sequence (5 – 3) Forward Reverse	Degree of annealing (C^{o})	Size (bp)	Reference
ETH10	5	F:GTTCAGGACTGGCCCTGCTAACA R:CCTCCAGCCCACTTTCTCTTCTC	58.2	185 - 221	(31)
CSSM66	14	F:ACACAAATCCTTTCTGCCAGCTGA R:AATTTAATGCACTGAGGAGCTTGG	59	168 - 207	(10)
ILSTS34	5	F:AAGGGTCTAAGTCCACTGGC R:GACCTGGTTTAGCAGAGAGC	55	138 - 212	(9)

Table 2. Materials used and their volumes in the PCR Technique

reaction materials		reaction volume
Master mix (Promega , USA)		12.5 µl
Primers	Forward	1 µl
	Reverse	1 µl
Nuclease Free Water		7.5 µl
DNA		3 µl
Total volume		25 µl

RESULTS AND DISCUSSION

PCR product amplification

The results of the migration PCR that were obtained revealed the presence, diversity, size and distribution of alleles for each of the three studied markers in the local and imported cattle breeds located in Iraq. 6 alleles of different sizes were found for the marker CSSM66 in the studied cattle groups, 7 alleles for the marker ETH10 and 5 alleles for ILSTS34 as shown in Table (3) Figure (2)(3)

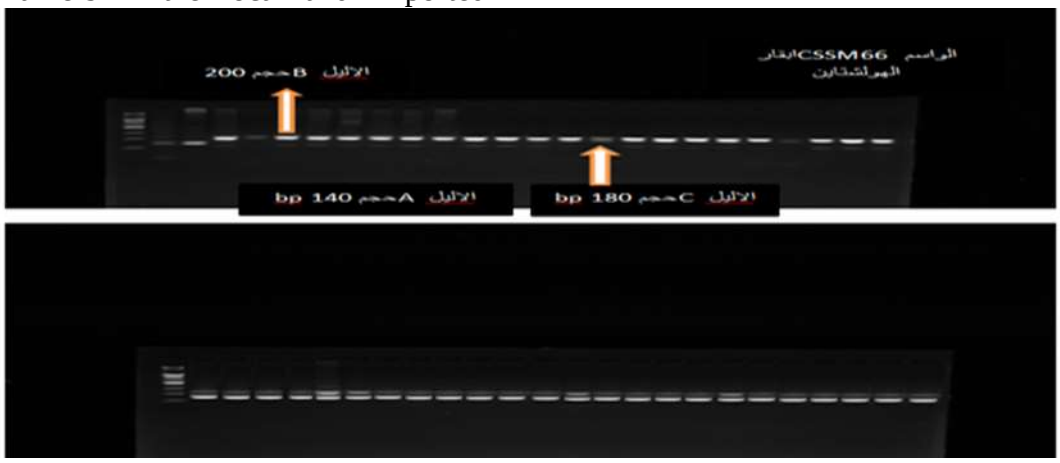

Figure 2.Results of PCR migration and allele size for CSSM66 markers of Holstein cow

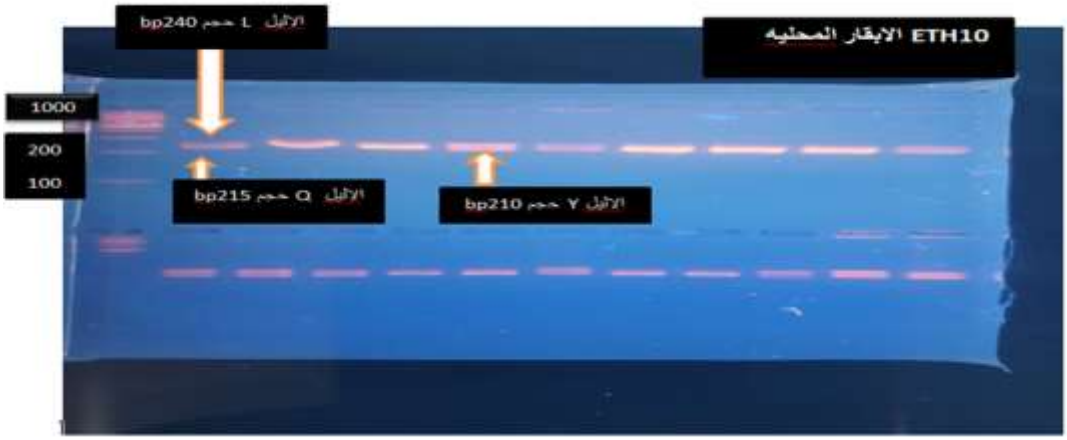

Figure3 Results of the migration of PCR results and allele size for ETH10 marker in domestic cows

Table 3. The distribution, allele code and size resulting from PCR and electrophoresis of the markers CSSM66, ETH10 and ILSTS34 in the studied cows

Marker	Code Allele	bp allele size
CSSM66	E	170
	B	200
	F	205
	C	180
	D	210
	A	140
EHT10	L	240
	Q	215
	Y	210
	H	205
	K	235
	G	225
ILSTS34	P	200
	T	200
	M	210
	S	240
	Z	170
	W	190

Genetic phenotypes of CSSM66, ETH10 and ILSTS34 markers studied in local cattle breed.

Table (4) shows that local cows possessed the same genotypes for the marker ETH10, being LQ and LY, with a different distribution rate within each species. 30 and 70%, respectively. In Restaki, the proportions were somewhat inverse, as the LQ genotype was superior to LY, and the distribution ratio reached 60 and 30%, respectively. For the marker ILSTS34, it was more genetically diverse, as it possessed Sharabi genotypes ZZ and ZT with a percentage of 80 and 20%, respectively. Restaki and Janobi cows both had one genotype, ZM and ZS, with a distribution of 100%. The third marker, CSSM66, showed more diversity, as the BB and EB genotypes appeared for Sharabi cows with a percentage of 60 and 40%, respectively, and the genotypes EE, EB, EF, and FF Restaki were 40, 10, 40 and 10%, respectively, while the southerners came with two single combinations, CC and CD, and they were at a rate of 40 and 60%, respectively. The presence of the same phenotypes within the studied markers for local cattle breed may indicate a common origin among them or that there is blood that was introduced by cross-breeding, for example Holstein. The analysis results of the three markers mentioned are the The results show the analysis of the three mentioned markers, the type of similarity and contrast between the studied local cows, as we notice similarities between the Sharabi, Rustaki and Southern cows carrying the phenotypes of the ETH10 marker with different rates of presence due to the small sample of cows and the variation that exists between cows carrying the different phenotypes of the two markers CSSM66 and ILSTS34. In Iraq, it was not raised in stations

Table 4.Phenotyp of the studied markers in a sample of local cattle breeds

local cows	ETH10		ILSTS34		CSSM66	
	phenotypes	(%) N	phenotypes	(%) N	phenotypes	(%)N
SHARABI	LQ	(%30) 3	ZZ	(%80) 8	BB	(%60) 6
	LY	(%70) 7	ZT	(%20) 2	EB	(%40) 4
	Chi-square (value (χ2	NS 1.600	---	* 3.60	---	NS 0.400
RESTAKI	LQ	(%60) 6	ZM	(%100)10	EE	(%40) 4
	LY	(%40) 4			EB	(%10) 1
					EF	(%40) 4
					FF	(%10) 1
	Chi-square (value (χ2	NS 0.400	---	-	---	NS 3.600
	LQ	(%30) 3	ZS	(%100)10	CC	(%40) 4
	LY	(%70) 7			CD	(%60) 6
	Chi-square (value (χ2	NS 1.600	---	-	---	NS 0.400

.non -significant :NS , (P<0.05) *

Genetic phenotypes of CSSM66, ETH10 and ILSTS34 markers in Holstein cattle breed.

The Holstein breed had two genotypes for each of the three studied markers, namely GP and HK for ETH10, with a distribution of 92 and 8%, respectively, TT and WM of ILSTS34, with a distribution of 94 and 6%, and genotypes AB and CB for CSSM66, with a distribution of 92 and 8%, respectively. Significant differences among the genotypes of each of the three markers as show in Table (5). These structures, if compared to Table (4) with Iraqi cows, revealed that there is no similarity between them and the Holstein cows, which indicates that there is no common ancestry between the Iraqi and Holstein breeds, as well as indicates to some extent the absence of Holstein blood in the studied sample, knowing that it is one of the breeds spread in Iraq and in turn indicates the presence of a good percentage of purity in the studied Iraqi cows, and explains Results The high moral difference of genetic phenotypes about the presence of selection intensity and internal breeding to stabilize specific traits and long-term improvement in Holstein cows by the German Holstein Friesian Association, especially from the It is known that these cows are specially imported as milk cows to Iraq.

This agreed with the study (9) in which micro-seed markers are used on French Hoechstein Friesian cows and explain the results of the small difference between the animals not due to genetic drift but by selection applied to this breed.

Table 5. Phenotyp of the studied markers in a sample of Holstein cows

Marker	Phenotypes	N	(%)
ETH10	GP	46	92.00
	HK	4	8.00
	Chi-square value (χ2	---	** 35.28
ILSTS34	TT	47	94.00
	WM	3	6.00
	Chi-square value (χ2	---	** 38.72
CSSM66	AB	46	92.00
	CB	4	8.00
	Chi-square value (χ2	---	** 35.28

.(P<0.01) **

Genetic phenotypes of CSSM66, ETH10 and ILSTS34 markers in a sample of Brahman cattle

It appears from Table (6) that the Brahman bulls possess the LQ and LY genotypes for the ETH10 marker in a sample of Brahma cows with a significant superiority for the LY genotype, which amounted to 73.68% at the expense of the LQ genotype, and its rate was 26.32% , that This marker shows a great similarity in its eye to the Iraqi and Brahman cows, and this may be the result of the phenotypic similarity between the two local breeds and the Brahman to some extent. As for the marker ILSTS34, the results revealed the presence of one genetic phenotype, WM, and its percentage was 100% in the sample of Brahman cows. The marker CSSM66 came with three genotypes: BB, CC and CD, with percentages of 5.29, 42.11 and 52.26%, respectively, and the results showed the limited and existing diversity of the genetic phenotypes of the markers. The three are in the Brahman cows, and the reason may return to is the process of selection and improvement on them, and they are animals imported specifically for slaughter and not for breeding to Iraq, which is consistent with the study of (16) on the Malaysian Brahman cows.

Table 6. phenotyp of the studied markers in a sample of Brahman cows

Marker	Phenotypes	N	(%)
ETH10	LQ	5	26.32
	LY	14	73.68
	Chi-square value (χ2	---	* 4.263
ILSTS34	WM	19	100
	Chi-square value (χ2	---	-
CSSM66	BB	1	5.26
	CC	8	42.11
	CD	10	52.26
	Chi-square value (χ2	---	* 7.125

(P<0.05) *

Relationship of the genetic phenotypes of ETH10 marker with the productive traits in a sample of Restaki cattle breed

Table (7) shows that the results of the current study showed a significant effect (P≤0.05) according to the genotype of ETH10 marker on the number of inseminations needed for fertilization. The LQ genotype has a rate of 2.00 ± 0.57 inoculation, and this trait is considered one of the important reproductive traits that affect the high-productivity cows. The ETH10 site is located in exon 1 of the STAT6 signal transducer and activator gene located on bovine chromosome 5(15). STAT proteins are involved in the influx of cell signaling after cytokines or hormones that bind their receptors and play an essential role in mammary gland development and differentiation. (4,25,27). It can be considered the first site for quantitative traits in Iraqi cows, and this agrees with the study of (11,23,31) . The genotypes of ETH10 is useful in QTL detection and phenotypic association studies in cattle. (21) showed that it is possible to achieve the mapping of these sites by analyzing their association with known

molecular markers, microsatellite are useful for identifying and mapping their QTL that are associated with variation in traits of economic importance by increasing the rate of genetic improvement using genetic information., the results showed that there was no significant difference between the genetic patterns of other productive and economic traits (length of milk season, daily milk production, fat, protein, lactose percentage, non-fat solids percentages, and the period between two births.

Table 7. Relationship of the genetic phenotypes of ETH10 markers to the studied production and economic traits in a sample of Restaki cows

Marker / Breed	Trait	mean ± standard error Phenotypes LQ	LY	significantly level
ETH10 Restaki	The length of the milk season is a day	a 18.87 ±172.50	a 12.04 ±175.00	NS
	Daily milk production kg	a 0.28 ±4.50	a 0.42 ±3.50	NS
	fat percentages	a 0.10 ±3.28	a 0.18 ±3.44	NS
	protein percentages	a 0.078 ±2.93	a 0.05 ±2.90	NS
	lactose percentages	a 0.09 ±4.39	a 0.06 ±4.40	NS
	(%) The percentage of non-fat solids	a 0.57 ± 8.03	a 0.11 ±7.99	NS
	The number of inseminations required for fertilization	a 0.57 ±2.00	b 0.16 ±1.16	*
	The period between two births day	a 22.50 ±337.50	a 20.11 ±326.66	NS

The mean with different letters within the same row differ significantly from each (P<0.05) **,(P<0.01 ,(NS :insignificant.

Relationship of the genetic phenotypes of cssm66 markers to the productive and economic traits of a Holstein cows

The results in Table (8) shows that there was a significant difference (P≤0.05) according to the genotype of CSSM66 markers, as the AB genotype recorded the highest total average in persistence in milk production for Holstein cows (1.44 ± 0.01) at the expense of the CB genotype, with 1.29 ± 0.06, persistence in production is considered one of the important traits that is affected by many factors, as it is possible to select animals carrying the genotype AB, which obtained the highest average because they are heritable and that fall within the marker CSSM66 and its location is close to the location of the quantitative traits on chromosome 14 in cows , This is in agreement with the study of (13,7,30) of the CSSM66 locus on chromosome 14 the region close to the bovine chromosome 14BTA14 (Bos taurus autosome) quantitative traits and genes related to economically important traits, and (24) demonstrated Bovine chromosome 14 (BTA14) is one of the most extensively studied chromosomes for quantitative trait loci (QTL) related to several economically important traits in cattle, studied by (32) The majority of QTL maps on BTA14 correlate with milk production traits

Table 8. Relationship of the genetic phenotypes of CSSM66 markers to the studied productive traits in a sample of Holstein cows

Marker / Breed	Trait	mean ± standard error Phenotypes AB	CB	significantly level
Restaki ETH10	The length of the milk season is a day	a0.26 ±303.02	a1.18 ±303.25	NS
	Daily milk production kg	a0.50 ±13.72	a1.93 ±12.75	NS
	persevere to produce	a0.01 ±1.44	b0.06 ±1.29	*
	pinnacle of production	a0.54 ±15.89	a1.17 ±14.86	NS
	fat percentages	a0.027 ±3.48	a0.08 ±3.39	NS
	protein percentages	a0.01 ±2.68	a0.05 ±2.90	NS
	lactose percentages	a0.03 ±3.97	a0.08 ±4.19	NS
	(%) The percentage of non-fat solids	a0.06 ±7.27	a0.06 ±7.44	NS
	The number of inseminations required for fertilization	a2.51 ±469.00	a4.79 ±470.00	NS

The mean with different letters within the same row differ significantly.* (P<0.05), NS: Not significant

Relationship of the genetic phenotypes of ETH10 marker with the productive and economic traits studied in a sample of Holstein cattle

The results in Table (9) shows that there was a significant difference (P≤0.05) according to the genotype of the ETH10 marker, as the GP genotype recorded the highest total average in the persistence of milk production for Holstein

cows (1.44 ± 0.01) at the expense of the HK genotype, with 1.29 ± 0.06, the trait of persistence in production is considered one of the important traits, as it is possible to select animals carrying the GP genotype, which obtained the highest average and associated in the cow genome, the ETH10 site in exon 1 of the signal transducer and transcription activator gene (6STAT) located on bovine chromosome No. 5 Studies (4,25,28) indicate that STAT proteins are involved in the signaling flow of cells after cytokines or hormones that bind their receptors and play an essential role in brown gland growth and differentiation. This is reflected in the differential expression of genes during development. The brown gland and through different stages of lactation.

Table 9. Relationship of the genetic phenotypes of ETH10 markers to the studied productive traits in a sample of Holstein cows

Marker Breed	Trait	mean ± standard error Phenotypes GP	HK	significantly level
Holstein ETH10	The length of the milk season is a day	a 0.26 ±303.02	a 1.18 ±303.25	NS
	Daily milk production kg	a 0.50 ±13.72	a 1.93 ±12.75	NS
	persevere to produce	a 0.01 ±1.44	b 0.06 ±1.29	*
	pinnacle of production	a 0.54 ±15.89	a 1.17 ±14.86	NS
	fat percentages	a 0.027 ±3.48	a 0.08 ±3.39	NS
	protein percentages	a 0.01 ±2.68	a 0.05 ±2.90	NS
	lactose percentages	a 0.03 ±3.97	a 0.08 ±4.19	NS
	The percentage of non-fat (%) solids	a 0.06 ±7.27	a 0.06 ±7.44	NS
	The number of inseminations required for fertilization	a 2.51 ±469.00	a 4.79 ±470.00	NS

The mean with different letters within the same row differ significantly * (P<0.05), NS: Not significant

Relationship of the genotypes of ETH10 and CSSM66 to weight in a sample of Brahman cows

It is clear from the Table (10) that there is a significant difference (P≤0.05) in the mean weight according to the genotype of CSSM66 markers among the males of the Brahman, where the genotype CC recorded the highest mean of the total weight of the males, which amounted to 502.75 ± 38.55 kg at the expense of the genotype CD At a rate of 392.30 ± 22.45 kg, which is one of the very important economic traits for beef cattle, as it is possible to select animals carrying the CC genotype, which obtained the highest average because it is heritable and close to the location of the quantitative traits on chromosome 14 in cows, which fall within the marker CSSM66. Considering the result important and adding it to previous research that supports the importance of the marker for mapping the quantitative traits in beef cattle. This is in agreement with the study of(13,7,30)of the CSSM66 locus on chromosome 14, the region close to the bovine chromosome 14BTA14 (Bos taurus autosome) quantitative traits and genes related to economically important traits. (32) studied and contributed The markers include CSSM66 marker with QTL mapping in beef cattle, mainly on growth traits, carcass and meat quality. The studies included target phenotypes, hot carcass weight, eye area, average daily increase ADG, muscle fat deposition, and depth of subcutaneous fat. In the same context, it appears from the same table that there are no significant differences between the genetic features of the marker ETH10 in relation to the weight of Brahman males.

Table 10. Relationship of genetic phenotypes of EHTL10 and CSSM66 to weight in a sample of Brahman cattle

Marker	Phenotypes	Weight kg	significantly level
ETH10	LQ	a19.96 ± 380.200	
	LY	a 30.25 ± 455.07	NS
CSSM66	CC	a 38.55 ± 502.75	
	CD	b 22.45 ± 392.30	*

The mean with different letters within the same row differ significantly.* (P<0.05), NS: Not significant

C0NCLUSION

In conclusion, studying more than one of the markers recommended by the World Food Organization to identify the variations and drawing the genetic map of the important quantitative traits of Iraqi cows and genetic diversity.

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