A study of the Relationship of Polymorphisms of the PPARGC1A Gene with Milk Production and its Components, in Holstein Friesian Cows

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A study of the Relationship of Polymorphisms of the PPARGC1A Gene with Milk Production and its Components, in Holstein Friesian Cows

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Abstract. This research was done at a Molecular Genetics lab at the College of Agriculture at the University of Basrah. The objective of this study is to determine how Holstein Friesian cows' PPARGC1A genetic polymorphism affects milk production and its constituent parts. For milk production and quality standards, 59 Holstein Friesian cows from Taj Al-Nahrain station in Al-Diwaniyah are employed. Blood was drawn for genomic DNA, and the PPARGC1A gene was amplified using PCR. The samples were then transferred to the Korean firm Macrogene for DNA sequencing analysis. Morning milk 50ml samples were collected once each week., and after taking the samples during the milking process, they were evaluated for quality using the Julie Z7 milk analyzer. Association analysis was performed using SPSS statistical software. According to the current study's findings, the stage of milk production had a significant impact on both daily and total production ($p \leq 0.05$). The results also shows that there is a significant differences ($p \leq 0.05$) of stage of milk production on the chemical composition of milk, while there are no significant differences between Genetic Polymorphisms in daily and total milk production. The results show that hap1 is superior on hap2 in the chemical contents of milk, except for the percentage of milk fat, which has not reach a significant level.

Keywords. Polymorphisms, PPARGC1A, Milk Production, Holstein Friesian Cows.

1. Introduction

The genetic basis of complex features, such as cattle productivity traits, has studied using the candidate gene technique. [1]. With the development of modern times, it became possible to predict the origin of local strains found in different regions of the world, and used many studies to uncover the whole genome sequence [2] . This technique has shown to be an efficient for determining the effects of genetic variations affecting economic variables [3] . The understanding of the gene function and biological mechanisms underlying the trait under investigation is necessary for the candidate gene strategy to be successful [4].

The peroxisome proliferator-activated receptor gamma coactivator-1 alpha (PPARGC1A) gene, also known as PGC-1a, is found on chromosome 6 of the bovine [5]. The nuclear hormone receptors and metabolic pathways connected to adipogenesis and adaptive thermogenesis are activated by the

PPARGC1A protein [6] . PGC-1a gene expression is increased in tissues that engage in active oxidative metabolism, such as brown adipose tissue (BAT), cardiac muscle, and skeletal muscle [7]. [8], identified a 420 kb region on bovine chromosome 6 that contains a QTL for milk production traits between the ABCG2 and LAP3 genes. The osteopontin (OPN) gene is one of just six genes housed in this compact area. Several research teams, encouraged by the results of the aforementioned QTL analyses, looked into the probable links between genes in this area and milk production attributes in dairy cattle. [9] a single nucleotide polymorphism (SNP) in microsatellite BM143 in the peroxisome proliferator-activated receptor gamma coactivator 1 alpha (PPARGC1A) gene was found to have a significant effect on milk fat yield.
The PPARGC1A gene in cattle has been proposed as a candidate gene for traits related to milk production [10]. Milk production, milk fat production, and protein production are all correlated with PPARGC1A gene polymorphism in Czech Fleckvieh cattle, however [11] . The current study aims to show how Holstein cows' milk production and its contents are affected by polymorphisms of the PPARGCIA gene.

2. Materials and Methods

2.1. Populations and Data
59 cows were sampled in the morning at various levels of production. After carefully mixing the milk produced by the cow until it becomes homogeneous and in an amount of about (50 ml), a sample of milk was obtained, and then it is immediately brought to the analysis laboratory. After samples were obtained during the milking process, they were preserved, kept out of direct sunlight, and kept away from high temperatures to estimate the milk's components of fat, protein, total solids, and lactose every two weeks for three months. 59 cows' milk veins were used to collect blood samples, which were then placed in a 10 ml vacuum tube containing K3 EDTA. Genomic DNA was isolated from the whole blood sample using a kit made available by the Korean Geneaid Company and kept at 4 °C. Primers :
F5′-CATAGCCGGCGCCCCAGG TAAGATGCACGTTGGC-3′
R5′-CTGGTACT CCTCGTAGCTGTC-3′ are used to amplify 195 bp in intron 7 at position 1892 of PPARGC1A, Primers were used by [9] .
25 ng of genomic DNA, 25 ng of each primer, 100 M of each dNTP, 1.5 l of 10 PCR solution (Promega, Madison, WI), and 0.2 U of Taq DNA polymerase (Promega) were added to a 15 l reaction volume. We start with 95 °C for 5 minutes, then cycle through 32 times at 94 °C for 45 seconds, 50 °C for 45 seconds, 72 °C for 45 seconds, and finally an extension at 72 °C for 7 minutes. Electrophoresis on an agarose gel of 2.5% is used to separate digestion products.

3. Results and Discussion

3.1. Analysis of Polymorphisms with PCR Amplification
Both genetic groups amplified a single band with a length of 195 bp (Fig. 1). Two banding patterns were found in the intron 7 region (195 bp), which were identified by digestion as hap1 and hap2. PPARGC1A's haplotypes are represented by the view numbers hap1(20) and hap2(39) in table 1, whereas the production stages are represented by the view numbers: Table (2), stage 1(11), stage 2(16), and stage 3(32).

Figure 1. PPARGC1A gene amplification of intron 7 (195 bp) Lane.

Table 1. Holstein Friesian cattle haplotypes.

Haplotype	N.
Hap1	20
Hap2	39

Table 2. Lifecycle of a Holstein-Friesian Cow.

Production stage	N.
Stage 1	11
Stage 2	16
Stage 3	32

3.2. Effect of PPARGC1A Haplotypes and Production Stage on Milk Production

Table (3) shows that there are significant differences (p≤ 0.05) in the production stages, pertaining to both the daily and overall milk yield , The 1st stage production is superior on the 2nd and 3rd stages in daily production , while no statistical differences have showed between the 2nd and 3rd stages , the results were showed : 7.389, 5.625 and 4.779 / kg respectively, even if the average daily production was 5.931/ kg.

The same table explains that the 1st stage of milk production has a higher total production rate than the 2nd and 3rd stages, while no significant differences are showed between the 2nd and 3rd stages, the values are recorded as follows 665.833, 514.125 and 429.123 / kg respectively, while the mean total milk production is 536.360 / kg . These findings did not agree with [12] it showed that the production stages on both the daily and total milk production do not change significantly , and the results agreed with [13], who indicated that the highest rate of milk production was showed in the 1st stage of production, and that regional and climatic variables might be the cause of the variance between researchers [14].

The results show that the highest daily production rate is observed for hap1 in the first stage, as it is 8,000 / kg, and the lowest daily production rate is observed for hap2 in the third stage, as it is 4.727 / kg, and the highest total milk production has shown for hap1 in the first stage, as it is 712,000 / kg, and th e lowest daily production rate is observed for hap2 in the third stage, as it is 4.727. The results of this study agreed with A[15] , who demonstrated that PPARGC1A haplotypes are not statistically associated with milk production. The results also agreed with [7] , who showed that there is no significant the effect of the PPARGC1A gene at site 1892 on the rate of milk production.

Table 3. Effect of PPARGC1A haplotypes and production stage on milk production.

Haplotype/ Stage		Haplotype		
		Hap 1	**Hap 2**	**The mean Stage**
Daily production	Stage1	8.000±1.202	6.778±.566	7.389±.664 a
	Stage2	5.375±.601	5.875±.601	5.625±.425 b
	Stage3	4.830±.537	4.727±.362	4.779±.324 b
The mean hap		6.068±.482	5.793±.301	5.931±.284
Total production	Stage1	712.000±106.488	619.667±50.199	665.833±58.863 a
	Stage2	498.000±53.244	530.250±53.244	514.125±37.649 b
	Stage3	436.700±47.623	421.545±32.107	429.123±28.718 b
The mean hap		548.900±42.743	523.821±26.637	536.360±25.182

Different letters in the columns don't differ significantly at the 5% level.
Similar letters in the columns don't differ significantly at the 5% level.

3.3. Effect of PPARGC1A Haplotypes on Milk Traits

Table (4) shows that there are statistical differences (p≤0.05) in production stage in each of the percentages of fat , protein, lactose and total solids in milk, where the 1st stage of milk production outperformed the milk fat percentage on the 2nd stage, while no statistical differences have showed between the 1st and 3rd stages and no significant differences have showed between the 2nd and 3rd stages, the values are recorded as follows : 3.268, 2.867 and 2.953%, respectively. While the mean percentage of milk fat is 3.029 %. Table (4) also shows that the 3rd stage of production is superior on the 2nd stage in the percentage of milk protein, while no significant differences have showed between each of the 1st and 2nd stages, as well as between the 1st and 3rd stages, the values are recorded as follows : 3.044, 3.022 and 3.107 % respectively, in when the mean percentage of milk protein is 3.058 %. The results show that the 3rd stage of production is superior on the 2nd stage in the percentage of lactose , while no significant differences have showed between each of the 1st and 2nd stages, as well as between the 1st and 3rd stages, as the results are : 4.466, 4.385 and 4.509% respectively, while the mean lactose percentage is 4.453 %. The same table also shows the superiority of the 3rd stage on the 2nd stage in the percentage of total solids, while no significant differences have showed between each of the 1st and 2nd stages, as well as between the 1s^t and 3rd stages, as the results are 8.091, 7.982 and 8.205% respectively, in when the mean percentage of total solids is 8.093 %.

The results of this study differed with [16] , who indicated that the highest percentages of protein, fat and total solids are in the 2nd stage, while they stated that there are no statistical differences in lactose in all stages of milk production. It also did not agree with [17] , who did not record a statistical effect between production stage and each of the production rate and the chemical composition of milk.

As shown in Table (4), there are statistical differences ($p \leq 0.05$) between haplotypes in different of production stages in the proportions of protein, lactose and total solids. For milk protein, the results affair that superiority of hap1 on hap2, as the results are : 3.111 and 3.004 %, respectively. Also, it is appears the superiority of hap1 over hap2 in the percentage of lactose , as the results are : 4.546, 4.360 %, respectively. These study also notice the superiority of hap1 on hap 2 in the percentage of total solids, as the results are 8.238 and 7.948 % respectively, While no significant differences have showed in the percentage of milk fat, and despite the lack of significance, the highest percentage of fat has showed for hap1 in the 1st stage of production, as it is 3.461 % , while the lowest percentage of fat has showed for hap 2 in the 2nd stage of production as it is 2.635 % , These results partly agreed with [18] , that there are no significant differences in milk fat during the different stages of production. The results also agreed with [19] , that there is no statistical effect of the PPARGC1A gene on the milk fat percentage, and disagreed with them about the rest of the milk components , and did not agree with [20] , about the percentage of fat. These findings agreed with [10] , who showed that there are no statistical differences in locus 1892 of the PPARGC1A gene with milk fat. It agreed with [21], regarding milk protein.

Table 4. Milk trait (percent) variation due to PPARGC1A haplotypes and lactation stage ±s.e.

Haplotype/ Stage		Haplotype		
		Hap 1	Hap 2	The mean Stage
Fat	Stage1	3.461±.301	3.075±.142	3.268±.166 a
	Stage2	3.099±.150	2.635±.150	2.867±.106 b
	Stage3	2.941±.139	2.965±.091	2.953±.083 ab
The mean hap		3.167±.121 a	2.892±.075 a	3.029±.071
Protein	Stage1	3.107±.082	2.980±.038	3.044±.045 ab
	Stage2	3.071±.041	2.973±.041	3.022±.029 b
	Stage3	3.154±.038	3.061±.025	3.107±.022 a
The mean hap		3.111±.033 a	3.004±.020 b	3.058±.019
Lactose	Stage1	4.595±.128	4.337±.060	4.466±.071 ab
	Stage2	4.465±.064	4.304±.064	4.385±.045 b
	Stage3	4.579±.059	4.440±.039	4.509±.035 a
The mean hap		4.546±.052 a	4.360±.032 b	4.453±.030
T.S.D	Stage1	8.261±.227	7.922±.107	8.091±.126 ab
	Stage2	8.128±.114	7.836±.114	7.982±.080 b
	Stage3	8.324±.105	8.086±.069	8.205±.063 a
The mean hap		8.238±.092 a	7.948±.057 b	8.093±.054

Different letters in the columns don't differ significantly at the 5% level.
Similar letters in the columns don't differ significantly at the 5% level.

Conclusion

Production stage has a statistical effect on daily and total production and the chemical composition of milk in the Holstein Friesian strain. Haplotypes also have a significant effect on protein, lactose, and total solids in milk, but the PPARGC1A haplotype has no effect on milk production.

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