ISSN NO: 2771-8840
Date of Publication: 30-03-2023

A Study of the Relationship of Genetic Polymorphisms of the PRL Gene with Milk Production and its Components in Holstein Friesian Cows

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Abstract

Prolactin is a key regulator of milk production, mammary gland development, and milk protein gene expression . This study has been conducted in a laboratory Molecular Genetics of the College of Agriculture / University of Basrah. It aims at measuring the genetic polymorphism of milk production and its components, in Holstein Friesian cows. 59 Holstein Friesian cows from Taj Al-Nahrain station in Al-Diwaniyah have been characterized for milk production and quality characteristics. Genomic DNA has been extracted from the blood and PCR has been used to amplify the PRL gene, then the amplification product has been purified and the samples have been analyzed by the Korean company Macrogene for DNA sequencing analysis. 50ml samples of morning milk have been collected on weekly basis and analysed for quality using the Milk analyzers Julie Z7 after collecting samples during the milking process. SPSS Statistical software has been used for association analysis. Gene shows the presence of a three genetic polymorphism in the breed of Holstein Friesian cows. The results show a significant the effect ($p \leq 0.05$) of the stage of milk production on each of the daily, and total milk production rate, while no differences have been shown of haplotypes on the rate of milk production.

There have been no significant differences in the production stage in the proportions of fat, protein and milk sugar, while there has been a significant difference for the production stage on the total solids. In addition, no significant differences have been between shown of the haplotypes of the chemical composition of milk, except for the total solids.

Key words: Genetic Polymorphisms, PRL, Milk Production, Holstein Friesian Cows.

Introduction

Polymorphic milk secretion genes are relevant as candidate genes and can be used in indirect livestock selection because of their ties to quantitative traits (Alfonso *et al.*, 2012) . Prolactin (PRL) is crucial for controlling mammary gland growth, protein expression in milk, also milk production (Brym *et al.*, 2005). As a result, the PRL gene has the potential to be a powerful genetic marker for improving livestock. The gene, which is 10 kb in size and codes for all 199 amino acids in the BTA23, has 5 exons and 4 introns (Dybus, 2002) . Pituitary gland and cells, primarily lymphocytes, release PRL, which has an immunostimulatory impact and encourages autoimmune disease(Orbach and Shoenfeld, 2007). One of its most crucial features was highlighted, about animal breeding , is its critical role in starting and maintaining lactation. This function is achieved by stimulating mammary alveoli for the synthesis and secretion of milk proteins. Moreover, it is in charge of creating all the major components of milk (Horseman *et al.*, 1997) . Previous research have reported on a number of bovine prolactin gene polymorphisms, mainly focusing on the association between milk production attributes and prolactin gene RsaI/PRL variations (Bayıl Oğuzkan and Bozkurt, 2019) . The current study's objective is to demonstrate the degree to which some economic and physiological features in Holstein cows are correlated with the prolactin gene.

Materials and Methods

During the morning arena, milk samples are obtained from 59 cows from different stages of production . A sample of milk is taken after thoroughly blending the milk produced by the cow such that the sample is homogeneous and in an amount of around (50 ml), and is then transported immediately to the analysis laboratory. The milk components are estimated from Fat, protein, and lactose every two weeks for three months using the Milk analyzers Julie Z7 after samples are taken during the milking process, along with preservation and not exposing them to sunlight or high temperatures.

Blood samples are taken from 59 cows' milk veins and put in a 10 ml vacuum tube containing K3 EDTA . Then, using a kit provided by the Korean Geneaid Company, genomic DNA is extracted from the complete sample of blood and stored at 4 °C. For the gene PRL:

F: 5´CCAAATCCACTGAATTATGCTT 3´

R: 5´ACAGAAATCACCTCTCTCATTCA 3′

Amplification is performed in Macrogene Company and as follows: 500 ng of template DNA, 1 unit of Taq DNA polymerase, 200 µM of each dNTP, 50 ng of each primer, and a final reaction volume of 25 µl are all included in each PCR experiment. MgCl2 is present in each 10X buffer. Denaturation at 94°C for two minutes, followed by 36 cycles of annealing at 57°C for thirty seconds, extension at 72°C for forty-five seconds, and a final extension step at 72°C for ten minutes, are the conditions for the amplification cycling . The reaction products are electrophoresed on a 1.5% agarose gel and stained with ethidium bromide to determine the success of the PCR reaction. Thermo Scientific's Rsa I is used to digest the PCR results in a final reaction volume of 25 l for an hour at 37 °C. After restriction digestion, the restricted fragments are examined electrophoretically on 3% agarose gel stained with ethidium bromide. The digested products are seen using a transilluminator with UV light. The banding patterns are manually evaluated, and the gels are documented in a system called a gel documentation system (Ruksana M Shah *et al.*, 2021).

Statistical Analysis

In order to determine the impact of production stage and haplotype on milk production and its constituent parts, the results of this study are analyzed using factional analysis based on the General Linear Model SPSS (2012).

Results and Discussion

PCR amplification and polymorphism analysis

A unique band of 294 bp length that corresponds to each genetic group. After digestion, the exon IV region (294 bp) showed three distinct banding patterns, which were given the names hap1, hap2, and hap3 (Plate 1). The number of haplotypes of prolactin are as follows : hap1(12) , hap2(16) and hap3(31) (table 1) , While the numbers of views for the stages of production are as follows : Stage 1(169),stage2(75) and stage3(31) (table 2) .

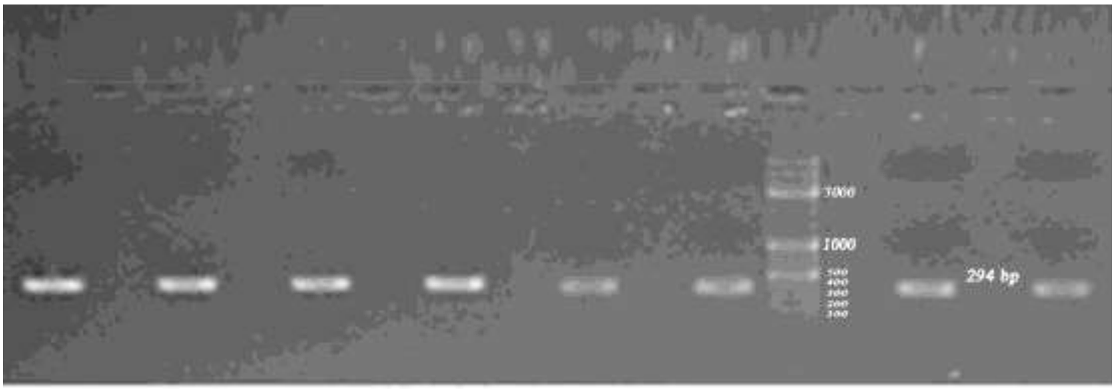

Plate 1: Amplification of Exon 4 (294 bp) , PRL gene Lane

Table 1: haplotypes in Holstein cows

Haplotype	N.
Hap1	12
Hap2	16
Hap3	31

Table 2: production stage in Holstein cows

ISSN NO: 2771-8840
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production stage	N.
Stage 1	169
Stage 2	75
Stage 3	51

The effect of PRL haplotypes and stage production on milk production

Table (3) demonstrates that the production phases for each of the daily and total milk production have a significant differences , as we notice from the table the superiority of the first stage of milk production over the second and third stages in the daily production rate, and the second stage outperformed the third stage as the results reached 7.056. 5,500 and 4,128 / kg , respectively, while the mean daily milk production is 5,561 / kg , as table (3) shows the superiority of the first stage of total milk production over the second and third stages, and the second stage outperformed the third stage as the results reached 639.444 , 504.444 and 378.351 / kg respectively, while the mean total milk production during three months is 507.413 / kg . These results did not agree with (Al-Muhja, 2014), which did not find a significant the effect of the production stage on the rate of milk production And agreed with (A. V. Capuco *et al.*, 2001) who indicated that the decrease in milk production with the progression of the lactation process is caused by a decrease in the number of epithelial cells by 50% than it is in the early stage.

Table (3) also shows that there are no significant differences for the haplotype in all stages of production , and despite the absence of significant differences, we note that the highest daily production rate is shown for the hap3 in the first production stage, as it reached 8,000 / kg and the lowest daily production rate is shown for the hap3 in the third production stage amounting to 2.750 / kg . The highest total milk production is shown for hap3 in the first production stage, which amounted to 715.333 /kg , and the lowest total production is shown for hap3 in the third production stage, amounting to 265,750 / kg . The results agreed with Brym *et al.* (2005), which shown that the highest rate of milk production is shown for genotype AG in Jersey cows for the prolactin gene, and also agreed with (Bayıl Oğuzkan and Bozkurt, 2019), which shown that genotype AA is the highest mean of milk production compared to genotypes AB and BB. This results agreed with Yayied, A. and Fzaqeer, B. (2018) , which shown that the highest rate of daily milk production is shown for hap3.

Table 3: The effect of PRL haplotypes and stage production on milk production ±s.e

Haplotype / Stage		Hap 1	Hap 2	Hap3	Mean Stage
Daily production	Stage1	6.166± .635	7.000 ± .898	8.000 ± .898	7.056±.473 a
	Stage2	6.000± .518	4.500 ± .778	6.000 ± .898	5.500±.432 b
	Stage3	5.384± .357	4.250 ± .550	2.750 ± .778	4.128±.339 c
Mean hap		5.850± .298	5.250± .436	5.583± .496	5.561± .242
Total production	Stage1	555.333±58.047	647.667±82.090	715.333± 82.090	639.444±43.265a
	Stage2	544.333±47.395	420.000±71.092	549.000± 82.090	504.444±39.496b
	Stage3	473.053±32.619	396.250± 50.270	265.750±71.092	378.351±30.993c
Mean hap		524.240 ±27.243	487.972 ±39.889	510.028± 45.377	507.413±22.092

The similar letters in the -The different letters in the columns show significant differences at 5% level. -columns show no significant differences at 5% level .

The effect of PRL haplotypes and stage production on milk quality traits

Table (4) indicated that there are no significant differences in the production stage in the proportions of fat, protein and milk sugar. As for the total solids, the results shown significant differences (p≤0.05), as the third stage outperformed the first and second stages, while no significant differences are shown between the first and second stages as the results are7.98, 8.044 and 8.287%. Despite the lack of significance in the rest of the milk components, we notice the superiority of the first stage in the percentage of milk fat over both the second and third stages, as the results are 3.208, 2.904, and 2.982 % respectively, and the results shown that with regard to milk protein, the superiority of the third stage over all From the first and second stages, as the results are3.004 , 3.043 and 4.937%, respectively, and with regard to milk sugar, it is noted that the third stage outperformed both the first and second stages, as the results are 4.385, 4.417 and 6.006 % respectively. These results partly agreed with (Gajbhiye *et al.*, 2019) who shown that there is no significant effect of the production stage on the percentage of protein and sugar and differed with it with regard to both the percentage of fat and minerals, and agreed with (R.B. Kayastha *et al.*, 2008) regarding the percentage of minerals, and our results did not agree with (Al-Muhja, 2018) . The ability to prolong lactation may be improved by increasing cell replacement or reducing apoptosis during lactation. (A. V. Capuco *et al.*, 2001)

As shown in Table (4) there are no significant differences for the haplotype in the production stages with regard to fat, protein and milk sugar. As for the total solids, significant differences (p≤0.05) are shown between the haplotype and the stage of milk production , hap3 outperformed Hap1, and Hap2 outperformed hap1. While no significant differences are shown between hap3 and hap2, where the results are 7.920 8.208 and 8.190 % respectively. In spite of the absence of significant differences in the rest of the milk characteristics, we note that the highest percentage of fat is shown for hap1 in the first stage of production 3.479% and the lowest percentage is in the second stage of production 2.776% , also evident in Table (4) recording the highest percentage of protein is shown for hap1 in the third stage of production 8.438% and the lowest in the second stage of production 2.970% . The same table also shows that the highest milk sugar percentage is recorded for hap1 in the third stage of production 8.790% , while the lowest milk sugar percentage is recorded in the second stage of production 4.304% . These results agreed with (Ruksana M Shah *et al.*, 2021), which shown that there are no significant differences in the genotype of the prolactin gene in belligerent cows on all components of milk except for milk minerals. These results also agreed with (Bayıl Oğuzkan and Bozkurt, 2019).

Table 4: The effect of PRL haplotypes and stage production on milk quality traits ±s.e

Haplotype / Stage		Hap 1	Hap 2	Hap3	Mean Stage
%Fat	Stage1	3.479± .219	3.021± .247	3.123± .247	3.208±.137
	Stage2	2.776± .142	3.081± .213	2.855± .247	2.904±.119
	Stage3	2.880± .093	3.078± .151	2.984± .208	2.982± .091
Mean hap		3.046±.092	3.060±.120	2.988±.135	3.031±.068
%Protein	Stage1	2.980± 5.387	3.013± 6.062	3.019± 6.062	3.004±3.375

	Stage2	**2.970± 3.500**	**3.142± 5.250**	**3.017 ±6.062**	**3.043± 2.917**
	Stage3	**8.438± 2.291**	**3.122± 3.712**	**3.250± 5.124**	**4.937± 2.243**
Mean hap		**4.796± 2.273**	**3.093± 2.946**	**3.095± 3.329**	**3.661±1.664**
%Lactose	**Stage1**	**4.335± 6.206**	**4.374± 6.984**	**4.446 ±6.984**	**4.385± 3.888**
	Stage2	**4.304± 4.032**	**4.575± 6.048**	**4.374± 6.984**	**4.417± 3.360**
	Stage3	**8.790± 2.640**	**4.513± 4.277**	**4.714± 5.903**	**6.006± 2.584**
Mean hap		**5.810±2.619**	**4.487± 3.394**	**4.511± 3.835**	**4.936 ±1.917**
%T.S.D	**Stage1**	**7.907± .162**	**8.051± .183**	**8.001± .183**	**7.986± .102 b**
	Stage2	**7.830± .105**	**8.333± .158**	**7.969± .183**	**8.044± .088 b**
	Stage3	**8.023± .069**	**8.238 ±.112**	**8.601± .154**	**8.287± .068 a**
Mean hap		**7.920± .068 b**	**8.208± .089 a**	**8.190± .100 a**	**8.106±.050**

-The different letters in the columns show significant differences at 5% level.
-The similar letters in the columns show no significant differences at 5% level .

Conclusion

In the Holstein Friesian cows populations, a significant the effect of the stage of milk production is shown on the daily and total milk production mean and total solids, and a significant difference is shown for haplotypes on the total solids, while no significant the effect of is shown on milk production.

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