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PROLACTIN AND RECEPTOR GENE POLYMORPHISM AND THEIR ASSOCIATION WITH SOME PRODUCTIVE TRAITS OF HOLSTEIN COWS

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ABSTRACT

This study was conducted at Al-Khalis Dairy Cattle Station in Diyala Governorate during the productive season during 2021–2022. 63 Holstein cows were selected randomly to study the effect of prolactin gene (PRL) and prolactin gene receptor (PRLR) polymorphism on milk production, milk components, and reproductive traits in Holstein dairy cows. The results showed three SNPs of PRL gene (TT, TC, and CC). There are highly significant differences (P<0.01) between the percentages of the genotypes, which reached (79.37, 17.46, and 3.17% , respectively) and three SNPs for PRLR gene (TT , TA and AA with percentage 47.62 , 41.27 and 11.11 respectively) in Holstein dairy cows in Iraq. the results showed there were a significant effect of TC genotype (4187.82 kg) of total milk yield compared with TT (4013.02 kg) and CC (3505.00 kg) The polymorphism for PRL gene The total milk production was higher in TA genotype (4321.04 kg) followed than TT genotype (4025.53 kg) and AA genotype (2877.33 kg) for PRLR gene. The polymorphism of the PRL and PRLR genes did not significantly effects on the reproductive traits and milk components except for fat percentage in the PRL gene and lactose percentage in the PRLR gene. It can be concluded, a highly significant relationship between the prolactin gene and the prolactin receptor gene with milk production, so it can be used for genetic improvement of milk cows.

Key words: milk production, genotypes, lactation period, persistency period, reproduction

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مجلة العلوم الزراعية العراقية- 2025 :56 (4):1305-1315 العبيدي والجنابي

تعدد المظاهر الوراثية لجين البرولاكتين ومستقبله وعلاقتها ببعض الصفات الإنتاجية لأبقار الهولشتاين

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المستخلص

أجريت الدراسة في محطة أبقار الخالص بمحافظة ديالى اثناء الموسم الإنتاجي 2021–2022. تم اختيار 63 بقرة هولشتاين عشوائياً لدراسة تأثير جين البرولاكتين (PRL) ومستقبلات جين البرولاكتين (PRLR) على إنتاج الحليب ومكونات الحليب والصفات التناسلية في أبقار هولشتاين الحلوب. أظهرت النتائج وجود ثلاثة مظاهر وراثية لجين (PRL) هي TT وTC و CC ، وهناك فروق ذات دلالة إحصائية (P>0.01) بين النسب المئوية للتراكيب الوراثية والتي بلغت 79.37 و 17.46 و 3.17٪ على التوالي ووجود ثلاثة مظاهر وراثية لجين (PRLR) وهي TT و TA و AA بنسبة 47.62 و 41.27 و 11.11 على التوالي في أبقار الهولشتاين الحلوب في العراق. أظهرت النتائج وجود تأثير معنوي في التركيب الوراثي TC (4187.82) كغم إنتاج الحليب الكلي مقارنة مع التركيب الوراثي TT(4013.02) كغم التركيب الوراثي, CC (3505.00) كغم لجين PRL . كان إنتاج الحليب الكلي أعلى في التركيب الوراثي TA (4321.04) كغم يليه التركيب الوراثي TT (4025.53) كغم والتركيب الوراثي AA (2877.33) كغم لجين PRLR . لم يكن لتعدد المظاهر الوراثية لجينات PRL و PRLR تأثير معنوي على الصفات التناسلية ومكونات الحليب باستثناء النسبة المئوية للدهن في جين PRL والنسبة المئوية اللاكتوز في جين PRLR. يمكن الإستنتاج وجود علاقة ذات دلالة إحصائية بين جين البرولاكتين وجين مستقبل البرولاكتين مع إنتاج الحليب، لذلك يمكن استخدامه للتحسين الوراثي لأبقار الحليب.

الكلمات المفتاحية: إنتاج الحليب، التراكيب الوراثية، طول موسم الحلب، طول فترة المثابرة

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INTRODUCTION

Dairy cows play a crucial role in the global economy and food supply. They are the primary source of milk, consumed by millions of people worldwide. The importance of dairy cattle stems from the fact that they are efficient converters of feed into a nutritious and valuable product (2, 12, 14). In 2022, the global production of milk is projected to increase to 849 million metric tons from 820 million metric tons in 2020 and global milk production will reach 1020 million tons in 2030, and dairy cows will produce 80% or more of the milk consumed globally (25). The increasing in milk production is driven by factors such as population growth, rising incomes, and urbanization. Dairy farming has become a major source of income for many farmers in both developing and developed countries. However, dairy farming also has its challenges, including managing herd health, maintaining milk quality, and meeting environmental regulations. Strategies such as improved Genetics, better feeding protocols, can help farmers increase efficiencies and improve sustainability (10, 14, 21, 31, 38). PRL and PRLR are both critical for milk production in dairy cows. Prolactin is a hormone that is produced in the pituitary gland in and is responsible for lactation and stimulates milk production, PRL codes for the synthesis of prolactin protein, while the PRLR gene codes for the synthesis of prolactin receptors. The receptor is necessary for prolactin to bind to the mammary gland cells and initiate milk production (25, 26, 34, 40). PRL is a polypeptide composed of five exons and four introns, encodes the 199 amino acid groups of the mature protein found on chromosome 23 in the bovine genome and is about 10 kb in size (25, 26) and PRLR was mapped on chromosome 20q17, in cattle, there are two distinct prolactin receptor isoforms: long PRLRR with 557 amino acids and short PRLRR with 272 amino acids (1). Polymorphism in the PRL and PRLR has been linked to differences in milk yield and composition, as well as various reproductive traits (4, 5, 9, 11, 15, 16, 36) and immune regulation (6, 7, 8). Understanding the genetic basis of these traits provides a means for selection and breeding programs that can improve milk production and quality in dairy cows (1, 39). Knowledge of the most probable producing ability (MPPA) values for cows helps in the conduct of selection programs through the application of the method of culling and replacement of cows (28, 33, 37), so this study was aimed to investigate the relationship between polymorphism of PRL and PRLR gene with milk production and reproductive traits of Holstein dairy cows in Iraq.

MATERIALS AND METHODS

This study was conducted at AL- Khalis Dairy Cattle Station in Diyala governorate during productive season 2021 to 2022 to aimed the effect of PRL and PRLR polymorphism on milk production, milk components, and reproductive traits in Holstein dairy cows. A total 63 Holstein cows were selected randomly from the herd for this research, the chemical composition of feed for cows during the experiment period was contains 20.213% Crud Protein, 11.595% crud fiber, and 47.015% carbohydrate, 8.81% moisture, 11.536% Ash, 0.831% ether extract addition hay and alfalfa hay, the water was always available all times.

DNA Extraction and Polymerase chain reaction (PCR): Blood samples were collected from the jugular vein and stored in EDTA tubes at -20°C Genomic DNA was isolated from blood sample according to the protocol ReliaPrep™ Blood gDNA Miniprep System, and Quantus Fluorometer was used to detect the concentration of extracted DNA in order to detect the quality of samples for downstream applications. PCR amplifications were performed with 20µl volumes containing 10µl GoTaq Green Master Mix (2X); 1µl for each primer (10pmol); 6µl nuclease free water and 2µl of template DNA, PCR cycling was performed with PCR Express (Thermal Cycler, BioRad, USA) with the following temperature program: denatured at 94oC for 4 min followed by 30 cycles of denaturation at 94°C for 30 sec; annealing at 60°C for 30 sec; and extension at 72°C for 30 sec. A final extension incubation of 7 min at 72°C was included, followed by a 10 min incubation at 4oC to stop the reactions, After PCR amplification, agarose gel electrophoresis (1.5%) was adopted to confirm the presence of amplification according to Sambrook et al., (34), 5µl from PCR product was directly loaded to well than electrical power was turned on at 100 volt/ 50mAmp for 60min and DNA moves from Cathode to plus Anode poles. The Ethidium bromide-stained bands in gel were visualized using Gel imaging system. PCR products were sent for Sanger sequencing using ABI3730XL, automated DNA sequences, by Macrogen Corporation – Korea. The results were received by email then analyzed using Geneious Software.

Studied traits: Daily milk yield from dairy cows during the 2021-2022 season was recorded in the morning (5 AM) and evening (4 PM) in the milk production records for each cow, and the cows were milked by automated milking machines inside the station. As well as the length of the milking season, the days needed to reach peak milk production, and the length of peak production. Also, samples of milk were taken at the beginning of each month for each cow, after weighing the milk and mixing it well in clean plastic containers (50 ml), and analyzed by an ultrasonic milk analyzer (Master LM2) to determine the ratio of milk components such as portion, lactose, and fat, etc.

Statistical analysis

The data were analyzed statistically by using SAS (35) to study the effect of polymorphism of the PRL and PRLR gene on the traits studied on a sample of Holstein cows. The significant differences between means were compared by using the Duncan multiple range (13) by applying the least square means method, as well as using the Chi-square test to determine the significant differences between percentages of genotypes and their allelic frequencies, and also to compare the percentages of the distribution of genotypes.

RESULTS AND DISCUSSION

Results of the amplification of PRL gene of Blood samples were fractionated on 1.5% agarose gel Electrophoresis stained with Eth. Br. M: 100bp ladder marker, Lanes 25-43 resemble 982bp PCR products as shown in Figure 1 and DNA Concentration range was 20-35 ng/µl.

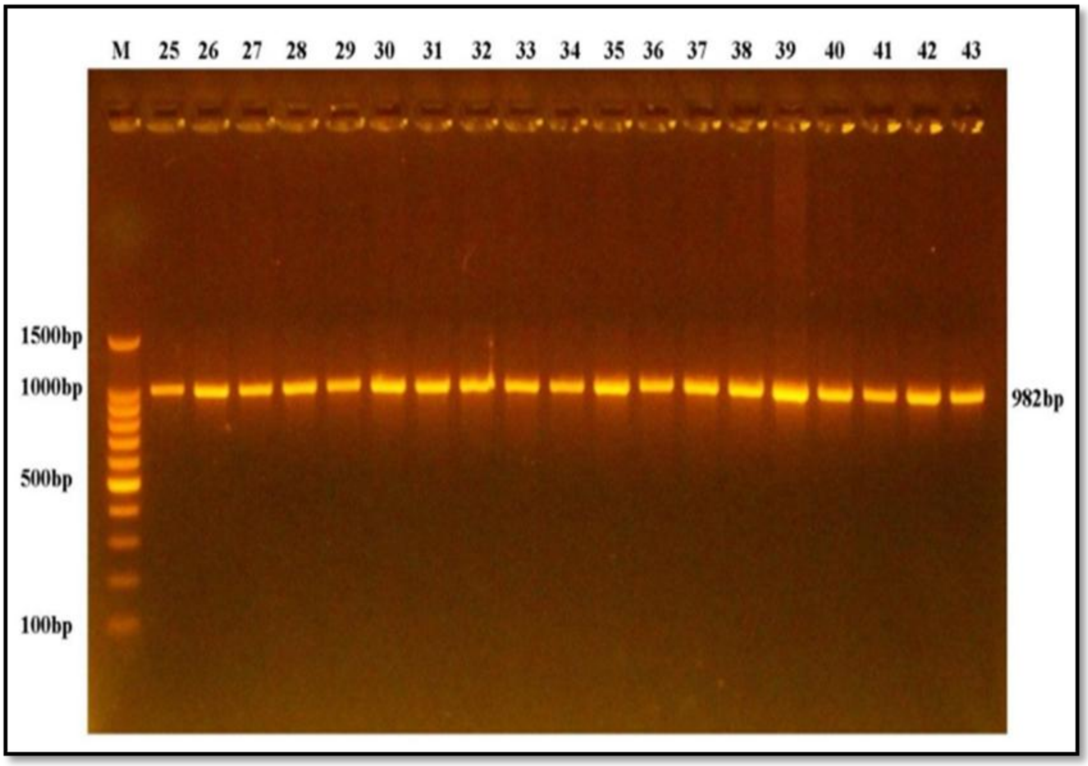

Figure 1. Results of the amplification of PRL gene in Holstein cow by agarose gel electrophoresis (1.5%), power (40 mA), volt (70 v), mint (70), stain=5µl

The results of the amplification of PRLR gene of Blood samples Figure 2, Lanes 25-43 resemble 732bp PCR products and DNA Concentration range was 20-35 ng/µl.

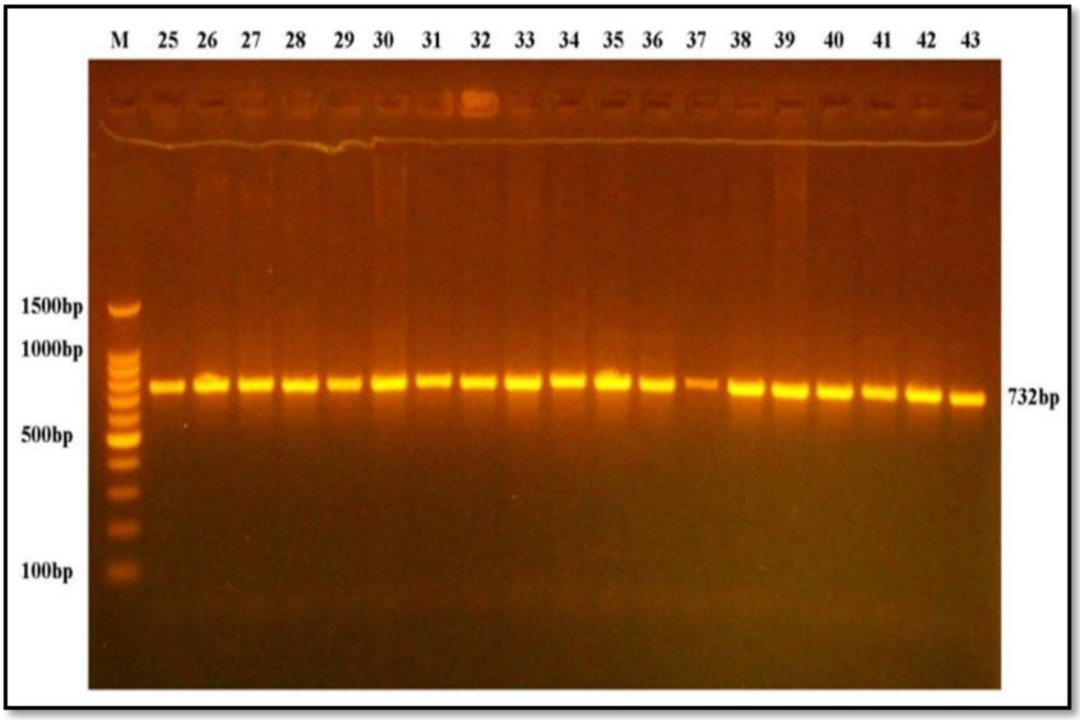

Figure 2. Results of the amplification of PRLR gene in Holstein cow by agarose gel electrophoresis (1.5%), power (40 mA), volt (70 v), mint (70), stain=5µl

The analysis of SNP of PRL and PRLR gene using Sanger sequencing, Single T peak indicative of a T homozygous allele. Single C peak indicative of a C homozygous allele. Presence of the T and C peak indicative of T/C heterozygous allele for PRL gene as shown in Figure 3 and in PRLR gene. The Single T peak indicative of a T homozygous allele. Single A peak indicative of a A homozygous allele. Presence of the T and A peak indicative of T/A heterozygous allele (Fig. 4).

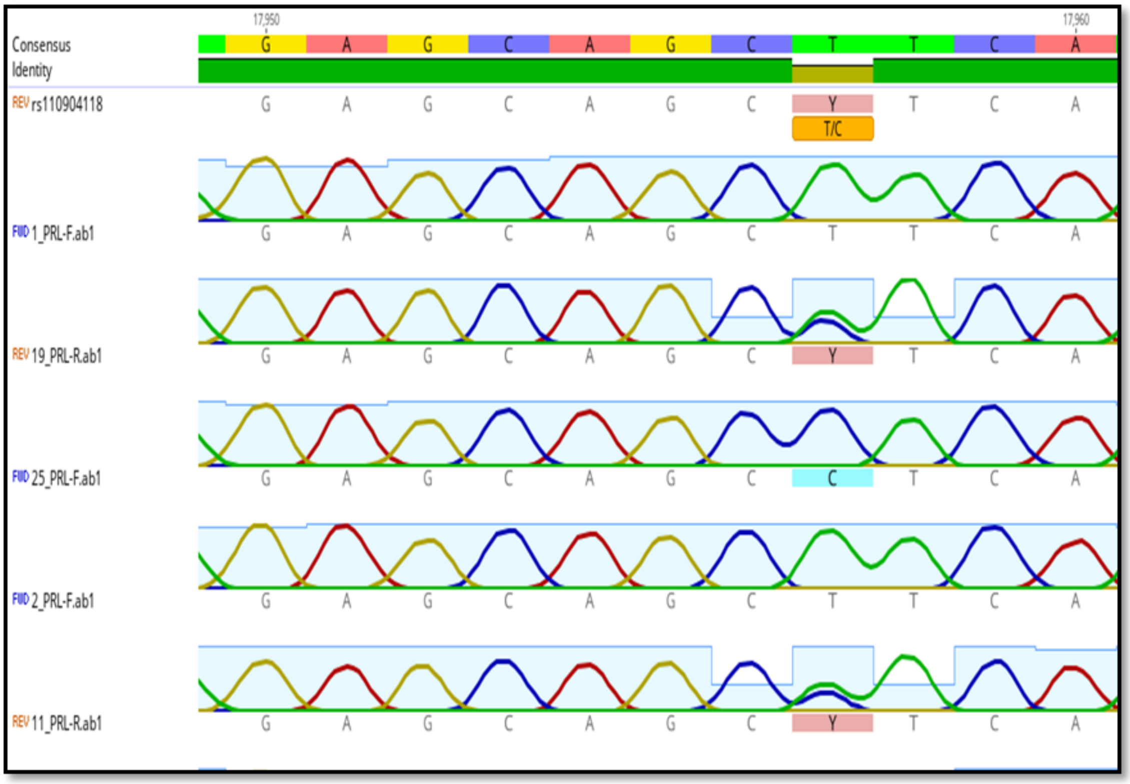

Figure 3. Analysis sequencing of rs110904118 SNP of PRL gene in Holstein cow

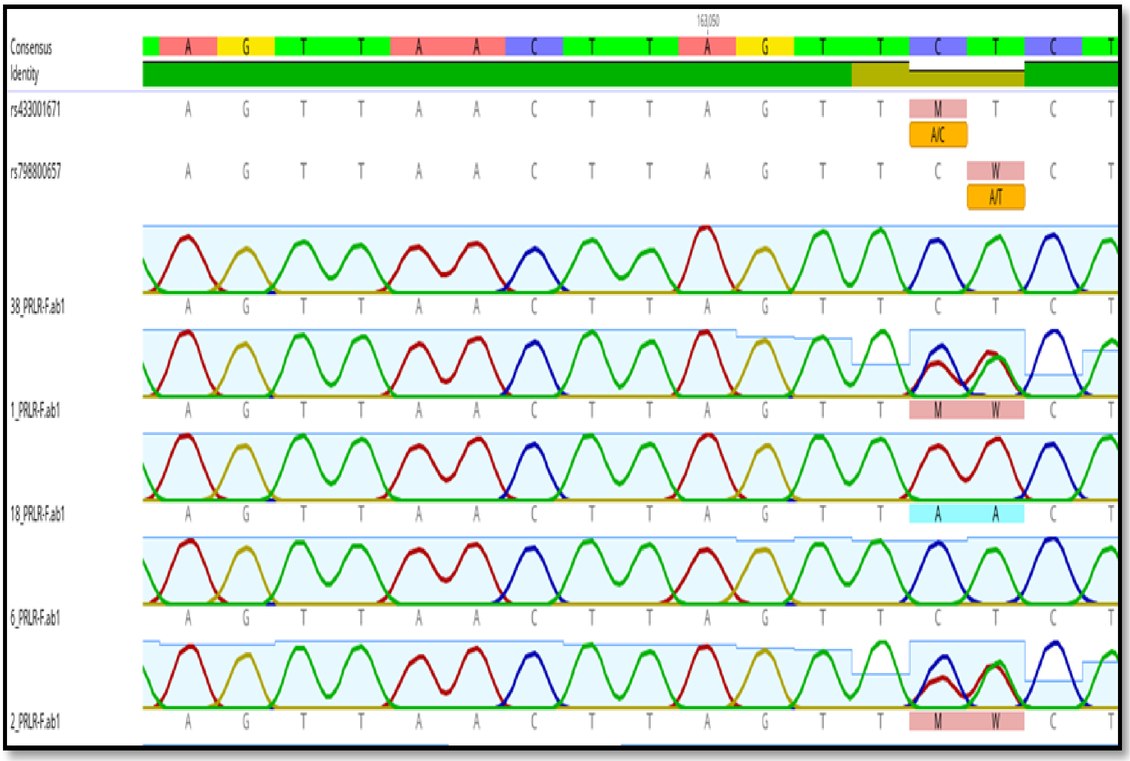

Figure 4. Analysis sequencing of rs433001671 SNP of PRLR gene in Holstein cow

Table 1 shows the number and percentage of genotypes and the allelic frequency of PRL gene. Three genotypes of Holstein dairy cows in Iraq, namely TT, TC, and CC and there is highly significant differences (P<0.01) between the percentage of the genotypes, which reached 79.37, 17.46 and 3.17% for TT (n = 50), TC (n = 11) and CC (n = 2) genotypes respectively, The frequency of the T allele of the prolactin gene was 0.88, while the frequency of the C allele was 0.12, and for PRLR gene Three genotypes TT, TA and AA with percentage 47.62, 41.27 and 11.11 respectively and, the frequency of the T allele was 0.68 compared with 0.32 in A allele this result reflects the prevalence of the T allele in PRL and PRLR gene in imported Holstein dairy cows.

Table 1.Number, percentage and the allelic frequency of the PRL and PRLR genotype in Holstein cows

Genotype	Number	Percentage	Genotype	Number	Percentage
	PRL gene			PRLR gene	
TT	50	79.37	TT	30	47.62
TC	11	17.46	TA	26	41.27
CC	2	3.17	AA	7	11.11
Total	63	100	Total	63	100
Chi-square	99.825**		Chi-square	18.714 **	
Allele	Frequency		Allele	Frequency	
T	0.88		T	0.68	
C	0.12		A	0.32	
Significance level	**		Significance level	**	

** : P≤0.01

Table 2 showed the effect polymorphism of the PRL and PRLR gene on milk production during early, Persistency and late lactation period as well as total milk production, in PRL gene the highest milk production was recorded in TC than TT genotypes compared with CC genotype in milk production during early, Persistency and late lactation period, In addition, total milk production was significant (P≤0.01) higher in TC genotype (4187.82 kg) followed than TT genotype (4013.02 kg) compared with 3505.00 kg in CC genotype and for PRLR gene highest milk production was recorded in TA genotype and reach 852.50 and 1918.42 and 1491.65 kg flowed by TT genotype which recorded 805.10, 1811.73 and 1408.70 kg during early, Persistency and late lactation period respectively, Total milk production was significant (P≤0.01) higher in TA genotype (4321.04 kg) followed than TT genotype (4025.53 kg) and CC genotype (2877.33 kg). The reason for the significant difference in the rate of total milk production between the genotypes may be attributed to its effect on gene expression and then the product of the gene (PRL and PRLR) and its direct or indirect effect on milk production.

Table 2. Effect of PRL and PRLR gene polymorphism on milk production kg in Holstein cows (mean ± SE)

Genotype	Early lactation period	Persistency Period	Late lactation period	Total milk production
		PRL gene		
TT	796.52±44.43 B	1792.44±99.96 B	1393.66±77.75 B	4013.02±221.63 B
TC	837.54±115.24 A	1884.73±259.30 A	1465.55±201.66 A	4187.82±576.21 A
CC	701.00±31.00 C	1577.50±69.50 C	1226.50±54.50 C	3505.00±155.00 C
Significance level	**	**	**	**
		PRLR gene		
TT	805.10±55.17 B	1811.73±124.12 B	1408.70±96.54 B	4025.53±275.84 B
TA	852.50±67.37 A	1918.42±151.58 A	1491.65±117.90 A	4321.04±333.51 A
AA	589.00±91.58 C	1325.43±206.11 C	1030.43±160.30 C	2877.33±536.00 C
Significance level	**	**	**	**

**: P≤0.01

During the early persistent, and late lactation periods, the CC genotype of PRL gene had the highest percentage of fat in milk , followed by the TC genotype which reach 4.30 , 5.29 and 4.25 % in CC genotype followed by 3.80 , 4.11 and 3.83 % in TC genotype and 3.70 , 3.73 and 4.10 % in TT genotype in early, persistent, and late lactation periods respectively as well as the PRL gene polymorphism had no effect on Protein , Lactose and Solids nonfat The polymorphism of PRLR gene did not significantly effect on milk components except lactose percentage during late lactation and the highest percentage of lactose was recorded in the TT (4.13 %) and TC (4.16%) genotypes compared with AA genotype (3.70%) as shown in Table 3, note that genetic variants of milk proteins affect milk production, composition and quality (17). Our results in Table 4 showed there's no significant effect of PRL gene polymorphism on reproductive traits of Holstein dairy cows, as well as an insignificant decrease in the Calving interval, the days open, and number of services per calving (NSPC) in the TT genotype compared with the TC and CC genotypes. as well as there's no significant effect of PRLR gene polymorphism on reproductive traits of Holstein dairy cows as shown in Table 4.

Table 3. Effect of the PRL and PRLR genes polymorphism on milk components in Holstein cows (mean ± SE)

Genotype	Early lactation period	Persistency Period	Late lactation period
PRL gene			
Fat %			
TT	4.10±0.18 b	3.73±0.16 c	3.70±0.15 c
TC	3.89±0.27 c	4.11±0.28 b	3.80±0.21 b
CC	4.30±0.60 a	5.29±0.60 a	4.25±0.25 a
Significance level	*	*	*
Protein %			
TT	2.77±0.07	8.57±5.73 a	2.78±0.04
TC	2.78±0.07	2.83±0.12 b	2.72±0.08
CC	2.76±0.06	2.76±0.06 b	2.85±0.15
Significance level	NS	*	NS
Lactose %			
TT	4.16±0.10	4.16±0.05	4.11±0.06
TC	4.16±0.10	4.11±0.16	4.08±0.13
CC	3.80±0.10	4.22±0.08	4.10±0.00
Significance level	NS	NS	NS
Solids nonfat %			
TT	7.63±0.17	7.84±0.09	9.08±1.37
TC	7.68±0.18	7.79±0.29	7.68±0.22
CC	7.04±0.16	7.73±0.13	7.55±0.05
Significance level	NS	NS	NS
PRLR gene			
Fat %			
TT	4.05±0.15	3.94±0.21	3.78±0.17
TA	3.88±0.32	3.73±0.21	3.58±0.20
AA	4.86±0.44	3.81±0.44	4.06±0.53
Significance level	NS	NS	NS
Protein %			
TT	2.82±0.10	2.08±0.27	2.79±0.05
TA	2.68±0.08	2.88±0.07	2.76±0.05
AA	2.76±0.04	2.80±0.05	2.65±0.19
Significance level	NS	NS	NS
Lactose %			
TT	4.23±0.12	4.15±0.06	4.13±0.06 a
TA	4.04±0.11	4.04±0.09	4.16±0.09 a
AA	4.15±0.08	4.15±0.06	3.70±0.18 b
Significance level	NS	NS	*
Solids nonfat %			
TT	7.75±0.23	7.79±0.11	9.74±1.98
TA	7.42±0.17	7.94±0.18	7.78±0.15
AA	7.68±0.13	7.55±0.18	6.99±0.32
Significance level	NS	NS	NS

* : $P \leq 0.05$; NS : Non-significant

Table 4. Effect of the PRL and PRLR genes polymorphism on reproductive traits in Holstein cows (mean ± SE)

Genotype	Calving interval	days open	Services per conception
		PRL gene	
TT	**374.00±4.63**	**97.72±3.20**	**1.70±0.10**
TC	**381.45±8.47**	**102.00±8.19**	**1.90±0.28**
CC	**400.00±10.00**	**120.50±7.50**	**2.50±0.50**
Significance level	**NS**	**NS**	**NS**
		PRLR gene	
TT	**382.30±3.99**	**101.96±3.89**	**1.83±0.32**
TA	**367.03±8.06**	**94.53±5.20**	**1.65±0.16**
AA	**382.00±7.49**	**101.50±7.49**	**1.66±0.33**
Significance level	**NS**	**NS**	**NS**

NS : Non-significant.

Prolactin, one of the pituitary hormones, regulates important physiological functions, ranging from mammary gland development to the initiation and maintenance of lactation. A previous study reported the associations between polymorphisms in the coding regions of the bovine prolactin gene and economically important traits (19, 20, 22, 23, 29, 39), PRL codes for the synthesis of prolactin protein, while the PRLR gene codes for the synthesis of prolactin receptors. The receptor is necessary for prolactin to bind to the mammary gland cells and initiate milk production (3, 18, 25, 26, 32, 34, 40). Further, our findings that there were significant associations between PRL and milk yield, fat percentage could be a potential QTN (quantitative trait nucleotide) affecting milk yield, fat yield, in dairy cattle. Further studies in a larger population are needed to confirm this result, also PRLR gene plays an important role in milk production and reproduction traits in dairy cow, our results showed association between polymorphism in the prolactin receptor gene and milk yield during early, persistent, and late lactation periods and total milk yield and there is no significant effect of the polymorphism of the prolactin receptor gene on protein , lactose percentages in milk and reproduction traits of dairy cows. Our findings are consistent with those of Ilie et al., (19) and Kale et al., (20) whose found a significant association between genetic variability in prolactin gene and milk fat content also agreement with Zhang et al. (40) used PCR-SSCP to detect polymorphisms in the PRLR gene in Chinese Holstein dairy cattle and found an association between PRLR variants and milk performance traits Many factors, such as genetics, especially the breed and the regions of the quantitative trait, have an impact on the quantitative traits of farm animals (20, 24, 27, 30).

CONFLICT OF INTEREST

The authors declare that they have no conflicts of interest.

DECLARATION OF FUND

The authors declare that they have not received a fund.

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