The Relationship Between Polymorphism of The Prolactin Gene and The Production of Milk, its Components, and the Reproductive Performance of Holstein Cows

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Article info	Abstract
  	The study was conducted at the Al-Khalis dairy production station in Diyala Governorate during the 2021-22 production season on 63 Holstein cows to determine the effect of polymorphism of the prolactin gene (PRL) on their milk production, its components, and their reproductive traits. The results showed that the three TT, TC, and CC genotypes had highly significant different ratios at 77.78%, 19.05%, and 3.17%, respectively. The frequency of the T and C alleles was 0.87 and 0.13, respectively. Total milk yield was significantly higher in the TC genotype at 4398.00 kg followed by the TT and CC genotypes at 3957.98 kg and 3505.00 kg, respectively. The polymorphism of PRL had a significant effect on milk composition but did not affect the reproductive traits of the cows. Therefore, the selection of hybrid individuals according to the prolactin gene will help increase milk production, and economic returns, and can thus be used for improving the genetics of dairy cows.

علاقة المظاهر الوراثية لجين البرولاكتين (rs208498963) مع انتاج الحليب ومكوناته والصفات التناسلية

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الخلاصة

أجريت الدراسة في محطة أبقار الخالص الكبرى لإنتاج الحليب في محافظة ديالى خلال الموسم الإنتاجي 2021–2022، على 63 بقرة هولشتاين بهدف دراسة تأثير تعدد المظاهر الوراثية لجين البرولاكتين (PRL) على إنتاج الحليب ومكوناته والصفات التناسلية لأبقار الهولشتاين. أظهرت النتائج وجود ثلاثة تراكيب وراثية لجين PRL وهي TT، وTC، وCC، وسجلت فروق عالية المعنوية بين نسب التراكيب الوراثية بلغت 77.78%، 19.05% و3.17% على التوالي، وبلغ تكرار الأليل T 0.87 بينما بلغ تكرار الأليل C 0.13. كان إنتاج الحليب الكلي أعلى معنويا في التركيب الوراثي TC 4398.00 كغم يليه الطراز الوراثي TT 3957.98 كغم مقارنة مع التركيب الوراثي CC 3505.00 كغم، وكان لتعدد أشكال PRL تأثير كبير على مكونات الحليب ولكن لم يؤثر على الصفات التناسلية لأبقار الهولشتاين. لذلك فإن انتخاب الأفراد الهجينة وفق جين البرولاكتين سوف يزيد من إنتاجية الحليب مما يزيد من العوائد الاقتصادية لذا يمكن استخدامه في التحسين الوراثي لأبقار الحليب.

كلمات مفتاحية: التركيب الوراثي، جين البرولاكتين، الحليب، الهولشتاين.

Introduction

In addition to energy, protein, and fats, milk provides vital minerals including calcium, vitamin D, and potassium, and is the staple diet of eight billion people worldwide. By 2030, total global milk production is forecast to reach 1020 million tons, with dairy cows generating at least 80% of the milk consumed (16). The genetic selection of key qualities such as milk output, milk fat, and protein has revolutionized and expanded the dairy cattle business. With the help of technological advancements and trait recording programs, specific characteristics evaluated for selection in dairy cattle populations have evolved as a response to changes in the needs of producers, consumers, and society (11). Due to its ability to regulate milk supply, milk protein expression, and mammary gland growth, and is a significant genetic marker that can be utilized in dairy cow genetic selection programs, prolactin is a crucial lactation hormone.

The 199 amino acid groups of the mature protein found on chromosome 23 in the bovine genome are encoded by the polypeptide known as the prolactin gene (PRL), which is approximately 10 kb in size and has five exons and four introns. To date, the

PRL gene has more than 20 single nucleotide polymorphisms (SNPs), the majority of which have been identified as silent mutations in the intron region (10, 13 and 14). Many studies have shown that PRL polymorphism is associated with reproductive traits and milk production (1) in mice (17), as well as in dairy cows, goats, sheep, and another farm animals (2, 3, 6, 12 and 18). Therefore, PRL is among the most important markers used in selection assistance to improve milk production traits as well as improve reproductive performance and the immune responses of dairy cows (7).

The aim of this study was to examine the impact of PRL gene polymorphism on reproductive parameters in Holstein dairy cows as well as the relationship between PRL polymorphism and milk components and productive performance.

Materials and Methods

This study investigated the effects of PRL polymorphism on milk production, milk components, and the reproductive traits in Holstein dairy cows. It was conducted at the Al-Khalis Dairy Cattle Station in Diyala governorate during the 2021-22 productive season. A total of 63 Holstein cows was randomly selected from the herd and fed a diet comprising 20.2% crude protein, 11.6% crude fiber, 6.20% fat, and 47.0% carbohydrate in addition to alfalfa hay and a constant supply of water.

DNA Blood Sample Extraction and Polymerase Chain Reaction (PCR): Blood samples of 5ml were taken from the jugular vein and stored at -20 °C in EDTA k2 tubes. Genomic DNA was extracted from a blood sample using the ReliaPrepTM Blood gDNA Miniprep System technique and measured using a quantum fluorometer to assess the quality of the samples for further use. The 20µl volumes containing 10µl GoTaq Green Master Mix (2X), 1µl primer (10 pmol), 6µl nuclease-free water, and 2µl template DNA were used for PCR amplifications. PCR Express (Thermal Cycler, BioRad, USA) was used for PCR cycling based on the following program: 30 cycles of denaturation at 94 °C for 30 seconds, annealing at 55, 58, 60, 63, or 65 °C for 30 seconds, and extension at 72 °C for 30 seconds after denaturation at 94 °C for 4 minutes.

To stop the reactions, a final extension incubation of 7 minutes at 72 °C was done, followed by 10-minute incubation at 4 °C. After PCR amplification, agarose gel (1.5gm) electrophoresis was done to confirm the presence of amplification, according to from the PCR product was directly loaded (100-1500 bp) to the well and electrical power was turned on at 100 volt/ 50mAmp for 60 min and DNA moved from the cathode to the anode poles. The 5µm ethidium bromide-stained bands in gel were visualized using Gel imaging system, use TBE. The PCR products were transferred to Macrogen Corporation in Korea for Sanger sequencing utilizing the ABI3730XL automated DNA sequencer. Following email receipt of the results, Geneious software was used for analysis.

Traits Studied: Milk production of the dairy cows during the 2021-22 study period was documented twice daily at 5 am and 4 pm. The cows were milked by automated milking machines located within the station. The duration of the milking season, number of days required to achieve maximum milk production, and peak production period according to (15), as well as the reproductive traits, were also recorded. Milk samples were also taken at the beginning of each month for each cow, and after

weighing and mixing them well in clean plastic containers (50 ml), they were analyzed using an ultrasonic milk analyzer (Master LM2) to determine the proportions of their composition.

Statistical Analysis: The data were analyzed statistically using SAS (18) to study the effect of polymorphism of the PRL gene on the traits of the Holstein cows. The significant differences between means were compared using the Duncan multiple range (10) by applying the least square means method. The Chi-square test was used to determine the differences between genotype percentages and their allelic frequencies, and to compare their percentage distributions.

Results and Discussion

The PRL gene fragment (982bp) was obtained using polymerase chain reaction after DNA extraction (Figure 1), the PRL gene amplification product was electrophoresed, and the DNA concentration range was 20-35 ng/μl.
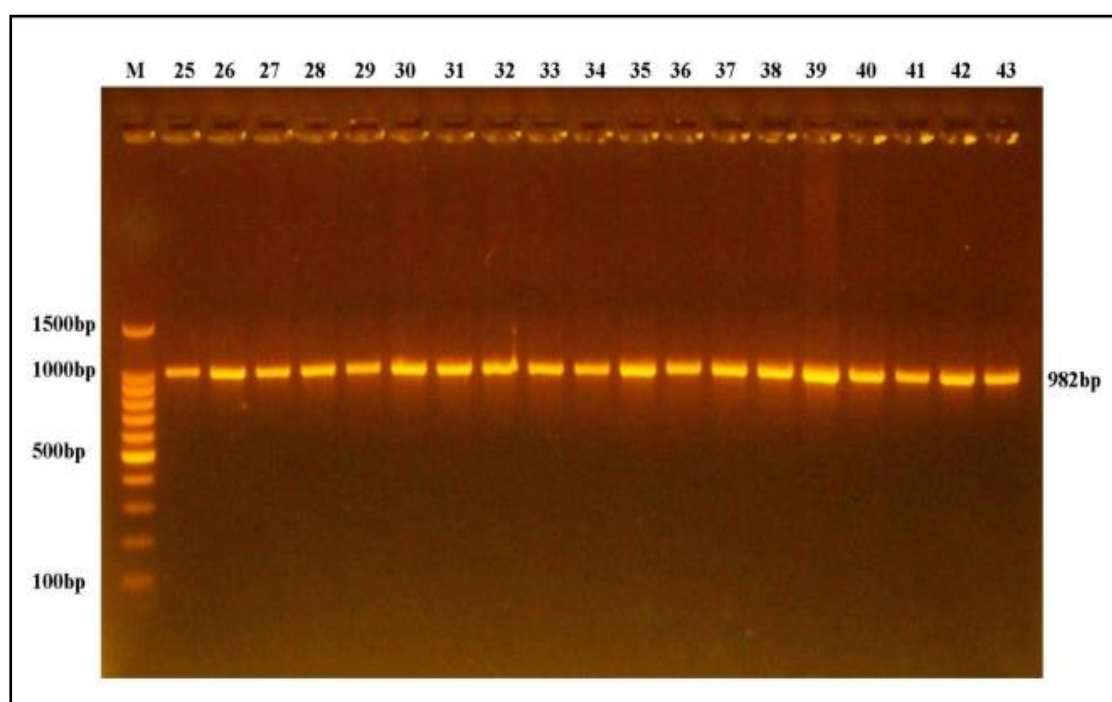

Fig. 1: Amplification of the prolactin gene from agarose gel electrophoresis.

Sanger sequencing was used to analyze the PRL gene SNP. Both T and C homozygous alleles are indicated by single "T" and "C" peaks, respectively. Figure 2 illustrates the presence of the peaks, which are suggestive of a T/C heterozygous allele.
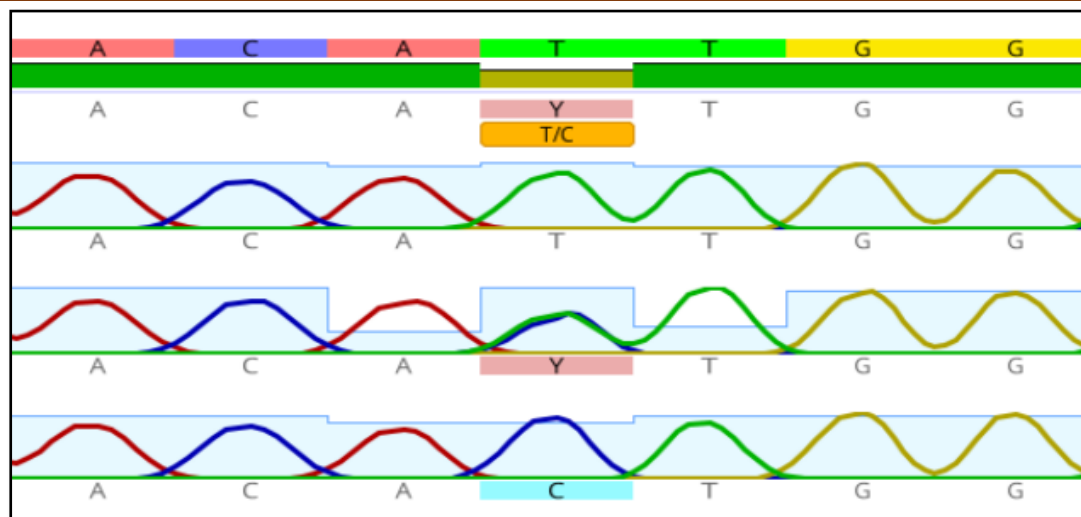
Fig. 2: Sequence analysis of the prolactin gene.

Table 1 shows the number and percentage of genotypes and the allelic frequency of the prolactin gene. The three genotypes of the Holstein dairy cows studied, namely TT (n = 49), TC (n = 12), and CC (n = 2) had highly significant differences (P < 0.01) in their genotype percentages at 77.78, 19.05, and 3.17%, respectively. The T and C allele frequencies of the prolactin gene were 0.87 and 0.13, respectively indicating the prevalence of the former in Holstein dairy cows imported into Iraq.

Table 1: Data on genotypes and allelic frequencies of the prolactin gene.

Genotype	Number	Percentage
TT	49	77.78
TC	12	19.05
CC	2	3.17
Total	63	100
Chi-square	94.269**	
Allele	Frequency	
T	0.87	
C	0.13	
Sig.	**	

** denotes a highly significant effect (P≤0.01).

Table 2 shows the effect of polymorphism of the prolactin gene on milk production during the early, persistency, and late lactation periods as well as total milk yield. Highest milk yield (P≤ 0.01) was recorded in the TC genotype at 4398.00 kg followed by the TT and CC genotypes at 3957.98 kg and 3505.00 kg, respectively.

Table 2: Impact of polymorphism of the prolactin gene on milk production.

Genotype	Milk yield in kg/cow (mean ± SE)			
	Early lactation period	Persistency period	Late lactation period	Total
TT	823.38±53.13 A	1852.85±119.54 a	1440.71±92.98 A	3957.98 ±219.11 b
TC	825.78±70.10 A	1858.35±157.72 a	1444.87±122.67 a	4398.00 ±566.44 a
CC	575.50±107.18 b	1295.00±241.20 b	1006.83±187.61 b	3505.00±155.00 c
Sig.	**	**	**	**

Different letters in the column indicate significant differences between the averages.
** denotes a highly significant effect (P≤0.01).

As shown in Table 3, the CC genotype had the highest percentage of fat in milk during the three lactation periods while that for TT and TC varied. The TT and TC genotypes recorded the highest percentage of protein during the early and persistency periods. The prolactin gene polymorphism had no effect on lactose percentage during the early and late lactation periods with the highest percentage recorded in the TT genotype during the persistency period. The higher percentage of nonfat solids was recorded in the TT followed by the TC genotypes during the early and late lactation periods.

Table 3: Impact of polymorphism of the prolactin gene on milk components (mean ± SE).

Genotype	Early lactation period	Persistency Period	Late lactation period
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
Sig.	*	*	*
Protein %			
TT	4.16±0.10 a	4.16±0.05 b	4.11±0.06
TC	4.16±0.10 a	4.11±0.16 b	4.08±0.13
CC	3.80±0.10 b	4.22±0.08 a	4.10±0.00
Sig.	*	*	NS
Lactose %			
TT	2.77±0.07	5.87±3.57 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
Sig.	NS	*	NS
Nonfat solids %			
TT	7.63±0.17 a	7.84±0.09	9.08±1.37 a
TC	7.68±0.18 a	7.79±0.29	7.68±0.22 b
CC	7.04±0.16 b	7.73±0.13	7.55±0.05 b
Sig.	*	NS	*

Different letters in the column indicate significant differences between the averages.
** denotes a highly significant effect (P≤0.05).

Table 4 shows no significant effect of prolactin gene polymorphism on the reproductive traits of Holstein dairy cows. Also, there was an insignificant decrease in the periods between two births, between birth and fertilization, and the number of pollinations required for fertilization in the TT compared with the TC and CC genotypes. When compared to the TC and TT genotypes, the CC genotype had a marginally higher period for milk yield.

Table 4. Impact of polymorphism of the prolactin gene on reproductive traits (mean ± SE).

Genotype	Calving interval	Days open	Services per conception	Period of milk yield (day)
TT	373.87±4.68	97.20±3.23	1.67±0.10	262.06±9.53
TC	383.41±7.89	103.75±7.68	2.00±0.27	271.91±19.39
CC	400.00±10.00	120.50±7.50	2.50±0.50	306.50±1.50
Sig.	NS	NS	NS	NS

NS indicates no significant differences.

One of the pituitary hormones, prolactin controls a number of vital physiological processes, from the development of the mammary gland to the start and continuation of lactation. Polymorphisms in the bovine prolactin gene's coding regions have been linked to economically significant features, according to studies by (5, 12 and 18). Further, this study reveals significant associations between PRL and milk yield, and fat and protein percentages. These traits imply that PRL could be a potential quantitative trait nucleotide (QTN) affecting milk, fat, and protein yields, and protein percentages in dairy cattle. Further studies using a larger population are needed to confirm this result.

The findings of this study are consistent with (7), who noted that cows with the AB genotype have highest milk, milk fat, and milk protein yield compared to the AA and BB genotype animals. Additionally, it is in agreement with (8), which found that AA genotype Holstein cows had statistically higher milk yield compared to those with AB and BB genotypes.

Conclusions

As the results show that there is a highly significant relationship between the prolactin gene and milk production, it can therefore be used in enhancing the genetic makeup of milk cows.

Supplementary Materials:
No Supplementary Materials.

Author Contributions:
Author 1: methodology, writing and preparation of the first draft, and writing the review and editing. The published version of the manuscript has been read and approved by all authors.

Institutional Review Board Statement:
The study was carried out in compliance with the guidelines approved by the Department of Animal Production, College of Agricultural Engineering Sciences, University of Baghdad.

Informed Consent Statement:

Not applicable.

Data Availability Statement:

Data available upon request.

Conflicts of Interest:

The authors declare no conflict of interest.

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References

1. Agrawal, V. K., Gahlot, G., Joshi, R., Dhakad, G. S., and Thakur, S. (2020). Assessment of genetic variability in prolactin gene and its impact on milk composition in Bos indicus cattle. Int. J. Curr. Microbiol. App. Sci, 9(6): 1514-1524. https://doi.org/10.20546/ijcmas.2020.906.187.

2. Al-Delemi, D. H., Al-Gewary, A. A., and Abd Ali, B. H. (2016). The Prolactin Receptor Gene Expression Variance in Marshes and Riverine Buffalos in Iraq. Agricultural Research and Technology: Open Access Journal, 2(1): 29-34.

3. Al- Thuwaini, T. M. (2022). The pleiotropic effects of the prolactin gene on milk production and reproductive traits in cattle: a review. CABI Reviews, 2022. https://doi.org/10.1079/cabireviews202217032

4. Ali, O., and Al-Azzawi, S. (2022). Detection of polymorphism in growth differentiation factor 9 gene (GDF9) Exon1 and its association with litter size in local Iraqi goats. Revis Bionatura 7(4): 49. http://dx.doi.org/10.21931/RB/2022.07.04.49.

5. Al-Thuwaini, T. M. (2021). Novel single nucleotide polymorphism in the prolactin gene of Awassi ewes and its role in the reproductive traits. Iraqi Journal of Veterinary Sciences, 35(3): 429-435. https://doi.org/10.33899/ijvs.2020.126973.1423.

6. Bangar, Y. C., Magotra, A., Patil, C. S., and Jindal, N. (2021). Meta-analysis of genetic structure and association of prolactin gene with performance traits in dairy cattle in India. Biochemical genetics, 59(3): 668-677. https://doi.org/10.1007/s10528-021-10031-4.

7. Bayıl Oğuzkan, S., and Bozkurt, A. S. (2019). A study on the effect of Prolactin gene variants on milk production traits of Holstein cattle. Russian Journal of Genetics, 55(4): 480-486. https://doi.org/10.1134/S1022795419040082.

8. Brito, L. F., Bédère, N., Douhard, F., Oliveira, H. R., Arnal, M., Peñagaricano, F., ... and Miglior, F. (2021). Genetic selection of high-yielding dairy cattle toward

sustainable farming systems in a rapidly changing world. Animal, 15: 100292. https://doi.org/10.1016/j.animal.2021.100292.

9. Duncan, D. B. (1955). Multiple range and multiple F tests. biometrics, 11(1): 1-42. https://doi.org/10.2307/3001478.

10. Elsahookie, M. M., Cheyed, S. H., and Dawood, A. A. (2021). An overview on mechanism of gene expression regulation. Iraqi Journal of Agricultural Sciences, 52(2): 454-460. https://doi.org/10.36103/ijas.v52i2.1307.

11. Lopdell, T. J. (2023). Using QTL to identify genes and pathways underlying the regulation and production of milk components in cattle. Animals, 13(5): 911. https://doi.org/10.3390/ani13050911.

12. Mao, Y., Yang, H., Ma, X., Wang, C., Zhang, L., and Cui, Y. (2023). Prolactin regulates RANKL expression via signal transducer and activator of transcription 5a signaling in mammary epithelial cells of dairy cows. Cell Biology International, 47(5): 920-928. https://doi.org/10.1002/cbin.11988.

13. Motmain, Z., Özdemir, M., Ekinci, K., Saygili, E., and Bilgin, E. (2022). A meta-analysis of the associations between prolactin (PRL) gene polymorphism and milk production traits in cattle. KAFKAS Üniversitesi Veteriner Fakültesi Dergisi, 28(5): 627-631. DOI: 10.9775/kvfd.2022.27857.

14. Oliveira, H. R., Cant, J. P., Brito, L. F., Feitosa, F. L. B., Chud, T. C. S., Fonseca, P. A. S., ... and Schenkel, F. S. (2019). Genome-wide association for milk production traits and somatic cell score in different lactation stages of Ayrshire, Holstein, and Jersey dairy cattle. Journal of dairy science, 102(9): 8159-8174. https://doi.org/10.3168/jds.2019-16451.

15. OECD, FAO. (2021). OECD-FAO Agricultural Outlook OECD Agriculture statistics (database).

16. Rizzoli, R. (2014). Dairy products, yogurts, and bone health. The American journal of clinical nutrition, 99(5): 1256-1262. https://doi.org/10.3945/ajcn.113.073056.

17. Salam, A., Harbeya, M. S., and SALM, A. H. (2021). Studying the effect of the genotypes of the prolactin gene (PRL) on the productive performance of Holstein cows. University of Thi-Qar Journal of agricultural research, 10(2): 43-51. https://doi.org/10.54174/utjagr.v10i2.132.

18. SAS. (2018). Statistical Analysis System, User's Guide. Statistical. Version 9.4th ed. SAS. Inst. Inc. Cary. N.C. USA.

19. Zghairand, Z. S., and Hassooni, H. A. (2021). The relationship between the PIT-1 gene at the rate of daily milk production and some of its components in buffaloes. In IOP Conference Series: Earth and Environmental Science, 923(1): 012029. DOI" 10.1088/1755-1315/923/1/012029.