

## RESEARCH ARTICLE

# A Meta-Analysis of the Associations Between Prolactin (PRL) Gene Polymorphism and Milk Production Traits in Cattle

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**Abstract:** The meta-analysis method is used to combine results from many independent studies conducted in a different region or area on a specific subject qualitatively and quantitatively and achieve a consensus. The current meta-analysis was conducted to investigate the association between prolactin gene polymorphism and milk production traits in dairy cattle. The data were collected between 2002 and 2021, and a total of 26 published studies were included in this study. The standardized mean difference (SMD) between genotypes was obtained for statistical evaluation, and to this end, the genetic models (dominant; AA + AB and BB, recessive; AB + BB with AA, co-dominant; AA, BB and AB genotypes) were compared with each other. In the analysis results, cattle with the AB versus BB genotype (SMD=0.289, 95% CI 0.005, 0.573) were found to have a statistically significant higher protein yield in comparison with the AA versus AB and AA versus BB genotypes. The relationships between prolactin gene polymorphism and milk yield, fat yield, fat content, and protein content traits were not found to be statistically significant.

**Keywords:** PRL gene, Polymorphism, Co-dominant, Meta-analysis, Standardized mean difference

## Sığırlarda Prolaktin (PRL) Geni Polimorfizmi İle Süt Verim Özellikleri Arasındaki İlişkilerin Meta Analizi

**Öz:** Meta Analizi, belirli bir konu üzerinde farklı bölge veya alanlarda yapılmış birçok bağımsız çalışmanın sonuçlarını niteliksel ve niceliksel olarak birleştirmek ve fikir birliğine varmak amacıyla kullanılan bir yöntemdir. Meta-analizi, süt sığırlarında prolaktin geni polimorfizmi ile süt verim özellikleri arasındaki ilişkiyi araştırmak için uygulanmıştır. Veriler 2002-2021 yılları arasında yayınlanmış 26 çalışma analizlere dahil edildi. İstatistiksel değerlendirme için genotipler arasında standartlaştırılmış ortalama farkı (SMD) elde edildi ve bu amaçla genetik model: baskın; AA + AB ile BB, çekinik; AA ile AB + BB, eş baskın; AA, BB ve AB genotipleri birbirleriyle karşılaştırıldı. Analiz sonuçları, AB ile BB genotipine sahip sığırların (SMD=0.289, %95 CI 0.005, 0.573), AA ile AB ve AA ile BB genotipli sığırlara kıyasla istatistiksel olarak anlamlı bir yüksek protein verimine sahip olduklarını göstermiştir. Prolaktin geni polimorfizmi ile incelenen diğer süt verimi, yağ verimi, yağ içeriği ve protein içeriği özellikleri arasındaki ilişkiler istatistiksel olarak anlamlı değildi.

**Anahtar sözcükler:** PRL geni, Polimorfizm, Ko-dominant, Meta-analizi, Standartlaştırılmış ortalama farkı

## INTRODUCTION

Meta-analysis is a method used to combine and re-evaluate results from at least two or more studies for the purpose of estimating or evaluating the joint effect size. It is applied to the results of further research in a particular field and ensures a strong analysis and sample effect <sup>[1]</sup>. It is necessary to conduct a meta-analysis in case of the repeating sample number <sup>[2]</sup>. The research on genetics in farm animals mainly focuses on identifying genes with an effect on economically important traits. Most

studies on dairy cows have identified genes determining or specifying variations in milk traits. In livestock, milk genes and hormones are researched as perfect candidate genes because of their biological significance and linkage with important quantitative traits.

The prolactin gene is a quantitative trait locus and is considered a potential genetic marker that can be utilized in enhancing production traits in dairy cattle. The prolactin (PRL) gene polymorphisms are associated with milk yield traits <sup>[3]</sup>. The process of milk production is very

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complicated with numerous interrelated factors (genetic and hormonal factors), and prolactin is one of the most essential hormones in this process [4,5].

Prolactin is one of the polypeptide hormones produced by the anterior pituitary gland and plays a very important role in lactogenesis, synthesis, regulating the growth of the udder, and secretion of milk proteins in dairy cows [6-10]. The prolactin (PRL) gene is located on chromosome 23, is approximately 10 kb in size, includes 5 exons and 4 introns, and encodes a 199 amino-acid polypeptide [6,11,12]. This site plays an essential role as a genetic marker for the genetic characterization of cattle populations [13]. There are significant associations between the PRL-*RsaI* locus and milk and fat yield in dairy cows. The RFLP technique can detect a small change naturally occurring through deletions or insertions of one or more pairs of nucleotides in the genome [5,11]. A shift of adenine to guanine (A103G) in the PRL gene causes point mutations, resulting in two alleles (A and B). The adenine at position 103 is referred to as the A allele. The restriction site for the *RsaI* restriction enzyme was caused by a mutation from adenine (A) to guanine (G), and the allele was referred to as the B allele. Three genotypic patterns were revealed from the digestion of a 156 bp fragment of exon 3 region in the prolactin gene by *RsaI*. The first uncut pattern fragment of 156 bp was referred to as the AA genotype, the second pattern fragments (82 and 74 bp) were denoted as the BB genotype (the presence of a restriction site), and the third one with three fragments (156, 82, and 74 bp) was referred to as the AB genotype [12,14]. Several studies have demonstrated that polymorphisms in the genomic sequence of the prolactin (PRL) gene have significant associations with different economic traits in dairy cattle [6]. The present meta-analysis was conducted to combine results from many independent studies on prolactin gene polymorphism and its association with milk production traits in dairy cattle.

## MATERIAL AND METHODS

Scientific journals were searched for meta-analysis on prolactin (PRL) gene polymorphism and its association with milk production traits in dairy cattle. To obtain the standardized mean difference, the random effect model and the fixed effect model were used. The model selection was performed based on whether the effects of studies were homogenous or heterogeneous: when the study effects were homogeneous, the fixed model was employed, and when the study effects were heterogeneous, the random model was utilized. Q statistic was employed with the objective of determining heterogeneity between studies, and the index was used for its further quantification. Cohen's method [15] was used for the standardized mean difference.

## Data Extraction

The authors extracted data independently, and the Microsoft Excel format was used for data collection. The researchers carried out discussions to avoid mistakes or errors with regard to studies and data collection. At first, 51 studies were searched, and a total of 26 studies on the same genotypes of the prolactin gene were reached and selected for this study. The important information, involving the author's name, the year of publication, country, sample size, genotypes, breed, lactation milk yield (LMY), fat yield (kg), and protein yield, was collected from the related studies.

## The Search Strategy of Sources

To carry out the meta-analysis, essential criteria for systematic reviews and meta-analyses were used for study selection. A comprehensive search was done among studies published in different languages, journals, and databases on the associations between prolactin gene polymorphisms and milk production traits between 2002 and 2021. Google Scholar, ResearchGate, Wiley, Springer, Taylor & Francis, PubMed, and Elsevier were searched for studies, and a number of keywords (meta-analysis, milk production traits, polymorphism, PRL, association, cattle) were used to find the required studies.

## Selection and Exclusion of Studies

During study selection, certain criteria were applied, including: (I) the number of animals of each genotype, (II) association between the gene polymorphism and milk production traits, (III) the reported least square means for milk production traits, and (IV) standard deviation/errors and average means of the involved trait for every genotype (if it is a standard error, it is converted to a standard deviation with the presence of the animal number).

Studies were rejected or excluded if they had the following characteristics;

- Publications as a summary,
- No indication of the number of animals of each genotype,
- Studies did not have standard deviation/errors and average means per genotype, and
- Duplicate studies.

From a total of 51 studies, 25 studies were excluded since they did not have the above-mentioned criteria, and accordingly, 26 studies were selected for analysis.

## Statistical Analysis

The meta-analysis was carried out using the Stata 11.2 software (StataCorp 2001; Stata Statistical Software), and  $P < 0.05$  was accepted as a significance level. For the gene locus, the database was arranged individually, and the four methods below were followed;

- Concerning differences between means, two effect models (random and fixed) were used in the meta-analysis. According to the  $I^2$  statistics, the fixed model was employed in case of the homogenous study results, while the random model was employed in case of the heterogeneous study results (the levels of significance for  $I^2$  were defined as 0.10 in the heterogeneity analysis)<sup>[2,16]</sup>.

- In the study, the following four genetic models were used: dominant; AA + AB versus BB, recessive; AA versus AB + BB, co-dominant; AA versus BB versus AB.

- The standardized mean differences (SMDs) and standard deviation were computed at a 95% CI (Confidence Interval) to estimate the capabilities between the three genetic models for every trait.

- For standardized mean differences, Cohen's method<sup>[15]</sup> was used to assess the effect size.

### Estimation of Heterogeneity

In this study, the  $I^2$  test was conducted with the objective of estimating heterogeneity between the studies. The random effect model was applied due to the high heterogeneity<sup>[2,16]</sup>.

## RESULTS

In this study, three performance traits (lactation milk yield, fat yield, and protein yield) were analyzed in a total of (26) studies by the meta-analysis technique, using the

random and fixed effect models. Each trait was analyzed separately. *Table 1* and *Table 2* contain the results for heterogeneity, standardized mean difference (SMD) with a 95% CI, and significances.

The following alleles were considered and used in this study: dominant (AA+AB versus BB), recessive (AA versus AB+BB), complete over-dominant (AA+BB versus AB), and co-dominant (AA + versus AB, AA versus BB, and AB versus BB). The statuses of the heritage pattern of alleles are shown in *Table 1*.

Upon analyzing 26 studies for lactation milk yield (LMY), all genetic models showed no significant ( $P>0.05$ ) association between the genotypes of the prolactin gene and milk yield ( $P>0.05$ ).

When 14 studies were analyzed in terms of fat yield, the results showed no significant ( $P>0.05$ ) association between prolactin gene polymorphisms and fat content. Concerning fat content from the analysis of 21 studies, no significant association ( $P>0.05$ ) was detected between prolactin gene polymorphisms and all genetic models.

With regard to protein yield ( $n=14$ ), a significant association was found between prolactin gene polymorphisms and the AB versus BB genotype ( $P<0.05$ ). No significant association ( $P>0.05$ ) was identified between protein yield and the other genetic models. Concerning protein content ( $n=18$ ), no significant association was determined between prolactin gene polymorphisms and all genetic models ( $P>0.05$ ).

**Table 1.** Genetic model analysis of the associations between PRL polymorphism and milk production traits

Traits	n	AA+AB versus BB Dominant Model						AA versus AB+BB Recessive Model						AA+BB versus AB Complete over Domn. Model					
		$I^2$	Model	SMD	95%	CI	P	$I^2$	Model	SMD	95%	CI	P	$I^2$	Model	SMD	95%	CI	P
Lactation Milk Yield	26	14.6	F	-0.009	-0.102	0.084	0.850	46.3**	R	-0.01	-0.067	0.095	0.727	27.9*	R	-0.003	-0.068	0.063	0.937
Fat Yield	14	2.5	F	-0.040	-0.186	0.105	0.587	0.0	F	-0.03	-0.099	0.033	0.329	0.0	F	-0.025	-0.090	0.040	0.451
Fat Content	21	74.0**	R	-0.226	-0.466	0.014	0.065	47.8**	R	0.03	-0.061	0.120	0.522	54.0**	R	0.093	0.000	0.186	0.050
Protein Yield	14	6.8	F	0.025	-0.121	0.170	0.739	3.1	F	-0.05	-0.116	0.016	0.140	0.0	F	-0.054	-0.119	0.011	0.106
Protein Content	18	0.0	F	0.013	-0.101	0.126	0.823	0.0	F	0.009	-0.048	0.067	0.753	0.0	F	0.001	-0.057	0.059	0.974

\*  $P<0.10$ ; \*\*  $P<0.01$ ; n: number of publication; F: variation in SMD attributable to heterogeneity; F: Fixed; R: Random

**Table 2.** Results of the meta-analysis on the associations between PRL polymorphism and milk production traits

Traits	n	AA Versus AB						AA Versus BB						AB Versus BB					
		$I^2$	Model	SMD	95%	CI	P	$I^2$	Model	SMD	95%	CI	P	$I^2$	Model	SMD	95%	CI	P
Lactation Milk Yield	26	47.1**	R	0.011	-0.074	0.096	0.796	32.7*	R	0.1	-0.010	0.022	0.470	39.9*	R	0.087	-0.063	0.238	0.254
Fat Yield	14	0.0	F	-0.034	-0.102	0.034	0.328	15.8	F	-0.09	-0.269	0.090	0.327	8.6	F	0.004	-0.147	0.156	0.956
Fat Content	21	45.1*	R	0.041	-0.052	0.133	0.388	76.8**	R	-0.3	-0.610	0.001	0.051	74.3**	R	-0.218	-0.477	0.041	0.099
Protein Yield	14	4.3	F	-0.057	-0.125	0.011	0.101	15.8	F	-0.03	-0.213	0.146	0.717	62.2**	R	0.289	0.005	0.573	<b>0.046</b>
Protein Content	18	0.0	F	0.006	-0.054	0.066	0.851	0.0	F	-0.01	-0.145	0.118	0.846	0.0	F	0.030	-0.096	0.156	0.642

\*  $P<0.10$ ; \*\*  $P<0.01$ ; n: number of publication; F: variation in SMD attributable to heterogeneity; F: Fixed; R: Random

## DISCUSSION

The meta-analysis method is used to combine results from numerous independent studies conducted in a different region or area on a specific subject qualitatively and quantitatively and assist in achieving a consensus [17]. Meta-analysis represents a test method using the results of numerous studies conducted in the area, offering a more powerful analysis. There is a need for meta-analysis if a lot of similar studies have been performed on a particular subject, and there has been a difference of opinion in some of these studies [2]. Therefore, the current research was conducted for a meta-analysis on PRL gene polymorphism and its association with milk traits in dairy cattle. The meta-analysis showed that the relationships of the prolactin gene with other factors must be studied by means of the co-dominant genetic model in general.

In the analysis of 26 studies for lactation milk yield (LMY), all genotypes showed no significant ( $P>0.05$ ) association between PRL gene polymorphism and milk yield. This result is similar to the studies carried out by many researchers [12,18-25]. They suggested that the polymorphisms of the prolactin gene did not affect milk yield. However, the obtained results contradict the findings obtained by some researchers [7,8,11,26,27], indicating that the polymorphisms of the prolactin gene had an impact on milk yield.

Upon analyzing 14 studies for fat yield, no significant association was identified between prolactin gene polymorphisms and fat content ( $P>0.05$ ). Similar results have previously been reported by various authors [8,18,21,28] for different dairy cattle breeds. Moreover, these results are in disagreement with the findings of other authors [7,19,26]. When 21 studies were analyzed with regard to fat content, no significant ( $P>0.05$ ) association was detected between prolactin gene polymorphisms and all genetic models. While similar results were reported by some authors [8,11,21,25-27], contradictory results were indicated by the others [7,28].

Considering protein yield, there was a significant ( $P<0.05$ ) association between prolactin gene polymorphisms and the AB and BB genotype. Similar results were reported by some authors [7,8,18,19,21,28]. However, this result is in disagreement with the studies by [25,26].

Considering protein content, no significant association was revealed between prolactin gene polymorphisms and all genetic models ( $P>0.05$ ). Similar results were reported by a number of authors [7,8,18,19,21,22,25,28].

It is thought that the results obtained in this study will make a contribution to developing new strategies necessary for breeding dairy cattle.

In accordance with the results obtained from the current meta-analysis, a single significant association was found

between prolactin gene polymorphism and protein yield in the AB and BB genotypes. No significant associations were observed between milk yield, fat yield, fat content, protein yield, and protein content and PRL genotypes. Since various conflicting results have been reported by the authors on this subject, more studies should be conducted to determine the PRL gene polymorphism and investigate its impact on milk yield traits. Moreover, to obtain more reliable results, the genotype x environment interaction should be taken into account, group comparisons should be made to minimize outcome variability with more articles, and the usability of the results obtained by applying the correct statistical model for breeding purposes should be discussed.

## AVAILABILITY OF DATA AND MATERIALS

Data sets are not deposited in different repositories, and data from a third party were not used. The data are original, and users can get it from corresponding author (M. Özdemir).

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## COMPETING INTEREST

We certify that there is no conflict of interest with any financial organization regarding the material discussed in the manuscript.

## AUTHOR'S CONTRIBUTIONS

MÖ and ZM designed the study, ZM, KE, ES, and EB collected all the data and arranged them for analysis. MÖ conducted the statistical analysis, and MÖ and ZM wrote the paper.

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