Prolactin Polymorphism Effect over Production Traits Types at Transylvanian Merino Sheep

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Abstract
Worldwide were developed animal selection programs assisted by molecular markers at many local breeds, targeting biodiversity and intrinsic value preservation.
Until now, were considered molecular markers associated with milk (beta-lactoglobulin, caseins) and meat production (calpastatin, miostatin etc.).
Another important marker for milk production is *prolactin.* Previous studies prove that prolactin depletion is associated with milk secretion decrease, making codifying gene for prolactin a candidate gene for milk production variation. Correlation of this marker effect with meat quality parameters was not studied yet.
Objective of this study is estimation of *prolactin* gene polymorphism effect over two traits types (for milk and meat quality) with a view to simultaneous use of both types in selection programs.
Using genomic DNA isolated from blood, we evaluate *prolactin* genetic polymorphism of 50 Transylvanian Merino sheep through PCR-RFLP method. The effect of *prl* polymorphism over production traits was estimated using animal model.
The research results reveal an important effect of *prl* gene over productive quality traits. The alleles effect is similar between those types; this fact sustain common selection program development in the same population.
In conclusion, our study proved that *prl* gene can be an important marker for selection programs in local sheep breeds.

Keywords: marker assisted selection, meat traits, milk traits, PCR-RFLP, prolactin.

1. Introduction

The pastoralist breeding system from Romania helps to use land resources in a cost effective manner. Local breeds are well adapted to this system and are very resistant to harsh conditions and walking on long distances grazing. On the other hand, local breeds are resistant to diseases and parasites, feed scarce and other environmental stressors [1].

Transylvanian Merino sheep was initially a wool breed, but now became more multi-purpose (meat, milk and wool). Selection for this breed was done using own performance records, with classical methodology. From that reason, production traits have large variability. For example, wool production is 3.5-7kg to females and 7-12kg to males, daily gain weight of 170-220g, and 70-90kg of milk over 4-8 weeks of lactation [2].
Genetic improvement of production traits is needed to keep the breed viable. Traditional breeding programs became more unfeasible due to difficulties on keeping records, low heritability of traits, etc.

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A solution can be implementation of Marker Assisted Selection program described by Marshal et al. [3].
Until now, molecular markers associated with milk (beta-lactoglobulin, caseins) and meat production (calpastatin, miostatin etc.). Another important marker for milk production is *prolactin (PRL).* Protein encoded by PRL gene seems to play a role in milk production [4]. PRL depletion in sheep, associated with reduction of milk secretion suggests that gene can be a functional candidate gene for milk yield variation [5].
PRL gene is located in ovine chromosome 20, in a putative region for milk, fat and protein yield [6] and for fat percentage [7].
Correlation of this marker effect with meat quality parameters was not studied yet.
Objective of this study is estimation of *prolactin* gene polymorphism effect over two traits types (for milk and meat quality) using PCR-RFLP technique with a view to simultaneous use of both types in selection programs.

2. Materials and methods

Animals, production traits and samples. Were analyzed 50 animals of Transylvanian Merino breed from Satu Mare County, Carei. Milk traits data were collected from National Agency for Zootechny. Meat traits were recorded on alive animals, using echo-graphical probe, measuring fat thickness, *Longissimus dorsi* muscle thickness, muscle eye area and muscle perimeter on two points of the animal. Blood samples were collected from jugular vein in heparinized tubes.
Ethical aspects. Blood and milk samples were collected with veterinary assistance, and cared for procedure for sample collection, handle and preservation techniques, in accordance with the Romanian Law 206/2004 for handling and protection of animals used for experimental purposes. The study protocol was approved by the Ethical Committee of the National Research Development Institute for Biology and Animal Nutrition, Balotesti, Romania.
DNA extraction. Genomic DNA was extracted from whole blood using a commercial kit (Wizard® Genomic DNA Purification Kit, Promega Corp., USA).
PCR conditions. PCR was carried out in a total volume of 20 µl, containing 75 ng genomic DNA, 200 µM dNTPs (Promega Corp., USA), 0.3 µM each primer and 1.25 units DNA polymerase (GoTaq Polymerase, Promega Corp., USA). PCR amplification was performed in a Corbett Research thermal cycler (Palm–Cycler, CG1-96 model).

Table 1. Nucleotide sequences of primers used for PCR-RFLP.

Primer sequence (5`→ 3`)	Tm (°C)	Amplicon length (bp)
ACCTCTCTTCGGAAATGTTCA	45	2500
CTGTTGGGCTTGCTCTTTGTC	49	

Amplification of *PRL* gene was carried out using the protocol described by Steiger [5].
The 2.5kbp amplified fragment was subsequently digested with HaeIII restriction enzyme (Promega Corp., USA). Digestion products were visualized on a 3% agarose gel stained with ethidium bromide.
Statistical analysis. First step was testing Hardy-Weinberg equilibrium condition, based on method cited by Giambra [8].
Genotype effect over production traits were estimated using Multiple Regression Least Square Method described by Gao et al [9]. The general model is defined as:

$$y_{ij} = \mu + h_i + m_j + e_{ij}$$

where y_{ij} is the trait value of sheep j in herd i, µ is overall mean, h is fixed effect of herd i, m is the effect of molecular marker j and e is residual of the model. In matrix notation, model will became:

$$\boldsymbol{y} = \mathbf{X}\boldsymbol{b} + \mathbf{M}\boldsymbol{u} + \boldsymbol{e}$$

y is the phenotypic values vector, b is a vector of various fixed effects (like sex, farm, breed etc.), u is a vector of marker effects (also fixed), X and M being incidence matrices for vectors b and u.
Marker codification were 101 allele coding system described by Strandén and Christensen [10].
For marker effect estimation we use production traits heritability described by Ugarte cited by Pelmus [11]: 0.19 for milk yield, 0.17 for fat

yield, and 0.17 for fat content, 0.18 for protein yield and 0.47 for protein content.
Statistical analysis and marker effect estimation was performed in R.

3. Results and discussion

The next steps were followed in marker analysis: gene polymorphism detection, allelic and genotypic frequencies estimation and marker genetic effect evaluation.
PRL polymorphism detection. DNA integrity was evaluated using UV transluminator (Figure 1).

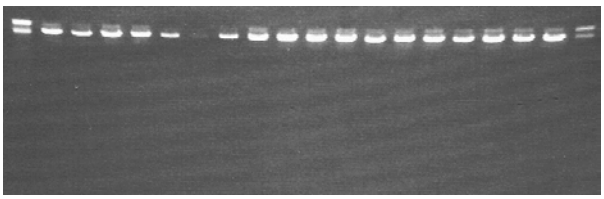

Figure 1. DNA integrity evaluation on 1% agarose gel

The fragment from *PRL gene*, of approximately 2.5kbp, was successfully amplified (Figure 2).
Digestion with restriction enzyme *Hae*III differentiated alleles *A* and *B*. Allele *A* contained 3 restriction sites for *Hae*III and resulted in 4 fragments of 1400, 530, 360, and 150 bp, whereas the presence of an additional restriction site in the *B* allele resulted in 5 fragments of 1400, 510, 360, 150, and 20 bp (Figure 3).

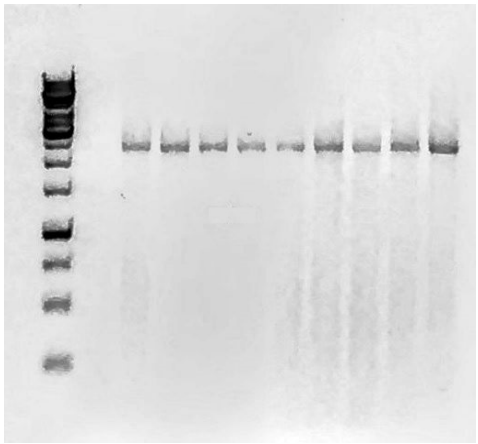

Figure 2. PCR product of a *PRL* gene segment amplification

Approximately 30% from genotyped animals show additional restriction sites that create a newer fragment of 700bp long.

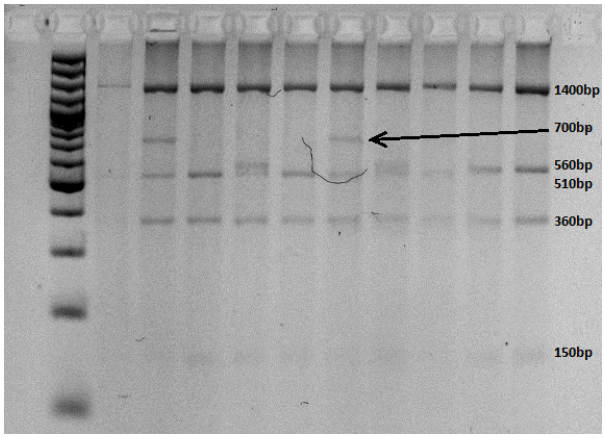

Figure 3. Digestion with *HaeIII* of PCR product

Genotypic and allelic frequencies for PRL gene. *PRL* genotypes recorded frequencies of 0.45 for *AA* genotype, 0.2 for *AB* and 0.35 for *BB* genotype (Figure 4).

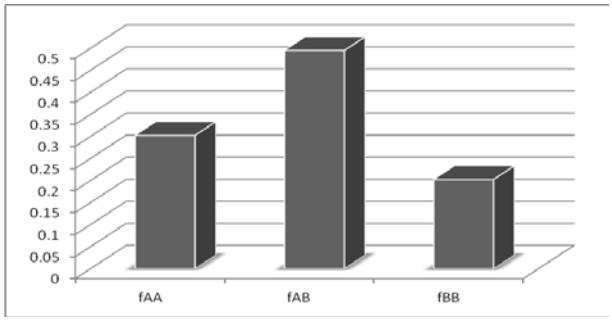

Figure 4. Genotype frequency for *PRL gene*

A allele recording a 0.55 frequency and *B* allele just 0.45 (Figure 5).

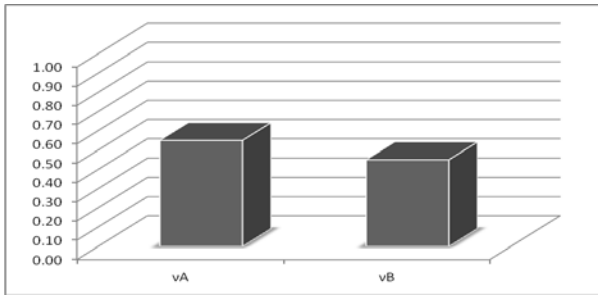

Figure 5. Allele frequency for *PRL gene*

In our study, *B* allele of *PRL* gene is slightly outnumbered by *A* allele. Ramos et al. [12] showed similar results with us regarding gene frequency in the Serra da Estrela and Merino breeds, *A* allele occurring more frequently than *B* allele in those populations. On the other hand, Staiger et al [5] found a different occurrence for *A* and *B* alleles.

Observed and expected genotypes for *PRL* genotype (Table 2) show a lack of Hardy-Weinberg equilibrium. Disequilibrium for *PRL* gene pointed some empirical selection in behalf of *AB* genotype and weighing against *AA* and *BB* genotypes.

Table 2. Observed and expected genotypes for *PRL* gene

Genotype	Observed	Expected
AA	9	6
AB	4	10
BB	7	4
	3.85	
χ^2 la 1 DF şi 5% degree of freedom		3.85 < 7.35*
χ^2 calculated	8.6	

In this research H-W disequilibrium work in favoring the genotypes containing *A* allele. Staiger et al [5] reported also an increase frequency for *A* allele in East Friesian breed. Those results point an empirical selection who leads over time in excess use of *A* allele selection. *Genetic effect on production traits.* In order to estimate the effect of *PRL* alleles on production traits, we used Multiple Regression Least Square Method and for meat production we obtained the values from Table 3.

Table 3. The effect of *prl* gene over few meat quality traits

Trait	AA	BB
Fat thickness 1 (cm)	0.121	-0.120
Fat thickness 2 (cm)	0.075	-0.075
Muscle thickness LD 1 (cm)	-0.001	0.001
Muscle thickness LD 2 (cm)	0.168	-0.168
Rib eye area 1 (cm)	0.067	-0.067
Rib eye area 2 (cm)	0.066	-0.066
Rib eye perimeter 1 (cm)	0.038	-0.038
Rib eye perimeter 2 (cm)	-0.509	0.509

In table 3 we can observe a small effect of *PRL* gene over meat quality traits but these effects seems to follow a similar trend with the effect of gene over milk production (Table 4).

Table 4. The effect of *prl* gene over milk traits

Trait	AA	BB
Milk yield (kg)	1.81	-1.81
Fat yield (kg)	0.12	-0.12
Fat content (%)	-0.10	0.10
Protein yield (kg)	0.08	-0.08
Protein content (%)	-0.06	0.06

The effect of *PRL* gene over production traits was poorly investigated, mostly over milk production. Serra da Estrela ewes [12] with *AB* and *BB* genotypes have highest production then those with *AA* genotype, those results being totally different than ours. In our investigation *A* allele of the *PRL* gene have a better effect in milk production. Similar results show Staiger et al [5] for East Friesian breed.

Regarding influence of *PRL* gene over meat quality, no one studied it. In our study we can see the positive effect of *A* allele over meat quality traits. A small exception is shown at rib eye perimeter measured at second point but further investigations are needed.

4. Conclusions

Positive association between *PRL* gene and production traits underlined in our study proved that *prl* gene can be an important marker for selection programs in local sheep breeds.

Acknowledgements

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