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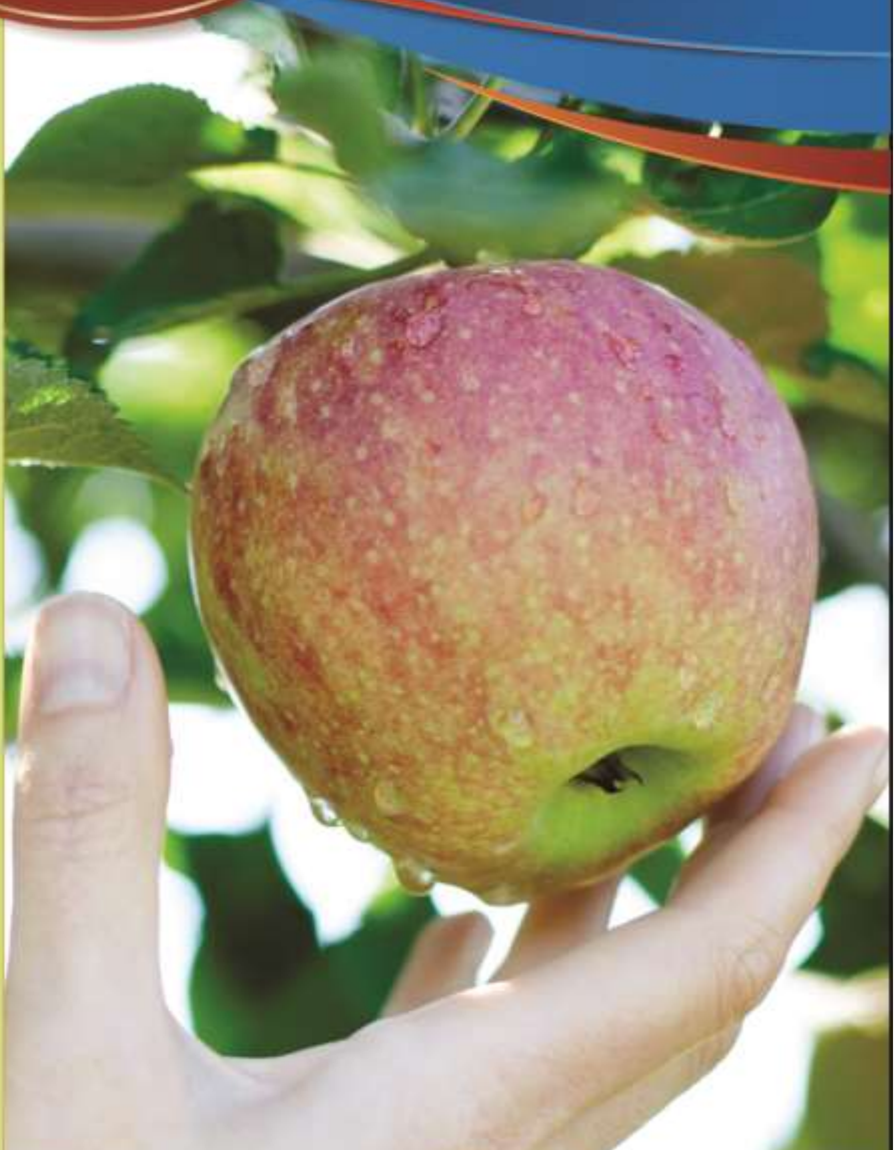

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"Agrosym 2025"
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THE INFLUENCE OF THE LEPTIN GENE ON MILK PRODUCTION IN THREE CONSECUTIVE LACTATIONS IN HOLSTEIN FRIESIAN CATTLE

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Abstract

This study uses a PCR method to detect allelic polymorphisms of the LEP gene. The study was conducted among 100 Holstein cows owned by private breeders. The study was conducted on Pelagonija farm, located in the Southwestern part of Republic of North Macedonia, over a period of three lactations or 915 days. The research focused on the health status of the animal and animal welfare. This study will identify all genotypes of the LEP gene and determine associations between the polymorphism of the leptin gene and the dynamics of milk production during three lactations of the cows. The best indicators for all three lactations will be identified in the group of animals with the TT genotype of the LEP gene. These individuals are characterized by increased milk yield, a high yield index and a tendency to increase the average daily milk yield during three lactations. In cattle, the LEP gene provides instructions for synthesizing leptin, a protein hormone predominantly expressed by white adipocytes (fat cells). Leptin's physiological roles include the regulation of energy intake, body mass, immune competence, and reproductive function. Its mechanism involves binding to receptors in the lateral hypothalamus to curb appetite and to those in the medial hypothalamus to induce satiety. Genetic variations within the LEP gene have been implicated in modulating circulating leptin concentrations and are associated with economically significant production characteristics, such as milk yield and quality.

Key words: *milk production, LEP gene, leptin polymorphism, dairy cattle.*

Introduction

Polymorphisms in the genes for leptin and leptin receptors provide powerfull additional tools for improved selection to meet the aspirations for increased global animal production within the comprehensive need to limit and reduce environmental impacts.
Animal fats are important throughout livestock production because they influence product quality and production efficiency, and they provide energy reserves during pregnancy and lactation. The discovery of leptin in cattle (Zhang et al., 2005) and its links to body fat in cattle [Frederich et al., 1995] have inspired scientists, who quickly recognized leptin's role as a predictor of reproductive readiness in female cattle (Hossner, 1998). Its involvement in inflammation and immune responses that help animals survive infections and trauma also has clear relevance, and attention is focused on research of leptin in the physiological functions of the body: puberty, mammary gland function, immune response, and reproductive pathways. Associations of production traits with polymorphisms in leptin and leptin receptor genes indicate potential further applications. Among many reviews of leptin, those based on cattle research (Hocquette et al., 2007). The leptin gene has been discovered in terrestrial and aquatic mammals, birds, and cold-blooded vertebrates, but not yet in any invertebrates.

The leptin gene and its polymorphic allelic variants and genotypes can be considered potential markers for milk production. Leptin is of interest for potential reproductive suitability because it significantly influences milk production in cattle, the content of milk components (proteins and fats), and, equally important, is linked to the productive profitability of livestock.
The leptin gene (LEP) is a non-glycosylated 16 kDa polypeptide that signals predispositions for adiposity and energy status in the hypothalamus. In cattle, the LEP gene is located on chromosome 4 (Stone et al., 2005), and on chromosome 18 in pigs [Sasaki S et al., 1996]. It consists of 3 exons and 2 introns, with only 2 exons translated into protein. Structurally, the leptin gene encodes a protein comprising 167 amino acids, including a signal sequence of 21 amino acids. A polymorphism associated with the LEP mutation (T→C) results in the substitution of cysteine with arginine in the α-helix of the leptin polypeptide. It has been identified at the coding region at position 73.
The aim of this study is to characterize cows with different genotypes of the leptin gene for milk production over three lactations (results from the first two lactations are expected to show minor variations). The increase in productivity occurs before the 4th-5th lactation. However, in recent years, due to changes in the genotype of animals (resulting from the improvement of indigenous breeds) and changes in the approach to animal use in industrial milk production technology, the average duration of productive longevity of dairy cattle has decreased to 2.6 lactations and continues to decline. All this relates to the rate of animal utilization. In this regard, the issue of how age influences milk production in cows becomes interesting to livestock breeders (Yu et al., 2017). The leptin gene and its polymorphic variants (alleles and genotypes) can be considered potential markers for milk and meat production (Machulskaya et al., 2017). Leptin is of interest for breeding because it largely determines milk production in cattle, the content of components in milk (proteins and fats), and, equally important, is linked to the productive longevity of livestock (Chizhova et al., 2017).

Material and methods

The research was conducted on 100 Holstein cows. The study was conducted on Pelagonija farm, located in the Southwestern part of Republic of North Macedonia, over a period of three lactations or 915 days. The research focused on the health status of the animal and animal welfare. This study will identify all genotypes of the LEP gene and determine associations between the polymorphism of the leptin gene and the dynamics of milk production during three lactations of the cows. During the experiment, all animals were kept under the same environmental conditions with standard feeding, proper care, and normal veterinary and technological attention at a farm free from infectious and invasive diseases.
Analysis of origin, physical development, body weight, milk production, and lactation activity of the experimental animals was fully performed. For DNA testing of allelic polymorphism of genetic markers related to economic traits in livestock, 100 blood samples were taken from the tail vein of the cows.
The biological material was collected in tubes with EDTA preservative. DNA extraction from the entire blood of the livestock was carried out using the commercial kit “Biogen” DNA-Sorb (Biogen, USA) for DNA purification [16].
The PCR method was used for analyzing SNPs in the second exon of the leptin gene (LEP), where both alleles were simultaneously amplified and then identified based on the size of their fragments in gel electrophoresis. (Merck, Corning Axygen, USA).

Protocol
A reagent mixture containing 3 µl of purified DNA, 3 µl of dNTPs, 0.2 µl of Taq DNA polymerase, 2 µl of buffer and a set of oligonucleotide primers with the given sequences was used for PCR:
F1: 5’ – GACGATGTGCCACGTGTGGTTTCTTCTGT – 3’
R1: 5’ – CGGTTTCTACCTCGTCTCCCAGTCCCTCC – 3’
F2: 5’ – TGTCTTACGTGGAGGCTGTGCCCAGCT – 3’
R2: 5’ – AGGGTTTGGGTGTCATCCTGGACCTTTCG – 3’

Replication of the fragments was performed using programmable thermal cyclers "T-100 Thermal Cycler" and "My Cycler" (BIO RAD, USA) under optimal temperature-time regime. Preliminary denaturation was performed for 5 minutes at 94 °C, followed by 40 cycles of denaturation at 94 °C for 10 seconds, annealing at 60 °C for 10 seconds, extension at 72 °C for 10 seconds, and final extension at 72 °C for 10 minutes. Electrophoretic separation was performed in a 2% agarose gel with a field strength of 20 V/cm for 20 minutes, using 0.5% ethidium bromide in 1xTBE buffer. The effect of electric current separated the amplified and restriction fragments by molecular weight. Visualization, video recording and documentation were performed using a UV transilluminator and the Gel&Doc system (BIO-RAD, USA). The identification of genotypes will be determined by the detectable polymorphism of the DNA sequences.
The analysis of the milk production and lactational activity of experimental animals was carried out according to data obtained from the official electronic file on the herd “SELEX w. 6.01” (AWS Plinor).

Results and discussion

Two alleles and three genotypes of the LEP gene have been identified as a result of polymerase chain reaction. The agarose gel electropherogram showed a combination of fragments depending on the genotype of the animal. The different genotypes were represented by the following base pairs: CC – 164 and 239 bp; TC – 131, 164 and 239 bp; TT – 131 and 239 bp. The CC genotype was present in about 32% of the population, the TC alleles in 51% of the population and the TT genotype in about 17% of the population.
According to the analyses performed, the chi-square value indicates the balance of Hardy-Weinberg equilibrium, Xcrit = 5.99, $p<0.05$, i.e. that the genetic balance in the population of interest for the LEP gene is not disturbed. Cows with the LEP TT genotype have a significant advantage in milk production during the 305-day lactation period (Table 4).

Table 1. Milk productivity for first lactation

Traits	Genotype CC	Genotype TC	Genotype TT
305-day milk yield, L	6053.5±115.4	5951.1±117.4	6832.3±191.5
Lactation milk yield, L	8095.8±248.7	7741.3±245.2	8387.2±263.8
Lactation length, days	365.9±10.1	363.1±10.1	363.3±13.9
One day lactation milk yield, L	20.7±0.3	2.1±0.2	23.2±0.4

In the first lactation (Table 1), individuals with the TT genotype had 14% higher milk production compared to individuals with the TC genotype and 12% higher milk production compared to individuals with the CC genotype. The difference between the TC and CC genotypes was small, and the highest milk production in the first lactation was observed in cows with the homozygous TT genotype of the leptin gene. The same group of individuals compared favorably during the second and third lactation. Heterozygous TC animals were characterized by minimal milk yield during all three standard lactations. The average milk yield for the 1st and 2nd lactation (305 days) between cows with different genotypes of the LEP gene differed slightly and had no statistical significance. Considering the results obtained, it then been noted that individuals with the TT genotype in the first lactation show higher values than those with the TC genotype and especially in contrast to those whith the CC genotype. In the first lactation, after 305 days, individuals with the CC genotype showed a milk production of 6053.5±115.4 L, which did not differ significantly from individuals with the TC genotype, which showed 5951.1±117.4L, but simultaneously showed a significant difference compared to individuals with the TT genotype 6832.3±191.5L. In terms of daily milk production, individuals with the CC 20.7±0.3 genotype also showed a significant difference compared to those with the TT 23.2±0.4 genotype and a small difference compared to those with the TC 21±0.2 genotype. The results are given in Table 1.

Table 2. Milk productivity for second lactation

Traits	Genotype CC	Genotype TC	Genotype TT
305-day milk yield, L	7823.0±161.5	7414.9±154.7	8172.9±277.5
Lactation milk yield, L	8657.0±277.9	8238.2±231.0	9051.7±405.5
Lactation length, days	1221.7±27.8	1280.1±31.9	1339.6±30.1
One day lactation milk yield, L	23.2±0.4	21.2±0.3	24.3±0.5

In the second lactation (Table 2), individuals with the TT genotype had 10% higher milk production compared to individuals with the TC genotype and 4% higher milk production compared to individuals with the CC genotype. The difference between the TC and CC genotypes was greater compared to the first lactation, but still in the second lactation the highest milk production was observed in cows with the homozygous TT genotype of the leptin gene.
In the second lactation, after 305 days, individuals with the CC genotype showed a milk production of 7823.0±161.5l, which did not differ significantly from individuals with the TC genotype, which showed 7414.9±154.7L, but simultaneously showed a significant difference compared to individuals with the TT genotype 8172.9±277.5L. In terms of daily milk production, individuals with the CC 23.2±0.4 genotype also showed a significant difference compared to those with the TT 24.3±0.5 genotype and a small difference compared to those with the TC 21.2±0.3 genotype. The results are given in Table 2.

Table 3. Milk productivity for the third lactation

Traits	Genotype CC	Genotype TC	Genotype TT
305-day milk yield, L	6771.8±326.8	7271.5±259.9	8213.0±321.6
Lactation milk yield, L	6983.7±329.8	7307.5±269.9	8303.0±326.6
Lactation length, days	1301.1±30.1	1377.9±29.9	1432.4±37.5
One day lactation milk yield, L	24.3±0.3	24.0±0.3	26.3±0.5

The same group of cows showed favorable performance during the second and third lactations. Animals with the CC and TC genotypes were characterized by lower milk yield during all three standard lactations. The average milk yield in the first lactation (305 days) in cows with the CC and TC genotypes differed slightly and was not statistically significant.
Comparatively, individuals with the TT genotype in the third lactation had 13% higher milk production compared to individuals with the TC genotype and 21% higher milk production compared to individuals with the CC genotype. Therefore, the highest milk production in the 1st, 2nd and 3rd lactations was observed in cows with the homozygous TT genotype of the leptin gene. The results are given in Table 3.
In the thitd lactation, after 305 days, individuals with the CC genotype showed a milk production of 6771.8±326.8L, which did not differ significantly from individuals with the TC genotype, which showed 7271.5±259.9L, but simultaneously showed a significant difference compared to individuals with the TT genotype 8213.0±321.6l. In terms of daily milk production, individuals with the CC 24.3±0.3 genotype also showed a significant difference compared to those with the TT 26.3±0.5 genotype and a small difference compared to those with the TC 24.0±0.3 genotype.

Table 4. Average milk productivity for three lactations

Traits	Genotype CC	Genotype TC	Genotype TT
Average 305-day milk yield, L	6942.1±201.2	6879.1±177.3	7739.4±263.5
Average milk yield for three lactation, L	7912.1±285.4	7762.3±248.7	8580.6±331.9
Milk yield for the three lactation, L	23736.3±154.1	23286.3±162.7	25741.8±217.7

The milk yield over 305 days for individuals with the CC genotype was 6942.1±201.2L, representing a slight difference from the amount of milk obtained from individuals with the TC genotype 6879.1±177.3L and a significant difference from individuals with the TT genotype 7739.4±263.5L. A very similar ratio is observed in the average milk production of three total lactations, where the average milk production of individuals with the CC genotype is 7912.1±285.4L, which is a minimal difference compared to individuals with the TC genotype 7762.3±248.7L, but we note a significant difference in milk production in individuals with the TT genotype 8580.6±331.9L.
In terms of total milk production over three lactations, we can observe the same tendency in individuals with a CC genotype of 23736.3±154.1L, almost no significant difference with individuals with a TC genotype of 23286.3±162.7L, and a significant deviation with individuals with a TT genotype of 25741.8±217.7L. The results are given in Table 4.

Conclusion

The results obtained indicate the genetic biodiversity of the Holstein cattle population. Three different genotypes were detected in the herd. The best indicators for all three lactations were found in the group of animals with the TT genotype of the LEP gene. These individuals are characterized by increased milk yield, and a tendency to increase average daily milk yield over three lactations. Thus, it can be concluded that the leptin gene will affect milk production, longer lactation, and productive duration of Holstein cows and can be used as a genetic marker in selective breeding.

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