



Associations Between IGF-II Gene Polymorphism and Milk Yield Characteristics In Brown Swiss Cattle[#]

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ABSTRACT

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This study was carried out on 114 Brown Swiss cattle reared in intensive conditions at Atatürk University Food and Livestock Application and Research Center and at the private cattle farm found in Erzurum province. Genotypic structures were examined in terms of Insulin-like growth factor (IGF)-II gene locus and the distribution of genotypes and allele frequencies of the cattle concerning the genes were determined. The identified Insulin-like growth factor (IGF)-II genotypes were associated with milk yield traits such as actual milk yield, 305-day, and daily milk yield. Insulin-like growth factor (IGF)-II genotypes were determined by using the Polymerase Chain Reaction (PCR)- Restriction fragment length polymorphism (RFLP) method from blood samples taken from the cattle. The CC, CT, and TT genotype frequencies of the Insulin-like growth factor (IGF)-II gene found in the population were 41 (34%), 65 (54%), and 14 (12%), and the frequency of the C allele and the T allele was found to be 0.61 and to be 0.39. The general averages of actual true milk yield, 305-day and daily milk yield were 4317±272.9 kg, 5277±240.7 kg and 18±0.9 kg, respectively, while CC, CT and TT genotypes 4168±515.8, 3756±321.7 and 5382±600.3 kg, respectively. As a result, correctly identified IGF-II genotypes were detected by using the PCR-RFLP method in the blood samples obtained from Brown Swiss cattle. Genotype and allele frequencies determined for IGF-II gene polymorphism can be considered sufficient to demonstrate the genotype diversity of the race. According to the Hardy-Weinberg genetic equilibrium test, the distribution of genotype frequencies of the cattle was observed in equilibrium.

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Introduction

Today, The importance of livestock is increasing day by day in terms of global warming that the world is facing, climate changes, destruction of natural areas, increasing industrial residues with increasing industrialization, increasing need for human nutrients in terms of quantity and quality, and providing adequate and balanced nutrition of human because of increasing of the world's population. The world annual population growth rate is approximately 0.5-1.0% in developed countries, 2-3% in developing countries (Çamurcu, 2005), and 1.27% (TUIK, 2021a) in our country. For this reason, in our country as well as in the world, the elimination of the nutritional requirements, especially animal protein needs, is of great importance to the number and yield potential of cattle. In the countries where developed agricultural techniques and livestock sector. Dairy cattle breeding has an important place in the livestock sector. Depending on the development level of countries, approximately 93% of milk production in the world consists of milk obtained from cattle (Hodoğlugil, 1996; Kopuzlu, 2003).

Brown Swiss, which has a good grazing ability in various rugged pastures, a strong body structure, a high ability to adapt to different care, nutrition and climatic conditions, is a late growing, combined yield, and a race with a huge structure among the daily breeds was brought to our country was brought from Australia to Karacabey stud farm, in 1925. This Breed has been used both in pure breeding and in the crossbred of domestic cattle. (Erdem, 1997; Akman, 1998; Özhan et al., 2012). According to the latest statistical data, the number of cattle and the culture of cattle breeds in Turkey is approximately 18.1 million and 8.8 million, respectively (TUIK, 2021b).

The first theme emphasized in the breeding studies based on yield is to provide the appropriate ideal environmental conditions to reveal the existing yield potential in the genotype of the animal. The implementation of efforts is more difficult, specific, and time-consuming. The yield characters obtained from the livestock are both under the control of a large number of genes and greatly affected by environmental factors.

Therefore, since the phenotypic value in these characters does not often reflect the genotypic value, the selection based on phenotype is observed to decrease productivity. Besides, as the phenotype is often not a good indicator of genotype, it is very important to estimate the genotypic value of the investigated character. Nowadays, genotype improvement studies have been taken to a more advanced level than classical breeding methods such as hybridization and selection with to the development of laboratory methods and techniques, and the advancement of technological conditions (Özdemir, 2001).

In recent years, many studies have been carried out to determine the existence of the relationship between some polymorphic properties of blood parameters and quantitative characters, and to benefit from these relationships in selection in cattle breeding. The studies show that the genotype is equivalent to the phenotype in its polymorphic characters, an autosomal inheritance path is followed, the simplicity of the genetic mechanism and the genetic structure are easily determined. Thus, the determination of blood characters at an early age by determining a relationship between polymorphic characteristics and quantitative characters is made the possible indirect selection, shortened the time between generations, directed breeding systems, analyzed the genetic structure of the population and observed changes in gene frequencies in the process (Ülker et al., 1999). Polymerase Chain Reaction (PCR) based on DNA and Molecular Techniques of Restriction Fragment Length Polymorphism (RFLP) analysis developed in recent years provides the opportunity to identify genotypes regardless of gender at a very early age. Therefore, PCR and RFLP molecular techniques are used as a process in the early identification of high-yielding animals, in the determination of breeding values for the yield characteristics of genotypes and the growth performance through several studies (Özdemir, 2001).

The IGF-II gene encoding the insulin-like growth factor-II protein is usually involved in the development of tissues and organs before birth and is expressed parentally by the activation of the gene transferred from the father (Zeric, 2012; Stinckens, 2010; Güngör and Ünal, 2015; Smith et al., 2015). It is also expressed in a wide variety of somatic tissues in early embryonic and fetal development. Adult IGF-II expression occurs in epithelial cells covering the liver and brain surface (Bergman et al., 2013). The gene is found on different chromosomes in different species. It is found on chromosome 2nd in pigs, 7th in mice, 11th in humans, 12th in horses, 21st in sheep, and 29th in cattle (Goodall and Schmutz, 2003; Güngör and Ünal, 2015). Research on the activation of the IGF-II gene has shown that this gene has important effects on fetal development in rats and many species, and increases growth and differentiation in many tissues after birth, and is associated with carcass characteristics in livestock (Harbili, 2008; Yaylalı, 2009; Arıkan, 2013; Güngör and Ünal, 2015). It has been reported that while IGF-II is associated with meat characteristics, quality, and body weight in beef cattle it affects milk yield in dairy cattle (Stinckens, 2010; Güngör and Ünal, 2015; Smith et al., 2015).

In a study conducted with cattle (Güngör and Ünal, 2015), it was reported that IGF-II might be effective in the development of mammary glands from the embryonic to

the adult period. The development of the mammary gland spreads over a wide period throughout the animal's life. Morphological development of mammary glands begins during embryonic development, and the period corresponds to the proliferation, differentiation and apoptosis periods of cells due to gene expression models during postnatal periods through puberty, pregnancy, lactation, and involution (O'Doherty et al., 2015).

In studies conducted with rats, the results obtained using a combination of mammary gland gene expression and comparative mapping revealed that IGF-II is a candidate gene for a QTL affecting milk production characteristics in cattle. (Ron et al., 2007; Bagnicka et al., 2009; O'Doherty et al., 2015). In another study, it was reported that there is a relationship between IGF-II and predicted reproductive values for milk yield, milk fat yield, and milk protein yield in Holstein-Friesian bulls (Zeric, 2012).

Bagnicka et al. (2009) previously defined g.8656 C> T-transduction (RFLP-BsrI) nucleotide in the 2nd exon region of IGF-II gene in 238 Holstein-Friesian dairy cattle and also the g.24507G> T transversion (RFLP-HaeIII) nucleotide at the 10th exon region as a result of the sequencing of the 237 bp DNA sequence in 6 animal DNA and then determined their single nucleotide polymorphisms by the PCR-RFLP technique. In another study by these researchers, It was concluded that CT/GT haplotype ($P \leq 0.001$) was directly related to milk yield characteristics and the most common CT/GG haplotype was found to be effective in milk and fat content of milk compared to other defined haplotype in terms of daily milk yields

This study aims to investigate genotypic structures of insulin-like growth factors-II (IGF-II) in terms of a gene locus, to determine the distribution of genotype and allele frequencies of the cattle in terms of relevant genes and to correlate the genotype and the allele frequencies with milk yield in Brown Swiss cattle reared at AUFLARC and in the private cattle farm in Erzurum province.

Material and Methods

Material

The animal material of the study consisted of 114 Brown Swiss cattle aged 3, 4, and 5 years raised at Ataturk University Food and Livestock Application and Research Center (AUFLARC) (60 cattle) and at a private cattle farm (54 cattle) in Erzurum province. The relationship between IGF-II genotypes and various milk yields of Brown Swiss cattle was investigated. However, only the milk yields records of the private cattle farm were used in the study of the relationships.

Method

The genotypic structure of the IGF-II gene locus from hair samples taken from 114 Brown Swiss cattle was determined using the PCR-RFLP method. Genomic DNA isolation was obtained by applying the orbitals performed in the method using the Purgene DNA kit (Gentra Systems, Minnesota, USA) Qualitative and quantitative controls of the obtained DNAs were determined by using Nanodrop (Drop Plate, Cat. No: 12391) spectrophotometry device.

In the PCR, the 217-bp DNA region was amplified by using primers, forward: 5'-5'CCTCAGCCTCATCCCCTCCTTGC-3' and revers: 5'-CTGTGCTCTATTT

GCTGTGTTGTCT-3', of growth hormone in the analysis (Goodall and Schmutz 2003). 1 µl of each primer and dNTP mix (D7595: Sigma, St. Louis, MO, USA), 0.5 units of Taq DNA polymerase (D1806: Sigma), approximately 300 ng of template DNA, 5 µl of 10× PCR Buffer (100 mM Tris-), HCl, pH 8.3, 500 mM KCl, 15 mM MgCl₂ and 0.01% gelatin), 1 µl 25 mM MgCl₂ and ddH₂O were used to complement the required total volume to 30 µl for PCR amplification of the IGF-II region. IGF-II gene amplification was identified using 300ng template DNA, 1 µl from each primer, 3 10l 10× PCR Buffer, 1.5 mM MgCl₂ and 100 ddM ddH₂O. PCR amplification conditions were determined as 5 min.-1 cycles at 94°C which is the initial denaturation temperature and 32 cycles at the second denaturation. Provided the times are the same, the elongation cycle times for the IGF-II gen were 94°C, 61°C and 72°C for 50 seconds, 5 minutes and 1 cycle at the final elongation temperature which is 72°C. Amplification products were visualized with UV light after electrophoresis for 15 minutes at 80 volts on 2.5% agarose gel.

To determine the IGF-II allele gene frequencies of each cattle breed, Firstly, approximately 10 µl from each amplification sample, each of which was placed in 0.2 ml sterile Eppendorf tubes, 3-5 U BsrI restriction enzyme (5'C^T3 '), 3-5 µl RE buffer and 6 µl ddH₂O were added to these tubes for the related region and then the prepared tubes were sealed with 8-10 ll of mineral oil and centrifuged at 13000 rpm for 1 minute. Later, the tubes were placed in the incubator and incubated at 38°C for 12-13 hours. After incubation, the samples were separately loaded on 3-3.5% agarose gel and subjected to electrophoresis at 60 volts for 2.5 hours. The electrophoresis gel was visualized under UV light and the band sizes were examined according to the cut regions (CC: 32-185; CT: 32-67-118,185; TT: 118, 67, 32). Finally, the IGF-II allele of each cattle breed was calculated by counting the gene frequencies. Whether the genotype frequencies were in Castle Hardy-Weinberg equilibrium was examined by Chi-square (χ^2) test (Özdemir, 2006; Sönmez, 2013).

Statistical Analysis

Allele gene frequencies were calculated by counting each sample separately. Hardy-Weinberg Genetic Equilibrium Test and Chi-square independence test (χ^2) were performed to determine whether the genotype frequencies were in genetic equilibrium. The data were analyzed based on the General Linear Model by the SPSS package program. The differences between the groups

were revealed by applying Duncan's multiple comparison tests (Duncan, 1955). In this study, environmental factors such as genotype, lactation order, cattle age, and calving season yield characteristics, the interaction of the cattle age with genotype, and interaction of the calving season with genotype were discussed. The following statistical model was used according to the yield characteristics in the study.

$$Y_{ijkl} : \mu + a_i + b_j + c_k + d_l + (ab)_{ij} + (ac)_{ik} + (ad)_{il} + e_{ijkl}$$

Y_{ijkl}: The value of any Simmental cow in terms of any of the characteristics of performance (real milk yield, 305 days milk yield, lactation period, and daily milk yield) considered;

μ : population means;

a_i : i. genotype effect (CC, CT and BB)

b_j : j. effect of the lactation order (1., 2., and 3.)

c_k : k. effect of calving season (Winter-spring and Summer-Autumn)

d_l : l. effect of maternal age (3., 4., and 5)

e_{ijkl} : random error.

Result and Discussion

DNA samples obtained from Brown Swiss cattle were amplified in the PCR Device and the IGF-II gene polymorphic regions were determined by cutting with the BsrI Restriction Indiviryrase enzyme (Figure 1).

The expression regions of the IGF-II gene were defined as CC; 32-185, CT; 32-67-118, TT; 118-67-32 bp genotype. The expression regions, genotype and allele frequencies of IGF-II, the results obtained from the genetic equilibrium test, and the X² independence test are shown in Table 1. CC, CT, and TT genotype frequencies of IGF-II, the frequency of the C allele and the frequency of the T allele in the population were determined as 41 (34%), 65 (54%) and 14 (12%), 0.61 and 0.39, respectively (Table 2). Zeric's study (2012) determined that the C allele frequency was high, that the T allele frequency was low, and that TT genotype was more effective. Our findings were found to be consistent with the findings determined by the researcher. According to the Hardy-Weinberg genetic equilibrium test, the distribution of genotype frequencies of 120 cattle was observed in equilibrium (P>0.05). The results obtained from the previous two similar studies (Bagnicka et al., 2009, Zwierzchowski et al., 2010) were found to be consistent with the results of this study.

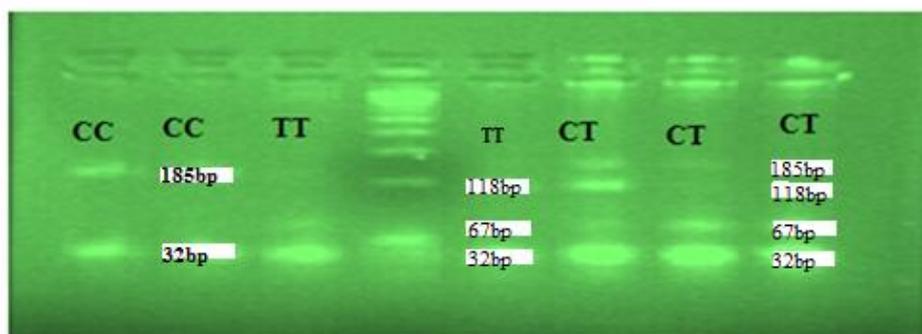


Figure 1. PCR-RFLP cut sites of the IGF-II gene: CC; 32-185, CT; 32-67-118, TT; 118-67-32 bp

Table 1. Genotype, allele frequencies and standard error and IGF-II genotype frequencies, the results of Hardy-Weinberg Genetic Equilibrium and χ^2 Independence Test in the Brown Swiss population

| Genotype % | Genotype (%) | | | Allel Frequency | | |
|------------|--------------|----|----|-----------------|------|-------|
| | CC | CT | TT | C | T | S. E. |
| | 34 | 54 | 12 | 0.61 | 0.39 | 0.059 |

*Standard Error

Table 2. IGF-II genotype frequencies, Hardy-Weinberg genetic balance and χ^2 independence test results

| N | Observed | | | Expected | | | H-W |
|-----|----------|----|----|----------|-------|-------|------------|
| 120 | CC | CT | TT | CC | CT | TT | χ^2 |
| | 41 | 65 | 14 | 45.02 | 56.96 | 18.02 | 0.1222 NS* |

*:NS: Not significant ($P>0.05$)

When Table 3 is examined, the overall mean of actual milk yield for the Brown Swiss cattle was determined as 4317 ± 272.9 kg. As a result of the assessment, the highest and the lowest mean milk yield among the IGF-II genotypes was found TT genotype (5382 ± 600.3 kg), respectively. At the same time, when the actual milk yield mean obtained from cattle was taken into consideration, the average actual milk yield of the cattle with TT genotype was 1214 and 1626 kg higher than the average actual milk yield of the cattle with the other genotypes, respectively. However, the differences among the means of genotypes belonging to IGF-II were not statistically significant.

In the study, when genotype x age interaction values are taken into consideration. In terms of actual milk yield, while the highest with 6100 ± 950.0 kg was found in the cattle with 5-year old-TT genotype, the lowest value with 2608 ± 767.7 kg was found in the cattle with CT genotypes at the same age. According to the results of the variance analysis, the differences among the actual milk mean values obtained in all genotypes were significant for the cattle ages ($P<0.05$), not significant for the number of lactation and the calving season. When genotype x calving season interaction is taken into consideration, the actual milk yield means of the cattle with TT genotype, which were born both in the winter-spring and summer-autumn season, had the highest values and these values were determined as 5878 ± 710.2 kg and 4886 ± 695.7 kg, respectively. When the studies on this subject are examined since there was no study to our best knowledge on the relationship of IGF-II genotype with actual milk yield. The comparison couldn't be made in terms of these traits.

305-days milk yield means of the Brown Swiss cattle and the standard errors of the means in the study are given in Table 3. According to the yield records of the 66 cattle that formed the study material, the overall mean of the 305-day milk yield was found as 5277.1 ± 240.7 kg. According to the significance test for the trait, in terms of the parameters mentioned, the effect of IGF-II genotype x age interaction was significant ($P<0.05$), and the effect of age was very significant ($P<0.01$).

The average 305-day milk yield values of the cattle with IGF-II genotype were determined as 5632 ± 529.3 kg for TT genotypes, 5420 ± 454.9 kg for CC genotypes, and 4898 ± 283.6 kg for CT genotypes. When the results of the interactions discussed for this trait are evaluated, For the IGF-II genotype x lactation order interaction, the maximum values of the calculated yields were CC genotype (5734 ± 822.2 and 5547 ± 635.5 kg) in the 1st and 3rd lactation and TT genotype in the 2nd lactation

(6641 ± 1013.2 kg). For IGF-II genotype x age interaction, the highest values were found in 5 aged cattle with CC (7184 ± 840.2 kg) and TT genotype (6328 ± 837.7 kg), in 4 aged cattle with CT genotype (5362 ± 298.3 kg). On the other hand, the lowest values were determined in 3 aged cattle with CC genotype (4199 ± 429.8 kg), in 5 aged cattle with CT genotype (4424 ± 676.9 kg) and in 3 aged cattle with TT genotype (4497 ± 523.0 kg). When the 305-day milk yield mean values determined for IGF-II genotypes in two different calving season groups were taken into consideration, the lowest mean values were 4880 ± 444.8 and 4916 ± 253.9 kg in CT genotypes in both calving seasons, respectively. While the largest mean values were determined as 6162 ± 626.2 kg in the TT genotype in the winter-spring birth season and as 5173 ± 396.4 kg in the CC genotype in the summer-autumn birth season. The TT genotype value was very close to the value of the CC genotype in the summer-autumn birth season. Since there was no study to our best knowledge on the association of IGF-II genotype with 305-day milk yield characteristics, no comparison was made with other studies in terms of this trait.

The overall value of the daily milk yield in the Brown Swiss herd studied was 18 ± 09 kg. These values of IGF-II genotypes were found as 19 ± 1.7 , 17 ± 1.1 and 19 ± 2.0 kg for CC, CT and TT genotypes, respectively. The mean daily milk yield of the herd varies from lactation to lactation, and the maximum mean value of this parameter was obtained in the 1st lactation. On the other hand, while the cattle age increases, it is observed that the mean daily milk yield increases as well. While the lowest mean daily milk yield in the herd was determined in 3 aged cattle (15.2 ± 0.81 kg) and the highest mean daily milk yield was found in 4 aged cattle (21.4 ± 1.72 kg).

In terms of this trait, although the mean in the winter-spring calving season was the highest value with 19.3 ± 1.52 kg, the difference between the calving season was not statistically significant. When the genotypes were taken into consideration, the mean daily milk yield values were found to be the highest in the CT genotype in 4 aged cattle, and in the CC and TT genotypes in 5 aged cattle. When the three genotypes were evaluated together, The TT genotype was found to have the highest value of the three genotypes.

According to the results of variance analysis, the effect of IGF-II genotype, number of lactation, calving season, IGF-II genotype x number of lactation and IGF-II genotype x calving season on the daily milk yield was statistically insignificant, the effect of IGF-II genotype x age on the daily milk yield was statistically significant ($P<0.05$) and the effect of the cattle age on the daily milk yield was statistically highly significant ($P<0.01$).

Table 3. Least squares means, standard errors and significance of mean differences for actual milk yield, 305-day milk yield and daily milk yield traits (kg)

| Factors | | N | Actual Milk Yield | | 305-Day Milk Yield | | Daily Milk Yield | | |
|---|---------------|----|--------------------------|-------------|-------------------------|--------------|------------------------|----------|----|
| | | | $\bar{X} \pm S\bar{x}$ | S | $\bar{X} \pm S\bar{x}$ | S | $\bar{X} \pm S\bar{x}$ | S | |
| Overall | | 66 | 4317±272.9 | | 5277±240.7 | | 18±0.9 | | |
| IGF-II Genotype | CC | 11 | 4168±515.8 | | 5420±454.9 | | 19.1±1.7 | | |
| | CT | 40 | 3756±321.7 | NS | 4898±283.6 | NS | 17.2±1.1 | NS | |
| | TT | 15 | 5382±600.3 | | 5632±529.3 | | 19.2±2.0 | | |
| The Number of Lactation | 1. | 7 | 1998 ^C ±544.9 | | 5282±480.5 | | 20.7±1.8 | | |
| | 2. | 24 | 5864 ^A ±446.0 | ** | 5641±393.3 | NS | 19.7±1.5 | NS | |
| | 3. | 35 | 4316 ^B ±320.8 | | 4910±282.9 | | 17.1±1.1 | | |
| Age | 3. | 31 | 4305±252.4 | | 4540±222.6 ^C | | 15.2±0.8 ^B | | |
| | 4. | 28 | 4269±355.4 | NS | 5357±313.4 ^B | ** | 19.6±1.2 ^{AB} | ** | |
| | 5. | 7 | 4377±516.7 | | 5935±455.6 ^A | | 21.4±1.7 ^A | | |
| Calving Season | Winter-Spring | 21 | 4836±443.4 | * | 5495±390.9 | NS | 19.3±1.5 | | |
| | Summer-Autumn | 45 | 3798±265.2 | | 5059±233.8 | | 17.5±0.9 | NS | |
| IGF-II Genotype X The Number of Lactation | CC | 1 | 2 | 2423±932.4 | | 5734±822.2 | | 21.2±3.1 | |
| | | | 5 | 1574±564.2 | | 4829±497.5 | | 18.3±1.8 | |
| | CT | 2 | 3 | 5071±632.3 | | 4978±557.6 | | 17.8±2.1 | |
| | | | 20 | 5576±265.7 | NS | 5304±234.3 | NS | 18.5±0.9 | NS |
| | TT | 3 | 1 | 6944±1149.0 | | 6641±1013.2 | | 22.9±3.8 | |
| | | | 6 | 5010±720.7 | | 5547±635.5 | | 19.2±2.4 | |
| | CC | 3 | 15 | 4117±499.2 | | 4561 ±440.2 | | 16.7±1.6 | |
| | | | 13 | 3820±397.0 | | 4623±350.1 | | 16.5±1.3 | |
| IGF-II Genotype X Age | CC | 3 | 5 | 4294±487.5 | | 4199±429.8 | | 14.1±1.6 | |
| | | | 4 | 3211±752.5 | | 4876±663.6 | | 18.5±2.5 | |
| | | | 2 | 4998±952.8 | | 7184±840.2 | | 26.7±3.1 | |
| | CT | 4 | 20 | 4500±243.2 | | 4909±214.5 | | 17.6±0.8 | |
| | | | 17 | 4159±338.3 | * | 5362±298.3 | * | 18.9±1.1 | * |
| | | | 3 | 2608±767.7 | | 4424±676.9 | | 16.0±2.5 | |
| | TT | 5 | 4 | 4028±593.1 | | 4497±523.0 | | 14.8±1.9 | |
| | | | 7 | 6020±699.6 | | 6071±616.9 | | 19.7±2.3 | |
| | | | 3 | 6100±950.0 | | 6328±837.7 | | 23.0±3.1 | |
| | | | 2 | 4647±958.8 | | 5666±845.4 | | 20.1±3.1 | |
| IGF-II Genotype X Calving Season | Winter-Spring | CC | 15 | 4330±504.4 | | 4880±444.8 | | 17.1±1.7 | |
| | | | 4 | 5878±710.2 | | 6162±626.2 | | 21.5±2.3 | |
| | | | 9 | 3689±449.5 | NS | 5173±396.4 | NS | 18.1±1.5 | NS |
| | Summer-Autumn | CT | 25 | 3181±287.9 | | 4916±253.9 | | 17.3±0.9 | |
| | | | 10 | 4886±695.7 | | 5102.4±613.4 | | 16.9±2.3 | |

NS: Inot significant ($P>0.05$), *: $P<0.05$ and **: $P<0.01$; Significant, ^{A, B and C}: Means in rows with different superscripts are significantly different at $P<0.01$ and $P<0.05$

The mean daily milk yield determined in the study show that showed that the value obtained from cattle born in the winter-spring season group was found to be more than 1.8 kg than the value obtained from cattle born in the summer-autumn season group and the increase was statistically insignificant (Table 3). When the result of this study was compared with the results of the study conducted with Holstein-Friesian cattle by Bagnicka et al., (2009), it was observed that the result of this study was not coherent with the result of the other study.

Conclusion

The genotypes (CC, CT, and TT) were determined by the PCR-RFLP method in blood samples taken from 114 Brown Swiss cattle raised in AUFLARC and on a private cattle farm. In the general population, IGF-II RFLP / MboII polymorphism for CC, TT, and CT genotypes was determined as 34%, 12% and 54%; the number of CC, CT,

and TT genotypes was found to as 41, 65 and 14; the frequency of the C allele and the T allele was determined as 0.61 and 0.39 for respectively. According to the Hardy-Weinberg genetic equilibrium test, the distribution of genotype frequencies in the population was found to be in equilibrium ($X^2: 0.1222$) ($P>0.05$).

In the study, TT genotype (5382±600.3 kg) was the highest value among the actual milk yield mean values of Brown Swiss cattle determined for IGF-II genotypes (CC, CT, and TT genotypes). This value determined for the TT genotype was found to be about 1547 kg more than the mean value of the actual milk yield (4317±272.9 kg). According to the results of variance analysis, the effect of IGF-II genotype × age interaction on the actual milk yield was statistically significant ($P<0.05$). The overall mean 305-day milk yield of the cattle forming the study material was found to be 5277.1±240.66 kg. 305-days milk yield means are generally evaluated for the parameters discussed. The highest overall mean values of the parameters were obtained

in the TT genotype (5632±529.3kg) for genotypes, in the second lactation (5641±393.3 kg) for the number of lactation, in the winter-spring season (5495±390.9 kg) for the calving season and the 5-year-old (5935±455.6 kg) for the cattle age. According to the significance test for the trait, the effect of IGF II genotype x age interaction was significant ($P<0.05$). When the mean milk yield of Brown Swiss cattle was evaluated, the highest value in terms of the mean of environmental factors was found for TT genotype (19±2.0 kg) and CC genotype (19±1.7 kg) in the genotype of IGF-II, for first lactation (20±1.8 kg) in the number of lactation, for 5 years (21±1.7 kg) in the cattle age and the winter-spring season (19±1.5 kg) in the calving season. As a result of the variance analysis applied for daily milk yield, on daily milk yield, the effect of IGF II genotype x age interaction was significant ($P<0.05$), the effect of age was very significant ($P<0.01$), and the effect of other factors was not significant.

According to the results of the variance analysis test of the actual milk yield, the effect of the number of lactation was highly significant ($P<0.01$), the effect of the calving season and IGF II genotype × age interaction were significant ($P<0.05$), and the effect of the other factors insignificant. As a result of the variance analysis test of 305-day milk yield and daily milk yield, the effect of the cattle age was highly significant ($P<0.01$), the effect of the calving season and IGF II genotype × age interaction were significant ($P<0.05$) and the effect of the other factors was not significant.

As a result of the study, IGF-II genotypes of the breed were accurately identified by the PCR-RFLP method from blood samples taken from Swiss Swiss cattle. Genotypes and allele frequencies determined in terms of IGF-II gene polymorphism were found to be sufficient to reveal the genotype diversity of the breed. In the analysis of variance, it was found that there was a relationship between IGF-II genotypes and the examined performance characteristics (actual milk yield, 305-day milk yield and daily milk yield) but these relationships were not statistically significant. In addition, we can say that it will be a need to carry out relationships studies with larger herds, different breeds, and different performance characteristics to better understand the relationships between IGF-II gene polymorphism and some performance characteristics.

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