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Original Research Article

EFFECTS OF SELECTION IN TERMS OF MEAT YIELD TRAITS ON LEPTIN RECEPTOR GENE IN JAPANESE QUAIL LINES

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Abstract: This study was carried out to investigate the effects of selection on the single nucleotide polymorphisms (SNPs) in coding sequence of leptin receptor (*LEPR*) gene and possible associations between SNPs' and some meat yield traits of Japanese quail lines. Fifteen generations divergently selected two lines (HBW and LBW) for 5-weeks of age body weight and a control were used as materials for this study. A 348-bp part of the *LEPR* coding region (18th exon) were sequenced in a total of 113 individuals from the three quail lines and shown that the fragments contained four SNPs loci (T490C, C528T, G537A, T571C) and five haplotypes (TTGT, CTGT, TCGT, TCAT, TCAC). T490C replacement caused the missense mutation of phenylalanine to convert to leucine (Phe>Leu). However, other SNPs were synonymous and there were no changes in transcripts. It was determined that the quails with higher phenotypic values were in the TT genotype at the T390C locus. Statistical analyses showed that there were significant differences among the quail lines, SNP alleles and haplotypes in terms of interested phenotypic traits (P<0.05), and also SNP and haplotype distributions changed depending on quail lines (P<0.001). When all results were evaluated together, it was concluded that the fifteen generations of selection caused significant changes in the *LEPR* gene in terms of economically important traits in Japanese quail lines (*C. coturnix japonica*).

Key words: SNP; haplotype; leptin receptor; selection; Japanese quail

Introduction

The control of feed intake, energy balance and fat deposition have high economic importance in farm animals. The accumulation of excess fat affects meat quality, fertility, productivity, and whole-body metabolism (1, 2). Leptin activity at specific receptors in the hypothalamus suppresses feed intake, which increases the use of energy, and leptin is a polypeptide hormone that controls the body's energy balance (3, 4). Leptin receptor (*LEPR*) is a member of the class 1 cytokine receptor family that mediates most of LEP central and peripheral effects (5). The presence of leptin in laying and broiler poultry genotypes decreases feed intake (6, 7). Quail chicks that were given recombinant mouse leptin on the fifth embryonic day were removed from incubation earlier (5-24 hours) and reached a higher body weight than the control group (8). DNA sequence analysis of leptin from several mammalian and avian species, including livestock animals, showed that leptin is highly conserved among vertebrate species. Among vertebrates, the leptin amino acid sequence shows more than 80% homology (9). The entire coding region of the leptin receptor (*LEPR*) in Japanese quail (*C. coturnix japonica*) was sequenced by Wang et al. (10). Also, four alternative *LEPR* spliced variants (one long and three soluble) were identified in Japanese quails

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(11). DNA sequencing methods have focused on single nucleotide polymorphisms (SNP) in recent years. SNPs of leptin and its receptors in relation to livestock yields has accelerated research efforts. Researchers are actively investigating the relationship between leptin and traits of high economic importance, such as feed intake (12, 13), fertility (14), milk production (15, 16) and meat production (17, 18). Tarabany et al. (19) has reported SNP haplotypes significantly associated with growth and egg production in Japanese quails. Also, a SNP of *LEPR* gene was significantly associated with abdominal fat in chickens (20).

Statistical analysis is an important part of scientific studies and there are different statistical tests and approaches have been developed for analyzing data sets which have been obtained the studies carried out for the same purpose. However, it is extremely important to use a statistical test or methods that will be able to give more detailed information about the effect of interested factor(s). Due to its advantages over classical methods (i.e., ANOVA and its parametric and nonparametric counterparts) Analysis of Mean Technique (ANOM) was used for analyzing data sets.

ANOM is not only a powerful tool for comparing means but also for comparing variances, proportions and other location and scale measures. This procedure can also be used efficiently as a multiple comparison test especially when there are a large number of groups (21). ANOM is accepted as a graphical counterpart to ANOVA for comparing group means. Since it presents the comparisons graphically, the researchers can easily see which treatment mean (s) are different. This is a big advantage especially for non-statisticians (21 - 23).

The aim of this study is to investigate the relationships between SNPs in the coding sequences (18^{th} exon) of the leptin receptor (*LEPR*) gene, which may occur as a result of 15 generations of long-term selection in Japanese quail (*C. coturnix japonica*) lines, and some phenotypic features.

Material and methods

All the experimental procedures were reviewed and approved by Akdeniz University Local Committee on Animal Research Ethics (Protocol number: 2012.02.02).

Animal

The material of this study was consisted of three different Japanese quail (C. coturnix japonica) lines (C: control, HBW: high body weight and LBW: low body weight). Control group was not selected previously while the quails in the treatment groups were divergently selected according to their high and low body weight at the fifth week for 15 generations. Quail lines were obtained in previous project which was supported by the Scientific Research Projects Coordination Unit of Akdeniz University (Project ID: 2003.03.121.004). The data sets used in this study were obtained from the projects supported by the Scientific Research Projects Coordination Unit of Akdeniz University (Project ID: 2012.01.0104.002) and the Scientific and Technological Research Council of Turkey (Project ID: 114O047).

Raising of Material

Fertilized eggs were collected from the HBW, LBW and C lines for a week and stored at 15-20 °C with 75-80% humidity. These eggs were incubated at 36.5 °C with 65% humidity for the first 14 days and at 36.0 °C and 55% humidity for the last 4 days. Hatching weight (HW) of chicks were weighed individually using 0.01 g precision scales, and an aluminum ID number was attached to the left wings of chicks after incubation. These chicks were fed 24% crude protein and 2900 kcal/kg ME (metabolic energy) during the first four weeks in a breeding cage. Sex determination was performed by observing the cloaca and breast feather color at the end of the fourth week. 50 males and 50 females were selected randomly from each quail line and transferred to individual breeding cages for 10 weeks. All birds were fed with 21% crude protein and 2800 kcal/kg ME for ten weeks. Lighting was applied continuously for the first four weeks and then 16 hours a day.

Feed Intake (FI)

Since the animals were not fed on the day of weighing the differences due to feed intake was fixed. Thus, 10 weeks of individual feed intake (FI) were recorded as grams per day. A total of FI for per individual was calculated from these records.

Sexual Maturity

The first ovulation day and the first day of release foam were considered as sexual maturity for females and males, respectively. Sexual maturity weight (SMW) of males and females were determined on the same day.

Body Weight and Carcass Weight

A total of 50 quails (25 males and 25 females) were selected randomly from the HBW, LBW and C groups, respectively. Then were introduced to the cutting process which was performed at the end of the 15th week. The body weights (BW) of the birds were measured just before cutting using 0.01 g precision scales from hatching to the 15th week on the same day each week. Low-voltage electrical current (100 mA, 50 Hz) was used to stun animals as recommended in the relevant scientific literature (24, 25), and then the jugular vein was cut. After the blood flow was over, the feathers were cleaned, and the internal organs were removed. Eventually, the carcass weights (CW) were measured using a precision scale of 0.01 g.

Tissue Samples and Total RNA Extraction

Liver tissue samples from each individual were isolated using sterile forceps and scissors immediately after cutting and placed into numbered tubes (Corning, New York, USA) containing RNAsave. These tissues were stored at -80 °C until use. RNA extractions from liver tissue samples were performed using a commercial kit (Axygen) after cellular degradation of the liver tissues using a lyser with tungsten beads. The concentrations of isolated RNAs were measured using a biodrop. Also, RNA gel analysis (Reliant Gel System) was performed to determine RNA quality. For this purpose, 3µL of each Total RNA sample from each quail and 3µL of formaldehyde were added to a microcentrifuge tube and centrifuged for 10 seconds. Then, the samples were heated at 65 °C for 15 min. Total RNAs were loaded individually into the RNA gel and run on an electrophoresis device for 2 hours. For staining, 5µL of ethidium bromide was added into 50 mL TE Buffer. The running RNA gel was immersed in this buffer for about 10 hours at slow speed on a magnetic stirrer. UV imaging device was used to visualize RNA gel results. Finally, RNAs obtained from 150 quails were stored at –80 °C until use.

cDNA Synthesis and PCR Amplification

cDNA contains only exons and is representative of the expressed genes of the cell. cDNA sequencing results are clearer because primers do not bind non-specifically. A commercial kit (Thermo Scientific #K1621) was used to synthesize cDNAs from total RNAs using the following protocol: 60 min at 42 °C, 5 min at 70 °C. Primers (forward, gcttgctcaggtagctcctg and reverse, tgcggcacgta tggcacgat) based on the recommendations of Dridi et al. (26) were used to PCR amplify a 348bp LEPR coding region (18th exon), from cDNA. PCR products (15 µL) were evaluated for a 348 bp length using 2% agarose gels (electrophoresed at 80 V/2 h) and stained with ethidium bromide. Separated fragments in the electrophoresis gel were cut with a sterile scalpel under UV light and transferred to individual 1.5 mL pre-numbered tubes. The PCR reactions were performed in 20 µL reaction volumes with 2 µL of genomic DNA (20 ng) as a template, 2 µL of buffer (NH2SO4), 0.4 µL of a dNTP mix (2.5 mmol/L), 0.5 mL of each primer (20 nmol/ mL), 1.25 µL of MgCl2 (25 mM) and 0.15 µL of EX Taq polymerase (Takara Bio Inc. Shiga, Japan). Amplifications were performed using a thermal cycler (Thermo Arktik) with the following conditions: 3 min for an initial denaturation at 94 °C, 35 cycles at 94 °C for 30 s for denaturation, 30 s for annealing at 60 or 62 °C, 45 s for extension at 72 °C, and a final extension for 5 min at 72 °C. β-actin gene primers (F:caaggagaagctgtgctacgtgc and R: ttaatcctgagtcaagcgcc) were used to determine that the PCR protocol worked (13).

Sequence Analysis and SNP Determination

cDNA samples were concentrated in the PCR and sequenced directly in a sequencing instrument (ABI-3730) after being purified from a gel and denatured at 94°C. LEPR gene fragments were sequenced for total of 150 individuals from the HBW, LBW and C lines. Firstly, in order to confirm the accuracy of the readings obtained as a result of sequence analysis, the nucleotides‘ peaks were examined using Chromas Pro software (version 2.1.3). Eventually, a total of 113 DNA sequences (HBW=33, LBW=44, and C=36) were used in this study. The BLAST online software (http://blast.

ncbi.nlm.nih.gov/Blast.cgi) was used to determine the location of 348 bp long fragment in the in the 18th exon of LEPR gene whose sequence was determined in this study. After this examining, the DNA sequences of the individuals belonging to the quail lines were aligned by using Mega6.0 software (version 6.06). According to SNP points seen in individuals, haplotype distributions of populations were determined in DnaSP software (version 5.10).

Statistical Analyses

SNP alleles were detected in each individual from each quail population and SNP haplotypes were coded numerically and phenotypic measurements (HW, BW, SMW, CW and FI) of these individuals were matched to these codes. Results of Kolmogorov-Smirnov test showed that the normality assumption was not fulfilled in data sets. Analysis of Means (ANOM) technique was used to compare quail genotypes, SNPs and SNP haplotypes in terms of measured phenotypic traits. Although, the ANOM is accepted as a graphical alternative to ANOVA, it has two advantages over ANOVA especially when researchers are interested in studying main effects. The advantages of the ANOM over the ANOVA are: a) if any of the group mean is statistically different from the others, it enables the researchers to see exactly which one is different easily and b) since the ANOM is a graphical technique, it presents the results visually that provides a quick way for researchers and readers to evaluate both practical and statistical significant differences between the treatment groups and the overall mean (21-23).

The results of the ANOM technique are based on confidence interval or decision lines (upper decision line (UDL) and lower decision line (LDL). The computation of the upper and lower decision lines is given as below (18,19). Computation of UDL and LDL for equal sample size:

$$UDL = \overline{Y}_{..} + h(\alpha, k, N-k)\sqrt{MSE}\sqrt{\frac{(k-1)}{N}}$$

$$LDL = \overline{Y}_{..} - h(\alpha, k, N-k)\sqrt{MSE}\sqrt{\frac{(k-1)}{N}}$$

Computation of UDL and LDL for unequal sample size:

$$UDL = \overline{Y}_{..} + h(\alpha, k, N-k)\sqrt{MSE}\sqrt{\frac{(N-n_i)}{Nn_i}}$$

$$LDL = \overline{Y}_{..} - h(\alpha,\ k,\ N-k)\sqrt{MSE}\sqrt{\frac{(N-n_i)}{Nn_i}}$$

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        10         20         30         40         50         60         70         80
GCTTGCTCAGGTAGCTCCTGGGAGCTGGGGAGCGAGGCATTCCTCCTGCTGCCTGACCAGCCTGACAGCCGGCCCTGCAG
GCTTGCTCAGGTAGCTCCCGGGAGCTGGGGAGCGAGGCATTCCTCCTGCTGCCTGACCAGCCTGACAGCCGGCCCTGCAG

        90        100        110        120        130        140        150        160
GACCCTTATATTTTCAGAGGGACTTTCAGAGCCTTCAGAGCAGGATGGTGCTTTCACAGCCGGAGGTCCGGAGCGAGGTC
GACCCTTATATTTTCAGAGGGACCTTCAGAGCCTTCAGAGCAGGATGGTGCTTTCACAGCTGGAGGTCCAGAGCGAGGTC

       170        180        190        200        210        220        230        240
TCTGTTACCTGGGGATGACATCATTGGGCAAAAGAGAAAATGGCATTTTTTTAACACAGAGCTCCAGACTGAGGTGCCAT
TCTGTTACCTGGGGATGACATCACTGGGCAAAAGAGAAAATGGCATTTTTTTAACACAGAGCTCCAGACTGAGGTGCCAT

       250        260        270        280        290        300        310        320
TTCCATACAGCTGATCTACTCAGAGGTGTGGGGTTTCTTCAGGATACACCTCCTAATTTAAATGCATTTATCCAGAGCAG
TTCCATACAGCTGATCTACTCAGAGGTGTGGGGTTTCTTCAGGATACACCTCCTAATTTAAATGCATTTATCCAGAGCAG

       330        340
CATTAAAGCCATCGTGCCATACATGCCG
CATTAAAGCCATCGTGCCATACATGCCG
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Figure 1: Sequenced 18th exon region of LEPR. Bold letters are SNPs, Underlined sequences are primer binding sites

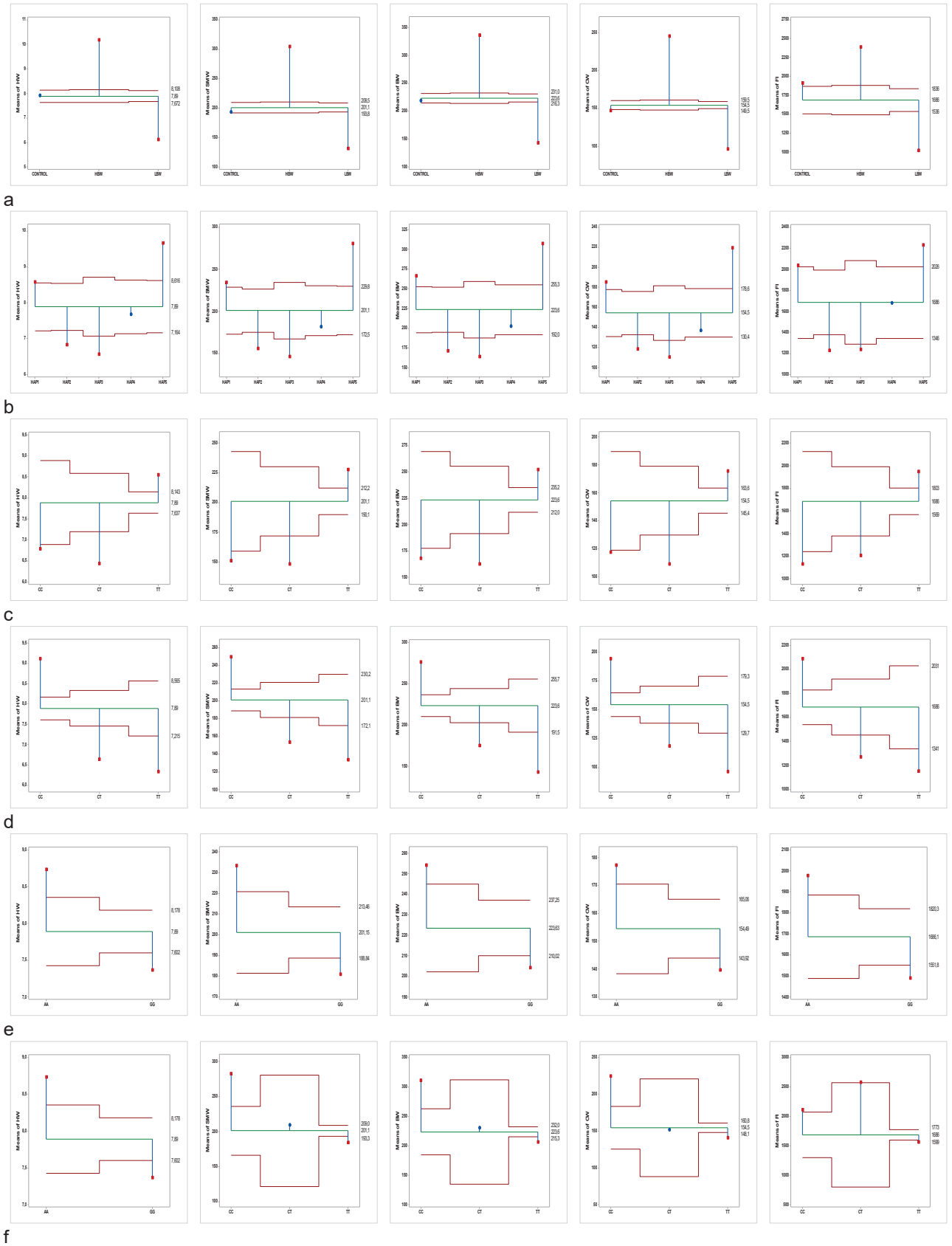

Figure 1: ANOM for comparing quail lines (a), haplotypes (b), SNP1 (c), SNP2 (d), SNP3 (e), and SNP4 (f) in terms of HW (Hatching Weight), SMW (Sexual Maturity Weight), BW (Body Weight), CW (Carcass Weight), and FI (Feed Intake), respectively

Where $\overline{Y}_{..}$, h, k, and MSE denote overall mean, critical table values for the ANOM technique based on α number of treatment group number and mean square error (21-23).

The decision is set as follows: if all means fall between the UDL and LDL, then the null hypothesis is accepted, and it is concluded that all means are equal. Any of the group mean, on the other hand, falls outside the decision lines, then the null hypothesis is rejected, and it is concluded that at least one group mean is significantly different from the overall or grand mean. In addition, Chi-square analysis was used on each of the haplotypes and SNPs to determine whether the ratio was due to genotype possession.

Results

A 348-bp fragment at the 18th exon of the *LEPR* gene was sequenced in 113 individuals from three quail lines. This fragment was BLAST searched against GenBank to confirm its identity as fragment of the *LEPR* gene. The 348 bp part of the sequence uploaded with KP674327.1 accession number that we identified exactly matched bases from 3163 to 3511 of the 3579 bp of whole *LEPR* sequence. Also, this region's position on the transcripts is between 811. codon and 926. codon in the entire gene (NCBI: XP_032302064.1). The sequenced fragments in this study were contained four SNPs loci (T490C, C528T, G537A, T571C) and five haplotypes (TTGT, CTGT, TCGT,TCAT, TCAC). When investigated the transcripts of the sequences, we found that the first SNP (T490C) caused with changing of phenylalanine to leucine (Phe-Leu) by occurring missense mutation. However, the other SNPs were synonyms and there were no changes on the transcripts. Loci of the four SNPs were marked in bold and locations of primers were underlined as shown Figure 1.

ANOM technique was used to investigate the effects of quail lines, SNPs and haplotypes on HW, SMW, BW, CW, and FI and the results have been presented in Figure 2a-f, respectively.

The results of the ANOM technique are based on confidence interval or decision lines (UDL: upper decision line and LDL: lower decision line). UDL and LDL are shown with red lines. Differences outside the UDL and LDL boundaries are indicated by the red dot.

When the results of ANOM for comparing quail lines in terms of HW, SMW, BW, CW, and FI were examined, it was clearly seen that the results were generally very similar for all traits except FI. As it can be seen from the Figure 2a, at least one mean falls outside the decision lines, that means there were statistical significance differences among the genotypes in terms of HW, SMW, BW, CW, and FI. The highest values for all traits have been observed for the HBW genotype while the least values observed for the LBW. The values of means from control population were generally located between decision lines. That means there were statistically significant differences among the quail lines in terms of studied phenotypic traits. Therefore, it was possible to conclude that there were statistically significant changes in the gene of the *LEPR* due to long-term selection. This suggests that long-term divergent selection for body weight in quail's results in different changes in the same locus of the *LEPR* gene. As seen in Figure 1b, all haplotypes except the fourth haplotype affected HW, BW, SMW, CW, and FI. The Hap1 and the Hap5 have positive affect while the Hap2 and the Hap3 have negative affect on interested traits in this study. The highest and the lowest HW values were obtained for the Hap5 and the Hap3, respectively.

For the effect of SNP1, obvious differences have been observed among the CC, CT, and TT genotypes. As it is seen from the Figure 2c, the HW, SMW, BW, CW and FI values for the TT were obviously higher than that of the CC and CT. Therefore, it can be concluded that the TT has a positive impact on the phenotypic traits while the CC and CT have negative. When the effect of SNP2 was examined, it was not difficult to observe that the CC genotype has positive impact, and the highest phenotypic values were obtained for the CC. The CT and TT genotypes, on the other hand, have negative affect and the lowest values were observed for the TT (Figure 2d). When Figure 1e is examined, it can be seen that the AA genotype has obviously positive impact while the GG genotype has negative affect on the phenotypic traits. The CC genotype at the SNP4 loci has positive affect on the HW, SMW, BW, CW and FI, while the TT has negative (Figure 2f). All means fall between lower and upper decision lines for the CT genotype that just has positive affect on the FI.

The distribution of the SNP genotypes and the haplotypes in quail lines are not the same

(Table 1, P<0.01). The allele numbers were found as almost same for the SNP1 locus in the LBW quails. However, this case was not valid for the control and HBW quail lines. It is possible to say that the selection results in favor of the TT at SNP1 locus in LBW because of there were no CC and CT genotypes in control line, and just 2 individuals in HBW (Table 1).

Table 1: Results of Pearson chi-square test of distributions of SNP genotypes in quail lines

SNPs LBW		Quail Lines C	HBW		Chi-square
T	CC	11	0	2	
103	CT	22	0	2	0.000*
C	TT	11	36	29	
C	CC	5	23	32	
141	CT	22	12	1	0.000*
T	TT	17	1	0	
G					
150	AA	5	23	16	0.000*
A	GG	39	13	17	
T	CC	0	4	14	
184	CT	0	4	0	0.000*
C	TT	44	28	19	

*p<0.01

Absence of the CC and CT and present of the TT in all individuals in LBW quail line suggests that negative selection in terms of low body weight effective in favor of the TT for SNP4. It was possible to claim that similar situation may be valid for the GG genotype at SNP3 locus. However, the numbers of TT at SNP1 and CC at SNP2 have increased by long term selection in terms of high body weight. The hap1 and hap5 were mostly observed in the HBW genotype while the hap 2 and hap 3 were mostly observed in the LBW. The most commonly observed haplotype for the control group was hap 4 (Table 2). As in the SNP alleles the haplotypes have also been formed in different quail lines resulting in long-term selection.

Table 2: Results of Pearson chi-square test of distributions of haplotypes in quail lines

	Quail Lines			
Haplotypes	LBW	C	HBW	Chi-square
Hap-1	5	7	13	
Hap-2	22	0	4	
Hap-3	12	6	0	0.000*
Hap-4	5	17	0	
Hap-5	0	6	16	

*p<0.01

Discussion

LEP and *LEPR* are excellent candidate genes for livestock production, as it is associated with features of economic importance. Indeed, in recent years, leptin gene polymorphism studies of several single nucleotide polymorphisms (SNPs) have been identified in cows and pigs, and several SNPs have been identified that are associated with important economic traits, such as milk yield, feed intake, adiposity, growth, and carcass quality (12-18, 27). The reasons for focusing on exon 18 in this study is that exon 18 polymorphisms are important on backfat thickness, feed yield and reproduction traits (28). When investigated the transcripts of the sequences, we found that the first SNP1 (T490C) caused with changing of phenylalanine to leucine (Phe-Leu) by occurring missense mutation. However, the other SNPs were synonyms and there were no changes on the transcripts in current study. El Moujahid et al. (29) found a significant association between 4 previously reported non-synonymous SNPs and the growth performance traits in meat-type chickens. In this study, the HW, SMW, BW, CW and FI values for the TT genotype were obviously higher than that of the CC and CT at SNP1 locus. When the effect of SNP2 was examined, it was not difficult to observe that the CC genotype has positive impact, and the highest phenotypic values were obtained for the CC. According to SNP3 the AA genotype has obviously positive impact while the GG genotype has negative affect on the phenotypic traits. The CC genotype at the SNP4 loci has positive relation on the HW, SMW, BW, CW and FI, while the TT has negative. Also, Hap1 (TTGT) and Hap5 (TCAC) have positive affect while the Hap2 (CTGT) and the Hap3 (TCGT) have negative affect on interested traits (HW, BW, SMW, CW, and FI).

De Vuyst et al. (30) found that both crossbred CT and TT beef cows wean significantly heavier beef calves than CC cows. There were several SNPs found in the porcine *LEP* and *LEPR* genes, suggesting that the SNPs lead to increased growth and fat (31). Buchanan et al. (32) revealed a SNP in the leptin gene of dairy cattle. This polymorphism, in which the first nucleotide is a thymine instead of cytosine in the 25th codon, changed arginine to a cysteine. However, homozygous animals carrying the T allele show no difference compared to animals carrying the C allele in terms of milk

fat and milk protein production and a daily milk yield of more than 1.5 kg. In addition, homozygous T allele-bearing animals developed higher fatty carcasses than those with the C allele (33). Also, El Moujahid et al. (29) reported relation of *LEPR* gene polymorphisms with growth and feed efficiency in meat-type chickens. Wang et al. (34) found a single nucleotide polymorphism (C/A) and three SNP genotypes in *LEPR* associated with fatness traits in chickens. Abbasi et al. (35) screened exons 9–11 of chicken *LEPR* but did not find any SNPs. Tarabany et al. (19) To the best of our knowledge, this is the first study to report the presence of two adjacent novel SNPs (A277G and A304G) in intron 8 of the *LEPR* gene of Japanese quail. GG/GG quails had significantly lower egg production and feed intake. Four SNPs loci (T490C, C528T, G537A, T571C) and five haplotypes (TTGT, CTGT, TCGT, TCAT, TCAC) were determined and the distributions of the SNP alleles and the haplotypes in quail lines were not the same in this study. This suggests that long-term selection for high body weight and low body weight in quail's results in different changes in the same locus of the *LEPR* gene.

Feed consumption, growth, development, energy metabolism and immune system functioning have a high economic importance in livestock. In this regard, there is a need for additional studies on genes that affect animal feed intake, the regulation of energy metabolism, yield and health. Leptin plays an important role in all of these mechanisms of economic importance in livestock. Therefore, studies of leptin will significantly contribute to animal nutrition, breeding and health. Yet, effects of the single nucleotide polymorphisms of leptin and *LEPR* genes in poultry animals are not adequately explained.

Therefore, it is possible to conclude that the effects of different genotypes, haplotypes, and SNPs on HW, SMW, BW, CW and FI are considerable. Since expectation of high correlation between the studied traits, that it is not a surprise to get these results of the long term bi-direction selection. These changes can be altered the function of the *LEPR*. However, to achieve a more accurate understanding of the role of leptin and its receptor, the DNA sequence of all of the SNP changes that benefit individuals and alter protein structure should be identified. However, conclusively demonstration of this effect requires the identification of all of the SNPs in the entire sequence of the *LEPR* in quails. Although it is very hard to interpret this data into livestock weight, surely these polymorphisms are worth a further investigation.

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

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UČINKI SELEKCIJE LINIJ JAPONSKIH PREPELIC NA GEN ZA LEPTINSKI RECEPTOR, POVEZAN S PRIREJO MESA

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Izvleček: Ta študija je bila izvedena z namenom raziskati učinke selekcije na polimorfizme posameznih nukleotidov (Angl., Single Nucleotide Polymorphism, SNP) v kodnem zaporedju gena za leptinski receptor (*LEPR*) in možne povezave med SNP in nekaterimi značilnostmi prireje mesa pri japonskih prepelicah. V študiji je bilo poleg kontrole uporabljenih petnajst generacij različno izbranih dveh linij (HBW in LBW) s telesno maso pri starosti petih tednov. 348-bp del kodne regije LEPR (18. ekson) je bil sekvenciran pri skupno 113 posameznikih iz treh linij prepelic, fragmenti pa so vsebovali štiri lokuse SNP (T490C, C528T, G537A, T571C) in pet haplotipov (TTGT, CTGT, TCGT, TCAT, TCAC). Zamenjava T490C je povzročila drugačnosmiselno mutacijo fenilalanina v levcin (Phe > Leu), vendar so bili drugi SNP-ji sinonimni in v transkriptih ni bilo sprememb. Ugotovljeno je bilo, da so prepelice z višjimi fenotipskimi vrednostmi imele genotip TT na lokusu T390C. Statistične analize so z vidika fenotipskih lastnosti pokazale značilne razlike med linijami prepelic, aleli SNP in haplotipi ($P < 0,05$), med linijami prepelic pa je bila različna tudi porazdelitev SNP in haplotipov ($P < 0,001$). Na podlagi vrednotenja vseh rezultatov smo ugotovili, da je selekcija petnajstih generacij linij japonskih prepelic (*C. coturnix japonica*) povzročila ključne spremembe v genu *LEPR*, povezanim z gospodarsko pomembnimi lastnostmi prepelic.

Ključne besede: SNP; haplotip; leptinski receptor; selekcija; japosnka prepelica