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Comparison of Some Carcass Components of Selected Japanese Quail Lines in terms of SNP Haplotypes[#]

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A B S T R A C T

In this study, we investigated the effect of SNP haplotypes on insulin-like growth factor gene (IGF-1) related with weights of body, back, chest, leg and wing in the fifteen generation selected Japanese quail (C. coturnix japonica) lines according to the 5th week live weight. 8 SNP haplotypes were identified in the 167-bp DNA sequence of the IGF-1 gene coding region identified in a total of 108 individuals from three quail lines (control, high body weight: HBW and low body weight: LBW). ANOM (Analysis of Means of Analysis) was performed to determine the relationships between carcass components with SNP haplotypes and to compare quail genotypes. There were significant differences between the quail lines in terms of SNP haplotypes. As a result, it was determined that fifteen-generation selection in Japanese quails resulted in differences in insulin-like growth factor-1 gene and these differences were reflected in carcass components.

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Seleksiyon Uygulanmış Japon Bıldırcını Hatlarının Bazı Karkas Bileşenlerinin SNP Haplotipler Bakımından Karşılaştırılması

M A K A L E B İ L G İ S İ

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Ö Z

Bu çalışmada, 5. hafta canlı ağırlığı göre on beş generasyon selekte edilmiş Japon bıldırcını (*C. coturnix japonica)* hatlarında insülin benzeri büyüme faktörü geni (IGF-l) üzerinde meydana gelen SNP haplotiplerin vücut, sırt, göğüs, bacak ve kanat ağırlıkları üzerine etkisi araştırıldı. Üç bıldırcın hattından (kontrol, yüksek canlı ağırlık: YCA ve düşük canlı ağırlık (DCA) toplam 108 bireyde belirlenen IGF-1 geni kodlama bölgesinin 167-bp'lik DNA dizisinde 8 SNP haplotip tanımlandı. Bu SNP haplotipler ile karkas bileşenleri arasındaki ilişkileri belirlemek ve bıldırcın genotiplerini karşılaştırmak için ANOM (Analyses of Means) analizi yapıldı. Bıldırcın hatları arasında SNP haplotipler bakımından önemli farklılıklar olduğu belirlendi. Sonuç olarak, Japon bıldırcınlarında on beş generasyon seleksiyonun insülin benzeri büyüme faktörü-1 geninde farklılıklara sebep olduğu ve bu farklılıkların karkas bileşenlerine yansıdığı tespit edildi.

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Introduction

Japanese quail is one of the economically important poultry species has been used as a model animal in scientific studies all over the world, and has been intensively selected for meat production due to advantages such as it is very short between generations, reaching to sexual maturity in a very short time, has high egg yield, short fattening period, being able to accommodate more animals in the unit area, more resistant to diseases, and need of simple tools in production. As a result of the breeding studies, commercial genotypes with high meat production have been developed. However, in these studies carried out with classical breeding methods, unwanted genes can be selected along with genes expressing desired properties.

Nowadays, there are quite rapid developments in the field of molecular genetics. These developments allow for the identification of genetic variation in various loci and to investigate the relationship between yield characteristics. With advances in DNA sequencing methods and technologies, molecular studies in recent years, including those in livestock species, have focused on single nucleotide polymorphisms (SNP). In parallel, determining the DNA sequences and SNPs of insülin-like growth factor (IGF) and its receptors in relation to livestock yields has accelerated research efforts.

Insulin is a approximately 7600 kDa polypeptide hormone containing 70 aminoacides with significant effects on growth and development (Baştürk, 2007). The major biological functions of IGF in birds include growth stimulation, protein synthesis, cell differentiation, and regulation of ovarian development (Ou et al., 2009). Lei et al., (2005) found 5 SNPs in selected chickens and reported that the resulting SNP haplotypes were associated with growth and carcass characteristics. Sato et al. (2012) reported that a SNP detected in the region of IGF-1 gene promoters the are significantly related to chest muscles and determined that the polymorphisms can be used in MAS (Marker Assisted Selection). All results show that the IGF-1 gene is a potential gene for breeding programs.

Statistical analysis is an important part of scientific studies and there are different statistical tests and approaches have been developed for analysing 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 non-parametric counterparts) Analysis of Mean Technique (ANOM) was used for analysing 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 (Nelson et al., 2005). 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 (Mendeş and Yiğit, 2013; 2018, Nelson et al., 2005).

The purpose of this study is to investigate the associations between some carcass components and SNP haplotypes in the coding sequences of IGF gene which possibility might be occurred as results of long-term selection of 15 generations in Japanese quail (*C. coturnix japonica*) lines using ANOM.

Materials and Methods

Animal

Three different Japanese quail (*C. coturnix japonica)* lines, which were namely Control (C) and treatment groups (HBW and LBW), were used as materials of this study. The Control group was not selected previously while the HBW and LBW lines were selected for 15 generations based on their 5. week body weight (low: LBW and high: HBW). This selection 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 Council of Turkey (Project ID: 114O047)

Raising of Material

To create the material of this project 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. An aluminum IDs were attached to the left wings of chicks after incubation. These chicks were fed 24% crude protein and 12.14 MJ metabolic energy during the first four weeks in a breeding cage. Sex determination was performed by observing the cloaca and breast feather colour 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 21% crude protein and 11.72 MJ for ten weeks. Lighting was applied continuously for the first four weeks and then 16 hours a day.

Measures of Carcass Components

A total of 50 quails (25 males and 25 females) were selected from each group (HBW, LBW and C group) on the same day at the end of the 15th week. Then were introduced to the cutting process was performed. The body weights (BW1) of the selected quails were measured before cutting. Low-voltage electrical current (100 mA, 50 Hz) was used to stun animals as recommended in the relevant scientific literature (Yalçin et al., 1995; Göksoy et al., 1999), and then the jugular vein was cut. After the blood flow was over, the feathers were cleaned and the internal organs were removed. So that the carcass component's weights (back weight: BW2, breast weight: BW3, wings weight: WW and legs weight: LW) were measured using a precision scale of 0.01 g.

Tissue Samples and Total RNA Isolation

Total RNA was isolated from liver tissue taken from each individual by using a commercial kit (Axygen). Isolated RNA samples were measured using the spectrophotometer to determine the total RNA concentration. Finally, RNAs obtained from 150 quails was stored at –80 °C until use.

cDNA Synthesis and PCR Amplification

A commercial kit (Thermo Scientific #K1621) was used to generate cDNA from total RNA using the manufacturer suggested protocol. Primers (forward, accggtctgagagcatccttg and reverse, gggaaaaagggtgtgcaaaag) were used to PCR amplify a 167 bp IGF-l coding region from cDNA. PCR products (15 µl) were evaluated for a 167 bp length using 2% agarose gels (electrophoresed at 90 V/2 h) and stained with ethidium bromide. Separated fragments in the gel were cut by using a scalpel under UV light and transferred to individual 1.5 ml pre-numbered tubes. The PCR reactions were performed in 20 µl volumes with 2 µl of genomic DNA (20 ng) as a template, 2 µl of buffer (NH_2SO_4), 0.4 µl of a dNTP mix (2.5 mmol/L), 0.5 ml of forward and reverse primers (20 nmol/ml), 1.25 µl of $MgCl_2$ (25 mM) and 0.15 µl of EX Taq polymerase (Takara Bio Inc. Shiga, Japan). PCR were performed using a thermal cycler (Thermo Arktik) with the following conditions: 3 min for an initial denaturation at 94°C, 30 cycles at 94°C for 30 s for denaturation, 30 s for annealing at 57°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 (Huang et al., 2011).

Sequence Analysis and SNP Haplotypes Determination

DNA 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. An IGF-l gene fragment of 167 bp was sequenced for total of 108 individuals from the HBW, LBW and C genotypes. First, 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 (v 2.1.3). Thus, DNA sequences 17 from the C group, 45 from the LBW group and 46 individuals from the HBW group were used in this study. To determine the location of the DNA fragment (167 bp) in the IGF-l gene that found in this study was used the online software of the BLAST (http://blast.ncbi.nlm.nih.gov/Blast.cgi). Then, the sequences of the individuals belonging to the population were aligned by using BioEdit software (v 7.0.5.3). According to SNP points seen in each individual, haplotype distributions of populations were determined in DnaSP software (v 5.10).

Statistical Analyses

SNP alleles were detected in each individual from each quail population and SNP haplotypes were coded numerically and phenotypic measurements (BW1, BW2, BW3, WW, LW) of these individuals were matched to these codes. Analysis of Means (ANOM) technique was used to compare quail groups, SNP haplotypes in terms of measured carcass components. 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 (Mendeş and Yiğit, 2013; 2018; Nelson et al., 2005).

Results

A 167-bp fragment of the insülin-like growth factor gene-1 was sequenced in 108 individuals from three quail lines. This fragment was BLAST searched against GenBank to confirm its identity as fragment of the IGF-1 gene. Fu et al. (2001) had uploaded the sequence of the entire IGF-l receptor mRNA (666 bp) of *Coturnix coturnix japonica* to GenBank under the accession number AF260702.1. The fragments we sequenced were contained three SNPs loci (G57A, C132T, G159A) and eight haplotypes (GTA, GCA, GTG, GCG, ATG, ACA, ATA, ACG) in a total of 108 individuals from three quail lines.

ANOM technique was used to investigate the effect of SNP haplotypes on body weight (BW1), back weight (BW2), breast weight (BW3), wings weight (WW), legs weight (LW) of Japanese Quails, and the results have been presented in Figure 1, respectively.

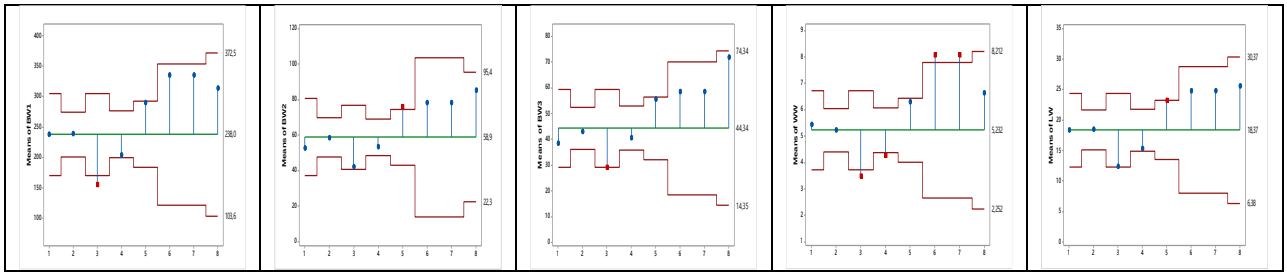

Figure 1 ANOM for SNP haplotypes in terms of BW1, BW2, BW3, WW, and LW respectively.
The results of analysis are based on confidence interval or decision lines (UDL: upper decision line and LDL: lower decision line). UDL and LDL are shown with red colour. Differences outside the UDL and LDL boundaries are indicated by the red point.

When the results of ANOM for comparing quail lines in terms of BW1, BW2, BW3, WW and LW were examined the results were generally very similar for all traits. As it can be seen from the figure 1, at least one mean falls outside the decision lines, that means there are statistical significance differences among the haplotypes in terms of BW1, BW2, BW3, WW and LW. All haplotypes except the Hap1 and Hap2 affected in studied traits. The highest values for all traits have been observed for the Hap6 and Hap7 while the least values observed for the Hap3. The values of means from Hap1 and Hap2 were generally located between decision lines. The Hap5, Hap6 and the Hap7 have positive affect while the Hap3 and the Hap4 have negative affect on interested traits in this study. That means there were statistically significant differences among the quail lines in terms of studied carcass traits. Therefore, it is possible to result that there were statistically significant changes in the gene of the IGF-l receptor due to long-term selection.

Discussion

Tang et al. (2010) reported that an SNP on the IGF-1 gene in chickens was significantly associated with body weight, shin length and diameter, and that detected polymorphisms could be used in MAS. Abbasi and Kazami (2011) reported that the IGF-1 gene polymorphism was associated with body weight, bone and muscle cells. Sato et al. (2012), IGF-1 gene promoters in the region of a SNP that they detected in the chest muscles are significantly related to the stated that the polymorphisms can be used in MAS. All results show that the IGF-1 gene is a potential gene for breeding programs.

In the present study, the fragments we sequenced were contained three SNPs loci (G57A, C132T, G159A) and eight haplotypes (GTA, GCA, GTG, GCG, ATG, ACA, ATA, ACG) in a total of 108 individuals from three quail lines. When the results of ANOM for comparing quail lines in terms of BW1, BW2, BW3, WW and LW were examined, it was clearly seen that the results were generally very similar for all traits.

Conclusion

In this study, statistical analyses showed that all studied phenotypic features related with some carcass components were affected by SNP haplotypes of the IGF-l gene resulting in long-term selection. Therefore, it is possible result that the effects of different haplotypes on BW1, BW2, BW3, WW, and LW are considerable. Because 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 IGF-l. 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 SNP haplotypes in the entire sequence of the IGF-l in japanese 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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