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Determining Gene Expression of MyoD, IGF-I and STAT5B Genes in the Japanese Quail Leg Muscles Which Reared Under Two Different Lighting Systems

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ABSTRACT

Light is a very important factor in growth and reproduction. This study aims to determine the effect of two different lighting systems on the expression of MyoD, IGF-I and STAT5B genes in the leg muscles of the Japanese quail. For this aim, two lighting systems were used; 16 hours' light: 8 hours' darkness (16 h L: 8 h D) group I and 8 hours' light: 16 hours' darkness (8 h L: 16 h D) group II. Quantitative real-time PCR was used for assessment of the gene expression levels in leg muscles. When the Real Time PCR results were evaluated, it was determined that MyoD gene expression was higher in group I than in group II. On the other hand, when IGF-I and STAT5B gene expressions were evaluated, the opposite situation was found, namely higher expression in group II compared to group I.

İki Farklı Aydınlatma Sistemi Altında Yetiştirilen Japon Bıldırcınların But Kaslarındaki MyoD, IGF-I ve STAT5B Genlerin İfadesinin Belirlenmesi

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ÖZ

Işık büyüme ve üremede oldukça önemli bir faktördür. Bu çalışma, Japon bıldırcınlarının but kaslarında MyoD, IGF-I ve STAT5B genlerinin ekspresyonu üzerine iki farklı aydınlatma sisteminin etkisini belirlemeyi amaçlamaktadır. Bu amaçla iki aydınlatma sistemi kullanılmıştır; 16 saat aydınlık: 8 saat karanlık (16 saat U: 8 saat G) grup I ve 8 saat aydınlık: 16 saat karanlık (8 saat U: 16 saat G) grup II. But kaslarındaki gen ekspresyon seviyelerinin değerlendirilmesi için kantitatif real-time PCR kullanılmıştır. Real Time PCR sonuçları değerlendirildiğinde grup I'de grup II'ye göre MyoD gen ekspresyonunun daha yüksek olduğu belirlenmiştir. Öte yandan IGF-I ve STAT5B gen ekspresyonları değerlendirildiğinde ise tam tersi durum yani grup II'de grup I'e göre daha yüksek ekspresyon saptanmıştır.

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1. Introduction

Light is a principal environmental factor in poultry production. It is an essential administrative measure, which is illustrated by its direct impact on growth and production traits (Mohammed et al., 2010; Wang et al., 2015; Retes et al., 2017). All lighting systems aim to reach the best production standards for poultry (Lien et al., 2008; Min et al., 2012). The growth trait is a significant factor in the

assessment of poultry, especially in meat production (Van Kaam et al., 1999; Al-Zghoul et al., 2016; Park et al., 2018). The quails gained faster weight under the continuous lighting system than during the 13 hours of daylight (De Jager, 2003). The reducing number of lighting hours in the early part of the birds' life led to a decrease in the rate of growth speed (Olanrewaju et al., 2006). The growth rate of birds reared under the intermittent lighting system was significantly higher compared to the birds reared under the continuous lighting system (Shariatmadari et al., 2007). The light directly affects the activity of myogenic regulatory factors (MRFs) (Sławińska et al., 2013; El Naby et al., 2018). The MRFs are basic helix-loop-helix transcription factors and consist of four genes; Myf5, myogenin, MyoD and MRF4 (Perry et al., 2000; Bhuiyan et al., 2009; Mok et al., 2015; Hernández-Hernández et al., 2017). The MRFs family stimulates embryonic muscle development because they affect the activity of genes that directly participate in muscle specification, differentiation, formation, and growth (Braun et al., 2011; Wood et al., 2013; Collins et al., 2019). MyoD is the first gene among the MRFs family that was identified, and it has a fundamental role in the development and growth of skeletal muscle fibers (Davis et al., 1987; Kim et al., 2017; Lassar et al., 1986; Weintraub et al., 1991; Yang et al., 2015; Zhang et al., 2018). The MyoD gene is expressed in tissues of the skeletal muscles and myoblast and is not expressed in smooth muscles and non-muscle tissues (Olson, 1990). Insulin-like growth factor 1 (IGF-1), also called somatomedin C, is similar to insulin in molecular structure. It is considered a candidate gene for growth, development, and metabolism in animals (Keating, 2008). The poultry mRNA for the IGF1 gene is expressed in the muscles, heart, liver, and brain (Kadlec et al., 2011). Signal transducer and activator of transcription 5b (STAT5b) belong to the STAT family of transcription factors. STAT5b is an important candidate gene for growth, metabolism, lactation, and reproduction in animals (Zhao et al., 2012). This study aims to determine mRNA levels of the MyoD, IGF-I, and STAT5b genes in quail leg muscles reared under two different lighting systems.

2. Materials and Methods

The birds were obtained from a breeding station at livestock development, Faculty of Agriculture, Selçuk University, Turkey. 7 weeks old female breeding of the Japanese quail layer was used in this study. The study consisted of two groups of the Japanese quail layers, where each group contained five birds. Animals were slaughtered at 13 weeks of age. Two different lighting systems were used. The group I applied for a conventional, static, and intermittent lighting program with 16 hours light: 8 hours darkness (16 h L: 8 h D) (mechanically open-close basis). During the lighting phase, the intensity of the coop light was standardized as 20 lux. In group II, which was planned as an innovative application, the lighting duration is planned as (8 h L: 16 h D). The lighting apparatuses' opening and closing are not mechanically open-close basis but organized according to the principle of automatic dynamic lighting with environmental interaction. A dynamic lighting program that varies according to time has been implemented without a constant light intensity. The study lasted six weeks and the diet containing 2850 Kcal/kg ME and 22% HP was used.

2.1. Collection of tissue samples

Tissue samples were collected in the sixth week. The samples were placed directly in the tubes and kept at -80°C.

2.2. İsolation of RNA and synthesis cDNA

After the applications, RNA isolation from the leg muscles tissues of ten individuals, each group was created with at least five individuals. RNA isolation was performed via Axygen RNA isolation kit, and the manufacturer's directions were carried out. After the RNA extractions, the quality and concentrations of samples were estimated via Nanodrop 2000 and validated by agarose gel electrophoresis. Purified RNA was then converted to cDNA using the Biorad cDNA synthesis kit.

2.3. Expression analysis

The mRNA expression levels of studied gene regions and housekeeping gene regions were measured via RT-PCR. The RT-PCR assays were performed in Biorad CFX Connect. The RT-PCR was done to detect expression profiles for MyoD, STAT5b, IGF-I, and GAPDH gene regions by using iTaq™ Universal SYBR® Green. The gene expression experiments were performed with RT-PCR in a total volume of 10 µl mix, which contains 5 µl SYBR green master mix (Bio-Rad), 1 µl primer (Table 1), 3 µl dH2O, 1 µl cDNA. The GAPDH gene was used as a housekeeping gene. Although the GAPDH gene was primarily selected based on previous reports (Vitorino Carvalho et al., 2019), the study was continued with GAPDH because we also obtained low ct values. A control sample, obtained via performing qRT-PCR with no template, was also assayed to confirm that the samples were not contaminated. The primer sequences are given in Table 1. Primer efficiency rates were calculated, and the primers' efficiency was above 85 %. The obtained data were analyzed by the comparative CT method, and $2^{-\Delta\Delta CT}$ calculated the fold change. Experiments were made at least in triplicate. Melting curve analyses were performed, and the presence of a single peak confirmed the specificity of the amplicons. BM SPSS Statistics software was used in statistical analyses. A t-test made comparisons between groups—the significance level was considered at ($p<0.05$).

Table 1. Primer sequences and RT-PCR conditions

Gene	Primer sequencing	Annealing °C	PCR conditions	Reference
MyoD	F: GATTTCCACAGACAACTCCACAT R: GAATCTGGGCTCCACTGTCACT	60	95°C 10m, 95°C 15s, 60°C 45s, Annealing temperature 45s, 40 cycles 72°C 10m	El Naby and Basha (2018)
IGF-I	F: CACCTAAATCTGCACGCT R:CTTGTGGATGGCATGATCT	60		Gasparino et al. (2013)
STAT5b	F: CTGCTGTGTGATGGAGTACC R: GACACTGAACTGCGACTCAA	60		El Naby and Basha (2018)

GAPDH	F: TCTCTGTTGTTGACCTGACCTG R: ATGGCTGTCACCATTGAAGTC	60	Vitorino Carvalho et al., (2019)

3. Results

The MyoD, IGF-I and STAT5B mRNA expression levels for leg muscles were given in (Figure 1). The mRNA expression levels of MyoD gene in leg muscles showed an increase in group I and decreased in group II who exposed to automatic dynamic lighting. Whereas, IGF-I and STAT5b mRNA expression levels were higher in group II.

4. Discussion

This investigation determined mRNA expression levels of IGF-I, MyoD, and STAT5B genes in Japanese quail leg muscles reared under two different lighting systems. MyoD is considered an essential factor in determining myoblast formation. It was found that the mRNA levels were high in group I, which was reared under a lighting system (16 h L: 8 h D). In contrast, the levels of mRNA expression of the MyoD gene decreased in group II. Studies have confirmed that the mRNA expression of the MyoD gene is high in embryonic stages and low in the major muscles. El Naby and Basha (2018) identified that mRNA expression levels of the MyoD gene were decreased in the group exposed to continuous artificial light (23 h L: 1 h D) in the major muscle of the Japanese quail. Fergany et al. (2017) revealed that mRNA expression levels of the MyoD gene were higher at embryonic day (ED) E7 and reached their peak at E16. Choi et al. (2014) noted that MyoD mRNA expression levels in quail birds were lower in the major muscles. Ban et al. (2013) confirmed decreased mRNA levels of quail and chicken breast muscles. Al-Musawi et al. (2011) indicated that mRNA levels at the MyoD gene decreased in pectoral muscles and gastrocnemius muscles from embryonic day (ED) 13 to 14 and ED14 to 15 in layers of chicken.

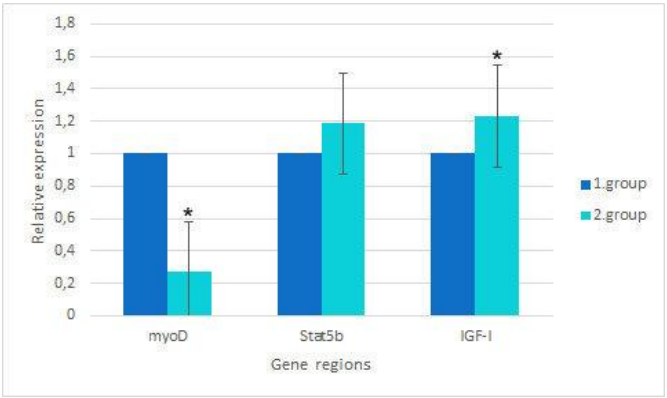

Figure 1. The levels of mRNA expression in MyoD, IGF-I and STAT5B genes in both group (* $p<0.05$, n=3)

IGF-I is classified as a critical regulatory system that plays a vital role in cell differentiation and proliferation in mammalian and poultry tissues like liver, muscle, bone, reproductive organs, and the

central nervous system (Collins et al., 2019). In this study, mRNA levels of IGF-I were found higher in group II than in group I. Fu et al. (2001) noted decreased mRNA levels in the IGF-I gene of quail testis, lung, liver, and heart. Gasparino et al. (2013) showed that mRNA levels for IGF-I were higher in the muscle of laying Japanese quail at different air temperatures. Silva et al. (2013) showed a high expression in quail breast muscles when birds fed 8 and 12% glycerol. Gasparino et al. (2014) found that high feed efficiency female quail under heat stress showed higher expression in the muscle and liver. Bhattacharya et al. (2015) Reported that chicken mRNA expression levels were high in breast muscle. STAT5B plays a vital role in growth, metabolism, reproduction and lactation. The study results showed a high expression of the STAT5B gene in group II and a low expression level in group I. El Naby and Basha (2018) determined that mRNA levels of the quail STAT5b gene increased in an artificial light group. Flisikowski et al. (2004) confirmed that the AA genotype has higher mRNA expression levels in cattle livers.

5. Conclusions

Understanding how the mechanism of genes works is crucial in terms of the economy. This study revealed a high expression of genes associated with growth and development in leg muscles of the Japanese quail. It can be said that a new generation of lighting systems interact with the environment, which can be an alternative to classical lighting applications in Japanese quails, where the automatic dynamic lighting systems with environmental interaction demonstrated an effect on the expression of genes. Considering animal welfare, it is seen that this system is much more compatible with natural life.

Statement of Conflict of Interest

Authors have declared no conflict of interest.

Author's Contributions

The contribution of the authors is equal.

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