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IGF-I, GHR and UCP mRNA expression in the liver and muscle of high- and low-feed-efficiency laying Japanese quail at different environmental temperatures

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ABSTRACT

In this study, we analyzed insulin-like growth factor I (IGF-I), growth hormone receptor (GHR) and uncoupling protein (UCP) mRNA expression in the muscle and liver of high- (0.23 g/g) and low- (0.17 g/g) feed-efficiency (FE) Japanese quail at three different air temperatures: comfortable (25 °C), heat stress (38 °C) for 12 h or cold stress (10 °C) for 12 h. Total RNA was extracted from the liver and breast muscle of each quail, and cDNA was amplified using specific primers for the target genes. Expression was analyzed using quantitative real-time PCR (qRT-PCR). IGF-I mRNA expression was higher in the livers of high-FE quail than in the livers of low-FE quail under both heat and cold stress conditions. High-FE birds also showed higher GHR mRNA expression independent of temperature. UCP mRNA expression in the liver was lower in high-FE birds and higher under heat stress compared with the other conditions. IGF-I mRNA expression was higher in the muscle of high-FE quail under the three conditions tested, and UCP mRNA expression was higher under cold stress. Our results suggest that air temperature affects the expression of genes related to growth and mitochondrial energy production, and quail with different feed efficiencies respond differently to environmental stimuli.

1. Introduction

Characteristics governing animal production, such as feed and reproductive efficiency, are expressed as a function of the animal's genetics, the environment to which the animal is exposed and the interaction between these two factors.

Birds are endothermic animals and thus require comfortable temperatures in order to channel all the energy they produce toward animal production (Macari et al., 2004). Changes in air temperature, above or below the comfortable range, can negatively affect animal performance. Animals exposed to low temperatures undergo cardiovascular system changes in order to meet their increased energy requirements (Blahová et al., 2007). The hormone T3 appears to be involved in regulating the growth rate at low air temperatures. The circulating level of T3 has been negatively correlated with temperature and positively correlated with feed ingestion in chicken (Yahav, 2000).

Low temperatures can also affect the bird performance via uncoupling protein (UCP). UCP is a protein located in the internal membrane of mitochondria that is responsible

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for redirecting energy from ATP production to heat production (Vidal-Puig, 2000). Because it causes uncoupling during energy production, studies have shown that chickens with high UCP mRNA expression also have lower feed efficiency (Ojano-Dirain et al., 2007). Low temperatures have been linked to higher UCP mRNA expression in several tissues and species (Dridi et al., 2008; Toyomizu et al., 2002). This higher level of expression has been attributed to the animal's attempts to maintain adequate body temperature.

Similar to low temperatures, heat stress also causes metabolic changes. In broilers, higher temperatures are correlated with decreases in feed intake, nutrient utilization efficiency, weight gain, egg production and feed efficiency (Akşit et al., 2006; Menten et al., 2006). Such decreased performance is mainly caused by reductions in T3 and T4 levels, changes in water and ionic balance, changes in the kinetics of important enzymes that control the concentrations of anabolic and catabolic products, depression of immune system function and changes in growth hormone concentration (Barbour et al., 2010).

In addition to air temperature (Gabillard et al., 2003, 2006), the expression of hormones, such as insulin-like growth factor I (IGF-I) and growth hormone receptor (GHR), can also be affected by many factors, including diet (Gasparino et al., 2012; Katsumata et al., 2002), tissue type and developmental stage (Berishvili et al., 2006). IGF-I mRNA expression also differs between chickens selected for high or low growth rates. Higher IGF-I mRNA expression was found in animals with high growth rates (Beccavin et al., 2001).

Confirming the hypothesis that IGF-I has a positive effect on bird growth, studies have shown increased protein deposition in the presence of higher levels of circulating IGF-I (Carew et al., 2003; Stubbs et al., 2002), possibly due to the effect of this hormone on the metabolic cycles responsible for protein synthesis (Tesseraud et al., 2007) and degradation (Sacheck et al., 2004).

Therefore, based on the hypothesis that air temperature can affect hormones and proteins important for bird performance, and that animals with different feed efficiencies respond differently to environmental stimuli, this study sought to analyze IGF-I, GHR and UCP expression in the muscle and liver of high- and low-feed-efficiency (FE) laying quail kept in three environments: comfortable, heat stress (38 °C) for 12 h and cold stress (10 °C) for 12 h.

2. Materials and methods

The experiment was conducted at the Iguatemi Experimental Farm at the State University of Maringá. First, 400 laying quails (*Coturnix coturnix japonica*), born from the same incubation, were conventionally raised for 28 days under the same experimental conditions. At this time, the birds were transferred to individual cages and underwent an adaptive period for 7 days. Feed efficiency was calculated as the increase in body weight relative to feed intake from 35 to 42 days of age. Feed consumption and weight gain during the test period were measured individually. During this time, the birds were kept at a comfortable temperature (25 ± 0.9 °C with $60 \pm 1.2\%$ relative humidity (RH)). The animals had free access to food and water throughout the experiment. The feed was formulated for the two phases of the birds' lives according to Rostagno et al. (2011). Starter feed was provided during the first 14 days of life, and growth feed was provided from 15 days of age. At 42 days of age, the animals were separated into two groups: the 36 birds with the highest FE (high-FE) and the 36 birds with the lowest FE values (low-FE) (Table 1). These groups were then divided into three environmental conditions: comfortable (25 °C, according to Pinto et al., 2003), heat stress (38 °C) for 12 h and cold stress (10 °C) for 12 h, with 12 animals in each group.

After the stress period, the animals were euthanized by cervical dislocation, and tissue from the breast muscle (*pectoralis superficialis*) and liver were collected and stored in RNA Holder® (BioAgency Biotecnologia, Brasil) at −20 °C until RNA extraction. Animals in comfortable conditions were sacrificed immediately after the groups were separated. Only 12 (6 high-FE and 6 low-FE) of the 24 animals submitted to each experimental condition were used for gene-expression analysis.

Total RNA was extracted using Trizol® (Invitrogen, Carlsbad CA, USA) according to the manufacturer's instructions (1 mL per 100 mg of tissue). All of the materials used had been previously treated with the RNase inhibitor RNase AWAY® (Invitrogen, Carlsbad, CA, USA). The tissue and Trizol mixture were triturated with a Polytron electric homogenizer until completely dissociated. Next, 200 μL of chloroform was added to the sample, and the mixture was manually homogenized for 1 min. The samples were then centrifuged for 15 min at 12,000 rpm and 4 °C. The aqueous phase was collected and transferred to a clean tube

Table 1
qRT-PCR primers.

Gene	Amplicon (bp)	Annealing temperature (°C)	Primer sequence (5′–3′)
GHR	145	60 °C	AACACAGATACCCAACAGCC AGAAGTCAGTGTTTGTCAGGG
IGF-I	140	60 °C	CACCTAAATCTGCACGCT CTTGTGGATGGCATGATCT
UCP	41	60 °C	GCAGCGGCAGATGAGCTT AGAGCTGCTTCACAGAGTCGTAGA
β-actin	136	60 °C	ACCCCAAAGCCAACAGA CCAGAGTCCATCACAATACC