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RESEARCH ARTICLE

Selection for High Body Weight and Its Association with the Expression Profiles of Somatotropic Axis and Mitochondrial Genes in Japanese Quail

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A B S T R A C T

Selection is the basic tool to exploit and improve the productive potential of livestock. The objectives of the present study were to evaluate the effect of selecting Japanese quail at 4-week for high body weight for four successive generations on the growth traits including body weight (BW) and average daily gain (ADG) and on the expression level of insulin-like growth factor I (IGF-I), growth hormone receptor (GHR), adenine nucleotide translocase (ANT), uncoupling protein (UCP) and Cytochrome C Oxidase subunit III (COX III) genes. Growth traits were evaluated for the base, 1st, 2nd, 3rd, and 4th generations**.** Total RNA was extracted from the liver then cDNA was synthesized. Real-time polymerase chain reactions were performed using the SYBR Green PCR Master Mix. The 4th generation of this study recorded the highest and significant values for body weight at zero (hatching), 2nd, 4th and 6th week of age (BW0 = 9.75, BW2 = 75.16, BW4 = 163.46 and BW6 = 253.13 gm, respectively), and for average daily gain (ADG0-2 = 4.67, ADG2-4 = 6.36 and ADG0-6 = 5.79 gm, respectively). Moreover, the 4th generation recorded the highest and significant mRNA expression of GHR and IGF-1 genes in the liver (1.80 and 1.66 fold change, respectively). In contrast, the base generation showed the highest and significant levels of hepatic mRNA expression of ANT, UCP, COX III genes (1.08, 1.24 and 1.05 fold change, respectively). We may conclude that these genes are promising candidate biomarkers for improving growth traits, leading to increased quail marketable meat.

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INTRODUCTION

Japanese quail is an important food source and is considered the fruit fly of avian species because they are characterized by short generation intervals and quick genetic progress. and excellent reproductive performance (Kar *et al.*, 2017). Their high protein and low-fat content, as well as their high amount of minerals and vitamins (Santhi and Kalaikannan, 2017), make 'quail's meat increasingly marketable and highly demanded. From a research perspective, quails are convenient and interesting models to understand the fundamental and molecular bases of growth performance that may be useful to other poultry and livestock species (Vitorino Carvalho *et al.*, 2020).

Growth performance is an essential trait for evaluating different livestock species, especially in meat-producing animals and birds. Because feed represents about 50 to 70% of the total cost, growth performance is considered an essential and vital trait for successful poultry production (Baracho *et al.*, 2019). Genetic selection for a high growth rate has been widely applied in poultry production without knowing the underlying molecular mechanisms. Although it has achieved remarkable progress in productivity and body weight, some of the undesirable changes, such as metabolic muscle disorders, appeared (Bailey *et al.*, 2020; Lake *et al.*, 2021). Thus, a deep molecular understanding of the vital breeding traits as growth performance may help avoiding unfavorable consequences. The growth

performance is affected by several factors, including the balance between protein synthesis and degradation, which is regulated by the expression of genes from the somatotropic axis such as insulin-like growth factor I (IGF-I) and growth hormone receptor (GHR) and the efficiency in energy production by mitochondria which is related to the expression of mitochondrial genes involved in energy production such as adenine nucleotide translocase (ANT), uncoupling protein (UCP) and cytochrome c oxidase subunit III (COX III) (Jia *et al.*, 2018; Hu *et al.*, 2019; Lassiter, 2019). The endocrine mechanisms that control avian growth are not well understood, as in the case of mammals. Gene expression level is a vital biological tool commonly used to characterize the response of body cells to biological and external factors (Ban *et al.*, 2013; Fu *et al.*, 2013; Khan *et al.*, 2016; Wang *et al.*, 2017). The GHR and IGF-I in birds need further studies, especially in their molecular basis, the translational and the transcriptional levels (Gasparino *et al.*, 2014; Xiao *et al.*, 2017). Although the pathways of the somatotropic axis in addition to energy metabolism and mitochondrial biogenesis are considered the significant controllers for growth rate and body size invertebrates, the effect of selection on the expression of the somatotropic axis (GHR and IGF-1) and mitochondrial (ANT, UCP and COX III) genes and their association with body weight and growth performance in farm animals is not fully understood (Jia *et al.*, 2018; Loongyai *et al.*, 2019). Studies have shown that variation exists among these genes, which could be useful for evaluating their effects on poultry growth and development (Hosnedlova *et al.*, 2020).

The objectives of this study were to evaluate the effect of selecting Japanese quail at 4-week for high body weight for four successive generations on a) body weight and gain traits and b) the mRNA expression of genes that are involved in the somatotropic axis (IGF-I and GHR) and mitochondrial energy metabolism (ANT, UCP, and COX III); this could be useful for a better understanding of the energy regulation and the molecular basis of quails with a high growth rate, with the long-term goal of maximizing growth performance and avoiding the unfavorable consequences in quail's production.

MATERIALS AND METHODS

All procedures performed in this study involving animals were under the ethical standards of the committee of Animal Care and Welfare, Benha University, Egypt, with an approval number: BUFVTM 01-09-2019.

Experimental period and breeding plan: This study was carried out at the Poultry Research Farm, Department of Animal Production, Faculty of Agriculture, Benha University, Egypt. It was started in February 2017 and ended in April 2018. The total number of Japanese quail in each generation was shown in Table 1. The quails were selected for increasing body weight at the 4th week of age for four successive generations.

The birds were labeled by wing bands and sire families were housed in wire cages (25 × 25 × 25 cm) with males to females sex ratio ranging from 1:1.46 to 1:1.77. The birds received standard requirements of lighting, ventilation, as well as vaccination programs. The birds were fed *ad libitum* on a diet containing 24% crude protein and 2975.8 Kcal ME/kg of feed, while during the laying period, a laying diet containing 20% crude protein, 2975.8 Kcal ME/kg, and calcium content of 3.5% were used (NRC., 1994). Eggs were collected daily after complete sexual maturity, tagged according to their sire families, then stored at 18°C for a week. Pedigreed eggs were set in the setting trays according to their sire families in a forced draft incubator at 37.5°C and 60-70% relative humidity (RH). Eggs were turned automatically every three hours. On the 14th day of incubation, eggs were transferred in pedigree baskets to the hatchers, where the temperature and RH were kept at 37.5°C and70%, respectively.

Studied traits: Growth traits were evaluated for the base, 1st, 2nd, 3rd, and 4th generations**.** Body weights were determined twice a week from one-day-old chicks until the 6th week of age. Average daily gain (ADG) was calculated by measuring the weight gain relative to the number of days as shown in the following equation: Average daily gain (ADG) = (W2-W1)/days. Where: W_1 = body weight at the beginning of the period, W_2 = body

Table 1: Number of sires, dams and pullets used in the selection experiment in different generations of Japanese quail.

Generation	Number of sires	Number of dams	Number of Pullets	Number of hatched birds
Base generation (G0)	158	231	342	988
1st generation of selection (G1)	145	246	344	1081
2nd generation of selection (G2)	134	238	357	984
3rd generation of selection (G3)	137	225	353	987
4rd generation of selection (G4)	134	223	348	1002
Total	708	1163	1744	5042

Table 2: Primers of candidate genes used in gene expression analysis

Target Genes		Primer sequence (5′-3′)	Annealing temperature (°C)	Amplicon (bp)
THE	Forward	AACACAGATACCCAACAGCC	60°C	145
	Reverse	AGAAGTCAGTGTTTGTCAGGG		
IGF-I	Forward	CACCTAAATCTGCACGCT	60°C	140
	Reverse	CTTGTGGATGGCATGATCT		
ANT	Forward	TGTGGCTGGTGTGGTTTCCTA	60°C	67
	Reverse	GCGTCCTGACTGCATCATCA		
UCP	Forward	GCAGCGGCAGATGAGCTT	60°C	41
	Reverse	AGAGCTGCTTCACAGAGTCGTAGA		
COX III	Forward	AGGATTCTATTTCACAGCCCTACAAG	60°C	71
	Reverse	AGACGCTGTCAGCGATTGAGA		
β-actin	Forward	ACCCCAAAGCCAACAGA	60°C	136
	Reverse	CCAGAGTCCATCACAATACC		

weight at the end of the period**,** Days = number of days between W_1 and W_2 (weighing biweekly so days =14 days).

Gene's expression was evaluated only for the base, 2nd, and 4th generations to determine the trend of gene expression in response to the selection effect and decrease the cost. This step was carried out at the Central Laboratory, Faculty of Veterinary Medicine, Benha University, Egypt. Liver specimens were collected from eight females at the 4th week of age from the base, second and fourth generations and kept at -80ºC till gene expression analyses. According to the manufacturer's instructions, total RNA was extracted using Trizol® (Invitrogen, Carlsbad, CA, USA). RNA quantity and quality were determined by using spectrostar Nanodrop. Single-stranded cDNA was synthesized from 1000 ng of total RNA according to the manufacturer's High Capacity cDNA Reverse Transcription Kits (Applied Biosystems) protocol. Cycling conditions were as follows: 25°C for 10 min, 37°C for 120 min, and 85°C for 5 min. Then total RNA and cDNA samples were stored at -80°C till the next step.

Real-time polymerase chain reaction assays were performed using the fluorescent dye SYBR Green (SYBR® Green PCR Master Mix, Applied Biosystems, USA). All of the reactions were analyzed under the same conditions and normalized to the ROX Reference Dye (Invitrogen, Carlsbad, CA, USA) to correct for fluctuations in the readings due to evaporation during the reaction. The primers used in the GHR, IGF-I, ANT, UCP, and COX III amplification reactions were designed based on the gene sequences deposited at GenBank (http://www.ncbi.nlm.nih.gov), and the β actin was used as endogenous housekeeping gene as shown in Table 2. A real-time polymerase chain reaction for each gene was carried out for each analyzed sample. Each PCR reaction consisted of 1.5 µl of 1µg/µl cDNA, ten µl SYBR Green PCR Master Mix (Quanti Tect SYBR Green PCR Kit, Qiagen), one µM of each forward and reverse primer for each gene and nuclease-free water to a final volume of 20 µl. The reactions were then analyzed on an Applied Biosystem 7500 Fast Real-time PCR detection system under the following conditions: 95°C for 10 minutes (first denaturation and 40 cycles of 95°C for 15 seconds (second denaturation stage) followed by 60°C for 1 min (annealing and extension stage). Changes in gene expression were calculated from the obtained cycle threshold (Ct) values provided by real-time PCR instrumentation using the comparative Ct method to the reference housekeeping gene (β actin) (Gasparino *et al.*, 2014).

Statistical analysis: Differences between the studied groups were analyzed in SAS (SAS Institute Inc, Cary, NC, USA) using the General Linear Model (GLM) procedure and Duncan's Multiple Range Test. Statistical significance between mean values was set at (P<0.05). The statistical model was

$$X_{ij} = \mu + G_i + + e_{ij}$$

X_{ij} is the observation (body weight, daily gain and fold change in gene expression), µ= overall mean, G_I is the fixed effect of i^{th} generation (i = 0, 1, 2, 3, 4), e_{ij} = random error.

RESULTS

The fourth-generation recorded the highest and significant values for body weight at hatching (zero), second, fourth, and sixth week of ages (9.75, 75.16, 163.46, and 253.13 gm; respectively). Our results showed that the genetic improvement for the bodyweight at 4-week was 2.4, 6.25, and 4.04 gm for the second, third, and fourth generations, respectively, as shown in Fig 1. Moreover, the fourth generation showed the highest values for ADG at 0-2, 2-4, and 0-6 week intervals (4.67, 6.36 and 5.79, gm; respectively), while the second generation showed the highest values for ADG at 4-6

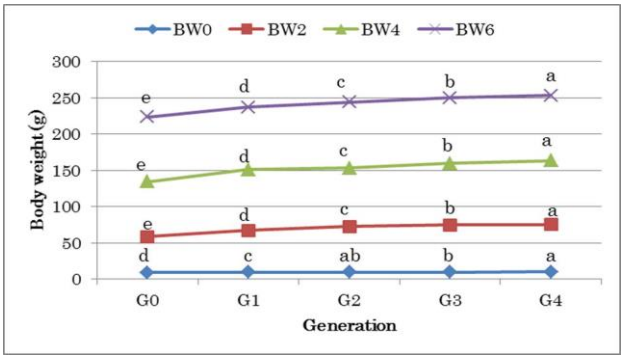

Fig. 1: The least-squares means for body weights (g) in different generations. Estimates with different letters within each trait are significantly different (P<0.05).

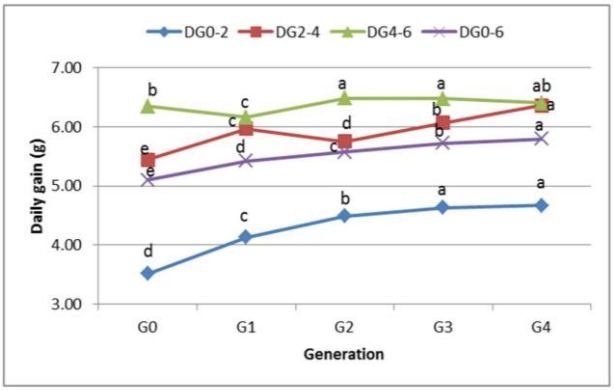

Fig. 2: The least-square means for daily body gains (g/d) in different generations. Estimates with different letters within each trait are significantly different (P<0.05).

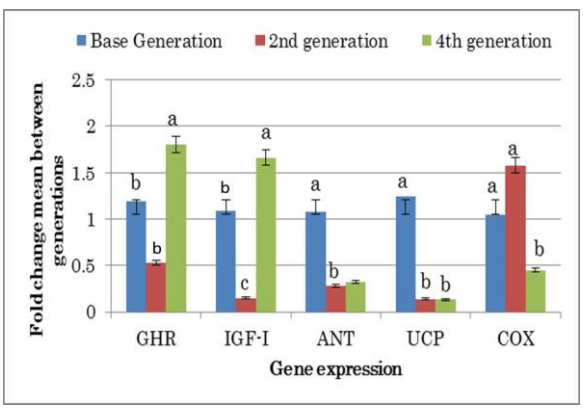

Fig. 3: The least-square means for liver GHR, IGF-I, ANT, UCP, COX III expression levels measured by real-time qPCR in the base, second and fourth generations. Estimates with different letters within each trait are significantly different (P<0.05).

week interval (6.48 gm) as shown in Fig 2. The fourth generation showed the highest hepatic mRNA expression of GHR and IGF-1 genes (1.80 and 1.66 fold change, respectively). In contrast, the base generation showed the highest levels of hepatic mRNA expression of ANT, UCP, COX III genes (1.08, 1.24, and 1.05 fold change, respectively), as shown in Fig. 3).

DISCUSSION

Selection is the primary tool to exploit and improve the productive potential of birds. Different selection strategies have been working worldwide, comprising mass selection to fully pedigree selection (Durmus *et al.*, 2017; Ahmad *et al.*, 2018). In order to investigate the effect of selection on body weight traits and expression level of genes involved in somatotropic axis and mitochondrial energy metabolism, we performed a selection of Japanese quail for increasing body weight at 4-week for four successive generations. The fourth-generation recorded the highest and significant values for body weight and gain traits in this study. Moreover, in all studied generations, the ADG increased from hatching till the sixth week of age. Our results agreed with Varkoohi *et al.* (2010), who reported that the genetic improvement in body weight was 4.7 and 6.2 gm/day for second and third generations, respectively. Moreover, our study was consistent with Momoh *et al.* (2014) and El-Attrouny *et al.* (2020), who recorded the increment in ADG from hatching till fifth and fourth weeks of age, respectively. On the other hand, our results disagreed with Jones and Hughes (1978), who recorded the maximum value of 4.8 gm/day for average daily gain in Japanese quail during the first period from the hatch till 3rd week of age then decreased during the second period from 3rd to 6th week of age.

As a primary step and to better understand the biological basis for differential gene expression of quails selected for high body weight, we sought to evaluate the expression profile of somatotropic axis and energy metabolism genes. In our study, the selection positively affected the hepatic mRNA expression of GHR and IGF-1 genes, where the fourth generation recorded the highest expression level for the two investigated genes. Our results coincided with Gasparino *et al.* (2013), who recorded higher hepatic expression of GHR and IGF-1 genes in Japanese quail with higher feed-efficiency than those with lower feed-efficiency. Moreover, Jia *et al.* (2018) recorded a higher and significant hepatic mRNA expression level for GH and IGF-1 genes in Wuding chickens compared with Daweishan mini chickens. Wuding chickens are significantly higher Chinese than Daweishan mini chickens for body weight, growth rate, and feed conversion efficiency traits.

On the other hand, the selection had a negative effect on the hepatic expression of mRNA for ANT, UCP, COX III genes in our study. Our results agreed with Iqbal *et al.* (2004), who reported higher protein expression of ANT gene in breast muscle of lower growth and feed efficiency chickens. Moreover, there were no differences in expression values of this gene between the high and low growth rate chicken groups in the liver (Iqbal *et al.*, 2005) and the duodenum (Ojano-Dirain *et al.*, 2005); this might be attributed to protein expression and mRNA levels do not always coincide because of other molecular processes such as mRNA stability and post-translational modification (Day and Tuite, 1998). Moreover, Gasparino *et al.* (2013) reported lower hepatic expression of UCP genes in Japanese quail with higher feed efficiency under comfortable and cold stress conditions, while hepatic expression of this gene was higher under heat stress conditions.

In contrast, the expression of hepatic UCP mRNA in quail was not affected by feed efficiency and environment temperature (Gasparino *et al.*, 2014). Similarly, Raimbault *et al.* (2001) recorded higher UCP mRNA expression of leg muscle from a line of chickens selected for low growth and feed efficiency. The researchers attributed the negative trend to the involvement of the UCP gene in controlling chicken body weight gain through increased energy dissipation via mitochondrial oxidation (Raimbault *et al.*, 2001; Dridi *et al.*, 2004). The difference in the observed trends might be a reflection of the inherent metabolism between different tissues. Moreover, the upstream regulation of mitochondrial protein expression and biogenesis might help understand mitochondrial function and biochemistry differences.

Conclusions: The selection for increasing body weight at the 4th week of age has resulted in positive changes in the expression profiles of the somatotropic axis genes and negative expression changes in mitochondrial energy genes in Japanese quail. Consequently, the somatotropic axis and mitochondrial genes are likely to be promising candidate biomarkers for more understanding of selection response for high growth rate and to improve these traits in Japanese quail.

Authors contribution: EA-M, MM-E, and SI-R planned and designed the study. MM-E collected the phenotypic data. EA-M, SH-B collected the blood samples and extracted the DNA. EA-M, SH-B performed the genotyping. EA-M, MM-E and SI-R performed the analysis and interpretation of the data. EA-M and SI-R drafted the article and revised it critically for important intellectual content. All authors read and approved the manuscript final version to be published.

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