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Laying performance, genetic parameters, and the expression of *FSHβ, LHβ, FSHR*, and *LHR* genes in Japanese quails selected for early egg production

Eman A. Manaa,[*,1,2] Mahmoud M. El-Attrouny,[†] Amal EL-Barbary,[‡] Ghadeer M. Albadrani,[§]
Muath Q. Al-Ghadi,[#] Mohamed M. Abdel-Daim,[‖,¶,1] Mahmoud S. El-Tarabany,[**] and
Sherif I. Ramadan[*]

[*]Animal and Poultry Production, Department of Animal Wealth Development, Faculty of Veterinary Medicine, Benha University, Moshtohor, Toukh 13736, Qalyubia, Egypt; [†]Department of Animal Productions, Faculty of Agriculture, Benha University, Moshtohor, Toukh 13736, Qalyubia, Egypt; [‡]Poultry Breeding Research Department, Animal Production Research Institute, Agriculture Research Center, Giza, Egypt; [§]Department of Biology, College of Science, Princess Nourah bint Abdulrahman University, Riyadh 11671, Saudi Arabia; [#]Department of Zoology, College of Science, King Saud University, Riyadh 11451, Saudi Arabia; [‖]Department of Pharmaceutical Sciences, Pharmacy Program, Batterjee Medical College, Jeddah 21442, Saudi Arabia; [¶]Pharmacology Department, Faculty of Veterinary Medicine, Suez Canal University, Ismailia 41522, Egypt; and [**]Department of Animal Wealth Development, Faculty of Veterinary Medicine, Zagazig University, Zagazig 44511, Sharkia, Egypt

ABSTRACT Investigating the impact of early egg production selection (the first 90 d of laying) on egg production features, cumulative selection response (**CSR**), and the mRNA expression of gonadotropins (*FSHβ* and *LHβ*), and their receptors (*FSHR* and *LHR*), in Japanese quails was the goal. The selection experiment involved 1293 females in all, 257 from the base group and 1036 from the 4 selected generations. Age and body weight at sexual maturity (**ASM, BWSM**), weight of the first egg (**WFE**), days to the first 10 eggs (**DF10E**), egg mass for the first 10 eggs (**EMF10E**), egg weight (**EW**), egg number at the first 90 d of laying (**EN90D**), and egg mass at the first 90 d of laying (**EM90D**) were all recorded. Most egg production traits had heritability estimates that were low to moderate and ranged from 0.17 to 0.33., where the highest estimates were reported for EN90D (0.33) and BWSM (0.32). With the exception of EN90D, low to moderate positive genetic correlations were observed between ASM and other egg production traits (0.17−0.44). The fourth generation showed significantly ($P < 0.05$) lower ASM and DF10E but higher BWSM, WFE, EN90D, EM10E, and EM90D when compared with the base generation. CSR were significant ($P < 0.05$) for ASM (−6.67 d), BWSM (27.13 g), WFE (0.93 g), DF10E (−1.25 d), EN90D (7.24 egg), EM10E (10.57 g), and EM90D (140.0 g). *FSHβ, LHβ, FSHR*, and *LHR* gene mRNA expression was considerably ($P < 0.05$) greater in the fourth generation compared to the base generation. In conclusion, selection programs depending on the efficiency of egg production (EN90D) could improve the genetic gain of egg production traits and upregulate the mRNA expression of *FSHβ, LHβ, FSHR*, and *LHR* genes in selected quails (fourth generation). These findings might help to enhance breeding plans and create commercial lines of high egg production Japanese quails.

Key words: egg production, genetic parameters, gonadotropins, selection, Japanese quails

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INTRODUCTION

Around the world, quail are raised for their meat and eggs (Minvielle, 2004). Meanwhile, during the past few decades, the genetics of poultry have changed from those with multiple purposes to those with a focus on producing meat or eggs (Siegel et al., 2006). The advantages of Japanese quail are their quick sexual maturation, quick growth rate, short generational intervals, small body sizes, and high egg output compared to other farm birds (Molino et al., 2015). These advantages suggest that quail could be used as an animal model for genetic studies on egg production (Mahmoud et al., 2015). Increasing the bird's genetic economic worth through multiple trait selection is the ultimate goal of all poultry breeders (Narinc et al., 2014).

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[1] These authors contributed equally.

[2] Corresponding author: eman.manaa@fvtm.bu.edu.eg (EAM); abdeldaim.m@vet.suez.edu.eg (MMA)

Selection progress for these birds to enhance certain traits of this bird can have a negative impact on other traits performance (Savegnago et al., 2011). Therefore, a desirable plan is needed to incorporate them into an index in order to maximize genetic progress simultaneously in all traits (Narayan et al., 2000). The majority of Japanese quail selection studies have concentrated on increasing body weight. On the other side, a small number of studies have focused on increasing egg production (Maeda et al., 1999). Alkan et al. (2009) found that Japanese quail selected over 11 generations for 120-day egg production had a first egg weight of 7.81 g and an average 3-mo egg output of 71.85 eggs. When a Japanese quail reaches sexual maturity, egg production begins, swiftly peaks, and then steadily declines over time. Japanese quail have a stated sexual maturity age of 35 to 45 d in a number of studies (Sezer et al., 2006; Alkan et al., 2009; Karabağ et al., 2010).

An essential economic characteristic of poultry is the number of days required to produce a specific number of eggs, as any reduction in this number will lead to a reduction in production costs. In Japanese quail, the early sexual maturity females recorded significant superiority in egg mass compared with the late sexual maturity group (Hassan and Hameed, 2019). In this context, the longer productive life that selection processes aim for would result from a reduction in the number of days needed, which in biological terms equates to an increase in the length of the clutch (Abou Khadiga et al., 2016). When days needed to produce the first 10 eggs (**DN10**) are taken into consideration as a selection criterion, it was found that the total merit of egg production and growth traits was improved in Japanese quail, and that there was favorable genetic correlation with production traits (Mahmoud et al., 2014).

Egg-laying productivity efficiency is an important economic characteristic in the worldwide poultry sector. The number of ovarian follicles advancing toward ovulation and the oviduct's capacity to transform the ova into a hard-shelled egg serve as indicators of excellent productivity (Hlokoe et al., 2022). Furthermore, ovarian functions and folliculogenesis are mainly controlled by various genes, such as follicle-stimulating hormone receptor (***FSHR***) (Li et al., 2019) and pituitary follicle-stimulating hormone subunit beta (***FSHβ***) (Zhao et al., 2010). The *FSHR* is one of the superfamily of G-protein-coupled receptors and is only found on the granulosa cells of the female ovary, which facilitate *FSH* signal transduction via the cAMP pathway (Simoni et al., 1997). *FSH* affects the appropriate somatic cells, which thus has an effect on oogenesis (Griswold et al., 1995). The development and growth of follicles are regulated by the luteinizing hormone receptor (***LHR***), which also boosts the production of steroid hormones that lead to ovulation and hence increase egg production (Zhou et al., 2022). In this context, Chen et al. (2020) reported that high levels of egg production or reproductive activity are accompanied by birds' high *FSHR* and *LHR* levels.

Despite numerous studies concerning the expression of gonadotropin mRNA in the pituitary gland and their receptors in avian gonads, there is no data regarding their measurement in laying birds selected for several generations. Therefore, the effect of selection for early egg production on laying performance, genetic parameters, and the mRNA expression of gonadotropins (*FSHβ* and *LHβ*) and their receptors (*FSHR* and *LHR*) in Japanese quails was the goal of the current study.

MATERIALS AND METHODS

Ethical Statement

The Animal Ethics Committee of Benha University in Egypt authorized our study's protocols, which were carried out in accordance with the Declaration of Helsinki (approval number BUFVTM 10-03-23).

Birds, Management, and Population Structure

The Faculty of Agriculture's Poultry Research Farm at Benha University in Egypt served as the origin and site for this study. The strain used in this study was the brown Japanese quail. A selection program was carried out for 4 generations and used 1293 females (257 base population and 1036 for selected generations). From the base population (random breed before applying the experiment), 133 male and 266female birds were chosen for the first generation based on the breeding value of the number of eggs laid within the first 90 d (Table 1). The selection ratio from each generation to the next was 20% for females and 8% for males. One selected male and 2 selected female birds were placed in each breeding cage, which measured 25 × 25 × 25 cm and had a sloped floor where eggs were collected. The bird house had a temperature of about 22°C, and the daylight was 16 h and the darkness was 8 h. A pedigree system based on the father and dam was used to gather eggs daily for the first 90 d of egg production for each family. The egg surface pattern on each cage's/dam's eggs served as means of identification. Small size plastic bands were used to band the wings of newly hatched chicks from each sire and dam. At 14 days old, the plastic bands were swapped out for metal ones. From hatching until they were 14 days old, the chicks were kept in brooder cages before being moved into rearing cages. The regular monitoring of bird comfort and housing conditions was meticulous. According to NRC (1994), the birds were fed a basal diet (30 g, every day) formulated to meet their nutritional requirements (Table 2). Ad libitum supplies of food and water were provided. All females were moved

Table 1. Numbers of quail sires, dams and hens for different generations of selection.

Generation of selection	Sires	Dams	Pullets
Base generation	132	182	257
First generation	125	185	266
Second generation	114	184	248
Third generation	107	173	259
Fourth generation	104	169	263
Total	582	893	1293

Table 2. Ingredients and chemical composition of the laying Japanese quail diet.

Ingredient	(%) Amount
Yellow corn	54.25
Soybean meal, 44% CP	34.80
Dicalcium phosphate	1.45
Calcium carbonate ($CaCO_3$)	5.25
Salt	0.20
Sodium bicarbonate ($NaHCO_3$)	0.17
Vegetable oil	3.23
DL-Methionine 99%	0.15
Mineral premix[a]	0.25
Vitamin premix[b]	0.25
Total 100	100
Calculated chemical composition	
Metabolizable energy (kcal/kg)	2900
Crud P (%)	20
Calcium (%)	2.5
Available phosphorus (%)	0.35
Sodium (%)	0.15
Lysine (%)	1.59
Methionine (%)	0.45
Methionine + Cysteine (%)	0.77
Threonine (%)	0.77

[a] each 1 kg contains of: iron (60,000 mg), copper (10,000 mg), zinc (120,000 mg), manganese (20,000 mg), iodine (1,000 mg), selenium (400 mg), cobalt (200 mg) and carrier up (calcium carbonate) to 1kg.

[b] each containing 1 kg composed of: Vit A (24000000 IU), Vit D (8000000IU), Vit E (80,000 mg), Vit K3 (6,000 mg), Vit B1 (4,000 mg), Vit B2 (12,000 mg), vitB6 (10,000 mg), Vit B12 (10gm), nicotinic acid (90,000 mg), pantothenic acid (24,000 mg), folic acid (3,000 mg), biotin (200 mg), BHA/BHT% (10,000 mg), and carrier (calcium carbonate) up to 1 kg.

to individual laying cages after 6 wk of age, where egg production was monitored. All the bird enclosures had the same management, hygiene, and environmental standards. Age at sexual maturity (**ASM**), body weight at sexual maturity (**BWSM**), weight of the first egg (**WFE**), days to the first 10 eggs (**DF10E**), egg mass for the first 10 eggs (**EMF10E**), egg weight (**EW**), egg number at the first 90 d of laying (**EN90D**), and egg mass at the first 90 d of laying (**EM90D**) were recorded.

Analysis of mRNA Expression of FSHβ, LHβ, FSHR, and LHR Genes

On day 90 of the laying period, 24 female quails (8 per generation) were randomly selected and sacrificed. The pituitary glands and ovaries were rapidly separated and then stored at −80°C for isolation of mRNA gene expression. TRIzol reagent (Invitrogen/Life Technologies, Carlsbad, CA) was used for RNA extraction, and NanoDrop was used for quantification. Using cDNA reverse transcription kits from Applied Biosystems, the cDNA was created from the RNA and kept in samples at −20°C.

Pituitary glands were employed to measure the expression of genes associated with eggs using real-time polymerase chain reaction (*FSHβ* and *LHR*), and from the ovaries (*FSHR* and *LHR*). As stated in Table 3, each gene's primers were chosen in accordance with the gene sequences deposited in GenBank (http://www.ncbi.nlm.nih.gov) and the reference housekeeping genes were *β actin* and *GAPDH*.

Real-time polymerase chain reaction with a final volume of 20 l, 1.5 l of 1 g/L cDNA, 10 l of SYBR Green PCR Master Mix (Quanti Tect SYBR Green PCR Kit, Qiagen), 1 M of each forward and reverse primer for each gene. Then, under the following circumstances, the reactions were measured using an Applied Biosystems 7500 Fast Real-time PCR detection system: First denaturation at 95°C for 10 min, followed by 40 cycles at 95°C for 15 s (second denaturation stage), and then annealing and extension at 60°C for 1 min. The comparative Ct method of target genes to the reference genes was used to calculate the changes in gene expression (Livak and Schmittgen, 2001).

Data Handling and Statistical Analyses

Using the univariate method in the SAS software (SAS, 2004), descriptive statistics of the egg production traits were computed. Duncan's multiple range test was used to assess for significant variations ($P < 0.05$) between means. The following model was used:

$$Yij = \mu + gi + eij$$

where Yij is the observation of the jth trait on birds, μ is the overall mean, g is the fixed effect of the ith generation (level 0−4), and eij is the residual random effect.

Using the following equation, a multitrait animal model (in matrix notation) was to analyze traits under the study:

$$\mathbf{y} = \mathbf{Xb} + \mathbf{Z_a U_a} + \mathbf{e}$$

Table 3. Primers of candidate gene for mRNA expression analysis.

Gene symbol	Gene name		Primer sequence (5′−3′)	Amplicon (bp)	Accession number
Reference genes					
B.ACT	β Actin	Forward	TGACCGCGGTACAAACACAG	167	XM_015876619.1
		Reverse	CATACCAACCATCACACCCTGA		
GAPDH	Glyceraldehyde-3-phosphate	Forward	TCTCTGTTGTTGACCTGACCTG	155	XM_015873412.2
	Dehydrogenase	Reverse	ATGGCTGTCACCATTGAAGTC		
Pituitary gland genes					
FSHβ	Follicle stimulating hormone	Forward	AAAAAGCCTTGCAGCACCAG	93	XM_015863527.1
	Subunit beta	Reverse	AGTCAGAACCTCAGCTAAGAGC		
LHβ	Luteinizing hormone	Forward	AGTCGTCGTGCACTTATGGG	90	S70834.1
	Beta-subunit	Reverse	CGGGTAGGATGACTTTGGGG		
Follicle granulosa genes					
FSHR	Follicle stimulating	Forward	ATTATGGCACGCGTTTTGGC	91	XM_015856892.2
	Hormone receptor	Reverse	TCAGGTTTGGGTGAGCAAAC		
LHR	Luteinizing hormone receptor	Forward	ACCAGCCACTACAAGCTCAC	133	HM588001.1
		Reverse	CGTGGTTGTAGTACTGCCCA		

where, **y** is the vector of observations hens, **b** is the vector of fixed effects of generation (5 levels), **U** is a vector of random additive genetic effects for each bird in the pedigree, **X** and **Z** is incidence matrices corresponding to the hen's fixed and additive random effects, **e** is a vector of random residual effects. A control line to compare with the selected line was the base generation as it was raised in parallel for all selected generations. VCE6 software was used to estimate the variance components of random effects and heritabilities (Groeneveld, 2010). The generation effects and their error variance-covariance matrix were solved by the PEST software using these estimations to answer the corresponding mixed model equations (Groeneveld, 2010). We also evaluated the genetic (r_g) and phenotypic (r_p) correlations among the studied traits (Becker, 1984). Estimated covariances were obtained by solving the corresponding mixed model equations for bivariate models.

To calculate the cumulated selection response (**CSR**) at generation, the following equation was used (Falconer and Mackay, 1996):

$$\mathbf{CSR_y = i_X h_x h_y r_g \sigma_{py}}$$

where CSR_y is the cumulated response in trait y, i is the selection intensity for trait x, h_x, and h_y is square roots of heritability for traits x and y, respectively, r_g is the genetic correlation of traits x and y, and σ_{py} is the phenotypic standard deviation of trait y.

RESULTS

Descriptive Statistics

Table 4 provides descriptive statistics of the studied traits. The average ASM of quails in this study was 53.9 d, and the BWSM was 271.4 g. Also, EWF and overall EW were 10.90 and 13.36 g, respectively. The DF10E was 14.8 d and EN90D was 59.9. The EMF10E and the EM90D were 112.2 and 804.3 g, respectively.

Table 4. Descriptive statistics of studies traits in Japanese quail.

Traits	Mean	[1]SD	Minimum	Maximum
[2]ASM (d)	53.9	3.96	49.6	62.5
[3]BWSM (g)	271.4	18.90	259.5	297
[4]WFE (g)	10.9	1.02	7.0	9.8
[5]DF10E (d)	14.8	1.43	12.3	18.4
[6]EMF10E (g)	112.2	9.3	99.2	117.1
[7]EW (g)	13.36	1.52	9.7	14.7
[8]EN90D (egg)	59.9	3.7	46	74
[9]EM90D (g)	804.3	73.7	695	865

[1] Standard deviation.
[2] Age at sexual maturity.
[3] Body weight at sexual maturity.
[4] Weight of the first egg.
[5] Days to the first 10 eggs.
[6] Egg mass for the first 10 eggs.
[7] Egg weight.
[8] Egg number at the first 90 d of laying.
[9] Egg mass at the first 90 d of laying.

Genetic Parameters

Table 5. Estimates of additive genetic ($\sigma^2{}_a$), remainder ($\sigma^2{}_e$) and phenotypic ($\sigma^2{}_P$) variances and heritability (h^2) for egg production traits in Japanese quails.

Traits	$\sigma^2{}_a$	$\sigma^2{}_e$	$\sigma^2{}_P$	h^2
[1]ASM (d)	3.7	10.2	13.9	0.27 ± 0.06
[2]BWSM (g)	69.1	148.0	217.1	0.32 ± 0.07
[3]WFE (g)	3.2	14.8	18.0	0.18 ± 0.01
[4]DF10E (d)	1.5	5.8	7.3	0.21 ± 0.05
[5]EMF10E (g)	3.9	9.5	13.4	0.29 ± 0.06
[6]EW (g)	7.2	35.0	42.2	0.17 ± 0.04
[7]EN90D (egg)	38.2	76.5	114.7	0.33 ± 0.07
[8]EM90D (g)	48.1	121.3	169.4	0.28 ± 0.09

[1] Age at sexual maturity.
[2] Body weight at sexual maturity.
[3] Weight of the first egg.
[4] Days to the first 10 eggs.
[5] Egg mass for the first 10 eggs.
[6] Egg weight
[7] Egg number at the first 90 d of laying.
[8] Egg mass at the first 90 d of laying.

Estimates of variance components and heritability for egg production traits in Japanese quails are described in Table 5. The estimates of heritability for most egg production traits were low to moderate and ranged from 0.17 to 0.33. The highest estimates were reported for EN90D (0.33) and BWSM (0.32), while the lowest estimate was reported for EW (0.17) and WFE (0.18).

Estimates of genetic and phenotypic correlations between age at sexual maturity and some egg production traits are presented in Table 6. Low to moderate positive genetic and phenotypic correlations were observed between ASM and BWSM (0.35 and 0.17, respectively), EW (0.41 and 0.26, respectively), EM90D (0.44 and 0.24, respectively), and WFE (0.18 and 0.22, respectively). Meanwhile, low negative genetic correlations were observed between ASM and EN90D (−0.28).

Selection Response

Least-square means for egg production traits in the random bred base population and the selected lines through 4 generations of selection in Japanese quails are presented in Table 7. The fourth generation showed

Table 6. Estimates of genetic (r_G) and phenotypic (r_p) correlations between age at sexual maturity and some egg productions traits in Japanese quails.

	[1]ASM	
Traits	r_G	r_P
[2]BWSM	0.35 ± 0.04	0.17 ± 0.03
[3]WFE	0.18 ± 0.03	0.22 ± 0.07
[4]DF10E	−0.15 ± 0.07	0.35 ± 0.07
[5]EMF10E	0.30 ± 0.04	0.19 ± 0.03
[6]EW	0.41 ± 0.05	0.26 ± 0.05
[7]EN90D	−0.28 ± 0.06	0.42 ± 0.06
[8]EM90D	0.44 ± 0.07	0.24 ± 0.03

[1] Age at sexual maturity.
[2] Body weight at sexual maturity.
[3] Weight of the first egg.
[4] Days to the first 10 eggs.
[5] Egg mass for the first 10 eggs.
[6] Egg weight.
[7] Egg number at the first 90 d of laying,
[8] Egg mass at the first 90 d of laying.

Table 7. Least-square means (±SE) for egg production traits in base, second, and fourth generations of selection in Japanese quails.

Traits	Base (G_0)	Second (G_2)	Fourth (G_4)	*P value*
[1]ASM (d)	56.8 ± 2.41^{a}	54.4 ± 2.15^{b}	49.7 ± 2.35^{c}	0.0001
[2]BWSM (g)	257 ± 10.1^{c}	273 ± 9.6^{b}	286 ± 11.3^{a}	0.0001
[3]WFE (g)	10.2 ± 1.3^{c}	10.8 ± 1.8^{b}	11.3 ± 2.2^{a}	0.0001
[4]DF10E (day)	15.1 ± 2.3^{a}	14.6 ± 2.4^{b}	13.7 ± 2.3^{c}	0.0001
[5]EMF10E (g)	108 ± 3.4^{b}	112 ± 4.6^{b}	119 ± 3.6^{a}	0.0001
[6]EW (g)	12.7 ± 2.3	13.3 ± 2.7	13.8 ± 2.4	0.0852
[7]EN90D (egg)	56.6 ± 4.6^{c}	60.1 ± 5.8^{b}	64.2 ± 5.4^{a}	0.0001
[8]EM90D (g)	735 ± 14.2^{c}	815 ± 15.8^{b}	885 ± 15.9^{a}	0.0001

[1] Age at sexual maturity.
[2] Body weight at sexual maturity.
[3] Weight of the first egg.
[4] Days to the first 10 eggs.
[5] Egg mass for the first 10 eggs.
[6] Egg weight
[7] Egg number at the first 90 d of laying.
[8] Egg mass at the first 90 d of laying.
[a,b,c] Values within a column with different superscripts differ significantly ($P < 0.05$).

significantly ($P < 0.05$) lower ASM and DF10E when compared with the random bred base generation and the second generation. Meanwhile, the fourth generation showed significantly $P < 0.05$) higher BWSM, WFE, EN90D, EM10E, and EM90D when compared with the random bred base generation and the second generation.

Cumulative selection response (**CSR**) as a result of selection for egg number at the first 90 d of laying is described in Table 8. Significant responses ($P < 0.05$) were obtained for ASM (−6.67), BWSM (27.13), WFE (0.93), DF10E (−1.25), EN90D (7.24), EM10E (10.57), and EM90D (140.0) between selected lines and random bred base generation.

Relative Gene Expression

Relative expression of *FSHβ* and *LHβ* genes among the random bred base population and selected quail lines is illustrated in Figure 1. The fourth generation showed significantly ($P < 0.05$) higher mRNA expression of *FSHβ* and *LHβ* genes compared to the random bred base generation and the second generation. Additionally, *FSHR* and *LHR* gene mRNA expression were considerably ($P < 0.05$) higher in the fourth generation compared to the base generation (Figure 2).

Table 8. Cumulative selection response (CSR) as a result of 4 generations of selection for egg number at the first 90 d of laying in Japanese quail.

Traits	[1]CSR
[2]ASM (d)	−6.67**
[3]BWSM (g)	27.13**
[4]WFE (g)	0.93*
[5]DF10E (d)	−1.25**
[6]EMF10E (g)	10.57**
[7]EN90D (egg)	7.24**
[8]EM90D (g)	140.0**

[1] Cumulative selection response.
[2] Age at sexual maturity.
[3] Body weight at sexual maturity.
[4] Weight of the first egg.
[5] Days to the first 10 eggs.
[6] Egg mass for the first 10 eggs.
[7] Egg number at the first 90 d of laying.
[8] Egg mass at the first 90 d of laying. ** = $P < 0.01$, * = $P < 0.05$.

DISCUSSION

Japanese quails had an average ASM of 53.9 d in the current study. This result is in agreement with those of Kocak et al. (1995) and Abou Khadiga et al. (2016), but higher than those of İnal et al. (1996) and Camci et al. (2002). Also, BWSM was higher than those recorded in previous trials (Karabağ et al., 2010; Özsoy and Aktan, 2011; Okenyi et al., 2013). The EWF was in line with those of Camci et al. (2002), but higher than those of Al-Kaisi and Al-Tikriti (2021) which may be attributed to a relatively higher BWSM in the current study. The days required to produce the first 10 eggs (**DF10E**) were in agreement with those of Abou Khadiga et al. (2016). They reported that DF10E in Japanese quail ranged from 13.63 d to 15.20 d. The EN90D in the current study was relatively lower than that reported in previous trials (Abou Khadiga et al., 2017).

The estimates of heritability in the current study were low to moderate for most egg production traits, and ranged from 0.17 to 0.33. The EN90D showed the highest heritability (0.33) indicating a moderate additive genetic variance for egg production traits and increased additive genetic variance over generations in Japanese quail. Consistent with these findings, El-Attrouny and Iraqi (2020) reported moderate heritability estimates for EN90D (0.26). Abou Khadiga et al. (2017) also suggested that the estimates of heritability for monthly egg production in Japanese quails were 0.17. In general, several studies agree with the estimations of heritabilities in the current study (Ani Nurgiartiningsih et al., 2005; Kranis et al., 2007; Farzin et al., 2011). However, heritability estimates of the current work were lower than those recorded by some authors (Punya Kumari et al., 2009; Narinc et al., 2013; Manaa et al., 2015) who reported egg numbers in Japanese quail were moderately to highly heritable (0.21−0.88). This variation in heritability estimates could be attributed to the differences in population structure, criteria of selection, method of estimation, and statistical models. Moderate heritability estimates were recorded for ASM (0.27), DF10E (0.21), and BWSM (0.32). Similar results were reported by Valente et al. (2011) (0.27) for ASM and Okenyi et al. (2013) (0.30) for BWSM. Meanwhile, higher direct heritability estimates for ASM were revealed by Sezer et al. (2006) (0.33), and Özsoy and Aktan (2011) (0.37). The latter authors reported higher estimates of direct heritability for BWSM (0.58) than those reported in the present study, which could be attributed to population structure and environmental factors.

Low positive genetic and phenotypic correlations were observed between ASM and some egg production traits (BWSM, EW, EM90D, and WFE). Consequently, positive genetic correlations may help breeders to improve several traits simultaneously. Meanwhile, low negative

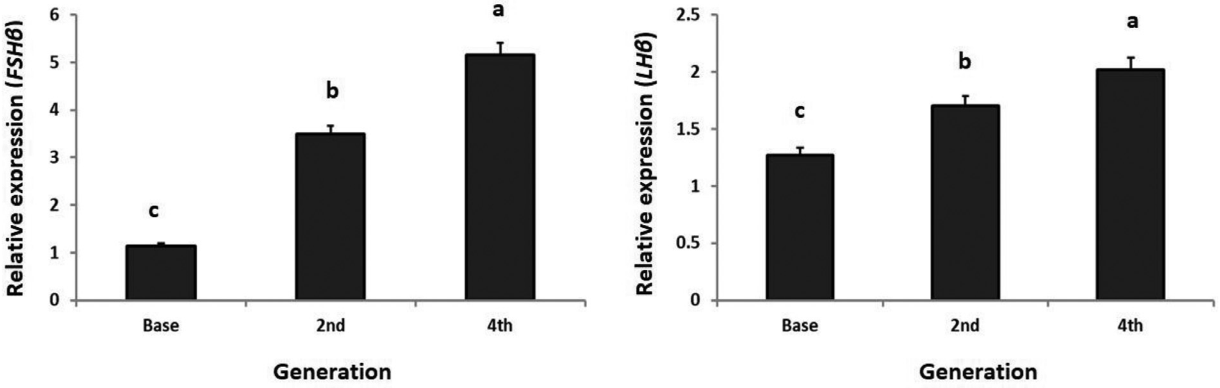

Figure 1. Relative expression of *FSHβ* and *LHβ* genes among random bred base generation and selected quail lines. ^{a,b,c}Different superscripts differ significantly ($P < 0.05$).

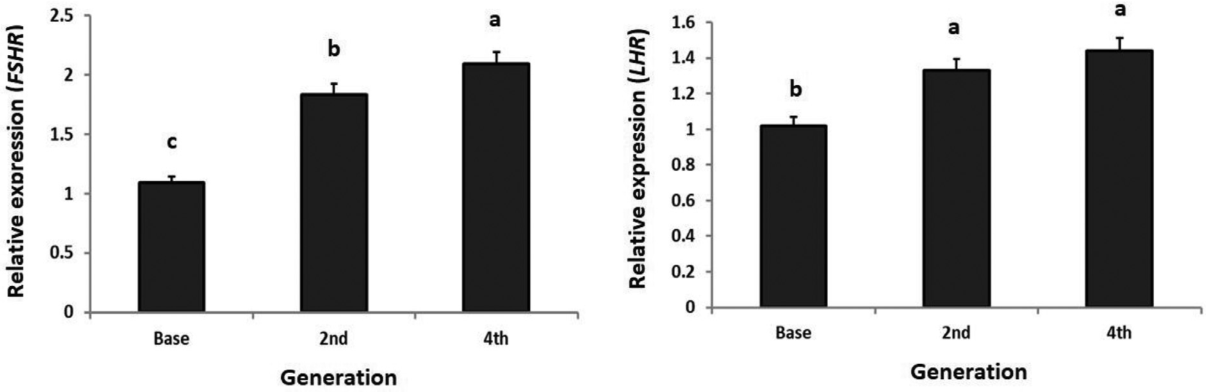

Figure 2. Relative expression of *FSHR* and *LHR* genes among random bred base generation and selected quail lines ^{a,b,c}Different superscripts differ significantly ($P < 0.05$).

genetic correlations were observed between ASM and EN90D. It means that early ASM selection will enhance egg production. In this context, Sezer et al. (2006) reported low positive genetic correlations between the Japanese quail females' age at sexual maturity and other characteristics that affect egg production. Moreover, Özsoy and Aktan (2011) demonstrated a weaker (0.17) positive genetic correlation between ASM and BWSM than what was observed in the present study. In a more recent study, El-Attrouny and Iraqi (2020) revealed that ASM was positively correlated with egg production traits (BWSM, WFE, and EW), with low to moderate r_g ranged from 0.18 to 0.43. They also demonstrated a negative genetic correlation between ASM and EN90D (−0.32). Lotfi et al. (2012) reported high negative r_g estimates between ASM and egg number from 9 to 12 wk of age in Japanese quail and ranged from −0.74 to −0.89. Also, high negative r_g estimates were recorded between age at the first egg and overall egg number in Japanese quail (Hidalgo et al., 2011).

The estimates of phenotypic correlation between ASM and other egg production traits were positive and ranged from low to moderate (0.17−0.42). This agrees with the previous reports of Karabağ et al. (2010) and Alkan et al. (2009), who found a moderate positive r_p between ASM and egg weight (0.32 and 0.22, respectively). Abou Khadiga et al. (2016) also reported low positive r_p estimates between ASM and BWSM (0.18). In a more recent trial, El-Attrouny and Iraqi (2020) recorded low to moderate positive r_p estimates between ASM and all egg production traits (0.16−0.30). In contrast, Özsoy and Aktan (2011) reported low negative r_p estimates (−0.10) between age at the first egg and egg weight. In addition, Farrag et al. (2011) reported high negative r_p estimates (−0.69) between ASM and egg production in the selected line of Japanese quail.

In the present study, ASM and DF10E decreased progressively with advancing generations, and this could reflect a negative correlated response due to the selection for increased egg production. Consistent with these findings, El-Attrouny and Iraqi (2020) suggested that age at the first egg and DF10E decreased progressively through 4 generations of selection. A similar trend was also reported for ASM in previous reports (Nath et al., 2011; Valente et al., 2011; Abou Khadiga et al., 2016). Meanwhile, a different trend was observed by El-Deen et al. (2015), who noticed that the overall means of ASM were 52.6 and 50.5 d for the random bred population and selected quail lines, respectively. For BWSM and WFE, there were significant changes between generations, with an upward tendency through generations. This finding would suggest that BWSM and WFE respond favorably to selection for increased egg production as stated previously by Özsoy and Aktan (2011) and Abou Khadiga et al. (2016). Egg number and egg mass during the first 90 d showed a progressive increase over generation, and this could reflect positive correlated responses due to selection for increasing egg number in Japanese quails. Similar findings were reported by El-Attrouny and Iraqi (2020), who recorded significantly higher EN90D in the base generation (58.9) compared to the second (63.5) and fourth generations (66.6). Durmuş et al. (2017) indicated that when a Japanese quail line was chosen for higher 120-day egg production, the egg production rate increased by 5.05%.

CSR after 4 generations of selection was favorable for all studied traits and exhibited the superiority of selected generations. According to the results of the current study, selection for egg quantity was linked to an increase in BWSM, WFE, EN90D, and EM90D. Meanwhile, ASM and DF10E were declined. In this context, this selection program depending on the superiority of egg production (EN90D) in Japanese quails could yield significant genetic improvement. Similar results were reported for ASM, BWSM, and DF10E (Nath et al., 2011; Abou Khadiga et al., 2016). Stivanin et al. (2019) also suggested that the first 60 d of production as a selection criterion is more advantageous to obtain more genetic progress for egg production traits in Japanese quails. In a more recent study, El-Attrouny and Iraqi (2020) reported negative correlated responses for ASM and DF10E after 4 consecutive generations of selection in Japanese quails (−3.1 and −1.6 d, respectively). On the other hand, Okenyi et al. (2013) reported lower BWSM in a short-term selection program in Japanese quails (30-day egg production for 2 generations).

As far as we know, no previous reports have discussed the effects of selection criteria on the mRNA expression of gonadotropins (*FSHβ* and *LHβ*) and their receptors (*FSHR* and *LHR*) in adult Japanese quails. In the current study, the fourth generation exhibited significantly higher mRNA expression of *FSHβ* and *LHβ* genes compared to the random bred base generation and the second generation. In this context, it has been established that the pituitary expresses both *FSHβ* and *LHβ* subunits during the late embryonic development of female chicks (Grzegorzewska et al., 2009). Furthermore, the activity of the *FSHβ* subunit determines its specific biologic activity and is responsible for interaction with the *FSHR* (Simoni et al., 1997), which is essential for combining the gonadal response with the pituitary *FSH* signal (Heckert, 2001). In order to maximize the function of the hypothalamic-pituitary gonadal axis in chickens, both the *FSH* and *FSHR* genes are crucial. Herein, the fourth generation exhibited significantly higher mRNA expression of *FSHR* and *LHR* genes compared to the base generation. It is well known that the *FSHR* gene is selectively expressed on granulosa cells of the ovary in laying geese (Kang et al., 2009). It is also obvious that *FSHR* plays a significant role in ovarian follicular formation, either on its own or in conjunction with particular signaling pathways (Kim, 2014). In this context, increased *FSHR* expression subsequently aids *FSH*-induced *LH* receptor mRNA expression, which in turn may enhance ovulation and progesterone synthesis in laying hens (Woods and Johnson, 2005). Consistent with our findings, Chen et al. (2020) suggested that the high level of *LHR* is associated in laying birds with egg production or high reproductive activity. In order to cause ovulation and hence enhance egg production, *LHR* stimulates steroid hormone synthesis and regulates follicular growth and development (Zhou et al., 2022). The increased mRNA expressions of *FSHβ, LHβ, FSHR*, and *LHR* genes may explain the significant improvement of egg production traits in selected quails compared to the base generation. The mRNA expression pattern in ovarian tissue was also reported to be linked to egg production (Wu et al., 2017). In this context, Kurnia et al. (2021) recorded a positive correlation between the polymorphism of *FSHR* gene promoter fragment and egg number and egg weight in hybrid chickens. Zhou et al. (2022) also observed that upregulation of the *FSHR* mRNA has a potential role in the regulation of chicken reproductive physiology and egg production ability.

CONCLUSIONS

Finally, the CSR was favorable for the majority of egg production traits as a result of selection for egg number at the first 90 d of laying in Japanese quails. Furthermore, this selection program increases the mRNA expression of the *FSHβ, LHβ, FSHR*, and *LHR* genes in chosen fourth generation quails. The results of the current study may be used to improve breeding programs, reduce breeding costs, and create more productive lines of egg-type Japanese quails.

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DISCLOSURES

The authors affirm that they do not have any competing interests.

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