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20SSOCIATION OF POLYMORPHISMS IN PROLACTIN RECEPTOR AND MELATONIN RECEPTOR 1C GENES ON EGG PRODUCTION AND EGG QUALITY TRAITS OF JAPANESE QUAILS *(COTURNIX COTURNIX JAPONICA)*

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ABSTRACT

In the present study, the association between a 24-bp Insertion-Deletion (Indel) in the prolactin receptor gene (PRL), and the polymorphic site NC_006091.5: c.25.144.C>A(T) in the melatonin receptor gene (MTNR-1C) was investigated in Japanese laying quails. In total, 396 female and 132 male quails were selected for the experiment. The number of eggs was recorded daily in individual quails for egg production and other reproductive traits. Every four weeks, one egg per quail was randomly selected for external and internal assessment. PCR-RFLP and PCR-SSCP were applied for genotyping the Indel, and the single nucleotide polymorphism in PRL and MTNR-1C genes, respectively. The data was continuously collected until 48 weeks of laying. For the effects of PRL Indel, it was found that DD genotyped quails provided a slightly higher egg yield in the first 24 laying weeks (155.1 eggs/quail); however, when the quails reached 36 and 48 weeks of laying, the difference in egg production was clear ($P<0.05$) in quails with II genotype (213.0 and 281.9 eggs/quail, respectively). In the MTNR-1C gene, six genotypes were detected at the polymorphic site, of which AA and AT genotyped quails were better for egg yield in comparison with those baring other genotypes. For egg quality, only albumen weight and albumen ratio differed from quails with II genotype to those of other quails in the 1-24 laying weeks. In the later period (25-48 weeks), yolk index was the highest in quails baring DD genotype. For the MTNR-1C polymorphism, the differences ($P<0.05$) were found in traits related to albumen and yolk; however, no genotype really stands out in terms of egg quality. In conclusion, the two polymorphisms studied may be utilized in breeding programs to enhance egg yield and egg quality traits in Japanese quails of which the PRL (II) and MTNR-1C (AA) alleles are suggested.

Key words: Association, egg traits, Japanese quails, MTNR-1C, PRL
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INTRODUCTION

Meat and eggs originating from poultry production have traditionally been the main food supply for human consumption, thus it is a necessary large industry all over the world. In addition to industrial production for chickens, Japanese quail (*Coturnix coturnix japonica*) has gained much preference in recent years. In fact, large-scale production of Japanese quails for commercial purposes has been widely established in several regions (Narinc *et al*. 2013). In quails, the selection of individuals based on genotypic information related to reproductive traits is preferred (Deeb and Lamont 2002). The research on candidate genes and their impact on economic value traits have laid the foundation for the use of molecular markers in breeding (Kulibaba and Podstreshnyi 2012).

Prolactin is a peptide hormone secreted by the anterior pituitary gland and it has a variety of roles for biological functions in all vertebrates. In birds, the increase of prolactin secretion leads to the regression of ovarian functions and is of crucial importance to egg production and brooding behavior (Sharp *et al*. 1984, Shimada *et al*. 1991). In Japanese quails, the molecular characterization of the PRL gene polymorphism is rare and the majority of the information recorded is restricted to indigenous or commercial chickens (Lotfi *et al*. 2013). In the 5'-flanking region of chicken PRL gene there was an abundance of single-nucleotide polymorphisms (SNP) (Liang *et al*. 2006), and the 24-bp Indel was considerably correlated with broodiness and egg yield (Cui *et al*. 2006, Jiang *et al*. 2005), which indicates its usefulness as a molecular marker for egg production.

Melatonin (N-acetyl-5-methoxytryptamine) secreted from the pineal gland is an important hormone that is involved in many functions in mammals, including feeding patterns and changing patterns of reproduction (Adachi *et al*. 2002, He *et al*. 2016). Also, melatonin helps activate many receptor mechanisms in the ovary and various cells, including theca and granulosa cells

(Ayre *et al*. 1992, Soares *et al*. 2003). Some studies have shown that the presence of melatonin in follicular fluid and its levels are positively correlated with the quality and maturity of oocytes (Nakamura *et al*. 2003, Shi *et al*. 2009, Tian *et al*. 2014). Recently, one of the ovarian melatonin receptors, melanin receptor 1C (MTNR-1C) has been defined in chickens, and its expression has shown a direct contribution to the reproductive process of hens and is therefore marked as a candidate gene for chicken reproduction (Li *et al*. 2013). A similar mutation was also detected in Noi chickens, a native chicken breed in Vietnam (Vu and Ngu 2016).

The current study was conducted to identify the polymorphisms of PRL and MTNR-1C genes and further investigate their association with egg production and egg quality traits in Japanese quails.

MATERIALS AND METHODS

Experimental quails: The quails studied were selected from a breeding farm in Tien Giang province. In total, 396 laying quails and 132 male quails were selected. The birds were fed a grower diet (ME: 2,950 kcal/kg; CP: 24%) from 0 to 6 weeks, and a laying diet (ME: 2,850 kcal/kg; CP: 21%) for the whole experiment. Clean water was provided at all times. During the laying period, the female quails were kept in individual compartments for egg collection and the male was alternatively introduced every 4 hours per day with the ratio of 1 male to 3 females. All quails were vaccinated against common diseases before the experimental period of 48 laying weeks. In detail, Newcastle virus vaccine was administered on day 1st and day 21st via eye drop and repeated every 3 months orally, and the vaccine against coccidiosis was applied on day 5th and day 30th through drinking water. In addition, vaccines for chronic respiratory disease (CRD) and salmonella were given on day 12th via drinking water and H5N1 vaccine was used on day 15th by intramuscular injection.

Egg production and egg quality measurements: Eggs were collected daily at 4 P.M. and numbered to monitor individual yield. The eggs were then marked and gathered in a storage room for 4 days before an incubation period of 17 days in an incubator. The following parameters were recorded as phenotypic data of reproductive capacity: total egg yield (per bird basis), total egg hatched, number of fertilized eggs and hatched chicks, laying rate and hatching rate.

For evaluation of the egg quality traits, one egg/quail was collected every four weeks with a total of 12 eggs/quail used for quality assessment for the whole experimental period. Eggs were weighed using an electronic scale. After breaking the outer shells, eggshells were washed and dried for 24 hours before weighing to obtain the ratio between eggshells and weight. For internal egg properties, album and yolk were evaluated using a tripod micrometer and a digital caliper. The yolk weight was recorded and the proportion was expressed in accordance with egg weight. Albumen weight was determined by the difference of the yolk and the shell weight from the whole egg weight. The yolk index was estimated from the ratio of yolk height to yolk width and the albumen index was calculated as the ratio of thick albumen to its thick diameter. In addition, the Haugh unit (HU) was determined from the formula of HU = 100 x log (AH + 7.57 – 1.7 x EW0.37), in which AH stands for albumen height (mm) and EW represents egg weight (g) (Nasr *et al*. 2016, Sari *et al*. 2012).

DNA extraction and genotyping: Genomic DNA isolated from quails' feathers (Bello *et al*. 2001) was subjected to amplification in a thermal cycler. In the prolactin gene (Genbank number AB011438.2), the primer pair was designed for amplifying the fragment in chickens (Cui *et al*. 2006). The primer sequences were 5'-TTT AAT ATT GGT GGG TGA AGA GAC A-3' (forward) and ATG CCA CTG ATC CTC GAA AAC TC (reverse). The polymerase chain reaction (PCR) was performed with an annealing temperature of 54^{o}C and the subsequence genotyping by PCR-RFLP (Restriction Fragment Length Polymorphism) of all birds was completed by following the protocol described by Cui *et al*. (2006).

For the MTNR-1C gene, forward (5'-TGCCAGATAAGTGGGTTCCT-3') and reverse (5'-AGCGTCCACGTCAGACAGAT-3') primers were designed by the primer3 tool (Untergrasser *et al*. 2012) to amplify a fragment of 164 base pairs (Genbank number NC_006091.5). Polymerase chain reactions were carried out with the following conditions: 95°C for 5 min, followed by 35 cycles at 94°C for 30 s, 55°C for 45 s, 72°C for 50 s, and ended with an extended cycle at 72°C for 5 min. Each PCR contained 10 µL, including 50 ng of genomic DNA, 0.25 M of each primer, 0.25 M of each dNTP, 1x PCR buffer, and 1U/µL Taq DNA polymerase. The polymorphic site was identified by sequencing with Sanger's method. For genotyping, the PCR-SSCP (Single-Strand Conformation Polymorphism) technique was applied. In brief, the mixture of 8 µl of PCR product and 10 µl loading buffer was heated to 95°C and maintained for 10 minutes to separate the DNA strands. The suspension was quickly transferred to ice and kept at -20°C for 10 minutes. Before electrophoresis, the gel was cooled at 7°C for 30 minutes. The DNA mixture was loaded on gel and run at 10°C for 6 hours at 30 W. After that, the gel was stained with 250 ml of TAE 1X solution containing 25 µl of ethidium bromide (25 mg) for 30 minutes and visualized under UV light of the gel imaging system (Gel Logic 212 - Kodak).

Statistical analysis: The association between genotype and egg yield and egg quality traits was analyzed based on the General Linear Model of Minitab software version 16.2.1 (Minitab 2010), Yij = μ + Gi + ξij (where Yij: traits observed; μ: general mean, Gi: influence of genotype; ξij: random error). Data is presented as Least square mean ± Standard error.

RESULTS

Total egg yield was associated with the insertion or deletion of nucleotides in the prolactin gene depending on laying stages (Table 1). Considering only in 12 laying weeks, all reproductive parameters remained similar (P>0.05); however, a tendency of difference occurred at six months of egg yield with a slightly higher rate (P=0.061) in quails with DD genotypes. In the next period, when the quails reached nine months of laying, egg yield was the highest in birds carrying the II genotype (213 eggs/bird; P<0.05). Due to similar fertilized and hatching rates, the number of fertilized eggs and hatched chicks was also higher in type II quails. The difference was even more apparent when eggs were collected up to 48 weeks of laying with the highest egg production in birds baring II genotype, while those with DD genotype were the lowest (P<0.01). However, it is worth considering that quails with DD genotype presented only at 3.9% in the population, whereas quails carrying II genotype occupied 45.1% in the population studied (396 individuals).

Table 1. Association between PRL polymorphism with egg production in different laying periods.

Parameters	Genotypes			P
	II (178)	ID (n=203)	DD (n=15)	
		1-12 laying weeks		
Total egg yield	71.3±0.6	70.6±0.5	73.2±2.1	0.364
Laying rate (%)	84.8±0.7	84.0±0.6	87.2±2.5	0.364
Total egg hatched	67.3±0.6	66.9±0.5	70.0±2.1	0.333
Fertilized eggs	57.7±0.7	56.7±0.6	66.3±2.3	0.121
Fertilized rate (%)	85.7±0.6	84.7±0.6	87.2±2.2	0.312
Hatched chick number	53.2±0.7	52.1±0.6	56.9±2.4	0.125
Hatching rate (%)	92.0±0.4	91.7±0.4	92.8±1.5	0.731
		1-24 laying weeks		
Total egg yield	148.4±1.0	146.6±0.9	155.1±3.8	0.061
Laying rate (%)	88.3±0.6	87.2±0.6	92.3±2.3	0.061
Total egg hatched	140.6±1.0	139.4±0.9	147.8±3.8	0.087
Fertilized eggs	122.5^{ab}±1.3	119.0^{b}±1.2	133.7^{a}±5.1	0.007
Fertilized rate (%)	87.1^{a}±0.7	85.3^{b}±0.6	90.6^{a}±2.6	0.039
Hatched chick number	111.0^{ab}±1.7	106.7^{b}±1.6	123.0^{a}±6.4	0.016
Hatching rate (%)	89.9±0.6	88.9±0.6	91.8±2.3	0.253
		1-36 laying weeks		
Total egg yield	213.0^{a}±2.4	204.7^{b}±2.2	205.2^{ab}±9.0	0.034
Laying rate (%)	84.5^{a}±0.9	81.2^{b}±0.9	81.4^{ab}±3.6	0.034
Total egg hatched	199.9^{a}±2.3	192.4^{b}±2.2	193.3^{a}±8.8	0.047
Fertilized eggs	172.4^{a}±1.9	165.0^{b}±1.8	171.2^{ab}±7.3	0.019
Fertilized rate (%)	86.6±0.5	86.2±0.5	88.6±2.0	0.447
Hatched chick number	150.3±1.8	145.1±1.6	151.5±6.7	0.056
Hatching rate (%)	87.4±0.5	88.1±0.5	90.2±2.0	0.298
		1-48 laying weeks		
Total egg yield	281.9^{a}±2.8	267.3^{b}±3.9	244.8^{b}±15.0	0.007
Laying rate (%)	83.9^{a}±1.4	79.6^{b}±1.2	72.9^{b}±4.5	0.007
Total egg hatched	272.9^{a}±4.2	258.3^{b}±3.9	235.8^{b}±15.0	0.007
Fertilized eggs	234.9^{a}±3.3	221.0^{b}±3.2	207.6^{ab}±11.9	0.003
Fertilized rate (%)	86.7±0.5	86.1±0.5	88.5±1.8	0.373
Hatched chick number	203.5^{a}±2.8	193.1^{b}±2.6	187.0^{ab}±9.9	0.014
Hatching rate (%)	86.9±0.5	87.8±0.5	90.1±1.9	0.174

^{a,b,c} Values within a row with different superscripts are significantly different (*P*<0.05)

At the polymorphic site of the MTRN-1C gene, the effects of the genotype were found in egg production when the yield was counted in 12 weeks or until 24 weeks of laying (P<0.01), but in the later stage, the difference was non-significant (Table 2). Superior egg yield was found in quails of AA genotype while those of in TT genotyped quails were inferior (153.4 vs. 144.6 eggs/24 laying weeks, respectively). A similar trend was also found in several fertilized eggs and hatched chicks.

In Table 3, egg quality is presented for different laying periods (1-24 weeks and 25-48 weeks). Among the external and internal egg traits, albumen weight and albumen ratio were found to differ (P<0.01) in the first 6 months of laying, with the highest values being in quails of II genotype (6.6 g and 54.3%, respectively). In the later stage (25-48 weeks), most of the parameters were similar, with the exception of yolk index, in which the highest was presented in DD genotype quails (P<0.05).

In the first 6 months of laying, many egg quality characteristics were associated with the MTRN-1C polymorphism, especially egg weight and other traits related to yolk and albumen (Table 4). The genotype CC showed lower egg weight than both CT and TT genotypes, which was also observed for the yolk index. Another internal trait, HU was the highest in birds with TT genotype. However, in the later stage, only yolk ratio was affected by genotype (P<0.05).

Table 2. Association between MTNR-1C polymorphism with egg production for extended laying periods.

Parameters	Genotypes						P
	AA (n=40)	AC (n=64)	AT (n=101)	CC (n=25)	CT (n=80)	TT (n=64)	
			1-12 laying weeks				
Total egg yield	72.4^{ab}±0.6	72.7^{a}±0.7	67.6^{c}±1.4	71.4^{abc}±1.0	71.6^{abc}±0.7	70.1^{bc}±0.6	0.002
Laying rate (%)	86.2^{ab}±0.8	86.5^{a}±0.8	80.4^{c}±1.7	85.0^{abc}±1.1	85.2^{abc}±0.9	83.4^{bc}±0.7	0.002
Total eggs hatched	68.6^{ab}±0.6	68.8^{a}±0.7	63.8^{c}±1.4	67.6^{abc}±0.9	67.7^{abc}±0.7	66.3^{bc}±0.6	0.002
Fertilized eggs	58.8±0.8	57.6±0.9	55.1±1.8	57.7±1.2	57.3±0.9	57.1±0.7	0.439
Fertilized rate (%)	85.8±0.8	83.6±0.9	86.3±1.8	85.3±1.2	84.6±1.0	86.0±0.7	0.342
Hatched chick number	54.6±0.9	52.7±1.0	51.0±2.0	53.1±1.4	52.5±1.0	52.7±0.8	0.477
Hatching rate (%)	92.6±0.7	91.0±0.7	92.4±1.5	91.9±1.0	91.5±0.7	92.0±0.6	0.661
			1-24 laying weeks				
Total egg yield	153.4^{a}±1.3	148.9^{ab}±1.5	153.9^{a}±2.8	149.7^{ab}±2.1	149.3^{ab}±1.5	144.6^{b}±1.2	0.000
Laying rate (%)	91.3^{a}±0.8	88.7^{ab}±0.9	91.6^{a}±1.7	89.1^{ab}±1.3	88.9^{ab}±0.9	86.1^{b}±0.7	0.000
Total eggs hatched	144.9^{a}±1.3	140.6^{ab}±1.5	145.4^{a}±2.7	141.4^{ab}±2.1	140.9^{ab}±1.5	136.3^{b}±1.2	0.000
Fertilized eggs	126.1^{a}±1.9	118.4^{ab}±2.1	127.1^{ab}±3.9	119.6^{ab}±2.9	121.8^{ab}±2.1	118.6^{b}±1.7	0.019
Fertilized rate (%)	87.0±1.0	84.0±1.1	87.4±2.0	84.5±1.5	86.2±1.1	86.9±0.8	0.211
Hatched chick number	114.8±2.4	105.7±2.7	117.6±5.1	106.0±3.8	109.6±2.7	107.4±2.1	0.052
Hatching rate (%)	90.5±0.9	87.9±1.0	92.1±1.9	87.7±1.4	89.3±1.0	90.0±0.8	0.196
			1-36 laying weeks				
Total egg yield	210.6±3.6	205.0±3.8	208.4±7.0	214.9±5.5	206.5±4.0	206.6±3.2	0.686
Laying rate (%)	83.6±1.4	81.3±1.5	82.7±2.8	85.3±2.2	82.0±1.6	82.0±1.3	0.686
Total eggs hatched	199.0±3.6	193.5±3.9	196.9±7.1	203.5±5.5	194.9±4.0	195.4±3.2	0.705
Fertilized eggs	172.8±3.0	164.8±3.2	173.3±5.9	172.7±4.6	167.5±3.4	168.1±2.7	0.445
Fertilized rate (%)	87.3±0.8	85.7±0.8	88.2±1.5	85.2±1.2	86.4±0.9	86.1±0.7	0.456
Hatched chick number	153.5±2.7	146.2±2.9	152.7±5.3	150.8±4.1	147.7±3.0	147.3±2.4	0.405
Hatching rate (%)	89.1±0.7	89.0±0.8	88.5±1.5	87.3±1.1	88.3±0.8	87.8±0.7	0.065
			1-48 laying weeks				
Total egg yield	266.0±6.4	264.9±6.8	246.1±14.8	268.3±9.4	265.2±7.1	270.3±5.7	0.783
Laying rate (%)	79.2±1.9	78.9±2.0	73.3±4.4	79.9±2.8	78.9±2.1	80.5±1.7	0.783
Total eggs hatched	250.6±6.3	249.6±6.8	230.7±14.7	253.0±9.4	249.6±7.1	254.6±5.7	0.784
Fertilized eggs	261.8±5.1	213.2±5.4	204.3±11.8	214.9±7.5	213.4±5.6	218.3±4.5	0.892
Fertilized rate (%)	87.3±0.7	86.1±0.8	85.3±1.7	85.6±1.1	86.3±0.8	86.0±0.7	0.555
Hatched chick number	191.6±4.1	188.0±4.4	183.6±9.5	187.3±6.0	186.3±4.6	190.2±3.7	0.936
Hatching rate (%)	89.0±0.8	88.7±0.8	90.0±1.7	87.5±1.1	87.8±0.8	87.5±0.7	0.494

^{a,b,c} Values within a row with different superscripts are significantly different (P<0.05)

Table 3. Association between PRL polymorphism and egg quality traits.

Parameters	Genotype			P
	II	ID	DD	
		1-24 laying weeks		
Total eggs checked	1.068	1.218	108	
Shape index	76.3±0.3	75.9±0.2	76.3±1.3	0.682
Egg weight (g)	12.2±0.1	12.0±0.1	12.2±0.3	0.240
Egg shell weight (g)	1.6±0.01	1.6±0.01	1.5±0.01	0.291
Albumen weight (g)	6.6^{a}±0.1	6.3^{b}±0.1	5.1^{c}±0.4	0.000
Albumen height (mm)	4.1±0.03	4.1±0.02	4.1±0.1	0.554
Albumen width (mm)	41.2±0.8	44.6±0.7	45.9±3.3	0.842
Yolk weight (g)	3.9±0.02	3.8±0.02	3.8±0.1	0.156
Yolk height (mm)	11.0±0.1	10.9±0.03	11.1±0.2	0.081
Yolk width (mm)	23.4±0.2	23.4±0.1	23.6±0.7	0.953
Egg shell ratio (%)	12.9±0.1	12.9±0.1	12.3±0.4	0.347
Albumen ratio (%)	54.3^{a}±0.7	52.1^{b}±0.6	41.9^{c}±3.0	0.000
Albumen index	9.5±0.1	9.5±0.1	9.1±0.5	0.780
Yolk ratio (%)	31.6±0.1	31.4±0.1	31.5±0.6	0.595
Yolk index	48.0±0.6	47.3±0.2	47.2±2.6	0.687
Haugh unit	87.0±0.2	86.9±0.1	86.9±0.7	0.849
		25-48 laying weeks		
Total eggs checked	1068	1218	108	
Shape index	76.3±0.3	75.9±0.3	75.2±1.2	0.548
Egg weight (g)	11.8±0.1	11.8±0.1	11.7±0.3	0.831
Egg shell weight (g)	1.6±0.01	1.6±0.01	1.4±0.1	0.097
Albumen weight (g)	6.6±0.1	6.6±0.1	6.5±0.2	0.929
Albumen height (mm)	4.4±0.03	4.4±0.03	4.3±0.1	0.268
Albumen width (mm)	42.9±0.4	42.3±0.4	42.2±1.8	0.585
Yolk weight (g)	3.7±0.03	3.7±0.03	3.8±0.1	0.364
Yolk height (mm)	10.8±0.1	10.9±0.1	11.2±0.5	0.494
Yolk width (mm)	26.5±1.3	23.2±1.1	22.9±5.4	0.148
Egg shell ratio (%)	13.2±0.1	13.3±0.1	13.3±0.5	0.115
Albumen ratio (%)	55.3±0.2	55.6±0.2	54.9±1.0	0.677
Albumen index	10.4±0.2	10.7±0.1	10.4±0.6	0.287
Yolk ratio (%)	31.4±0.2	31.1±0.2	32.8±0.8	0.084
Yolk index	45.8^{b}±0.4	46.7^{b}±0.3	49.1^{a}±1.2	0.033
Haugh unit	88.6±0.2	89.0±0.2	88.2±0.8	0.264

^{a,b,c} Values within a row with different superscripts are significantly different (*P*<0.05)

Table 4. Association between MTNR-1C polymorphism with egg quality traits.

Parameters	Genotypes						P
	AA	AC	AT	CC	CT	TT	
			1-24 laying weeks				
Total eggs checked	486	120	384	216	612	426	
Shape index (%)	76.7±0.5	75.9±0.6	75.7±1.1	76.4±0.8	75.9±0.6	75.6±0.5	0.727
Egg weight (g)	12.2^{ab}±0.1	12.0^{ab}±0.1	12.2^{ab}±0.2	11.7^{b}±0.1	12.3^{a}±0.1	12.2^{a}±0.1	0.025
Egg shell weight (g)	1.6±0.02	1.5±0.02	1.6±0.03	1.5±0.02	1.6±0.02	1.6±0.02	0.485
Albumen weight (g)	6.3±0.1	6.1±0.1	6.8±0.3	6.1±0.2	6.5±0.2	6.5±0.1	0.066
Albumen height (mm)	4.0^{b}±0.04	4.2^{ab}±0.04	4.1^{ab}±0.01	4.1^{ab}±0.1	4.1^{ab}±0.04	4.2^{a}±0.03	0.005
Albumen width (mm)	44.6±1.1	42.9±1.2	45.3±2.2	42.6±1.2	45.2±1.3	45.5±0.9	0.388
Yolk weight (g)	3.9±0.04	3.7±0.04	3.8±0.1	3.8±0.1	3.8±0.1	3.8±0.03	0.085
Yolk height (mm)	11.0^{abc}±0.1	11.1^{a}±0.1	11.1^{abc}±0.1	10.7^{c}±0.1	10.8^{bc}±0.1	11.0^{ab}±0.1	0.002
Yolk width (mm)	23.8^{a}±0.3	23.6^{ab}±0.3	23.6^{ab}±0.5	24.0^{a}±0.3	22.5^{b}±0.3	23.2^{ab}±0.2	0.003
Egg shell ratio (%)	12.8±0.1	12.9±0.1	12.8±0.3	13.1±0.2	12.7±0.2	13.0±0.1	0.525

Albumen ratio (%)	52.1±1.1	51.6±1.1	56.3±2.1	51.9±1.4	53.3±1.2	52.8±0.9	0.429
Albumen index (%)	9.1^{ab}±0.2	9.8^{a}±0.2	9.1^{ab}±0.3	9.6^{ab}±0.2	9.1^{b}±0.2	9.6^{ab}±0.1	0.013
Yolk ratio (%)	31.9^{ab}±0.2	31.1^{c}±0.2	30.8^{bc}±0.4	32.3^{a}±0.3	30.7^{c}±0.2	31.4^{bc}±0.2	0.000
Yolk index	46.3^{ab}±0.9	48.6^{ab}±0.9	47.2^{ab}±1.7	44.8^{b}±1.1	49.7^{a}±1.0	47.9^{ab}±0.7	0.015
Haugh unit	86.3^{b}±0.2	87.3^{a}±0.2	86.9^{ab}±0.4	86.9^{ab}±0.3	86.5^{ab}±0.3	87.3^{a}±0.2	0.004
				25-48 laying weeks			
Total eggs checked	486	120	384	216	612	426	
Shape index (%)	11.8±0.1	11.8±0.1	11.8±0.2	11.5±0.1	11.9±0.1	11.8±0.1	0.254
Egg weight (g)	11.8±0.1	11.9±0.1	11.9±0.2	11.5±0.1	12.0±0.1	11.9±0.1	0.198
Egg shell weight (g)	1.5±0.02	1.6±0.02	1.6±0.04	1.5±0.03	1.6±0.02	1.6±0.02	0.351
Albumen weight (g)	6.5^{ab}±0.1	6.6^{ab}±0.1	6.6^{ab}±0.1	6.3^{b}±0.1	6.7^{a}±0.1	6.6^{ab}±0.1	0.044
Albumen height (mm)	4.4±0.1	4.4±0.1	4.5±0.1	4.4±0.1	4.3±0.1	4.4±0.04	0.145
Albumen width (mm)	42.4±0.6	42.6±0.6	44.0±1.2	41.8±0.8	43.3±0.7	42.5±0.5	0.654
Yolk weight (g)	3.7±0.04	3.7±0.04	3.7±0.09	3.7±0.06	3.7±0.07	3.7±0.04	0.697
Yolk height (mm)	10.7±0.2	11.2±0.2	10.8±0.3	10.7±0.2	10.8±0.2	10.7±0.1	0.249
Yolk width (mm)	28.9±1.8	23.5±1.9	23.7±3.6	23.2±2.4	23.6±2.1	23.4±1.5	0.205
Egg shell ratio (%)	13.1±0.2	13.4±0.4	13.0±0.3	13.3±0.2	13.2±0.2	13.2±0.1	0.797
Albumen ratio (%)	55.3±0.3	55.8±0.3	55.9±0.6	54.8±0.4	56.3±0.4	55.4±0.3	0.161
Albumen index (%)	10.6±0.2	10.6±0.2	10.4±0.4	10.7±0.3	10.2±0.3	10.5±0.2	0.687
Yolk ratio (%)	31.6^{ab}±0.3	30.8^{ab}±0.3	31.1^{ab}±0.5	31.9^{a}±0.3	30.5^{b}±0.3	31.4^{ab}±0.2	0.015
Yolk index	45.1±0.7	47.5±0.8	45.9±1.4	46.2±1.0	46.6±0.8	46.1±0.6	0.345
Haugh unit	89.1±0.3	89.0±0.3	89.6±0.6	89.1±0.4	88.0±0.3	88.7±0.2	0.081

^{a,b,c} Values within a row with different superscripts are significantly different (P<0.05)

DISCUSSION

In the present report, a 24-bp Indel and a point mutation NC_006091.5: c.25.144.C>A(T) was detected in PRL and MTNR-1C genes of Japanese quails, respectively. The polymorphisms showed certain effects on egg production and internal egg quality traits, which may indicate that they can be of interest in the selection of reproductive traits in breeding programs.

In the PRL gene, the presence of the 24-bp Indel with three genotypes, including II (154 bp), ID (154 and 130 bp) and DD (130 bp), was found. The results also support the statement that in non-commercial breeds of chickens, the "I" allele frequency varies from low to medium (Begli *et al*. 2010, Cui *et al*. 2006, Rashidi *et al*. 2012), while in the commercial layer, this allele is commonly found. Besides, these results correlate favorably with the reports on chickens (Bagheri Sarvestani *et al*. 2013), fowls (Begli *et al*. 2010), and give further support to the conclusion found for quails (Lotfi *et al*. 2013, Yousefi *et al*. 2012). It has been demonstrated that II genotype has a positive effect on egg production efficiency compared with effects from ID and DD genotypes (Lotfi *et al*. 2013). In the present study, Japanese quails possessing II genotype had higher egg production, which is also in agreement with the report of Begli *et al*. (2010) in Fars native chickens. Supportably, the increase of allele I in the population has been reported to eliminate the incubation behavior in local Papua hens (Lumatauw and Mu'in 2016). However, the current results were in contrast with the previous report by Bagheri Sarvestani *et al*. (2013) that revealed genotype II and ID had higher hatching rates, whereas genotype DD gave a lower rate.

There has been evidence showing the linkage between the PRL polymorphic site with egg production. Jiang *et al*. (2005) informed that chickens with homozygote insertion (II genotype) could reduce prolactin expression leading to no broodiness in chickens. Also, the report from Shiue *et al*. (2006) revealed that mRNA expression of the prolactin gene was significantly higher in low egg-producing chickens, which indicates that egg production was related to prolactin mRNA expression. Then, a rise of prolactin level in plasma may induce incubation behavior and then terminate laying (Sockman *et al*. 2000) and thus decrease egg production (Reddy *et al*. 2002). The elevated levels of prolactin decrease the egg clutch lengths by increasing the inter-sequence pauses between the sequences of egg lay, particularly in native birds (Reddy *et al*. 2002, Reddy *et al*. 2006). Prolactin also plays a significant role in decreasing the number of follicles in the ovary, thus affecting egg production. In that respect, broodiness was adversely correlated with egg production and finally stimulated the reduction of egg production in domestic fowl (March *et al*. 1994).

It was also noted that, in the first months of egg-laying, the productivity of DD genotyped quails tended to be higher, then decreased rapidly during the 7-9 month period. In reality, if quails up to 24 weeks of laying are used for egg production, then it is reasonable to incorporate the selection of this genotype into breeding, yet with longer laying periods, productivity will decrease rapidly. However, in this study, there were only 15 individuals with genotypes DD out of a total of 396 individuals, so it is not possible to have a precise conclusion about the benefits and disadvantages of this genotype. Meanwhile, II genotype resulted in relatively stable egg production and a slow reduction in laying rate. Therefore, when considering the effectiveness of quails up to 48 weeks of laying, this would be the best choice in terms of productivity and flock development.

In the present study, a novel polymorphic site was detected in MTNR-1C gene and it was found to associate with egg yield and some egg quality traits. According to Sundaresan *et al*. (2009), the MTNR-1C gene is highly expressed in the follicular granulocytes; thus, it is thought to play an important role in egg formation, thereby affecting poultry egg production. The study by Li *et al*. (2015) showed that the expression of the MTNR-1C gene was related to chicken egg production, and the authors demonstrated that chickens with a high expression of MTNR-1C had the lowest number of eggs laid and vice versa. This was in line with the report of Abd El Naby and Basha (2016) that the MTNR-1C expression increased, which accompanied a delay in sexual maturity of Japanese quails. In an association study, it was found that the MTNR-1C polymorphic gene is linked with the age at which the first egg is laid and the number of eggs laid at 300 days of age in Erlang mountain chickens (Li *et al*. 2013). Recently, an association between MTNR-1C polymorphism with total egg yield at 34 weeks of age, and age at first egg, was reported and the expression of this gene was up-regulated in an early-age maturation group of Shaoxing duck (Feng *et al*. 2018). Although the exact role of melatonin receptors in different species during the reproductive process is not well understood, MTNR-1C is hypothesized to activate avian sexual mature by down-regulating its expression level (Feng *et al*. 2018).

Egg weight is an important indicator of egg quality and total egg production. If the total number of eggs is the same, but the egg weight varies, the entire egg production quality will be different. In quail breeding, high-quality eggs are favored, especially those close to the average value, because these eggs give better hatching probability than oversized or undersized eggs (Asuquo and Okon 1993). In addition, egg weight corresponds to the weight of albumen, egg yolk and eggshell. In Table 4, the genotypes at MTNR-1C polymorphism produced eggs with weights in the range of 11.7 to 12.2 g, in which quails of CC genotype had the lowest value. This result additionally implies that MTNR-1C polymorphism has certain effects on the egg weight of Japanese quails. The effect is supposed to relate to gene expression since MTNR-1C is negatively associated with egg weight, individuals with low MTNR-1C expression produce higher egg weight than those with high MTNR-1C expression (Abd El Naby and Basha 2016). Although there are differences between quails that carry different genotypes, no genotype really stands out in egg quality. Quails carrying AA genotype gave an average egg mass compared with those with other genotypes, and there is no difference in other indicators related to albumen and yolk. However, this is a homogeneous genotype for high yield, so it should be noted for breeding purposes.

Conclusion: Altogether, it can be concluded that the two polymorphisms studied may be used in breeding programs to improve egg production, and to some extent, egg quality traits in Japanese quails, of which the II genotype in PRL gene and the homozygous AA genotype in MTNR-1C genes are appropriate for selection.

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