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Genetic Variability in Growth Hormone Gene and Association between Restriction Fragment Length Polymorphisms (RFLP) Patterns and Quantitative Variation of Live Weight, Carcass, Behaviour, Heterophil and Lymphocyte Traits in Japanese Quails

Research Article

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ABSTRACT

Growth hormone gene plays a critical role in regulating growth and metabolism which leads to potential correlations between the polymorphisms of this gene and economic trait. A 776 bp fragment within the intron 1 region of the growth hormone gene from 346 individuals of an F2 population of Japanese quail was amplified. The polymerase chain reaction (PCR) product was digested using *MspI* restriction enzyme. restriction fragment length polymorphisms (RFLP) analysis revealed three restriction sites, which led to four different restriction fragments. Four distinctive alleles (A, B, C, and D) and four different genotypes (AA, AB, AC, and AD) were identified in this population. The results indicated that the population deviated from the Hardy-Weinberg equilibrium (P<0.005). The observed heterozygosity and Shannon's information indexes were 0.720 and 0.560, respectively, which demonstrated high diversity in this population. There was a significant association between RFLP patterns and live weight at four and five weeks of age, carcass weight, breast weight and proportion of the internal organs. Carcass weight of the AB birds was less than that of other genotypes (P<0.05). These results suggested that growth hormone gene can be used as a candidate gene for marker-assisted selection to improve performance in Japanese quails.

KEY WORDS growth hormone, heterozygosity, Japanese quail, Shannon's information index.

INTRODUCTION

In order to take advantage of breeding programmes and productivity of poultry and other domesticated animals, it is essential to assess genetic variability and study the strategies to preserve genetic diversity. Japanese quails (*Coturnix japonica*) is phylogenetically related to chickens and is reared in Asia for its meat and egg (Stock and Bunch, 1982). It thrives in small cages, is cheap to produce, and needs less feeding requirement (20-25 g/day) than chickens (120-130 g/day) Ani *et al.* (2009). Both the environment and genetic makeup influence the growth of an animal. One study showed that genetic correlations were higher than phenotypic and permanent environmental correlations in the study of genetic parameters of body weights in Japanese quails (Akbas *et al.* 2004). Another study reported a positively high correlation in body weight at different ages in two strains of Japanese quails (Vali *et al.* 2005). One source

provided a detailed statement concerning the relatively high genetic and phenotypic correlations between the growth curve parameters in this bird (Narinc *et al.* 2010).

Poultry growth hormone (cGH) is a peptide hormone that is synthesised, stored, and secreted within the lateral wings of the anterior pituitary gland. It stimulates many physiological functions such as growth, carcass weight, egg production, regeneration, and immune response and because of this it is crucial for growth and metabolism (Vasilatos-Younken *et al.* 2000). This hormone is encoded by a gene on chromosome 19 in cattle, 17 in human, 11 in sheep and mouse, 12 in pig, and 1 in chicken. This gene has 4101 base pairs and consists of five exons and four introns encoding a 191 amino acid mature growth hormone protein (Mou *et al.* 1995).

As far as digestion is concerned, one study reported the traces of polymorphism in GH gene fragment at 776 bp-sized in quails which produced two kinds of alleles (A and B) (Setiati *et al.* 2014).

Single nucleotide polymorphisms (SNPs), which include deletion, insertion, and substitution, have a significant role in the transcription as well as translation of genes and are considerably distributed along the chicken genome. A great number of SNP positions in the genome can be identified through DNA sequencing technique (El-Bayomi *et al.* 2016).

In a study to detect the single nucleotide polymorphism (SNP) in growth hormone (GH) and insulin-like growth factor -1 (IGF-1) genes, two lines of Japanese quails, distinguished by high body weight (HBW) and low body weight (LBW), were selected for body weight at four weeks of age. The results showed one nucleotide change (T/C) in the intron 2 of GH gene. There were no nucleotide differences, however, in IGF-1 gene between the two selected lines (El-Bayomi *et al.* 2016).

The result of a study on four breeds of chicken identified 46 single nucleotide polymorphisms (SNPs) in the cGH gene, consisting of 10 mutations in exons and 36 mutations in introns, some of which were associated with body weight (Nie *et al.* 2002). The study of Nie *et al.* (2002) also showed considerable diversity in the cGH gene between the Chinese native breeds and the commercial breeds. Most of the studies on the cGH gene have revealed the effect of this gene on the performance traits. Three SNPs are identified in chicken IGF1 and IGF2 genes which are associated with growth and feeding traits (Amills *et al.* 2003). On the other hand, other studies suggest that the intron region plays an important role in regulating the cGH gene expression (Nie *et al.* 2002).

In the present study, polymorphisms in the cGH gene were studied by restriction fragment length polymorphisms (RFLPs) technique in 346 individuals of Japanese quails.

MATERIALS AND METHODS

Blood samples were collected from 346 individuals of Japanese quails. This population was developed by two distinct Japanese quail strains, white (layer type) and wild (meat type). The blood samples were frozen at -20 ˚C. Genomic DNA of the individuals was extracted from ethylenediaminetetraacetic acid (EDTA) anticoagulated blood using the optimised and modified salting-out DNA extraction procedure (Miller *et al.* 1988). Quality and quantity of DNA were examined by electrophorese and spectrophotometry. The primers for the PCR assay of *MspI* RFLP were as follows (Ip *et al.* 2001): 5'-ATCCCCAGGCAAACATCCTC-3' (forward) and 5'-CCTCGACATCCAGCTCACAT-3' (reverse). The reaction conditions were 94 ˚C for 5 min, 35 cycles of 94 ˚C for 30 sec, annealing at 60 ˚C for 120 sec, and extension at 72 ˚C for 3 min. The PCR products were run on 1.50% agarose gels using electrophoresis. Individual PCR product fragments were determined by visualising the band pattern via ethidium bromide staining. The PCR products were digested with *MspI* at 37 ˚C for 3 h. The digestion mixture contained 10 µL PCR products, 2 µL digestion buffer (10X), and 1 µL of enzyme with the final volume of 30 µL containing dilution water. The digestion mixtures were tested by electrophoresis on 2.5% ethidium bromide stained agarose gels in 0.5x TAE buffer and the genotypes were scored. POPGENE 1.31 software was used for data analysis (Yeh *et al.* 1997). Presence of different alleles, allele and genotype frequencies and heterozygosities were estimated. Expected and Observed genotype frequencies were compared by chi-square test. Average expected theoretical heterozygosity from Hardy-Weinberg assumptions was calculated using the formula of Hedrick (2000):

$$1-\sum_{i=1}^{n} p_i^2$$

Where:

P_i: frequency of the i^{th} allele in the population.

Average heterozygosity at each locus was calculated according to formula:

$$H_{i=\frac{an}{an-1}} 1-\left(\sum_{j=1-p_j^2}^{ik}\right)$$

Where:

p_j: frequency of the j^{th} allele at i^{th} locus with k number of alleles in the population assumed as under Hardy-Weinberg equilibrium.

n: number of individuals in the population assumed as under Hardy-Weinberg equilibrium.

Allelic variation was determined based on one or more restriction enzyme sites. Genotype frequencies were calculated by combining different alleles. The deviation from the Hardy-Weinberg equilibrium was evaluated by Chi-square (χ^2) test. We also measured the observed homozygosity, expected homozygosity, average heterozygosity, expected heterozygosity, Nei's expected heterozygosity, observed number of alleles, effective number of alleles, and Shannon's information index. The statistical model used to test the association between the RFLP patterns and the traits included the mean of F_2 population for each trait, fixed effects of the bird genotype (four levels), hatch (five levels), sex (two levels), carcass weight as covariate, and residual random term.

RESULTS AND DISCUSSION

A 776 bp fragment within the intron 1 region of the growth hormone gene from 346 individuals of an F2 population of Japanese quails was amplified. The PCR product was digested using *MspI* restriction enzyme. *MspI* was used to cut the amplified growth hormone gene in the population of Japanese quails and its restriction site was as follows:

5'…C CGG…3'
3'…GGC C…5'

Electrophoresis showed four different RFLP patterns as demonstrated in (Figure 1). RFLP analysis revealed three restriction sites, which led to four different restriction fragments the RFLP patterns had the restriction fragments with the size of 414, 373, 241, and 125 bp (this pattern indicated the presence of the three restriction sites in 776 bp amplicon). Four distinctive alleles (A, B, C, and D) and four different genotypes (AA, AB, AC, and AD) were identified in this population. It was consistent with the result of another study on Chinese native chickens (Ip *et al*. 2001). The results indicated that the population deviated from the Hardy-Weinberg equilibrium (P<0.005). The observed heterozygosity and Shannon's information indexes were 0.720 and 0.560, respectively, which demonstrated high diversity in this population.

There was a significant association between RFLP patterns and live weight at four and five weeks of age, carcass weight, breast weight and proportion of the internal organs. Carcass weight of the AB birds was less than that of other genotypes (P<0.05).

In the present study, we identified four different genotypes, i.e. AA, AB, AC, and AD with the frequencies of 26, 21, 34, and 19%, respectively.

The observed heterozygosity was 0.742, which was higher than the common observed heterozygosity in the chicken population. The diversity parameters obtained for the studied population are given in Tables 1 and 2. Investigation of the Hardy-Weinberg equilibrium based on the observed and expected individuals showed that this locus deviated from the Hardy-Weinberg equilibrium (P<0.005) (Table 3). Body weight measurements were taken weekly and the significant effect of genotype on weight was found at four weeks of age (P<0.05). By measuring the least squares mean for different genotypes, AB (130±2.87) was determined to be significantly different from AA (120±2.67) (P<0.05). On the other hand, the AB birds were also heavier than the AC and AD birds (Table 4).

The breast weight is one of the most economically important traits in the poultry industry; it was measured and found that the beast weight in AB (39.2±0.98) was significantly better than the others. And although the breast weight in AA (37.7±0.92) and AC (38.3±0.87) was not significantly different, both AA and AC proved to be higher than AD (36.7±0.97) (Table 4).

For hot carcass weight, i.e. weight of a carcass just prior to chilling, the AB genotype (118±2.37) was better than the others and there was no significant difference between AC (116±2.11) and AD (112±2.34), but both of them were better than AA (113±2.23) (Table 4).

Feeding expenses represent about 70% of the costs in poultry. The main way to decrease these costs is to improve feeding efficiency. In rapidly growing species like Japanese quails, animal growth rate is related to the intestine development (Ruiz-Garcia *et al*. 2006). Intestine weight and percentage of intestine weight affect the food intake, so we measured these traits and found that the genotype had a significant effect on intestine weight (P<0.05). By comparing least squares mean, we found that AB (6.16±0.22) was better than AC (6.59±0.20) and AD (6.68±0.22) and significantly different from AC and AD (Table 4). The genotype had a significant effect on intestinal weight, (P<0.05). Least squares mean of the AC genotype was significantly different from that of the AB genotype, and the AC birds (7.354±0.20) performed better than the AB birds (6.846±0.21).

The other trait on which genotype had a significant effect was the average daily gain at three to four weeks (P<0.0082). By comparing least squares mean of genotypes, it was determined that AB (5.96) (P<0.01) was significantly different from AA (5.35); therefore, AC was better than AA.

It was also obvious that AB was better than AC (5.53) (P<0.05) (Table 4). Japanese quails had higher potentiality for rapid growth than chickens or turkeys (Wilson *et al*. 1961).

Table 1 Estimates of diversity parameters for growth hormone gene in Japanese quails

Locus	n	Obs. (Hom.)	Obs. (Het.)	Exp. (Hom.)	Exp. (Het.)	Nei	Average (Het.)
Growth hormone	346	0.257	0.743	0.444	0.557	0.556	0.556

Obs: observed; Hom: homozygote; Het: heterozygote; Exp: experimental and Nei: Nei's expected heterozygosity.

Table 2 Measured number of alleles and Shannon's information index for growth hormone gene in Japanese quails

Locus	n	Na	Ne	I	χ^2
Growth hormone	346	4.00	2.25	1.06	120

Na: observed number of alleles; Ne: effective number of alleles; I: Shannon's information index and χ^2: Chi-square.

Table 3 Estimates of the observed and expected individuals for growth hormone genotypes in Japanese quail

Genotype	Obs.	Expt.	(Obs.-Expt.)²/Expt.
AA	89	136	16.6
AB	74	46.6	16.1
AC	117	73.7	25.5
AD	66	41.5	14.4

Obs: observed individuals and Expt: expected individuals.

Table 4 Growth performance, biological incidences and carcass traits of Japanese quails based on different genotypes

Trait	Genotypes							
	AA		AB		AC		AD	
	LSM	SE	LSM	SE	LSM	SE	LSM	SE
Hatching weight, g	6.82	0.150	6.85	0.150	6.73	0.140	6.73	0.150
Weight at week 1, g	23.7	0.760	24.7	0.810	24.3	0.720	23.2	0.790
Weight at week 2, g	48.5	1.28	51.5	1.41	50.3	1.20	48.2	1.38
Weight at week 3, g	83.7	1.87	88.9	2.04	86.8	1.75	84.1	2.03
Weight at week 4, g	120^{b}	2.67	130^{a}	2.87	125^{ab}	2.53	124^{ab}	2.85
Weight at week 5, g	151^{ab}	2.86	156^{a}	3.12	155^{ab}	2.74	151^{b}	3.38
Hot carcass weight, g	113^{b}	2.23	118^{a}	2.37	116^{ab}	2.11	112^{ab}	2.34
Breast weight, g	37.7^{ab}	0.920	39.2^{a}	0.980	38.3^{ab}	0.870	36.7^{b}	0.970
Tonic immobility	74.0	9.050	76.5	9.73	70.7	8.59	86.7	9.62
Log tonic immobility	1.80^{a}	0.050	1.78^{ab}	0.05	1.76^{b}	0.050	1.85^{ab}	0.050
Rectal temperature ˚C	40.3^{ab}	0.080	40.2^{ab}	0.090	40.4^{a}	0.080	40.2^{b}	0.090
Heterophil %	32.2	1.36	33.2	1.57	33.8	1.28	37.3	1.54
Lymphocyte %	67.7	1.35	67.0	1.56	66.1	1.27	62.6	1.53
Small Intestine %	6.41^{a}	0.210	6.16^{b}	0.220	6.59^{ab}	0.200	6.68^{ab}	0.220
Pancreas %	0.420	0.010	0.400	0.020	0.410	0.010	0.430	0.020
Liver %	3.08^{a}	0.080	3.01^{b}	0.080	3.10^{ab}	0.070	3.20^{ab}	0.080
Spleen %	0.080	0.010	0.080	0.010	0.080	0.010	0.080	0.010
Heart %	1.12^{a}	0.030	1.11^{b}	0.030	1.11^{b}	0.030	1.11^{b}	0.030
Gizzard %	3.55	0.070	3.51	0.080	3.52	0.060	3.60	0.070
Pre-stomach %	0.590^{a}	0.020	0.560^{b}	0.020	0.580^{ab}	0.020	0.580^{ab}	0.020
Testis %	0.140	0.070	0.310	0.070	0.210	0.060	0.220	0.070
Ovary %	0.130	0.060	0.130	0.070	0.230	0.060	0.070	0.080
Carcass fat %	0.670	0.090	0.850	0.100	0.900	0.090	0.620	0.100
Uropygial gland %	0.250	0.010	0.250	0.010	0.260	0.010	0.260	0.010
Bursa of fabricius %	0.120^{a}	0.010	0.120^{a}	0.010	0.120^{a}	0.010	0.110^{b}	0.010
Breast %	35.03^{ab}	0.470	35.6^{a}	0.500	35.3^{ab}	0.440	34.7^{b}	0.490

LSM: least square mean and SE: standard error.

One study has reported that weight for age and growth rate are the parameters that are often used as the selection trait in many breeding programmes (Bakker *et al*. 1974). Research has proved that the polymorphisms of the avian GH gene can be detected both at intron 2 and exon in different regions of this gene as in ducks and geese. It has been reported that polymorphisms in intronic regions of the avian GH gene are detected at introns 1, 3 and 4 in chickens, intron 2 in ducks and intron 3 in geese (El-Bayomi *et al*. 2016).

In an investigation, the polymorphism of intron 2 of the quail GH gene was examined. Previous investigations had revealed that the polymorphisms of the avian GH gene could be detected not only at intron 2, but also at exon in different regions of this gene as in ducks and geese. Meanwhile, polymorphisms in intronic regions of the avian GH

gene were detected at intron 1, 3 and 4 in chickens, intron 2 in ducks and intron 3 in geese (El-Bayomi *et al.* 2016).

Through employing PCR-RFLP technique researchers have been able to notice a difference in such poultries as chickens, ducks, quail, rabbits, and turkeys, and reported different restriction analyses in the meat of these poultries (Abdel-Rahman *et al.* 2015). They also used PCR-RFLP to investigate the polymorphism of some loci in Japanese quails (*Coturnix coturnix japonica*), the result of which shows the SEMA3E and TLX loci can be used for studying recombination frequencies in the populations of Japanese quails. Out of the eight loci studied (SEMA3E, IFR1, HAL, LOC396025, UGP2, LOC396192, TLX and BMP5), polymorphism was detected in the SEMA3E and TLX loci; five loci were found to be monomorphic and one locus (HAL) could not be amplified by PCR (Bozkaya *et al.* 2013). One study on the RFLP analysis of Japanese quail's genomic DNA using chicken's MHC class I Probe showed the RFLP pattern of DNA digested with Pvu II in RWL strain (Ly1/Ly1), PNN strain (Ly2/Ly2) and SBPN strain (Ly3/Ly3) 18-19 pieces of DNA bands, and a band of 8.3kb was observed in RFLP pattern of RWL strain Shiina *et al.* (1995). They not only reported the RFLP analysis of mitochondrial DNA in 14 laboratory strains of Japanese quails, but also stated that the laboratory strains of Japanese quail could have been established from at least two different gene pools (Shen *et al.* 2000). Another study employed polymerase PCR-RFLP of mitochondrial 12S rRNA gene Girish to investigate the meat species (Girish *et al.* 2005). The PCR-RFLP has also been employed to analyse cytochrome b (cyt b) inheritance in the wild-type strain and laboratory population of Japanese quails (Shen *et al.* 1999).

CONCLUSION

One of the most important candidate genes is the chicken growth hormone (cGH) gene which can affect the performance traits because of its function in metabolism and growth (Vasilatos-Younken *et al.* 2000). On the other hand, after selection experiments conducted in the poultry industry for such traits as bodyweight, rate of growth, and rate of egg production, we found polymorphism in a Japanese quail growth hormone gene and its association with some economic traits in this population and some specific genotypes were shown to be important in the selection for the next generation. It can be concluded that growth hormone gene can be used as a candidate gene for marker-assisted selection to improve performance.

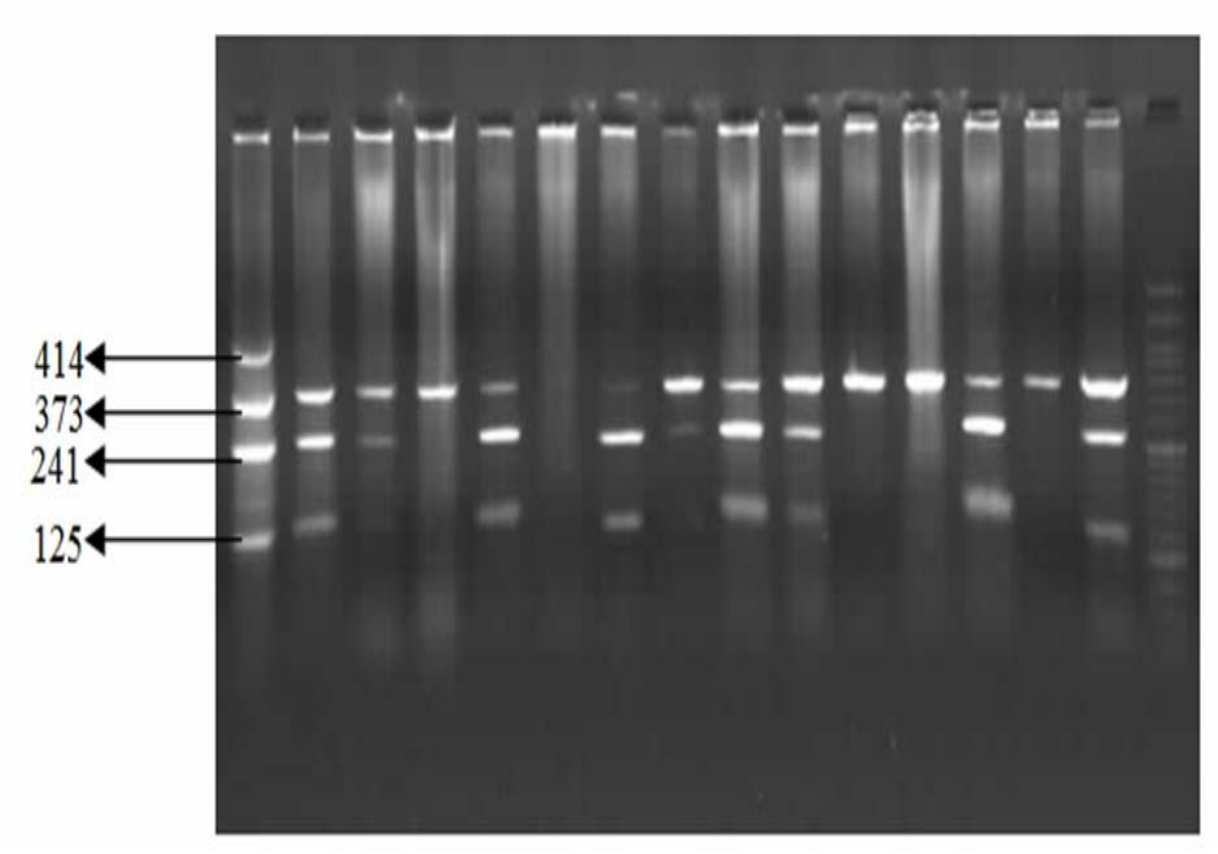

Figure 1 Digested PCR product using *MspI*

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