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Mob: +92 300 3008585, Fax: +92 41 8815544
E-mail: editorijps@gmail.com

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The Gene Effect of Growth Hormone on Body Weight and Egg Production in Divergent Selection for Five Generation of Japanese Quail (*Coturnix coturnix japonica*)

S. Johari[1], N. Setiati[2], J.H.P. Sidadolog[3], T. Hartatik[3] and T. Yuwanta[3]
[1]Faculty of Animal Science and Agriculture, Diponegoro University, Semarang, Central Java, Indonesia
[2]Faculty of Mathematics and Natural Sciences, State University of Semarang, Indonesia
[3]Faculty of Animal Science, Gadjah Mada University, Yogyakarta, Indonesia

Abstract: The aim of this study was to determine the gene effect of Growth Hormone (GH) on divergent selection of Japanese quail. Quails were grouped into high weight (Q-H), low weight (Q-L) and random weight (Q-R) females as a treatment for divergent selection. Parameter phenotype observed in each generation is the weight at four weeks of age and egg production at ten weeks of age for five generations. The results showed that the dominance level on body weight of Q-L was incomplete dominance, Q-R was over dominance and Q-H was lack of dominance. While the dominance level on egg production of Q-L and Q-H were over dominance and Q-R was lack of dominance. The gene effect of GH on body weight of Q-H is 1.53 times greater than the Q-L and 12.37 times greater when compared with Q-R. Whereas the gene effect of GH on egg production of Q-H is 1.53 times greater than the Q-L but only 4 times greater when compared with Q-R. Should be developed that to increase the low-weight (Q-L) is in the BB genotype and the high weight (Q-H) is in the AA genotype groups. Otherwise, to increase the low-weight (Q-L) and high-egg production are in the AA and BB genotypes and the high weight (Q-H) and low-egg production are in the AA and AB genotypes groups.

Key words: Gene effect, growth hormone, body weight, egg production, divergent selection, Japanese quail

INTRODUCTION

Genetic improvement has knew significant developments in quail performance during recent decades (Chang *et al*., 2001; Reddish *et al*., 2003; Sezer, 2007; Gasparino *et al*., 2012). The genetic evolution of quail and other poultry has been based on selection for growth (Reddish *et al*., 2003; Sezer, 2007), growth gene expression (Kuhnlein *et al*., 1997; Gasparino *et al*., 2012) and has been a important factor in obtaining good quality of poultry with phylogenetic study (Chang *et al*., 2001) and genetic diversity (Zhang *et al*., 2002; Chang *et al*., 2007).

Growth hormone gene polymorphisms have been reported in mice (Barta *et al*., 1981), Cattle (Woychik *et al*., 1982), sheep (Byrne *et al*., 1987), Pig (Vize and Wells, 1987), goats (Kioka *et al* ., 1989), chicken (Tanaka *et al*., 1992; Stephen *et al*., 2000) but there are no reports of the GH gene effect in quail. GH gene structure in these animals results of these studies are the same which consists of 5 exons and 4 introns. While the report by Kuhnlein *et al*. (1997) have analyzed the GH gene polymorphism in 12 strain Leghorn chickens using PCR-RFLP method with two kinds of enzymes, namely *Msp*1 and *Sac*1, the result is that the alleles in the intron may be the production traits, resistance for Marek's disease or avian leucosis.

The availability on good quality of quail remained a major constraint in the development of quail, because the product quality is one component that is essential for business success in quail farm. The birds quality improvement can be done through the selection of body weight and egg production followed by a planned mating. Unlike the divergent selection is the selection of the positive and negative direction can be made for the purpose of improvement of the genetic quality (Falconer and Mackay, 1996; Aggrey *et al*., 2003; Mehrgardi, 2012). Selection of livestock is an opportunities that have the best genes to the next generation of production quality that increases and decreases the frequency of the desired gene (Chang *et al*., 2001; Kinoshita *et al*., 2002). Individual values showed high phenotypic variation, because it is influenced by many pairs of genes, including the gene additive and non-additive (Aggrey *et al*., 2003; Balcioglu *et al*., 2005). The additive effects of genes change when the effect of the alleles of the same gene and no interactions between gene pairs (Warwick *et al*., 1995). Furthermore, the non-additive gene including dominant and epistasis, genes showing dominance when one gene of a pair of alleles close attendance and prevent the realization of the allele. Therefore, the aim of the present study was to determine the effect of GH Gene on divergent selection of body

Corresponding Author: S. Johari, Faculty of Animal Science and Agriculture, Diponegoro University, Semarang, Central Java, Indonesia

weight at four weeks and egg production at ten weeks for five generation of Japanese quail.

MATERIALS AND METHODS

Blood was collected from Divergent Selection based on body weight of Quail, Female birds grouped into 50 groups of high weight and 50 groups of low weight, likewise 20 birds of quail males grouped into 10 birds for high weight (Q-H), random weight (Q-R) and low weight (Q-L) as a treatment of divergent selection. Blood was taken through wing vein of 0.8 ml with syringe and put into tubes containing EDTA. Parameter phenotype observed in each generation is the Body Weight (BW) at four weeks of age and egg production or Hand Day Production (HDP) at ten weeks of age for five generations.

For the detection of Growth Hormone (GH) gene, electrophoretic separation of polymorphism was performed in chicken GH gene polymorphism research by Nei *et al.* (2002), GH (forward primer, 5'-ATCCCCAGGCAAACATCCTCGH-3' and reverse primer, 5'-CCTCGACATCCAGCTCACAT-3'). Restriction enzyme digestion, the enzyme PCR (Polymerase Chain Reaction) products were digested using RFLP (Restriction Fragment Length Polymorphism) method of Stephen *et al.* (2000) which restriction enzyme M*sp* 1 to detect the GH gene polymorhism, cut sequences were 5'.....C↓ CGG..... 3' and 3'.....GGC↓C..... 5' (Mulyadi, 1992).

Electrophoresis of PCR-RFLP, by using 1% of agarose (0.3 g agarose (Sigma) plus 30 ml TAE one time), then dissolved in the microwave and in warm plus 1 µl ethidium bromide (C21H2ON3Br2, 7-diamine-10-ethyl-9 pheme-henanthridinium-bromide), gel transferred to a tray that has been installed to create a sink. Gel inserted into the tank (Biorad), then poured TAE buffer until the gel is immersed. DNA samples as much 5 µl mixed with 1 µl of DNA loading buffer (blue juice) on parafilm and loaded into the gel wells carefully, on an electrophoresis system at 100 volts for 30 minutes. The results seen with UV and visualized with a Sony digital camera 6 mega pixel (Sulandari and Zein, 2003).

The weighting of genetic material, the genetic weights calculated are the population mean, dominance deviasion and gene effect. The absolute value of population mean calculated by a formula based on population means (Hardjosubroto, 1999), as follows:

$$\bar{M} = M + PM \quad (1)$$

where, $\bar{M}$ = Absolute mean, M = mean and PM = population mean:

$$PM = a(p-q) + 2pqd \quad (2)$$

where, a = genetic weight, p = frequency of gene A, q = frequency of gen B and d = level of dominance.

The GH gene polymorphism were used to analyses the effect of GH gene on Divergent Selection of Quail. The GH Gene effect was the deviation from the population mean which received one gene from a parent while the other genes of the parent which randomly selected. The GH gene effect was determined by calculating a gene from all genes of effect (Pichner, 1981 and Hardjosubroto, 1999). The GH Gene effect of each gene were calculated as described by Hardjosubroto (1999):

$$\alpha = \alpha_1 - \alpha_2 = a + d(q-p) \quad (3)$$

$$\alpha_1 = q[a + d(q-p)] \quad (4)$$

$$\alpha_2 = -p[a + d(q-p)] \quad (5)$$

where, α = The gene effect, α_1 = The effect of gene A and α_2 = The effect of gene B.

Genetic variation within the population was quantified by measuring the average heterozygosity, $\bar{H}$. The average heterozygosity was estimated from the expected proportion of heterozygosity per locus, using the following formula (Nei, 1978):

$$\bar{H} = \frac{1 - \sum_{i=1}^{m} X_i^2}{r} \quad (6)$$

where, X_i is the frequency of the allele at locus, m is the number of allele and r is the number of locus.

RESULTS AND DISCUSSION

GH gene polymorphisms: DNA fragment length was determined by comparing the DNA fragment samples with standard 1000 kb marker. Each DNA fragment profiles were analyzed to see the position of each restriction changes that occur. The results showed that three of the banding pattern in quail GH gene electrophoresis. GH gene polymorphisms are indicated by the results of digestion of PCR product size 776 bp to 3 profiles were AA genotype sized 536 bp and 237 bp, BB genotype sized 776 bp and AB genotype sized 776 bp, 536 bp and 237 bp (Fig. 1 and 2). DNA fragment length was determined by comparing the DNA fragment samples with standard 1000 kb marker. Each DNA fragment profiles were analyzed to see the position of each restriction changes.

Studies on the GH gene polymorphism of chicken using Restriction Fragment Length Polymorphism (RFLP) showed that the GH gene had very high polymorphisms in the intron area and alleles identified for selection abdominal fat (Fotouhi *et al.*, 1993). While the results by Stephen *et al.* (2000) stated that the PCR products obtained from genomic DNA chicken in China along the 776 bp, anyway by PCR-RFLP profiles obtained 6 pieces in intron 1, allegedly due to the success of amplification

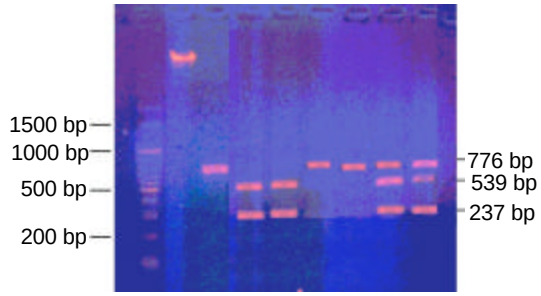

Fig. 1: The results of growth hormone gene pattern of divergent selection of Japanese quail by using restriction enzymes *Msp*1 and PCR-RFLP method; 1: DNA Genome, 2: PCR Product, 3-4: Genotype AA, 5-6: Genotype BB, 7-8: Genotype AB

Enzyme *Msp*1

Pattern-**I**

539	↓	237	A
539		237	A

↑

Pattern-**II**

539	↓	237	A
	776		B

Pattern-**III**

	776		B
	776		B

Fig. 2: Pattern pieces of genomic DNA restriction results by PCR-RFLP

is excellent and is probably due to the GH gene sequences chicken has a high similarity with GH gene sequences on quail but it is also the chicken and quail are included in one family. RFLP can indirectly serve as a genetic marker when connected with QTL, hence determining the contribution (direct/as a marker) RFLP at traits for the production of the selection map is very important.

Dominance level and gene effect of GH: The absolute mean and dominance level on body weight and egg production in divergent selection of quail is presented in Table 1. Results showed that the estimation of the gene effect of GH was polymorphic in the population, it was known that absolute mean of body weight for the low weight (Q-L), the random (Q-R) and the high weight (Q-H) were 57.137, 73.219 and 89.147 g, respectively. While the absolute mean of egg production or Hand Day Production (HDP) for the low weight (Q-L), the random (Q-R) and the high weight (Q-H) were 80.447, 87.165 and 50.598%, respectively.

It was known that the body weight and egg production of genotypes AA, AB and BB were the low weight (Q-L) for incomplete dominance (-0.76), the random (Q-R) for over dominance (3.60) and the high weight (Q-H) for lack of dominance (0.11), respectively. Meanwhile the dominance level of HDP was -6.67, 0.35 and 7.15, respectively.

Dominance deviation is the average effect of the gene, often said to be an additive genes effect and construct only GH gene. The result showed dominance when heterosygote AB genotype had a value greater than the mean between the two homozygous AA and BB of the gene pair. The calculations showed a low weight means was incomplete dominance and had a value less than one in which the genotype AB was superior than average but not as good as the two homozygous genotypes of homozygous genes showing dominance in the egg production. Through the dominance of the heterozygote was superior to both homozygotes. The role of the two pairs of additive genes with each gene added on a certain amount of weight and egg production traits.

The gene effect of GH on body weight and egg production in divergent selection of quail (Table 2). The effect of GH gene was 0.817 and 0.821, 0.101 and 0.313, 1.249 and 1.255 for Q-L, Q-R and Q-H, respectively. The gene effect of GH on body weight of Q-H is 1.53 times greater than the Q-L and 12.37 times greater when compared with Q-R. Whereas the gene effect of GH on egg production of Q-H is 1.53 times greater than the Q-L but only 4 times greater when compared with Q-R. The gene effect of GH on body weight and egg production properties of divergent selection arranged by one or two pairs of factors. Size weight from generation to generation for 5 generations variability change were not significant or was not extreme as the parent body weight that mechanism is not the same as the qualitative traits. The genetic variation of genes affected by GH genes A and B influenced by environmental factors working together that is difficult to control homogeneity. Many pairs of genes for hereditary factors that affect its weight will be difficult to determine how much of each gene play an important role.

The gene effect on average equal to the average deviation in the population that are additive with each gene that is adding a certain amount at a traits showed dominance, or there are interactions between pairs of gene A and gene B. Based on Table 2, showed that genes A and B affect on weight and production equal to

Table 1: The absolute mean and dominance level on body weight (BW) and egg production (HDP) in divergent selection of quail

			Absolute mean $(\overline{M})$		Dominance level (d/a)	
Population/groups	Genotype	n	BW (g)	HDP (%)	BW	HDP
Q-L :	AA	9	58.444	83.444		
	AB	9	56.889	78.222		
	BB	12	56.889	82.083		
			$\overline{M}$: 57.137	$\overline{M}$: 80.447	-0.76	-6.67
Q-R :	AA	38	72.000	90.250		
	AB	45	75.400	87.03		
	BB	17	69.400	80.34		
			$\overline{M}$: 73.219	$\overline{M}$: 87.165	3.60	0.35
Q-H :	AA	17	89.941	57.765		
	AB	9	88.778	40.111		
	BB	6	87.333	63.50		
			$\overline{M}$: 89.147	$\overline{M}$: 50.598	0.11	7.15

BW: Body weight at 4 weeks of age, HDP: Hand Day Production at 10 weeks of age

Table 2: The gene effect of growth hormone (GH) on body weight and egg production in divergent selection of quail

		GH-gene effect		
Genetic weight	Population/ groups	A Gene (α_1)	B Gene (α_2)	GH Gene (α)
BW:	Q-L	0.449	-0.368	0.817
	Q-R	0.040	-0.061	0.101
	Q-H	0.382	-0.867	1.249
		BV=AA = $2\alpha_1 = 2q\alpha$	BV=BB = $2\alpha_2 = -2p\alpha$	$\alpha = \alpha_1-\alpha_2$
HDP:	Q-L	0.452	-0.369	0.821
	Q-R	0.123	-0.189	0.313
	Q-H	0.411	-0.843	1.255
		BV=AA = $2\alpha_1 = 2q\alpha$	BV=BB = $2\alpha_2 = -2p\alpha$	$\alpha = \alpha_1-\alpha_2$

BW: Body weight at 4 weeks of age, HDP: Hand Day Production at 10 weeks of age, BV: Breeding value

the breeding valaue of Q-L, Q-R and Q-H respectively. According to Warwick *et al.* (1995) that the average effect of each gene are difficult to measure but the breeding value of an individual can be measured and equal to twice the average deviation of the offspring to the population mean.

The results of divergent selection on body weight, egg production and GH gene polymorphism detection in the population suggests that the low weight at 4 weeks of age were decreased and high weight has increased from generation to generation. The selection on growth in Quail, long-term divergent selection resulted in asymmetry of response in the low and high lines (Aggrey *et al.*, 2003). In addition, different genes may respond differently to the same selection pressure in opposite directions.

Heritability estimates of genetic variation were moderate to high often occurs at low weight and high weight (Sezer, 2007; Mehrgardi, 2012). Based on the GH gene polymorphism on divergent selection of Q-L groups shows role of 0.817 and 0.821 were lower than the Q-R groups and the high weight. GH gene in a population of Q-H were 1.249 and 1.255 which means that the selection process is over dominance obtained on quail with high body weight and egg production.

Divergent selection results that the body weight and egg production up to 5 generations of female quail obtained with genotype AA, AB and BB which showed that the GH gene couldn't control weight and egg production. The estimation of the genetic variation based on GH gene polymorphism with AA and AB genotypes in groups of high (Q-H) and low weight (Q-L) with a higher value than the AA and BB genotypes, suggesting that weight and egg production derived from parent to offspring is influenced by genetic factors of GH gene.

In the group of high weight, low and control by various additives can be known the potential heredity of genes are relatively higher on the AA genotype compared to AB and BB genotypes. The body weight of quail with AA genotypes derived in offspring are additive because it has a positive value while the egg production with genotype BB is a non additive because it has a negative value.

Heterozygosity of GH gene: The results showed the difference in average heterozygosity in the divergent selection for body weight and egg production of quail (Table 3). The average heterozygosity of the highest found in the Q-L was 0.495, followed by Q-R and Q-H, was 0.478 and 0.441 respectively. Warwick *et al.* (1995) and Chang *et al.* (2007) stated that heterozygosity is a measure of gene diversity and reflects the genetic variance of populations at polymorphic site. Furthermore, Chang *et al.* (2007) assessed the genetic variation and relationship of domestic quail and two wild quail species using 400 quails specific microsatellite

Table 3: The heterozygosity of GH gene on body weight and egg production in divergent selection of quail

		Quail		
		Q-L (n = 30)	Q-R (n = 100)	Q-H (n = 32)
Genotype:	AA	9	38	17
	AB	9	45	9
	BB	12	17	6
Frequency:	GH-A	0.45	0.60	0.67
	GH-B	0.55	0.40	0.33
Average heterozygosity $(\bar{H})$	0.495	0.478	0.441	0.471

markers and founded that the value for PIC and $\bar{H}$ were the highest, was 0.573 and 0.662, respectively.

The results (Table 3) indicated that heterozygosity in divergent selection population of quail was high at these sites. While more, Liu *et al.* (2006) indicated that there were positive correlations between microsatellite heterozygosity and body weight in Chinese native chicken. The average value of heterozygosity was higher when compared with the average heterozygosity reported by Okabayashi *et al.* (1999) that the average value of heterozygosity the local ducks in Asia, were 0.098-0.179 and Mahfudz *et al.* (2011) reported that the average heterozygosity of kedu chickens was 0.187-0.442. This may imply that the blood protein polymorphism can be used to identify the genetic variation, to analysis the difference of blood protein polymorphism in three breeds of Indonesian local chickens and ducks (Johari *et al.*, 2008; Mahfudz *et al.*, 2011).

Conclusions: It can be concluded that the dominance level on body weight of the Q-L is incomplete dominance, Q-R is over dominance and Q-H is lack of dominance. While the dominance level on the egg production of Q-L and Q-H are over dominance and Q-R is lack of dominance. The gene effect of GH on body weight of Q-H is 1.53 times greater than the Q-L and 12.37 times greater when compared with Q-R. Whereas the gene effect of GH on egg production of Q-H is 1.53 times greater than the Q-L but only 4 times greater when compared with Q-R. Should be developed that to increase the low-weight (Q-L) is in the BB genotype and the high weight (Q-H) is in the AA genotype groups. Otherwise, to increase the low-weight (Q-L) and high HDP are in the AA and BB genotypes and the high weight (Q-H) and low HDP are in the AA and AB genotypes groups. The body weight and egg production was not significantly different on effect of GH gene. The gene effect of GH on body weight and egg production has potential to determine the breeding development program of quail.

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