

Effect of divergent selection body weight to egg production during the six generation and GH gene polymorphism quail (*Coturnix coturnix japonica*)¹

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ABSTRACT: The research aimed to study the effect of divergent selection four weeks of age body weight to the egg production of quail eggs (*Coturnix coturnix japonica*) and GH gene polymorphism was done for six generations. Selection descendants for six generations done the same phenotype in each generation were divergent for high and low body weight. Identification of GH gene polymorphism in 72 male quail samples selected for six generations and 100 female quail samples as a control group at the end of the study. Data of body weight and egg production from generation to generation factor analysis of variance based, high and low weight with replication based on group of males. The results showed that the difference between high and low due to divergent selection were significantly different from earlier generations ($P < 0.05$) higher weight groups where egg production was lower compared with the low weight group. Effect of divergent selection on egg production was obtained from the value of genetic correlation and phenotypic correlation between body weight with egg production results negatively correlated. GH gene polymorphism effected on the characteristics of body weight at 100 male quail AA genotype control group of 38 (38%), genotypes AB 45 (45%) and BB genotypes in 17 (17%). The selection group of samples 72, genotypes AA 30 (41.6%), genotypes AB 22 (30.5%) and genotypes BB 20 (27.7%). Polymorphic genes GH was known from the acquisition of different fragment sizes among genotype AA was sized 539 bp and 237 bp with an average of 79 grams weight and grain egg production 10, genotype AB was sized 776 bp, 539 bp and 237 bp with an average weight 73 grams and egg production 14 and BB was sized 776 bp with an average of 65 grams weight and grain egg production 17.

Key words: body weight, GH gene, polymorphism, egg reproduction, divergent selection

INTRODUCTION

The ability of quail in generating growth and reproductive characteristics during certain periods are vary greatly due to the diversity of quail has a fairly high among individuals. The nature of growth and reproduction are generally influenced by the hormone called *Growth Hormone* (GH), steroids and peptides. In an effort to increase the weight and nature of quail egg production, one of the effort is to conduct continuous quail selection by implementing a planned marriage arrangement pattern followed by the action of selection to choose superior males and females as a source of genetic material for the next generation. In the last decade the role of biotechnology is increasingly growing in supporting the development of selection that can be improved with the use of molecular techniques through DNA material. The result of preliminary research has been done by Setiati (2007) GH gene polymorphism using PCR-RFLP method can be found on quail populations. Other research results about the selection that has been done, still based on morphological markers and less efficient utilization of information obtained is still less accurate and require an expensive cost. Progress in biotechnology has made it possible to get other markings other than morphological markers, ie protein and DNA markers. Fotouhi *et al.* (1993); Feng *et al.* (1997) and Dunn *et al.* (2003) in his research on the selection of chicken based on genetic markers of growth hormone receptor gene (GH gene) can speed up the selection. Many candidate genes have been known as a genetic marker that can be immediately known phenotype expression in chickens as leptin gene studied by Friedman *et al.* (1999), gene growth (*growth hormone*) by Kuhlein *et al.* (1997), prolactin gene by Shimada *et al.* (1991) and

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other genes associated with reproduction and production properties but there has been no research on GH gene polymorphism in quail that can affect weight and egg production.

MATERIALS AND METHODS

Research materials in the form of 100 male and female quail males performed 20 tail for divergent selection of high weight and low weight with between five females mated with one male. To identify gene polymorphisms GH samples come from the record results of quail body weight during the six generations of divergent selection that consists of 72 male quail populations selected females and 100 females aged four weeks as a control population. Quail kept in battery cages four-story. Food that is provided during the research there are two types of starting age of 4 weeks to 6 weeks fed BR1, age 7 weeks to 10 weeks of laying quail fed Comfeedbrand. Traits analyzed were age four-week weight females (BW4F) and male (BW4M) and egg production until the age of 10 weeks (Pr.T10). Data were analyzed based on the factor variance-generation, high and low weight with replication based on group of males. Recording done for six generations and each generation is determined five male quail high weight and low weight of five fish to be collected blood samples.

DNA isolated from blood samples quail, GH gene was amplified with the primer pair GH Forward 5'-ATCCCCAGGCAAACATCCTC-3 and GH Reverse 5'-CCTCGACATCCAGCTCACAT-3'. PCR performed with the program beginning predenaturasi conditions for five minutes at a temperature of 95 ° C, denaturation for 30 seconds at a temperature of 94 ° C, annealing at 56 ° C for 45 minutes, elongation at 72 ° C for 1 minutes, 35 cycles, extension at 72 ° C for 5 minutes. PCR products were digested with enzymes *MspI*. Polymorphism detection was conducted in two stages: (a) the identification of genotypes of GH gene and (b) calculate allele frequencies were found to know the relationship between divergent selection body weight on the performances of the production with the resulting genotypes.

RESULTS AND DISCUSSION

Effect of divergent selection weight for quail egg production for six generations to the criteria of high and low body weight gave a response that varies from generation to generation. Based on the measured properties ie weight females and males aged four weeks to give tangible effect to the accretion and weight loss from initial generation to generation six (Table 1).

The increase and decrease in body weight changes in gene frequency was believed to occur leading to improvement and weight loss due to selection. As a result of divergent selection of high body weight during the six generations of offspring obtained higher body weigh, on the contrary due to selection of low body weight of offspring obtained by low body weigh as well. This situation is consistent with Macha and Becker (1976) that the body weight of quail can be enhanced and reduced by holding a selection of high weight and low weight selection.

By looking at the development of body weight, age four weeks quail from generation to generation among the groups illustrates that each generation has the ability to grow was likely to increase and decrease. Increased body weight between male and female quail age of four weeks for six generations showed differences, namely average weight of female quail was relatively bigger than the male quail was intermittent weight of earlier generations for females 78.60 ± 3.71 g and 95.05 ± 5.62 a six-generation g, whereas the weight on the early generations of male quail 61.59 ± 3.02 g and 85.41 ± 3.16 a six-generation gateways. Besides weight was influenced by genes inherited from the elders to their children also by the environment that is sufficiently large. Genes can not be seen from the outside but his expression which was outside the influence can be seen. Gene expression in the form of outside influence was called the phenotype of a gene. Phenotype as all biological manifestations, including the chemical structure and behavior of individuals inherit from their parents and will be passed on to subsequent generations (Hardjosubroto, 1998).

Male superiority and the parent will be passed on to the offspring through a planned marriage that resulted in increased animal productivity is populated. Selection is based on recording only the interpretation of quantitative traits in livestock because it is almost impossible genotip determined

with certainty. This leads to the result of selection bias based on the recording qualitative and quantitative traits of cattle. Differences in growth between the female and males were due to genetic

Table 1. Effect of divergent selection weight age of four weeks for six generations

| Generation | Divergent selection body weight four week of age | | | | | | | |
|------------|--|---------------------------------------|-----|--|----------|--------------------------------------|----|-------------------------|
| | BW4F (g) | | | | BW4M (g) | | | |
| | n | HBW | n | LBW | n | HBW | n | LBW |
| 0 | 181 | 78.60 ± 3.71 ^d (4.72%) | 178 | 62.58 ± 2.85 ^c (4.565%) | 69 | 61.59 ± 3.02 ^{NS} (4.9%) | 72 | 59.22 ± 2.58 (4.36%) |
| 1 | 183 | 80.99 ± 3.11 ^c (3.845%) | 180 | 61.01 ± 2.50 ^c (4.10%) | 67 | 70.42 ± 2.64 (3.75%) | 70 | 61.01 ± 1.95 (3.20%) |
| 2 | 188 | 4.47 ± 83.14 ^c (5.38%) | 181 | 59.72 ± 2.74 ^{fe} (4.59%) | 62 | 71.79 ± 5.18 (7.22%) | 69 | 59.04 ± 2.92 (4.95%) |
| 3 | 184 | 88.70 ± 3.60 ^b (4.05%) | 180 | 57.58 ± 2.53 ^{fe} (4.39%) | 66 | 79.03 ± 3.41 (4.31%) | 70 | 57.30 ± 2.32 (4.05%) |
| 4 | 182 | 92.60 ± 3.78 ^{ab} (4.08%) | 185 | 56.87 ± 3.73 ^{fe} (10.56%) | 68 | 80.04 ± 3.12 (3.40%) | 65 | 56.65 ± 3.28 (5.79%) |
| 5 | 179 | 95.62 ± 2.78 ^{ab} (1.05%) | 184 | 54.98 ± 4.70 ^f (8.54%) | 71 | 82.21 ± 3.06 (3.72%) | 66 | 55.27 ± 3.39 (6.13%) |
| 6 | 186 | 95.05 ± 5.62 ^a (5.9%) | 189 | 53.69 ± 2.71 ^f (5.05%) | 64 | 85.41 ± 3.16 (3.70%) | 61 | 48.57 ± 2.29 (4.71%) |

BW4F : body weight four-week of age female

BW4M : body weight four weeks of age male

HBW : high body weight

LBW : low weight

Different superscript letters in the same column indicate significant differences

NS : not significantly different

factors which have a female quail estrogen affects Synthesis in the Oviduct development in preparation for laying eggs, the hormone progesterone with androgens regulate growth hormone secretion Synthesis in the Oviduct for albumin from magnum (Yuwanta, 2004), thus affecting body weight of female weight compared with male. Testicular development was influenced by hormones gonadotropins, namely FSH and JH was secreted from the anterior pituitary. Male quail testes produce androgen hormones that function to affect sperm production during spermatogenesis in the tubuli seminiferi, interstitial of Leydig cells.

Effect of divergent selection of weight during the six generations of quail egg production until the age of 10 weeks.

Production of eggs until the age of 10 weeks can be seen in Table 2. Results for six generations of divergent selection showed an increase and decrease in egg weight and production quite varied.

The age of 10 weeks of egg production was the number of eggs produced until the age of 10 weeks. In this study, egg production from selection of high weight age of four weeks on from generation to generation has decreased by the value of 11, 90 ± 2.4 to 9,74 ± 2.23, while its low weight selection four weeks of age increased as the value of 16.96 ± 3.86 to 24,11 ± 2.00. The results were consistent with the results of research Kuswahyuni (1989) that there was a positive correlation between the first egg weight with age was first quail egg-laying. Doran *et al.* (1973) suggested that egg production was influenced by genetic factors. Influence of parent age on egg production were observed for three generations of quail by Woodard *et al.* (1973) and obtained the result that the rate of egg production will decline sharply after the parent was 26 weeks old. Woodard *et al.* (1973) suggests that egg production can reach 250 to 300 rounds each year with a weight between 8 to 12 grams.

Based on the results of direct divergent selection weight and egg production of indirect selection for six generations still needed to be developed or researched more about the genetic markers that can ensure the quail as individual as the elders in order to obtain superior genetic quality of offspring a better and faster process. The selection was continuously can obtain high quality seeds to the purpose, but require a long time and cost expensive.

Table 2. Production of eggs until the age of 10 weeks (E.Pr-10) results for six generations of divergent selection

| Generation | Divergent selection body weight four week of age | |
|------------|--|--------------------------------------|
| | (E.Pr-10) | |
| | HBW | LBW |
| 0 | 11,90 ± 2,84 ^f (2,39%) | 16,96 ± 3,86 ^d (2,28%) |
| 1 | 10,28 ± 2,09 ^f (2,03%) | 17,92 ± 2,48 ^d (1,38%) |
| 2 | 9,53 ± 2,89 ^f (3,03%) | 19,88 ± 2,45 ^c (6,26%) |
| 3 | 10,76 ± 2,47 ^f (2,30%) | 20,57 ± 2,56 ^c (1,24%) |
| 4 | 9,65 ± 2,69 ^{ef} (2,79%) | 22,30 ± 2,59 ^b (1,16%) |
| 5 | 10,19 ± 1,65 ^{ef} (1,62%) | 23,01 ± 2,23 ^b (9,70%) |
| 6 | 9,74 ± 2,23 ^c (2,29%) | 24,11 ± 2,00 ^a (8,30%) |

E.Pr-10 : egg production until the age of 10 weeks

HBW : high body weight

LBW : low body weight

abcdef : superscript in the same column indicate differences

This question will be attempted to be further investigated using genetic approaches, which were expected to obtain for the acceleration of the selection marker. Selection of the molecular become a topic of interest to try, because the selection response will be faster and more accurate. The use of gene marker candidates will be selected as appropriate because it has a *blue print* for the genome research hash accuracy guaranteed and were registered in the database *Assession number* DNA bank. Therefore if the determination of the gene-controlled methodology, applications to produce seeds with molecular selection with the desired selection criteria will be more easily achieved.

GH Gene Polymorphism Detection

Digestion results showed the existence of polymorphism in GH gene fragment at 776 bp-sized quail research was to produce two kinds of alleles (A and B). Electrophoresis of each sample was compared to the DNA marker bands. Polymorphisms GH gene was indicated by a single band: 776 bp (genotype BB), two bands: 539 bp and 237 bp (genotype AA); three bands: 776 bp, 539bp and 237 bp (genotype AB).

Based on the calculation of genotype frequencies obtained from research quail AA genotype selection group numbered 30 (41.6%) samples, AB 22 (30.5%) samples and 20 (27.7%) samples genotype BB. From the results of the calculation of allele frequencies of genes derived GH was known that GH gene was polymorphic in quail research. GH gene was polymorphic effects on body weight as a material quail selected research studies to assess the nature of growth. Unselected

group of quail were found to have GH gene was more polymorphic than the control group. Weight divergent selection results provide significant effect on body weight four weeks of age and have the phenotype correlation (-0.886) and (-0.779) in the group selected in the control group. Genetic correlation was also obtained negative values of weight loss with a quail's egg production means low body weigh can produce lots of eggs, whereas low body weigh quail that can produce eggs slightly.

Table 3. Body weight and egg production based on the genotype groups in the quail number of genotypes within selection and control populations

| Genotype | Band numb | Population Selection | | | Population Control | | |
|----------|---------------|----------------------|-------------------|----------------------------|--------------------|--------------------|----------------------------|
| | | N | \bar{X} BW4 (g) | \bar{X} E.Pr-10 (no/hen) | N | \bar{X} BW4 (g) | \bar{X} E.Pr-10 (no/hen) |
| AA | 237; 539 | 30 (41,6%) | 79 ^{ab} | 10 ^a | 38 (38%) | 72,0 ^{ns} | 15,2 ^{ns} |
| AB | 776; 237; 539 | 22 (30,5%) | 73 ^a | 14 ^{ab} | 45 (45%) | 75,4 | 13,3 |
| BB | 776 | 20 (27,7%) | 65 ^a | 17 ^b | 17 (17%) | 69,4 | 16,0 |
| Jumlah | | 72 (100%) | | | 100 (100%) | | |

Description : Different superscript letters indicate significant differences
^{ns} : Non significantly

GH gene polymorphism may be found in the group of high weight quail (HBW) and low (LBW), the result of six generations of divergent selection. In this group found two alleles was allele A and allele B, three GH gene genotypes AA, AB and BB. Of the three alleles were dominated by the AA allele (high weight). Results of detection by PCR-RFLP and enzyme F *SPI* that the GH gene was found polymorphic in quail weight selection. AA genotype was known as much as 30 individuals (41.6%), genotype AB 22 rats (30.5%) and genotype BB 20 males (27.7%). Polymorphic genes of the acquisition of GH was known fragment sizes different between genotypes AA-size 536 bp and 237 bp with an average of 79 grams weight and egg production 10, genotype AB was sized 776 bp, 536 bp and 237 bp with an average weight 73 grams and 14 eggs and egg production of BB sized 776 bp with an average 65 grams weight and grain egg production 17. Indicated significant differences between the genotype quail AA with BB but not significantly different between genotype AA with AB and AB.

CONCLUSIONS

The conclusions from the research showed that the GH gene in quail (*Coturnix coturnix japonica*) was polymorphic. Effect of divergent selection body weigh four week age during six generation was obtained from the value of genetic correlation and phenotypic correlation between body weight with egg production was negatively correlated.

LITERATURE CITED

- Anonimus. 1969. *Coturnix (Coturnix coturnix japonica)* Standard and Guidelines for the Breeding, Care and Management of Laboratory Animals. National Academy of Sciences, Washington.
- Dunn, IC, YV Miao, A. Morris, MN Romanov, PW Wilson and D. Waddington. 2004. A Study of Association Between Genetic markers in candidate genes and reproductive traits in one generation of a commercial broiler breeder hen population. *Heredity* 92: 128-134.
- Feng XP, U. Kuhnlein, RW Fairfull, SEAggrey, J. Yao and D. Zadworny. 1998. A Genetic Marker in the Growth Hormone Receptor Gene Associated with Body Weight in Chicken. *J. Heredity* 89: 355-359.
- Fotouhi N., CNKaratzas, U. Kuhlein and D. Zadworny. 1993. Identification of growth hormone DNA polymorfisme Which response to divergent selection for abdominal in Chickens. *Theor. Appl. Genet.* 85 (1993) 931-936.
- Garret, RL, LZ Mc Farland and CE Franti. 1972. Characteristic of Selected Produced

- by Japanese Quail Egg. *Poultry Sci.* 31: 1370-1376.
- ME Friedman et al, 1999. The chicken leptin gene: Has it been cloned?. *Gen Comp Endocrinology* 115:354-363.
- Hardjosubroto, W., 1994. Pemuliabiakan Livestock Application in the Field, Faculty of Animal Husbandry, Gadjah Mada University, Yogyakarta.
- Kuhnlein U., L. Ni, S. Weigend, JSGavora, W. Fairfull and D.Zadwony.1997. DNA polymorphism in the chicken growth hormone gene: response to selection for Disease Resistance and association with egg production. *Animal Genetic* 28: 116-123.
- Kuswahyuni, IS 1989. Short-Term Selection Response Body Weight Age Four appearances Sunday on Production and Reproduction Quail Bird. Dissertation. Faculty of Graduate IPB, Bogor.
- Macha, AM and WA Becker.1976. Comparison of Predicted with Actual Body Weight Gain Selection of *Coturnix coturnix japonica*. *Theoretical and Appl. Genetica* 47: 251-255.
- Shimada K, Ishida H, Sato K, Seo H, Matsui N, 1991. Expression of prolactin gene in incubating-hens. *J Reprod fertile* 91 (1): 147-154.
- Setiati, Ning. 2007. GH gene polymorphism in quail using PCR-RFLP. UNNES Research Report Semarang. (Not published).
- Woodard, AE, H. Abplanalp, WO Wilson and P. Vohra. 1973. Japanese Quail Husbandary in the Laboratory (*Coturnix coturnix japonica*) Department of Avian Sciences, University -of California, Davis.
- Yuwanta, Tri.2004. *Poultry Association*. Publisher Canisius. Yogyakarta.