ISSN 1682-8356
ansinet.org/ijps

INTERNATIONAL JOURNAL OF

POULTRY SCIENCE

ANSInet
308 Lasani Town, Sargodha Road, Faisalabad - Pakistan
Mob: +92 300 3008585, Fax: +92 41 8815544
E-mail: editorijps@gmail.com

OPEN ACCESS International Journal of Poultry Science

ISSN: 1682-8356
DOI: 10.3923/ijps.2017.203.208

Research Article
Polymorphisms of Growth Hormone Gene Exon 1 and their Associations with Body Weight in Pitalah and Kumbang Janti Ducks

Yurnalis, Husmaini and Sabrina

Faculty of Animal Husbandry, Andalas University, West Sumatera, Indonesia

Abstract

Objective: The objective of this study was to determine Growth Hormone (GH) gene polymorphisms and their association with productive traits, including body weight, at ages 1, 2, 3, 4, 5, 6, 7 and 8 weeks. **Methodology:** Polymorphisms in exon 1 of the *GH* gene were evaluated in two duck populations in West Sumatra Province Indonesia (Pitalah and Kumbang Janti ducks). For this purpose, blood samples were collected and DNA samples were extracted using the Promega Wizard® Genomic DNA Purification Kit. For this purpose, a total 225 ducks blood samples were collected from 145 male and 80 female ducks. Genetic polymorphisms were determined with the Polymerase Chain Reaction-Restriction Fragment Length Polymorphism (PCR-RFLP) method using the *Eco*721 restriction enzyme and agarose gel electrophoresis. Direct sequencing of some samples was used to confirm the results. **Results:** Two alleles (GHG and GHA) and three genotypes (GH/GG, GH/GA and GH/AA) were found in the studied duck samples at locus GH/*Eco*721. In both groups of ducks, the dominant allele was GHG. The most frequent genotype in the examined ducks was GH/GA. Three genotypes were observed in the Pitalah ducks, whereas two genotypes (GH/GA and GH/GG) were identified in the Kumbang Janti ducks and in the males. Pitalah ducks with the GH/GA genotype were characterized by a higher ($p<0.01$) body weight than the ducks with the GH/GG and GH/AA genotypes. This same trend was observed in the female Pitalah ducks; individuals with the GH/GA genotype had higher body weights ($p<0.05$ and $p<0.01$) than the birds with the two other detected genotypes. Kumbang Janti ducks with the GH/TT genotype were distinguished by higher values of all evaluated traits compared to the ducks with the GH/CT and GH/CC genotypes; however, most of the recorded differences were not significant. The only trait that was markedly impacted ($p<0.05$) by the polymorphism of *GH* gene intron 1 was the body weight at 5, 6, 7 and 8 weeks. **Conclusion:** This study found that the GH/TT genotype was associated with a higher body weight at 5, 6, 7 and 8 weeks of age in Pitalah and Kumbang Janti ducks.

Key words: Pitalah ducks, Kumbang Janti ducks, GH gene, genetic polymorphism, body weight

Received: December 23, 2016 Accepted: February 14, 2017 Published: April 15, 2017

Citation: Yurnalis, Husmaini and Sabrina, 2017. Polymorphisms of growth hormone gene exon 1 and their associations with body weight in pitalah and kumbang janti ducks. Int. J. Poult. Sci., 16: 203-208.

Corresponding Author: Yurnalis, Faculty of Animal Husbandry, Andalas University, Padang, Sumatera Barat, Indonesia
Tel/Fax: +62 0751 71464/+62 0751 71464

Competing Interest: The authors have declared that no competing interest exists.

Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

Advances in the molecular genetics of livestock animals have led to the identification of genes or markers associated with genes that influence growth, carcass and meat quality traits and reproductive features[1,2]. The molecular bases of these characteristics are being revealed by functional genomics methods and are providing opportunities for the enhancement of genetic improvement programs in farm animals through Marker-Assisted Selection (MAS)[3].

Several genes have been used as candidate genes for marker-assisted selection for improved productive and reproductive performances of animals. One of these genes is the Growth Hormone gene (GH)[4]. The expressed product of the *GH* gene is the protein Growth Hormone (GH) (also called somatotropin), which is a member of the growth hormone/prolactin family produced in specific cells (somatotrophs) of the pituitary gland[5]. This gene has many physiological functions, such as promoting muscle growth[6], bone growth and development[7] and regulation of fat content[8] and metabolism[9]. Additionally, GH plays important roles in the innate and acquired immune systems. The GH has been shown to affect the proliferation of lymphoid cells, the activity of phagocytic cells, thymulin excretion and the growth of the thymus[10]. Studies in animals have indicated that GH is involved in the sexual differentiation and pubertal maturation processes and participates in gonadal steroidogenesis, gametogenesis and ovulation[11]. In birds, GH has an important function in growth but is also involved in a variety of secondary functions, including egg production, aging and reproduction[12].

Due to its functional importance, the *GH* gene has been studied in a wide range of species. The genomic structure of this gene has been examined in fish[13], rats[14], mice[15], bovines[16,17], sheep[18], pigs[19] and humans[20]. In birds, GH cDNA clones have been isolated and sequenced from chickens[21], turkeys[22] and ducks[23]. The genomic sequence of the avian *GH* gene was first reported in chickens[24].

In all mammals, the *GH* gene extends over 2-3 kb and comprises five exons split by four introns[25]. The duck *GH* gene is 5.25 kb in size, consists of five exons and four introns and is structurally similar to the mammalian and chicken *GH* genes[26]. Furthermore, the *GH* gene is highly polymorphic in a variety of livestock animals. Many polymorphisms have been identified in the *GH* genes from pigs[27], bovines[17], goats[28] and poultry[29-31]. In ducks, the effect of *GH* gene polymorphisms on important economic traits has been noted[32,33]. Moreover, a study conducted on ducks by Hiyama *et al.*[34] suggested that variations in the GH promoter might influence the laying performance by changing the GH mRNA expression levels in the anterior pituitary gland.

The objectives of this study were to estimate the allele and genotype frequencies of *GH/Eco*721 polymorphisms in Pitalah and Kumbang Janti ducks. Additionally, we investigated the possible associations of duck growth hormone gene polymorphisms with body weight to identify a potential marker for use as a complementary parameter in the selection of ducks.

MATERIALS AND METHODS

Animals: The experiment was conducted in the Faculty of Animal Husbandry Andalas University, Indonesia. A total of 225 samples were used to genotype the *GH* gene. The ducks used for this study were two Indonesian native breeds (the Pitalah duck and the Kumbang Janti duck from Payakumbuh district, West Sumatra, Indonesia). The ducks used consisted of 145 Pitalah ducks (100 males and 45 females) and 80 Kumbang Janti ducks (45 males and 35 females). During the trial, the ducks were fed complete commercial diets *ad libitum* according to age as follows: A starter diet (from the 1st day to 3rd week of age) containing 20.0% CP and 11.7 MJ of Metabolizable Energy (ME) and a grower/finisher diet (from the 4th week of age to the end of the experiment) containing 18.5% CP and 12.2 MJ of ME. The ducks were weighed at ages 1, 2, 3, 4, 5, 6, 7 and 8 weeks.

Detection of polymorphisms in exon 1 of the *GH* gene: Blood samples were collected from the wing vein of each individual in tubes containing EDTA as an anticoagulant. The blood samples were stored at -20°C prior to DNA extraction. Genomic DNA was extracted from whole blood using the Wizard® Genomic DNA Purification Kit, Promega, Madison, USA. Genomic DNA from each duck was stored at -20°C prior to the allelic discrimination assays.

The GH genotypes were analyzed using the RFLP-PCR method. The 801 bp *GH* gene PCR product was amplified using the Master Mix from Thermo Scientific. The PCR conditions included 25 µL of the Master Mix, 2 µL (20 ng) of genomic DNA, 1.5 µL (15 nM) of each primer (forward primer 5′-CTG GAG CAG GCA GGA AAA TT-3′ and reverse primer 5′-TCC AGG GAC AGT GA AC-3′) and 20 µL of nuclease-free water. The following cycles were applied: Denaturation for 5 min at 94°C, followed by 40 cycles of 45 sec at 94°C, annealing for 45 sec at 60°C and extension for 60 sec at 72°C and a final extension for 5 min at 72°C. The PCR product

(consisting of 801 bp) was digested with the *Eco*721 restriction enzyme for 4 h at 37°C. The digested fragments were separated on 2% agarose gels. The genotypes were identified against the molecular marker O' Gene Ruler Low Range DNA Ladder (Thermo Scientific).

Statistical analysis: The genotype and allele frequencies were calculated in each group of ducks. The data used to compare the effects of GH gene polymorphisms on duck body weight were tested with a model that included the effect of each genotype at the *GH/Eco*721 locus. The genetic effects of the GH gene polymorphisms on body weight were analyzed using a General Linear Model (GLM) procedure[35]. The model used the Eq. 1:

$$Y_{ijk} = \mu+G_i+D_j+S_k+g_{ijk} \quad (1)$$

where, Y_{ijkl} is the observed value of the dependent variable, μ is the overall mean, G_i is the fixed effect due to genotype *GH/Eco*721 (i = GH/CC, GH/CT or GH/TT), D_j is the fixed effect due to duck origin (j = Pitalah ducks or Kumbang Janti ducks), S_k is the fixed effect due to gender (k = males or females) and g_{ijkl} is the random residual error.

The Hardy-Weinberg equilibrium was assessed with the Chi-square test. The statistical significance of differences among the means was calculated in accordance with the SAS/STAT Software, Release 6.12 (SAS Institute Inc., Cary, NC, USA).

RESULTS

The allele frequencies of *GH* gene exon 1 in the two duck populations are listed in Table 1. The dominant allele of exon 1 of the duck *GH* gene was GHG for the two duck populations. No marked differences were observed between the frequencies of the GHG and GHA alleles in the Pitalah and Kumbang Janti ducks. The allelic distributions of the *GH/Eco*721 polymorphic sites in the Kumbang Janti duck populations followed a similar pattern. As a result of digestion of an 801 bp target region of the duck *GH* gene exon 1 by the *Eco*721 enzyme, the samples with an 801 bp fragment (uncut) were accepted as the GH/GG genotype, the samples with 801, 564 and 237 bp fragments were accepted as GH/GA and the samples with 564 and 237 bp fragments were accepted as the GH/AA genotype (Fig. 1). The genotype distributions of the *GH* gene in the two studied duck populations are presented in Table 2. The most frequent genotype in the examined duck groups was GH/GA. The highest degree of genetic polymorphism for the duck *GH* gene intron 1 was found in the Pitalah ducks. The genotype frequencies of the GH/*Eco*721 locus in this duck population were not in Hardy-Weinberg equilibrium ($p>0.05$).

Regarding the polymorphisms, 2 genotypes and 2 alleles were distinguished according to their restriction fragment lengths as follows: 801 bp (A allele) and 564 and 237 bp (G allele). The genotypes and alleles of the *GH* gene are shown in Fig. 1.

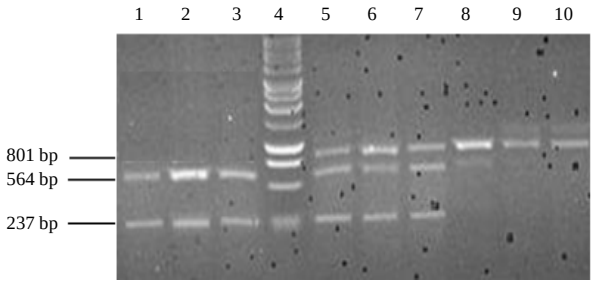

Fig. 1: *GH/Eco*721 genotype identification
Lane 1, 2 and 3: Genotype GH/GG, Lane 4: Molecular weight marker 250, 500, 750, 1000 bp, Lane 5, 6 and 7: Genotype GH/GA-801, 564, 237 bp, Lanes 8, 9 and 10: Genotype GH/AA-801 bp)

Table 1: Allele frequencies at the exon 1 locus of the *GH* gene

	Number of genotypes						
	Observed			Expected			
Duck populations	GH/GG	GH/GA	GH/AA	GH/GG	GH/GA	GH/AA	p-value
Pitalah duck							
Male	91 (0.91)	6 (0.06)	3 (0.03)	88.36 (0.88)	11.28 (0,11)	0.36 (0.01)	>0.05
Female	40 (0.89)	4 (0.09)	1 (0.02)	38.93 (0.90)	2.93 (0.07)	0.22 (0.03)	>0.05
Both	131 (0.90)	10 (0.07)	4 (0.03)	128.12 (0.88)	16.36 (0.11)	0.52 (0.01)	>0.05
Kumbang Janti duck							
Male	38 (0.84)	5 (0.11)	2 (0.04)	36.45 (0.81)	8.1 (0.18)	0.45 (0.01)	>0.05
Female	30 (0.86)	3 (0.09)	2 (0.06)	28.35 (0.81)	6.3 (0.18)	0.35 (0.01)	>0.05
Both	68 (0.85)	8 (0.10)	4 (0.05)	64.8 (0.81)	14.4 (0.18)	0.8 (0.01)	>0.05

Table 2: Association of *GH* gene polymorphisms in exon 1 with body weights at age 1-8 weeks in Pitalah ducks

	Body weight (g) at age (weeks)							
Genotypes	1	2	3	4	5	6	7	8
GH/GG	46.60±4.33	106.30±20.71	190.5±60.4	320.4±125.9	492.50±150.1	655.5±125.5	728.5±150.1	800.5±167.1
GH/GA	46.44±4.17	105.92±20.54	170.5±65.4	300.5±120.5	415.23±120.2	525.3±100.5	600.2±120.2	650.4±129.1
GH/AA	46.50±4.71	106.01±20.01	180.4±64.2	305.9±110.5	430.5±100.9	550.4±103.2	650.7±122.4	700.9±104.5
p value	NS	NS	NS	<0.05	<0.01	<0.01	<0.01	<0.01

NS: Non-significant

Table 3: Association of *GH* gene polymorphisms in exon 1 with body weights at age 1-8 weeks in Kumbang Janti ducks

	Body weight (g) at age (weeks)							
Genotypes	1	2	3	4	5	6	7	8
GH/GG	55.55±3.93	115.70±22.69	195.5±63.5	319.3±115.8	450.4±140.2	740.5±180.5	770.5±145.2	810.9±161.1
GH/GA	53.44±4.27	105.88±21.64	175.5±64.3	305.5±121.5	400.3±120.2	600.5±100.5	650.2±122.2	670.4±129.1
GH/AA	54.50±4.81	108.01±20.01	182.4±63.5	308.9±121.6	410.5±105.9	610.4±103.2	650.9±109.4	705.9±104.5
p value	NS	NS	NS	NS	<0.05	<0.01	<0.01	<0.01

NS: Non-significant

The results of the analysis of the associations between the *GH/Eco*721 polymorphisms and body weights in the Pitalah ducks are summarized in Table 2.

The association between different genotypes and body weights in the Pitalah ducks show no significant associations for 1, 2 and 3 weeks of age, significant associations for 4 weeks of age and highly significant association for 5, 6, 7 and 8 weeks of age ($p<0.01$). The ducks with the GH/GG genotype had the highest body weights, followed by the GG/AA and GH/GA genotypes.

The results of the analysis of associations between the *GH/Eco*721 polymorphisms and body weights in the Kumbang Janti ducks are summarized in Table 3.

The association between different genotypes and body weights showed similar results to those obtained for the Pitalah ducks. No significant association was found for 1, 2, 3 and 4 weeks of age, a significant association was found for 5 weeks of age and a highly significant association was found for 6, 7 and 8 weeks of age ($p<0.01$). The ducks with the GH/GG genotype had the highest body weights, followed by the GG/AA and GH/GA genotypes.

DISCUSSION

In the present study, polymorphisms of exon 1 of the duck GH gene were examined. Previous studies showed that polymorphisms of the avian *GH* gene could be identified not only at exon 1 but also in other regions. Polymorphisms in exonic regions of this gene were detected in ducks[1] and geese[31]. Additionally, polymorphisms in the intronic regions of the avian *GH* gene were found in chickens at intron 1[30], in ducks at intron 2[32], in geese[36] and chickens[8] at intron 3 and in chickens at intron 4[37]. There is no information in the literature concerning the identification of *Eco*721 polymorphisms in the first exon of the duck *GH* gene. However, we can compare the frequencies of the *GH* gene alleles and genotypes presented herein with the results described by Wu *et al.*[32]. The allelic frequencies reported in the above mentioned study, which was conducted in three duck populations, differed from the results of the present study. Wu *et al.*[32] reported that usage of the *Bsm*FI restriction enzyme in all duck populations enabled the detection of three genotypes. The observed differences may result from the origin of the experimental animals. Productive performances of poultry are affected by quantitative traits that can be influenced by many environmental factors and genes, such as the growth hormone gene. The *GH* gene polymorphisms have been studied in various poultry species, including chickens[8], quail[38], geese[36] and ducks[33]. In these poultry species, a high degree of polymorphism has been detected in the DNA sequence of the *GH* gene. Chang *et al.*[1] found 19 SNPs in a 2087 base pair (bp) region in the duck *GH* gene. This study indicated that each SNP was associated with at least one duck fertility-related trait. However, available literature shows that some SNPs in the duck *GH* gene also affect growth and carcass traits. The effect of the avian growth hormone gene on the above mentioned characteristics was demonstrated by Wu *et al.*[32], who first discovered the *Bsm*FI polymorphism in the second intron of the duck *GH* gene. This Chinese research group tested three different breeds of duck (Cherry Valley, Muscovy and Jingjiang) slaughtered after 56 days of life. Considering the body weights of the ducks on the day of slaughter, the results of the present study partially confirm the observations of Wu *et al.*[32]. The authors noted that in one of the evaluated duck breeds (Jingjiang), individuals with genotype GH/TT were heavier than individuals with the GH/CT and GH/CC genotypes. However, findings of the present study regarding two other breeds were not in agreement with the findings described by Wu *et al.*[32]. In the Cherry Valley and Muscovy groups, the heavier birds had the GH/CT genotype[32].

Due to a lack of any comparable results concerning the effect of the *GH* gene on body measurements in ducks, verification of our findings based on the results reported in previous studies is hampered. However, the results of present study showed that individuals with the GH/TT genotype displayed higher values of most of the assessed features compared to ducks of other genetic groups, which indicated that the *GH* gene might be a candidate marker for biometric traits in ducks. In conclusion, the highest degree of polymorphism in the first exon of the *GH* gene was observed in the Pitalah and Kumbang Janti ducks. The results of this study regarding the *GH/Eco*721 genotype showed a significant influence on BW.

SIGNIFICANCE STATEMENT

This study is the first attempt to explore the *GH* gene in Pitalah and Kumbang Janti ducks. This study discovered a new polymorphism (*GH/Eco*721) in intron 1 of the *GH* gene that was associated with body weight in Pitalah and Kumbang Janti ducks. These results can be beneficial for genetically assisted selection to improve these breeds. These findings are important for poultry farmers and policy makers when designing selection strategies for improving duck production and to ensure a protein supply for the general public.

ACKNOWLEDGMENTS

This study was funded and supported by the Directorate General of Higher Education of Ministry of Research, Technology and Higher Education through the Hibah Bersaing 2016 scheme with contract No. 01/H.16/HB/LPPM/2016. We also thank the Dean of the Faculty of Animal Science Andalas University, for housing support.

REFERENCES

1. Chang, M.T., Y.S. Cheng and M.C. Huang, 2012. The SNP genotypes of growth hormone gene associated with reproductive traits in Tsaiya ducks. Reprod. Domestic Anim., 47: 568-573.
2. Bhattacharya, T.K. and R.N. Chatterjee, 2013. Polymorphism of the myostatin gene and its association with growth traits in chicken. Poult. Sci., 92: 910-915.
3. Gao, Y., R. Zhang, X. Hu and N. Li, 2007. Application of genomic technologies to the improvement of meat quality of farm animals. Meat. Sci., 77: 36-45.
4. Supakorn, C. and W. Pralomkarn, 2013. Genetic polymorphisms of growth hormone (GH), insulin-like growth factor 1 (IGF-1) and diacylglycerol acyltransferase 2 (DGAT-2) genes and their effect on birth weight and weaning weight in goats. Philipp. Agric. Scient., 96: 18-25.
5. Wallis, M., 1988. Mechanism of Action of Growth Hormone. In: Hormones and Their Actions. Part 2: Specific Action of Protein Hormones, Cooke, B.A., R.J.B. King and H.J. Van Der Molen (Eds.). Elsevier, Amsterdam, New York, Oxford, pp: 265-272.
6. Ge, X., J. Yu and H. Jiang, 2012. Growth hormone stimulates protein synthesis in bovine skeletal muscle cells without altering insulin-like growth factor-I mRNA expression. J. Anim. Sci., 90: 1126-1133.
7. Ohlsson, C., B.A. Bengtsson, O.G. Isaksson, T.T. Andreassen and M.C. Slootweg, 1998. Growth hormone and bone. Endocr. Rev., 19: 55-79.
8. Zhang, X.L., X. Jiang, Y.P. Liu, H.R. Du and Q. Zhu, 2007. Identification of Ava II polymorphisms in the third intron of GH gene and their associations with abdominal fat in chickens. Poult. Sci., 86: 1079-1083.
9. Bauman, D.E., 1999. Somatotropin mechanism in lactating cows: From basic science to commercial application. Domest. Anim. Endocrinol., 17: 101-116.
10. Gala, R.R., 1991. Prolactin and growth hormone in the regulation of the immune system. Proc. Soc. Exp. Biol. Med., 198: 513-527.
11. Hull, K.L. and S. Harvey, 2001. Growth hormone: Roles in female reproduction. J. Endocrinol., 168: 1-23.
12. Kansaku, N., A. Nakada, H. Okabayashi, D. Guemene, U. Kuhnlein, D. Zadworny and K. Shimada, 2003. DNA polymorphism in the chicken growth hormone gene: Association with egg production. Anim. Sci. J., 74: 243-244.
13. Du, S.J., R.H. Devlin and C.L. Hew, 1993. Genomic structure of growth hormone genes in chinook salmon (*Oncorhynchus tshawytscha*): Presence of two functional genes, GH-I and GH-II, and a male-specific pseudogene, GH-Ψ. DNA Cell Biol., 12: 739-751.
14. Barta, A., R.I. Richards, J.D. Baxter and J. Shine, 1981. Primary structure and evolution of rat growth hormone gene. Proc. Nat. Acad. Sci., 78: 4867-4871.
15. Das, P., L. Meyer, H.M. Seyfert, G. Brockmann and M. Schwerin, 1996. Structure of the growth hormone-encoding gene and its promoter in mice. Gene, 169: 209-213.
16. Woychik, R.P., S.A. Camper, R.H. Lyons, S. Horowitz and E.C. Goodwin *et al*., 1982. Cloning and nucleotide sequencing of the bovine growth hormone gene. Nucl. Acids Res., 10: 7197-7210.
17. Yurnalis, Y., S. Sarbaini, A. Arnim, J. Jamsari and W. Nellen, 2013. Identification of single nucleotide polymorphism of growth hormone gene exon 4 and intron 4 in pesisir cattle, local cattle breeds in west sumatera province of Indonesia. Afr. J. Biotechnol., 12: 249-252.

18. Byrne, C.R., B.W. Wilson and K.A. Ward, 1987. The isolation and characterisation of the ovine growth hormone gene. Aust. J. Biol. Sci., 40: 459-470.
19. Vize, P.D. and J.R. Wells, 1987. Isolation and characterization of the porcine growth hormone gene. Gene, 55: 339-344.
20. Fiddes, J.C., P.H. Seeburg, F.M. DeNoto, R.A. Hallewell, J.D. Baxter and H.M. Goodman, 1979. Structure of genes for human growth hormone and chorionic somatomammotropin. Proc. Nat. Acad. Sci., 76: 4294-4298.
21. Lamb, I.C., D.M. Galehouse and D.N. Foster, 1988. Chicken growth hormone cDNA sequence. Nucl. Acids Res., 16: 9339-9339.
22. Foster, D.N., S.U. Kim, J.J. Enyeart and L.K. Foster, 1990. Nucleotide sequence of the complementary DNA for turkey growth hormone. Biochem. Biophys. Res. Comm., 173: 967-975.
23. Chen, H.T., F.M. Pan and W.C. Chang, 1988. Purification of duck growth hormone and cloning of the complementary DNA. Biochim. Biophys. Acta (BBA)-Gene Struct. Exp., 949: 247-251.
24. Tanaka, M., Y. Hosokawa, M. Watahiki and K. Nakashima, 1992. Structure of the chicken growth hormone-encoding gene and its promoter region. Genetics, 112: 235-239.
25. Li, J., X.Q. Ran and J.F. Wang, 2006. Identification and function of the growth hormone gene in Rongjiang pig of China. Sheng li xue bao:[Acta Physiol. Sinica], 58: 217-224.
26. Kansaku, N., A. Soma, S. Furukawa, G. Hiyama and H. Okabayashii *et al*., 2008. Sequence of the domestic duck (*Anas platyrhynchos*) growth hormone encoding gene and genetic variation in the promoter region. Anim. Sci. J., 79: 163-170.
27. Wenjun, W., H. Lusheng, G. Jun, D. NengShui, C. Kefei, R. Jun and L. Ming, 2003. Polymorphism of growth hormone gene in 12 pig breeds and its relationship with pig growth and carcass traits. Asian-Aust. J. Anim. Sci., 16: 161-164.
28. Malveiro, E., M. Pereira, P.X. Marques, I.C. Santos, C. Belo, R. Renaville and A. Cravador, 2001. Polymorphisms at the five exons of the growth hormone gene in the algarvia goat: Possible association with milk traits. Small Rumin. Res., 41: 163-170.
29. Xu, S.H., W.B. Bao, J. Huang, J.H. Cheng, J.T. Shu and G.H. Chen, 2007. Polymorphic analysis of intron 2 and 3 of growth hormone gene in duck. Hereditas, 29: 438-442, (In Chinese).
30. Ghelghachi, A.A., H.R. Seyedabadi and A. Lak, 2013. Association of growth hormone gene polymorphism with growth and fatness traits in Arian broilers. Int. J. Biosci., 3: 216-220.
31. Zhang, Y., Z. Zhu, Q. Xu and G. Chen, 2014. Association of polymorphisms of exon 2 of the growth hormone gene with production performance in huoyan goose. Int. J. Mol. Sci., 15: 670-683.
32. Wu, Y., A.L. Pan, J.S. Pi, Y.J. Pu, J.P. Du, Z.H. Liang and J. Shen, 2012. One novel SNP of growth hormone gene and its associations with growth and carcass traits in ducks. Mol. Biol. Rep., 39: 8027-8033.
33. Wu, X., M.J. Yan, S.Y. Lian, X.T. Liu and A. Li, 2014. *GH* gene polymorphisms and expression associated with egg laying in muscovy ducks (*Cairina moschata*). Hereditas, 15: 14-19.
34. Hiyama, G., H. Okabayashi, N. Kansaku and K. Tanaka, 2012. Genetic variation in the growth hormone promoter region of *Anas platyrhynchos*, a duck native to Myanmar. J. Poult. Sci., 49: 245-248.
35. SAS., 1996. SAS/STAT User's Guide, Software Release, Version 6.12. 1st Edn., SAS Institute Inc., Cary, NC., USA.
36. Zhao, W.M., R.X. Zhao, N. Qiao, Q. Xu and Z.Y. Huang *et al*., 2011. GH polymorphisms with growth traits in goose. J. Anim. Vet. Adv., 10: 692-697.
37. Nie, Q., C.Y. Ip, X. Zhang, F.C. Leung and G. Yang, 2002. New variations in intron 4 of growth hormone gene in Chinese native chickens. J. Hered., 93: 277-279.
38. Johari, S., N. Setiati, J.H.P. Sidadolog, T. Hartatik and T. Yuwanta, 2013. The gene effect of growth hormone on body weight and egg production in divergent selection for five generation of Japanese quail (*Coturnix coturnix japonica*). Int. J. Poult. Sci., 12: 489-494.