Identification of *Msp*I Polymorphism in the Forth Intron of Chicken Growth Hormone Gene and Their Associations with Growth Traits in Indonesia Native Chickens

MA Mu'in* and S Lumatauw

Faculty of Animal Science, Fishery and Marine Science, Papua State University
Jl. Gunung Salju, Amban, Manokwari 98314, West Papua, Indonesia
***Corresponding author email: muinunipa@gmail.com**

Abstract. The objective of this research was to identify *Msp*I polymorphism in the forth intron of chicken growth hormone (cGH) gene and their associations with growth traits in native Indonesian chickens. A total of 72 Indonesia native chickens were genotyped for a locus in the forth intron of cGH gene (cGH-I4/*Msp*I locus) by PCR-RFLP with *Msp*I restriction enzyme. The result showed two genotypes in this locus: AA and BB, with the frequency of 90.28% and 9.72%, respectively. Based on body weight average, B allele had a beneficial effect in increasing the live body weight. The result of General Linier Models analysis indicated that the polymorphism of this locus had significant association (P<0.05) with body weight at 4 months of age and so did the daily gain between 2 to 4 months of age. Therefore, these results suggest that there is a possibility of cGH-I4/*Msp*I locus acting as a genetic marker for growth traits of native Indonesian chickens, especially for body weight at 4 months and daily gain between 2 to 4 months of age.

Keywords: polymorphism, cGH gene, growth traits, Indonesia native chickens.

Abstrak. Tujuan dari penelitian ini adalah untuk mengidentifikasi polimorfisme MspI intron ke-4 pada gen hormon pertumbuhan ayam (cGH) dan hubungannya dengan sifat pertumbuhan pada ayam asli Indonesia. Sebanyak 72 ekor ayam asli Indonesia diidentifikasi gentotipenya untuk lokus pada intron ke-4 dari gen cGH (cGH-I4/MspI lokus) dengan PCR-RFLP mengguakan enzim restriksi MspI. Hasil penelitian menunjukkan adanya dua genotipe pada lokus ini: AA dan BB , dengan frekuensi masing-masing 90.28 % dan 9,72 %. Berdasarkan rata-rata bobot badan, alel B memiliki pengaruh yang menguntungkan dalam meningkatkan bobot badan hidup. Hasil analisis *General Linier Model* menunjukkan bahwa polimorfisme lokus ini memiliki hubungan yang nyata (P<0,05) dengan bobot badan pada umur 4 bulan dan begitu pula dengan pertambahan bobot badan harian antara umur 2 sampai 4 bulan. Oleh karena itu, hasil ini menunjukkan bahwa ada kemungkinan cGH-I4/MspI lokus bertindak sebagai penanda genetik untuk sifat pertumbuhan ayam asli Indonesia, terutama untuk bobot badan pada umur 4 bulan dan pertambahan bobot badan harian ayam antara umur 2 sampai 4 bulan.

Kata kunci: polimorfisme , gen cGH , sifat pertumbuhan , ayam asli Indonesia.

Introduction

Currently, technology of molecular biology has been used in many fields, including farm animals to determine chicken productivity improvement through a molecular approach. Gene mapping has resulted many discoveries of polymorphic loci in the chicken's genome. Single nucleotide polymorphism (SNP) in the chicken genom has been identified with a frequency of 1 SNP per 225 bp, which is 5 times in humans (Vignal et al., 2002). When a polymorphic locus is associated with the economic traits of animals, therefore that polymorphic loci can be used as genetic markers for the trait. Furthermore, that specific loci becomes useful as a selection criterion for genetic improvement of these properties.

The chickens growth hormone (cGH) is a polypeptide hormone, consisting of 216 amino acids (Tanaka et al., 1992). The cGH synthesized in and secreted by pituitary gland, is involved in a variety of physiological functions such as growth, body composition, egg production, ageing and reproduction (Stephen et al., 2001; Wardecka et al., 2005; Thakur et al., 2006;

Jafari et al., 2009). The cGH gene located on chromosome number 19 in avian (Stephen et al., 2001), and contains 5 exons and 4 introns with an overall length of 4.1 kb (Kansaku et al., 2008).

Some studies have found polymorphisms along the cGH gene and several polymorphisms correlated with the growth traits in some breeds of chickens (Stephen et al., 2001; Bingxue et al., 2003). Zhang et al. (2007) also found that AvaI polymorphism in intron 3 of the cGH gene in the chickens was significant associations with abdominal fat weight and abdominal fat percentage. Kuhnlein et al. (1997) analyzed 12 strains of non-inbred White Leghorn chickens using PCR-RFLP *Msp*I in intron 1, 3 and 4, and *Sac*I PCR-RFLP in intron 4, demonstrating that alleles in the intron were associated with egg production traits. Bingxue et al. (2003) found a *Msp*I polymorphism in intron 4 in the cross breed of chicken (broiler Star x Silky) associated with breast muscle weight.

Screening results of cGH genes from four groups of chickens found 46 single nucleotide polymorphisms (SNPs). Four of them (4 SNP) were found in the 5'UTR (untranslated region), 1 SNP in the 3'UTR, 5 SNPs in exon, and 36 SNPs the intron regions (Nie et al., 2002; 2005). It is also informed that some of them found to be significantly correlated with body weight, shank length and weight gain (0 to 4 months).

More recently, Lumatauw and Mu'in (2011) revealed non-significant (P>0.05) association of polymorphism locus in the intron 3 of cGH genes (cGH-I3/EcoRV locus) with body weight at age 1, 2, 3 and 4 months in native Indonesian chickens. This study aimed to detect single nucleotide polymorphisms in intron 4 of the cGH gene (cGH-I4/*Msp*I locus) using PCR-RFLP and its association with growth traits in native Indonesian chickens.

Materials and Methods

Data on body weight and DNA samples. Data of body weight at 1, 2, 3, and 4 months of age in 72 chickens and each of its genome DNA were used as materials in this study. Both of these materials were obtained from previous studies (Mu'in et al., 2009). In this study, genomic DNA were isolated from blood samples of native Indonesian chickens using phenol-chloroform extraction method (Sambrook et al., 1989). The genomic DNA samples were stored at -70^{0}C. DNA analysis was conducted at the Centre for Biotechnology, Gadjah Mada University, Yogyakarta from August to September 2010.

DNA amplification. Amplification of specific DNA fragments conducted in intron 4, size 713 bp, spanning from the 2479 base to 3192 of the chicken cGH gene (GenBank: D10484.1). The amplification process was performed using a pair of primers (forward: 5'-cgc-tct-gct-att-tct-ctt-ac-3' and reverse: 5'-atg-gaa-ccg-tgg-aat-gat-gg-3'). A total of 19 µl dH2O, 2 µl of DNA solution (± 50 ng) and a pair of specific primers, each with 2 µl (16 pmol) were inserted into a 0.2 ml tube of Ready-To-Go PCR Beads (Amersham Biosciences), mixed until homogeneous, then inserted into the PCR machine. PCR conditions was programmed as follows: initial denaturation 94^{o}C for 5 min, 30 cycles with each cycle, denaturation 94^{o}C for 60 sec, annealing 60°C 60 sec, extension 72^{o}C for 60 sec, and final extention 72^{o}C for 10 min (Bingxue et al., 2003).

PCR product digestion. PCR products (amplicons) obtained were digested with *Msp*I restriction enzyme (sequence: c|cgg). A total of 10 µl PCR product, 1 µl restriction *Msp*I enzyme, 1.5 µl buffer and 2.5 µl aquabides (dH_2O) were put in a 1.5 ml micro tube, and incubated at 37°C temperature for 2 hours.

The digests with the *Msp*I enzyme were electrophoresed through 1.5% agarose gel (contain GoldView Nucleic Acid Stain) in TBE buffer. The procedure: 5 µl digestion was mixed with 2 µl product loading buffer, and then inserted into the gel wells. The running gel was performed at 100 volts for ± 30 minutes. The electrophoresed gels were vizualized under ultraviolet light and photographed.

Genotypes identification. The Identification of genotypes procedure used in this study was adopted from Tanaka et al., 1992 as follows: AA genotypes were those shown by a DNA fragment (size 713 bp), genotypes AB were shown by the three DNA fragments (sizes 713, 519 and 194 bp), and BB genotypes were shown by two DNA fragments (sizes 519 and 194 bp).

Statistical analysis. Genotypic and allelic frequencies of cGH-I4/*Msp*I locus were calculated according to the formula of Nei and Kumar (2000), below:

$$X_{ii} = (n_{ii}/N) \times 100\%$$

$$X_i = (2n_{ii} + \sum_{i \neq j} n_{ij})/2N$$

where:

X_{ii} = ii^{th} genotype frequency; n_{ii} = number sample of ii genotype; n_{ij} = number sample of ij genotype; N = total sample; X_i= i^{th} allele frequency. If allele frequencies of the locus studied were not exceed 0.99 (Harris, 1994) means that locus was categorized polymorphic.

The associations between the *Msp*I genotypes and the growth traits were analyzed using *General Linier Models* (GLM) from *MINITAB Release 13.20 for Windows*. Analysis of the relationship between the polymorphic loci (GH-I4/*Msp*I) with the chicken growth traits (weight age of 1, 2, 3, and 4 months), and the body weight gain at the age of 1-2 months, the age of 1-3 months, age 1 - 4 months, age 2-3 months, the age of 2-4 months and the age of 3-4 months), were studied through the model: $Y_{ijk} = \mu + S_i + G_J + \varepsilon_{ijk}$ where: Y_{ijk} = specific weight age or weight gain during the period observation of a certain age were observed in this study on the type of sex (S) the i^{th} genotype (G) to j^{th}, and k^{th} replicates; μ = general mean; S_i = effect of the i^{th} sex; G_J = effect of the j^{th} genotype; ε_{ijk} = random error of sex (S) the i^{th} genotype (G) to-j, and the k^{th} test.

Results and Discussion

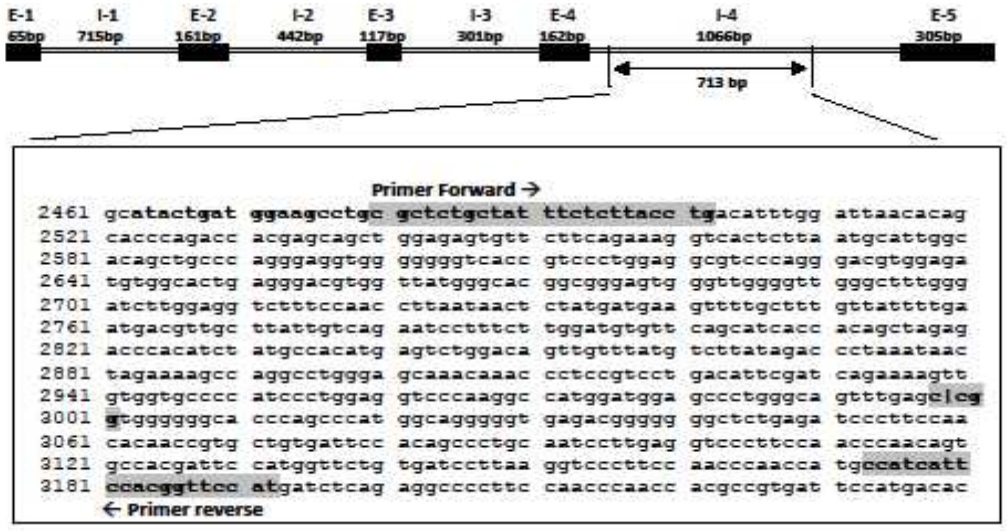
***Msp*I polymorphism.** Detection of single nucleotide polymorphisms in intron 4 in the chicken GH gene studied had been successfully performed using the PCR-RFLP/*Msp*I technique. In this study, the target DNA fragment size of 713 bp was located in intron 4 of the chicken GH gene, extending from the base 2479 to 3192 of the chicken GH gene (Figure 1). Previous studies indicated this DNA fragment contained mutation point. The mutation was caused by a substitution of cytosine nucleotide (c) with thymine nucleotide (t) on the 2998 base sequences of the chicken GH gene that could be detected by using *Msp*I (Bingxue et al., 2003).

Amplification of specific DNA fragment (713 bp) was successfully performed using the primer pair, ie forward: 5'-gtccgtgctcttctcttatc-3 'and reverse: 5'-gcgcaggcttccatcagtat-3' in PCR conditions as recommended by Bingxue et al. (2003) . The results of electrophoresis of PCR products obtained in this study (Figure 2) appeared as a clear single band, and occupied the appropriate position (sized 713 bp).

Digestion with *Msp*I to the PCR product (713 bp) obtained in this study resulted in two kinds of alleles, B allele (the two bands, 519 and 194 bp size) and A allele (single band sized 713 bp, and its position parallel to the PCR product). B allele of cGH-I4/*Msp*I locus was demonstrated by the success of the *Msp*I (5 '-c↓cgg-3') found DNA sequences that were recognized throughout the PCR products and success to cut them into two fragments sized 519 bp and 194 bp. In contrast, the A allele is indicated by the

failure of *Msp*I find a recognizable DNA sequences along the PCR products. As a result the size of the PCR products before and after digested remain the same, which is 713 bp. The *Msp*I failed to find DNA sequences that were recognized due to the-2998 nucleotide sequence (Figure 3) occupied by thymine nucleotide (t). The results were consistent with the previous research that had been reported by Tanaka et al. (1992) and Bingxue et al. (2003).

These results indicate that the frequency of alleles A and B of the GH-I4/*Msp*I loci studied in chicken were 0.9028 and 0.0972 respectivelly. This means that the A allele is a common allele. Thus the GH-I4/*Msp*I locus in chicken can be categorized as polymorphic loci, because the most common A allele frequency is not



Note: E-1: Exon-1; I-1: Intron-1; E-2: Exon-2; I-2: Intron-2; E-3: Exon-3; I-3: Intron-3; E-4: Exon-4; I-4: Intron-4; E-5: Exon-5.
ccgg: sekuen *Msp*I, restriction site: 5'-...c|cgg...-3'
cgctctgctatttctcttac: primer forward; ccatcattccacggttccat: primer reverse

Figure 1. The chickens growth hormone gene (exons and introns) and the sequence of DNA fragment in the forth intron of chicken growth hormone (size: 713 bp) extending from 2479 to 3192 nucleotides of chicken growth hormone (source: GenBank No.D10484.1) and restriction site at position 2998 (c to t).

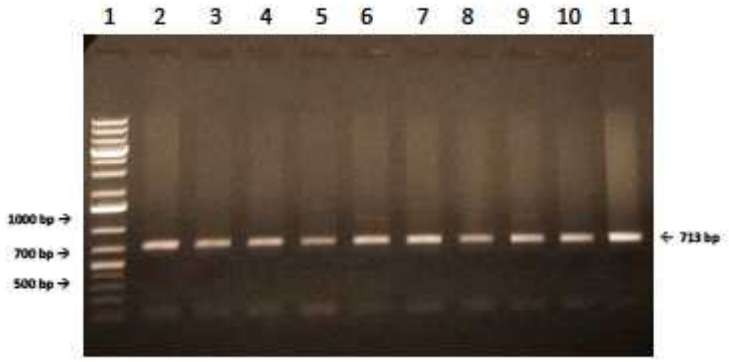


Figure 2. PCR product in the forth intron of Indonesia native chicken growth hormone gene analysed in a 1.5% agarose gel containing GoldView Nucleic Acid Stain. Lane 1: DNA marker, lanes 2 – 13: PCR product (713 bp)

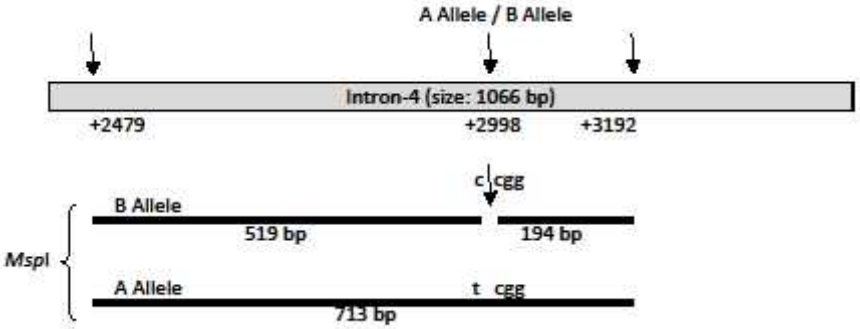


Figure 3. *Msp*I restriction pattern of cGH-I4 primer (forward and reverse primer) using 713 bp PCR product illustrated in Fig 1.

exceed 0.99 (Harris, 1994). Pipalia et al. (2003) found that A allele also showed higher frequency (0.8878) in Bantamised White Leghorn, while the allele frequencies of A dan B alleles were found intermediate in Bantam genetic groups, and in White Leghorn group showed lack of polymorphism with only A allele present.

*Msp*I genotypes found in this study were AA and BB, whereas AB genotype was not found. AA genotype was found more (65 chickens as compared to BB genotype (only 7 chickens). This is in contrast with the research results done by Bingxue et al. (2003) reporting three genotypes: AA (253 chickens), AB (71 chickens), and BB (9 chicken) in a population of hybrid chickens (broilers Star x Silky). These studies, however demonstrated that the AA genotype is a genotype commonly found in poultry populations. Figure 4 shows the genotypes of the GH-I4/*Msp*I locus were found in this study.

Association of *Msp*I polymorphism with the growth traits. Table 1 shows that the average body weight and the body weight gain of BB genotypes were higher than of the AA genotype at any age or period of observation. These indicates that the B allele has a positive effect on body weight and weight gain of Indonesia native chickens until the age of 4 months. The results of the analysis as presented in Table 1 in this study showed that the average body weight and weight gain of BB genotype is higher than of the AA genotype. Significant differences in body weight ($P < 0.05$) was found in chicken at the age of 4 months while the significant differences in weight gain was found only in the period of 2 to 4 months. In contrast, Pipalia et al. (2003) reported that non-significant effect of genotypes at this locus on body weight 8, 20, and 40 weeks of age in Bantam and Bantamised White Leghorn.

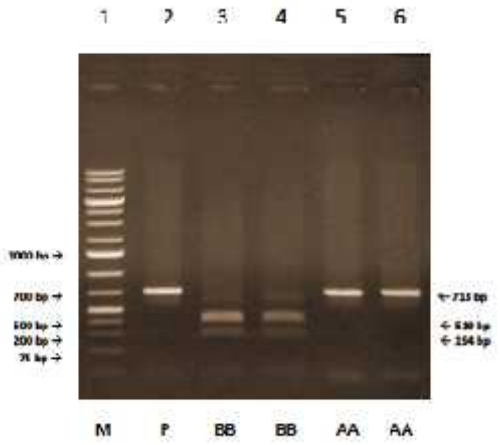


Figure 4. The PCR-RFLP electrophoresis product of chickens growth hormone locus (cGH-I4/*Msp*I locus) in Indonesia native chickens using *Msp*I enzyme. Lane 1: DNA markers (M), lane 2: PCR product, P (713 bp); lanes 3 dan 4: BB genotype (519 dan 194 bp); lanes 5 and 6: AA genotype (713 bp).

Table 1. Average body weight and weight gain (g) of Indonesia native chickens aged 1, 2, 3, and 4 months by sex and genotypes of the cGH-I4/*Msp*I locus

Traits	Genotypes		P
	AA (n=65)	BB (n=7)	
Body weight (1 month)	322.1± 61.3[a]	354.3± 52.6[a]	0.202
Body weight (2 months)	646.0±166.9[a]	721.4±139.7[a]	0.257
Body weight (3 months)	1038.5±311.7[a]	1247.1±260.7[a]	0.063
Body weight (4 months)	1474.9±452.1[a]	1793.0±365.0[b]	0.047
Weight gain (1-2 months)	323.8±130.7[a]	367.1±122.2[a]	0.439
Weight gain (1-3 months)	761.3±284.4[a]	892.9±259.0[a]	0.092
Weight gain (1-4 months)	1152.8±423.0[a]	1439.0±366.0[a]	0.060
Weight gain (2-3 months)	392.5±183.6[a]	525.7±227.0[a]	0.061
Weight gain (2-4 months)	828.9±331.4[a]	1071.0±300.0[b]	0.045
Weight gain (3-4 months)	436.5±187.6[a]	545.7±138.7[a]	0.133

P = probability; Different superscripts in the same row indicate significant differences (P <0.05).

Shahnaz et al. (2008) also found that frequency of RFLP patterns in this locus did not differ significantly for body weight at 20 and 36 weeks of age in three breed groups (Bantam, Bantamised White Leghorn, and White Leghorn birds). Instead of the body weight and weight gain, other researchers found that the B allele is positively associated with breast meat weight. The presence of B alleles in a genotype is more and more breast meat (Bingxue et al., 2003).

Conclusions

Two *Msp*I genotypes in intron 4 of cGH gene (locus cGH-I4/*Msp*I) were found in native Indonesian chicken: AA (90.28%) and BB (9.72%). A common allele is the one with the frequency reaching 0.90 in the population. B allele was positively related to the body weight and weight gain during the observation period. *Msp*I genotype showed a significant effect (P <0.05) on body weight at the age of 4 months and on the weight gain during the period of 2 to 4 months. Accordingly, BB genotype of the cGH-I4/*Msp*I locus can be used as a molecular marker for the age of 4 months of weight loss and weight gain at the growth period of 2 to 4 months.

Acknowledgement

This research was funded by the grant provided by of DP2M DIKTI, Ministry of Education and Culture, Indonesia through "DIPA Universitas Negeri Papua" (Contract: No: 247/H42/KU/2010, April 26, 2010).

References

Bingxue Y, D Xuemei, F Jing, H Xiaoxiang, W Changxin and L Ning. 2003. Single nucleotide polymorphism analysis in chicken growth hormone gene and its associations with growth and carcass Traits. Chinese Sci. Bulletin 48(15):1561-1564.

Harris H. 1994. Dasar-Dasar Genetika Biokemis Manusia. Edisi ketiga, diperbaharui Penerjemah: dr. Abdul Salam M. Sofro, Ph.D. Gadjah Mada University Press, Yogyakarta.

Jafari A, A Pakdel and KS Esmail. 2009. Study of polymorphism in the introns 1 and 4 chicken growth hormone gene in the native fowls of Isfahan and Mazandaran. Modern Genetics Fall 4(3):37-43.

Kansaku N, G Hiyama, T Sasanami and D Zadwomy. 2008. Prolactin and growth hormone in birds: Protein structure, gene structure and genetic variation. J. Poultry Sci. 45:1-6.

Kuhnl U, L Ni and S Weigend. 1997. DNA polymorphism in the chicken growth hormone gene: response to selection of disease resistance and association with egg production. Anim. Gen. 28:116-123.

Lumatauw S and MA Mu'in. 2011. Identifikasi polimorfisme EcoRV dalam intron 3 gen penyandi hormon pertumbuhan dan hubungannya dengan berat badan ayam buras. In: Proceeding of National Seminar on Zootechniques for Indogenous Resources Development. Semarang, 19-20 Oktober 2011. (ISBN: 978-602-097-243-5). Jointly published by Faculty of Animal Agriculture Diponegoro University, Indonesian Society of Animal Agriculture. p. 183-188.

Mu'in MA, A Supriyantono and HT Uhy. 2009. Polimorfisme gen Insulin-like growth factor-I (IGF-I) dan efeknya terhadap pertumbuhan ayam lokal. Jurnal Ilmu Ternak dan Veteriner 14(4):288-294.

Nei M and S Kumar. 2000. Molecular Evolution and Phylogenetics. Oxford University Press, New York.

Nie Q, SCY Ip, X Zhang, FC Leung and G Yang. 2002. New variations in Intron 4 of growth hormone gene in Chinese native chickens. http://jhered.oxfordjournals.org. Access: February 10, 2012.

Nie Q, B Sun, D Zhang, C Luo, NA Ishag, M Lei, G Yang and X Zhang. 2005. High diversity of the chicken growth hormone gene and effect on growth and carcass traits. J. Heredity. 96(6):698-703.

Pipalia DL, CG Joshi, K Khanna, DN Rank, KM Thakkar, BP Brahmkshtri and JY Solanki. 2003. Growth hormone gene polymorphism in Bantam, White Leghorn and Bantamised White Leghorn. Indian J. Poultry Sci. 38(3):206-211.

Sambrook J, Fritcsh and T Maniatis. 1989. Molecular Cloning Laboratory Manual. Second Edition, Vol. 3. Cold Spring Harbor Laboratory Press. USA.

Shahnaz S, F Shadma, DN Rank, K Khanna and CG Joshi. 2008. Growth hormone gene polymorphism and its correlation with different traits in Bantam and White Leghorn Chicken. Indian J. Poultry Sci. 43(2):123-127.

Stephen CY Ip, X Zhang and FC Leung. 2001. Genomic growth hormone gene polymorphism in native Chinese chickens. Experimental Biology and Medicine. 226:458-462.

Tanaka M, Y Hosokawa and M Watahiki. 1992. Structure of the chicken growth hormone-encoding gene and its promoter region. Gene. 112:235-239.

Thakur MS, SNS Parmar, TC Tolenkhomba, PN Srivastava, CG Joshi, DN Rank, JV Solanki and PVA Pillai. 2006. Growth hormone gene polymorphism in Kadaknath breed of poultry. Indian J. Biotechnol. 5:189-194.

Wardecka B, K Jaszczak, R Parada, M Korczak and G Zieba. 2005. Growth hormone gene polymorphism in chicken divergently selected for axial skeleton malformation. J. Anim. and Feed Sci. 14(3):561-570.

Vignal A, D Milan, M SanCristobal and A Eggen. 2002. A review on SNP and other types of molecular markers and their use in animal genetics. Genet. Sel. Evol. 34:275-305.

Zhang XL, X Jiang, YP Liuy, HR Du and Q Zhu. 2007. Identification of AvaI polymorphism in the third intron of GH gene and their associations with abdominal fat in chickens. Poul. Sci. 86:1079-1083.