



Polymorphism and sequence analysis of growth hormone gene and its relationship with body weight in meat type of chicken

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ABSTRACT

The growth hormone gene is a candidate gene controlling the metabolic and physiologic actions. Blood and feather samples were collected from 200 individuals of birds and genomic DNA was extracted using salting out technique. DNA fragment with 776 bp at growth hormone gene was amplified using polymerase chain reaction (PCR). The products from each strain (New Genotype, Punjabi Broiler-2, Indian Curnish-3 and University Male Line) were digested with *MspI*. In total, 4 RFLP pattern were observed. The genotypic frequencies obtained for different strains were tested using chi-square test. The differences among genotypes for all the 4 strains were significant. The overall allele frequency for A, B and C alleles were 0.495, 0.110 and 0.395 respectively. Correlation of cGH/*MspI* patterns with sixth week body weight was estimated. There were significant differences among 4 strains for body weight. Nucleotide sequencing of the amplified fragment of GH gene of 4 strains were done and submitted to the NCBI Genbank (accession nos JN403372 and JN403373). The nucleotide sequence analysis indicated that the per cent similarity of GH gene fragment of 4 strains were more than 99. In this study result also showed that there is a scope for using growth hormone gene as a candidate gene for growth traits.

Key words: Body weight, cGH gene, Polymorphism, PCR-RFLP, Sequencing

Growth hormone (GH), a protein-based polypeptide hormone, stimulates growth and cell reproduction in poultry and other animals. Growth hormone gene has 4098 base pairs with 5 exons and 4 introns in poultry (Lechniak *et al.* 1999). The chicken growth hormone gene is similar to mammalian growth hormone genes. However, the introns of cGH were significantly larger in chicken. Restriction fragment length polymorphism (RFLP) studies showed that the GH gene is highly polymorphic in the intron region (Fotouhi *et al.* 1993). The chicken growth hormone (cGH) gene is considered one of the most important candidate genes that can influence chicken performance traits because of its crucial function in growth and metabolism (Byatt *et al.* 1993, Vasilatos-Younken *et al.* 2000). It supports growth through enhanced formation of protein and nucleic acids (anabolic action) and of other constituents of lean body mass (Payne 1998). Polymorphism knowledge can be useful in phylogenetic analysis and in designing of breeding programmes (Bostein *et al.* 1980). A 50 bp deletion in intron 4 of the cGH gene was found in Chinese native Taihe Silkies chickens (Nie *et al.* 2002). Tanmankaur *et al.* (2008) showed that GH gene was highly

polymorphic in exon and intron regions and identified alleles that had significant association with body weight. Nie *et al.* (2005) found 46 SNPs and reported that, among other correlations, G + 1705A was significantly associated with body weight at all ages measured. The cGH gene in Yellow Wai Chow, has one silent substitution, 31 insertions, and other substitutions spread among the introns (Ip *et al.* 2001). In this study, we identified allelic patterns of growth hormone gene in 4 chicken strains by PCR-RFLP method and nucleotide sequencing of 4 strains of meat type chicken and conducted associations between growth hormone gene and chicken body weight.

MATERIALS AND METHODS

Experimental birds: Chickens (200) from 4 strains (50 from each strain) were used. Samples collected randomly from the 4 chicken genetic groups (New Genotype, Punjabi Broiler-1, Indian Cornish-3 and University Male Line) produced and maintained at poultry farm of the veterinary college. For this study, data were recorded for body weight at 6 weeks.

DNA extraction and DNA amplification: About 1 ml of venous blood was collected from vein of the wing of every bird and mixed with 37 µl anticoagulant and 50 µl of whole blood was transferred into 1.5 ml microcentrifuge tubes and

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basal tip of the calamus from the superior umbilicus were separated from each feather and processed for DNA isolation by phenol/chloroform method as described by Sambrook *et al.* (2001) and Horvath *et al.* (2005) from blood and feather samples respectively. DNA samples were checked for its quality and concentration. The PCR reaction conditions were approximately 100 ng of genomic DNA, 20 pmol of each primer, 200 μ M of each dNTP, 1 $\times$ PCR buffer [50 mM of KCl and Tris-HCl (pH 8.4)] and 3 U of Taq polymerase in a total volume of 25 μ l. The PCR was conducted on gradient thermalcycler, using a preliminary denaturation at 94°C for 5 min, 62°C for 1 min and 72°C for 10 s, 35 cycles of amplification were performed as followed: denaturation at 94°C for 40 s and a final extension at 72°C for 10 min. A 776 bp fragment of cGH consisting of exon-1 and partial intron-1 was amplified using forward (5'-ATCCCCAGGCAACATCCTC3') and reverse (5'-CCTCGACATCCAGGTC ACAT 3') primers (Kuhnlein *et al.* 1997). An aliquot of 10 μ l of PCR product was digested overnight with 3 Units of *MspI* restriction enzyme. The restriction enzyme digested PCR products were electrophoresed in 2.2% agarose gel containing ethidium bromide as staining agent in 1 TBE buffer for 60–75 min at 100V. The digested products were visualized and documented under gel documentation system. The polymorphisms are detected by PCR-RFLP, and then confirmed by sequencing. The PCR products amplified by primer are defined as genotype AA, BB, CC and AC respectively.

For sequencing, the PCR products were run in 0.8% agarose gel and the products band were eluted for purification. The purified PCR products were sequenced using automated dye-terminator cycle sequencing method with Ampli Taq DNA polymorphism in ABI PRIZM 377 DNA sequencer. The sequences analyses were performed using DNASTAR programme.

Statistical analysis: The gene and genotype frequencies were estimated by applying standard procedure (Falconer and Mackey 1996).

The genotype frequencies were compared by Chi-square test. Association between genotypes and body weights was tested using the ANOVA procedures of SAS version 8.1 with the following model: $Y_{ij} = \mu + BW_6 + M_j + e_{ij}$, where Y_{ij} is the dependent variable, μ is the population mean, e_{ij} is the random error and M_j is the fixed effect of j^{th} genotype ($j = 1$ (AA), 2 (CC), 3 (AC), 4 (BB)). Significant differences between least squares means of the different genotypes were calculated using a contrast test (p value).

RESULTS AND DISCUSSION

The fragment of cGH gene (776 bp) was characterized and successfully amplified from the DNA of each sample (200 samples) used in this study (Fig. 1). Digestion of 776 bp target region of GH gene exon- 1 was carried out with *MspI* endonuclease enzyme. Digestion of the PCR product

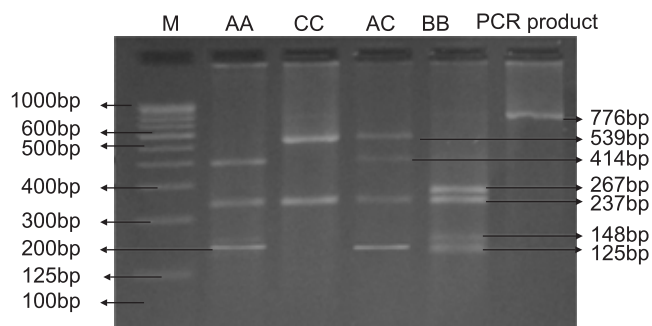


Fig. 1. PCR-RFLP pattern of GH locus by *MspI* restriction enzyme. M, Molecular marker (100bp); AA, NG, PB1; CC, PB1, IC3; BB, IC3, UM1; AC, NG, IC3.

Table 1. Genotype frequencies of GH/*MspI* polymorphism in four strains of chicken

Genotype	Strain				Overall	χ^2 value (df=3)	Result
	NG	PB1	IC3	UM1			
AA	0.46	0.38	0	0.30	0.28	60.71	S
BB	0	0	0.20	0.24	0.11		
CC	0.16	0.26	0.32	0	0.18		
AC	0.38	0.36	0.48	0.46	0.42		

S, significant.

with *MspI* restriction endonuclease which has C[^]CGG as its restriction site revealed four different patterns (AA, BB, CC and AC) created by a combination of polymorphic *MspI* cut sites present in exon-1 (one site) and in intron-1 (2 sites). The presence of restriction sites both at exon-1 and intron-1 region for *MspI*, resulted in AA genotype and resolved three fragments of sizes 125, 237 and 414 bp in NG, PB1 and UM1 strains (57 birds). BB genotype was created by all the three *MspI* cut sites with fragments of sizes 267, 237, 147 and 125 bp in IC3 and UM1 strains (22 birds), however, due to the absence of the restriction site in intron 1, the CC genotype was formed which had only 2 fragments of sizes 539 and 237 bp in NG, PB1 and IC3 strains (37 birds) (Fig.1). The birds with all 4 fragments (125,237,414 and 539 bp) were designated as AC genotype and observed in 4 strains (84 birds) (Fig. 1). The BB genotype observed in this study was observed by Thanmankaur *et al.* (2008) in Punjab Broiler 2 strain. The overall genotype and allele frequency of the GH/*MspI* locus ranged from 0.11 to 0.42 and 0.110 to 0.495 respectively. The highest genotype frequency was observed in IC3 for AC (0.48) genotype (Table 1). Lowest frequency was observed in NG strain (0.16) for CC genotype. The genotypes AA, BB and CC were not found in IC3, NG and PB1 and UM1 strains respectively. Singh *et al.* (2008) revealed high frequency of AC and CC genotype, and genotype AA was absent in pure layer chicken. Kuhnlein *et al.* (1997) observed BB genotype with least frequency as also observed by Singh *et al.* (2008). Chi-square test revealed

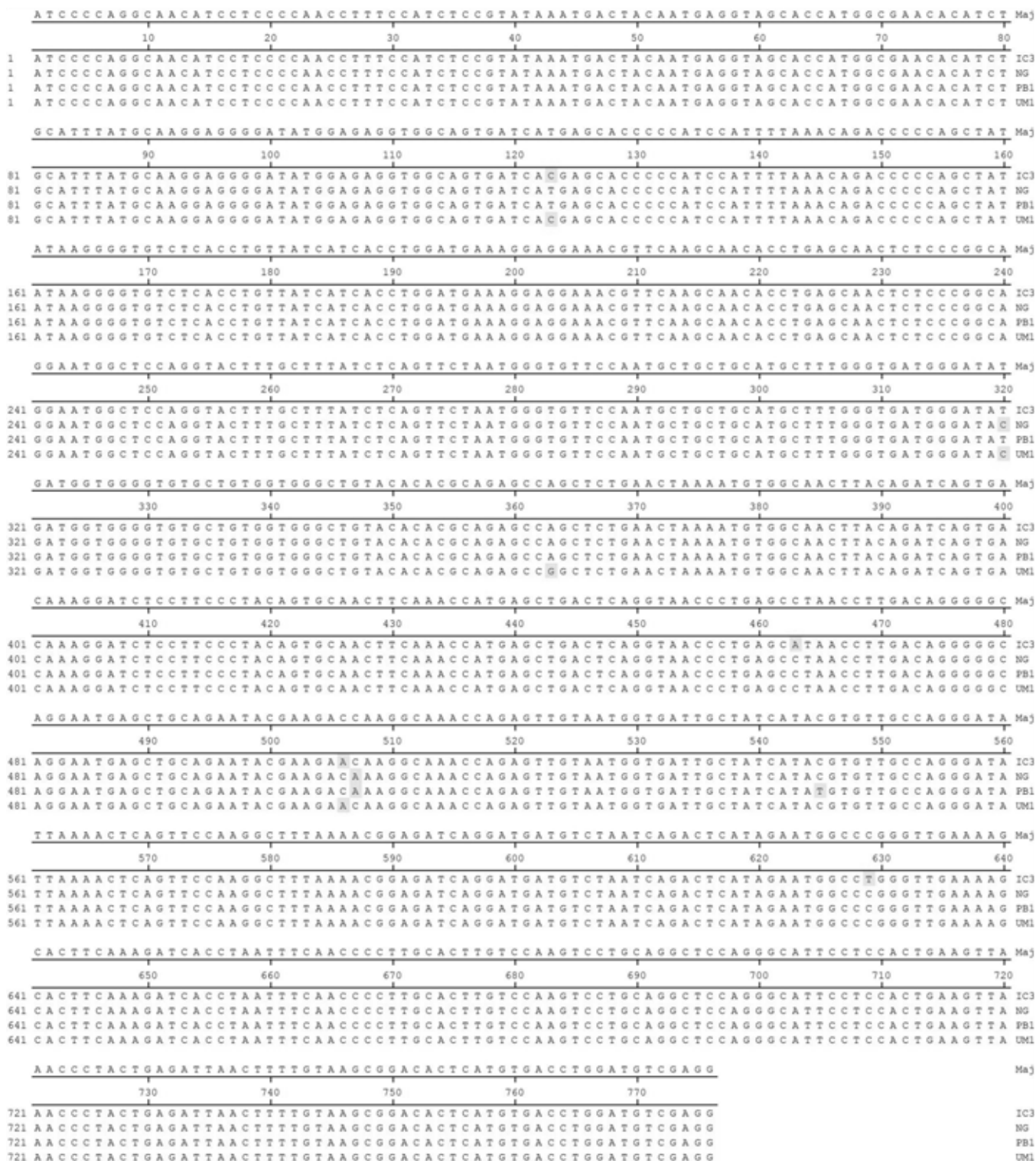


Fig. 2. Nucleotide sequence comparison of amplified GH gene of four strains of chicken.

significant difference in genotype frequencies between strains.

The highest allelic frequencies for A, B and C alleles were observed in NG strain (0.65), UM1 strain (0.24) and IC3

strain (0.56) respectively (Table 2). Darabi *et al.* (2010) reported the allelic frequencies of 0.685 and 0.315 for A and B alleles respectively in Iranian native chicken.

The mean sixth week body weight under each of the

Table 2. Frequencies of GH/*Msp*I alleles in four strains of chicken

Allele	Strain				Overall
	NG	PB1	IC3	UM1	
A	0.65	0.56	0.24	0.53	0.495
B	0	0	0.20	0.24	0.110
C	0.35	0.44	0.56	0	0.395

Table 3. Mean±SE of 6-week-body weight of GH/ *Msp*I RFLP genotypes

Strains	Genotypes	n	Mean±SE (g)	P-value
NG	AA	23	1347±25.55	0.1807 ns
	CC	8	1273±39.87	
	AC	19	1364±25.28	
PB1	AA	19	1260±35.42b	0.0001 ****
	CC	13	1438±32.21a	
	AC	18	1476±32.8a	
IC3	BB	10	930±23.03	0.8920 ns
	CC	16	926±19.26	
	AC	24	913±28.70	
UM1	AA	15	1007±36.50a	0.0019 **
	BB	12	1206±44.38c	
	AC	23	1122±26.49b	

N, 200; means in a column within a strain with no common superscript differ significantly ($P \leq 0.05$).

genotypes in different chicken strains were estimated. In the population of NG strain, mean body weight at 6 weeks in birds with AC heterozygote was higher compared to both AA and CC homozygotes (Table 3). Although there were no differences in 6 weeks body weight between genotypes, the weight gain from 5 to 6 weeks was higher in the AC heterozygote compared to AA and CC genotypes. Among the 3 genotypes, birds of AC genotype of PB1 strain were heavier 1476±32.8 g at 6 weeks than both the homozygotes (AA and CC). However, there was a significant difference in the sixth week body weight in UM1 strain. BB genotype had highest mean value 1206±44.38 g and AA genotype had the lowest mean value 1007±36.50 g (Table 3). Bingxue *et al.* (2003) have reported that *Msp*I polymorphism in indigenous Chinese chicken GH gene had no significant association with 6 weeks body weight.

Nucleotide sequence analysis: The samples which showed different RFLP profiles were selected for sequencing. Four samples, from each of the gene regions studied, were selected for sequencing. The nucleotide sequencing of amplified fragments of GH gene in four strains were done and submitted to the NCBI GenBank with accession no. JN403372 (AA) and JN403373 (CC genotype). The PCR amplified products of GH gene were sequenced and selected sequences were aligned using MEGALIGN programme of 'DNA STAR' software, Laser gene, USA (Fig. 2). The nucleotides

Table 4. Nucleotide sequence variation of GH gene in 4 strains

Position	Strain	Nucleotide changes
123	IC3	T→C
	UM1	T→C
320	NG	T→C
	UM1	T→C
363	UM1	A→G
463	IC3	C→A
506	IC3	C→A
	UM1	C→A
507	NG	C→A
	PB1	C→A
545	PB1	C→T
629	IC3	C→T

sequences of A and B alleles had all the 3 restriction sites hence A and B alleles in homozygote conditions (AA) and (BB), produced 3 bands 125, 237 and 414 bp in NG, PB1 and UM1 strains and 4 bands of 267, 237, 147 and 125 bp sizes in IC3 and UM1 strains respectively. However, in other allele C, *Msp*I restriction sites absence at positions 363 and 629, hence the CC genotype had only 2 fragments of sizes 539 and 237 bp in NG, PB1 and IC3 strains and accordingly the AC genotype (heterozygote) exhibited bands from both of the alleles with all 4 fragments (125, 237, 414 and 539 bp) observed in 4 strains. From the alignment of all the genotypes in the different strains, it was found that there are 12 nucleotide variations in 8 positions. Transition at positions 123 and 320 (T to C), 363 (A to G) and 545 and 629 (C to T) were observed (Table 4). At 463, 506 and 507 positions, transversions from C to A were identified among the sequences.

Sequence homology and phylogenetic analysis: Per cent similarity study was carried out between nucleotide sequences by using DNASTAR software (Table 5) which indicated the per cent similarity of GH gene fragment of all the 4 strains were more than 99% similarity among themselves. The strains NG and PB1 had highest similarity of 99.7%. The strain characters are large body size and high rate growth. Comparison of the nucleotide sequences from this study with that of sequence already published (GenBank accession no. EF452679.1) of *Gallus gallus* showed 99% similarity at the exon 1 (base pair 189–254) of the present study sequence. Phylogenetic analysis was conducted using

Table 5. Per cent similarity (above diagonal); per cent divergence (below diagonal) of GH gene between different strains of chicken

Strains	IC3	NG	PB1	UM1
IC3	6	99.2	99.2	99.5
NG	0.8	6	99.7	99.5
PB1	0.8	0.3	6	99.2
UM1	0.5	0.5	0.8	6

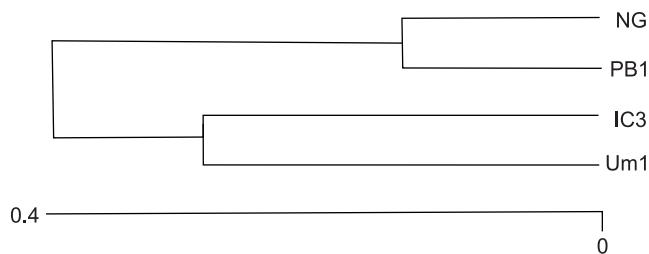


Fig. 3. Phylogenetic studies of growth hormone gene on nucleotide sequence of four strains of chicken.

MegAlign programme of DNASTAR Software. Based on respective GH gene sequences the phylogenetic tree was constructed among 4 strains showing their relative distance (Fig. 3). Phylogenetically, New Genotype and Punjabi Broiler-1 were in one sub-cluster, while Indian Cornish-3 and University Male line strains of chicken in another sub cluster (Fig. 3). Clustering analysis showed that NG had a close genetic relationship with PB1, whereas IC3 is more closely related with UM1. Singh *et al.* (2008) showed the phylogenetic analysis between Punjab Broiler-2, chicken and other poultry species.

In conclusion, growth hormone gene was successfully amplified in New Genotype, Punjab Broiler-1, Indian Cornish 3 and University Male Line varieties of chicken, indicating that this region is conserved and candidate gene was polymorphic and thus suitable for use as genetic marker. The present study revealed that certain growth hormone genotypes are in higher frequencies than the other, possibly indicating that the genotypes CC and AC are favourable in broiler chicken. And there could be selection pressure against some of the low/absent genotypes. The nucleotide variations were observed randomly among the different strains and genotypes and hence could not be linked to the performance. The nucleotide sequence analysis indicated that the per cent similarity of GH gene in four strains of chicken of this study was nearly 99%. The phylogenetic analysis in four chicken strains revealed that the highest genetic similarity was found between NG and PB1. The result also showed that there is a scope for using growth hormone gene as a candidate gene for growth traits. However, extensive study involving F_1 and F_2 generation data are required for confirmation of these results.

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REFERENCES

Bingxue Y, Deng X, Fei J, Xiaoxiang H, Chagxin W and Ning I. 2003. Single nucleotide polymorphism analysis in chicken

- growth hormone gene and its associations with growth and carcass traits. *Chinese Science Bulletin* **48** (15): 1561–64.
- Botstein D, White R L, Skolnick M and Dabis R W. 1980. Construction of a genetic linkage map in man using restriction fragment length polymorphism. *American Journal of Humanities and Genetics* **32**: 324–33.
- Byatt J C, Staten N R, Salsgiver W J, Kostele J C and Collier R J. 1993. Stimulation of food intake and weight gain in mature female rats by bovine prolactin and bovine growth hormone. *American Journal of Physiology* **264**: 986–92.
- Darabi A, Fayazi J, Roshanfekr H and NASIRI M. 2010. Investigation of growth hormone gene polymorphism using PCR-RFLP technique in native poultry in Khuzestan province. *Journal of Animal and Veterinary Advances* **9** (2): 255–57.
- Falconer D S and Mackay T F C. 1996. *Introduction to Quantitative Genetics*. 4th edn, pp. 1–12, Addison Wesley Longmann Ltd. Essere, England.
- Fotouhi N, Karatzas C N, Kuhnlein U and Zadworny D. 1993. Identification of growth hormone DNA polymorphisms which respond to divergent selection for abdominal fat content in chickens. *Theory and Applied Genetics* **85**: 931–36.
- Horvath M B, Martin-cruz B, Negro J J, Kalmar L and Godoy J A. 2005. An overlooked DNA source for non-invasive genetic analysis in birds. *Journal of Avian Biology* **36** (1): 84–88.
- Ip S C, Zhang X and Leung F C. 2001. Genomic growth hormone gene polymorphism in native Chinese chicken. *Experimental Journal of Biology and Medicine* **226**: 458–62.
- Kuhnlein U, Ni L and Zadworny D. 1997. DNA Polymorphisms in the chicken growth hormone gene: Response to selection for disease resistance and association with egg production. *Journal of Animal Genetics* **28**: 116–23.
- Lechniak D G, Machnik M, Szydowski M and Switonski M. 1999. Growth hormone gene polymorphism and reproductive performance in AI bull. *Theriogenology* **52** (3): 1145–12.
- Payne J W. 1998. Peptides and micro-organisms. *Advances in Microbial Physiology* **13**: 55–113.
- Sambrook J and Russel D W. 2001. *Molecular Cloning. A Laboratory Manual*. 3rd edn. Cold Spring Harbor Laboratory Press. Cold Spring Harbor, NY.
- Nie S, Ip S C, Zhang X, Leung F C and Yang G. 2002. New Variations in intron 4 of growth hormone gene in Chinese native chickens. *Journal of Heredity* **93** (4): 277–79.
- Nie Q, Sun B, Zhang C, Luo N, Ishag M, Lei G and Zhang X. 2005. High diversity of the chicken growth hormone gene and effects on growth and carcass Traits. *Journal of Heredity* **96** (6): 698–703.
- Tanmankaur G V P, Ravikumar P S, Bajwan I S and Trehan P K. 2008. PCR-RFLP of growth hormone gene in meat type chick. *Indian Journal of Poultry Science* **43** (2): 129–33.
- Sing S P, Brash G S, Amrita C and Ravikumar G V P P S 2008. PCR-RFLP of chicken growth hormone gene in egg-type chickens. *Indian Journal of Poultry Science* **43** (1): 1–4.
- Vasilatos-young R, Zhou Y, Wang X, Mcmurtry J P, Rosebrough W, Decuypere E, Buys N, Darras V M, Geyten V and Tomas F. 2000. Altered chicken thyroid hormone metabolism with chronic GH enhancement in vivo: consequences for skeletal muscle growth. *Journal of Endocrinology* **166**: 609–20.