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Polymorphism in the IGF-1 and NPRY gene associated with important economic traits in 'Zo-ar' chicken of Mizoram, India

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ABSTRACT

Background: *Molecular genomic selection on candidate genes is proving to be a promising method to genetically improve economically important traits in chickens. These molecular markers have provided new opportunities and also have accelerated the process of selection and obtaining the desired genetic gain or selection for new traits that are costly or difficult to record in animals to improve animal production. The Neuropeptide Y (NPY) gene is known to influence the release of gonadotropin-releasing hormone (GnRH) from the median eminence and is also critical in controlling feed intake in birds. Similarly, insulin-like growth factor-1 (IGF-1) gene acts as a potent paracrine modulator in a variety of tissues and regulates tissue-specific cell differentiation. Ths study aimed to identify single nucleotide polymorphisms (SNPs) in these two candidate genes (NPY and IGF-1) in a native chicken population of Mizoram, India which is known as 'Zo-ar'.*

Methods: *Isolation of genomic DNA from blood samples. Spectrophotometric and electrophoretic evaluation of extracted DNA samples was done. The samples were then subjected to Polymerase Chain Reaction (PCR) amplification and Restriction endonuclease (DraI and PstI for NPY and IGF-1, respectively) digestion by using Restriction Fragment Length Polymorphism (RFLP) method. Determination of alleles and genotypes along with statistical analysis of population genetics data were compiled and analyzed using POPGENE® 3.1 software for proper conclusion.*

Results: *Both the genes under the study were found to be polymorphic and yielded variable restriction patterns. The NPY locus revealed three genotypes, viz. AA, Aa and aa with the highest frequency of a genotype (0.44) and an allele (0.62). The IGF-1 locus also showed the presence of three genotypes BB (0.16), Bb (0.44) and bb (0.40) with an allelic frequency of 0.62 and 0.38 for b and B alleles, respectively. The population was found to be conforming to Hardy-Weinberg equilibrium for both the studied loci. The presence of different genotypes in each locus indicated the presence of genetic variation in this chicken population, which may be exploited for their genetic improvement by suitable selection and breeding programmes.*

Keywords: *Neuropeptide Y, Insulin-like growth factor-1, 'Zo-ar' chicken, PCR-RFLP, genetic variation*

Introduction

The local chickens of Mizoram are medium-sized and generally scavengers by nature. They are usually reared in a free range system or backyard system by the villagers of the state for table and game purposes without any health care facilities[1]. The eggs of these chickens are small to medium in size with light brown shells, brooding behaviour and good mothering ability[2]. The eggs of the native chicken of Mizoram were found to contain superior quality when compared to eggs of improved varieties such as Vanaraja and Gramapriya. The body weight gain of these chickens and their Feed Conversion Ratio were also found to be higher than Black and white Nicobari fowl, Naked Neck and Frizzle fowl[3].

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However very little work has been done to know the genetic potential of this chicken population and to increase production further. There has been a huge demand for meat and eggs from the native chicken population among consumers in recent times due to the flavourful meat of local chicken which contains less fat percentage as compared to that of commercial broilers chickens. Therefore, if proper attention is given to finding out the genetic polymorphism and the presence of variability in important candidate genes that affect both egg production and body weight gain, an overall improvement can be made in increasing the production performance in these chickens.

The Neuropeptide Y (NPY) gene has a size of about 8 kilobases consisting of four small exons and is located in chromosome no. 7 of the avian genome[4]. The NPY plays a critical role in controlling feed intake in birds influencing body growth which might possibly match the satiety level to reproductive activity and is also known to induce precocious time of puberty[5]. NPY level in the body might also play a significant role in the plasma concentration of luteinizing hormone (LH), growth hormone (GH), prolactin hormone (PRL), gonadotropin-releasing

hormone (GnRH), adrenocorticotropic hormone (ACTH) and vasopressin[6]. In a study done on a broiler breeder hen population[7], a 4 bp indel mutation was detected which gave rise to a polymorphism in the NPY gene with the presence of two alleles. Moreover, a correlation between this polymorphism with total egg production (NE) and age at first egg was observed. In West-Azarbaijan native chicken was found to be polymorphic when subjected to digestion with restriction enzyme *Dra*I and the results showed a 4 bp deletion in the transcription starting site (TSS) giving rise to three genotypes AA, Aa and aa[8]. Similar results were obtained when observed in Chinese Wenchang chicken[9] out of which the AA genotype was seen to be producing more numbers of eggs during 300 days, which is also known as NE (300d).

The Insulin-like growth factor-1 (IGF-1) gene has been mapped to 165.95 centimorgan on chromosome 1 in chicken[10]. It consists of four exons and is about 70 – 90 kb long in humans, pigs, goats and rats[11, 12]. Using two chicken strains of Black Pendesenca breed (PN and MN)[13], 4 SNPs in the IGF1 gene were detected and significant association between two of these SNPs were found with average daily weight gain at 107d and feed efficiency at 44, 73 and 207 days of age. In a Korean Native chicken breed called Ogol[14], an association of the IGF1 genotypes was observed with egg productivity. In another study conducted on a native chicken breed of China, genetic polymorphism was found in the 5'-untranslated region (UTR) of the IGF-1 gene which showed to have three genotypic variants (bb, Bb and BB) upon digestion with *Pst*I restriction enzyme and significant association of this polymorphism was found with total egg production in 300 days (NE 300d), total egg production in 400 days (NE 400d) and average days of continual egg laying (ADCE)[9]. IGF-1 gene polymorphisms were stated to have significant association with skeletal measurements like length and weight of bone, growth, breast muscle weight, abdominal fat and skeletal integrity in chicken[10].

Therefore, the principle objective of this study has been concentrated on the investigation of polymorphism within the NPY and IGF-1 genes in the local chicken 'Zo-ar' of Mizoram along with the observation of genetic variation present within the population as candidate gene approach has been seen to be a powerful method for understanding the genotypic constitution and variety present in a population which will help to recognize the quantitative difference between individuals and use them in breeding programmes accordingly for the improvement of traits like egg production, egg numbers and body weight gain.

Materials and Methods

Experimental chicken and blood samples

A total of 50 unrelated, randomly chosen 'Zo-ar' chickens of Mizoram, India astaken for collection of blood (about 1 mL) from the backyard flocks reared in different districts of the state (Kolasib, Mamit and Aizawl) by the natives. The samples were collected aseptically from the wing vein in EDTA vials and kept in an ice pack immediately. Thereafter, the blood samples were brought directly to the laboratory and stored at -20˚C until further use.

Polymerase Chain Reaction-Restriction Fragment Length Polymorphism (PCR-RFLP) Assay

DNA extraction from the collected samples was done by using GeneJET Genomic DNA Purification Mini Kit (K0782, Thermo Fisher Scientific) following the attached protocols. Quantity and quality checking of the isolated DNA was done using a Nanodrop Spectrophotometer (Thermo Scientific, USA) and gel electrophoresis with 0.75% agarose gel. The optical density ratio (OD_{260}/OD_{280}) of the DNA samples was checked and only those samples having an OD_{260}/OD_{280} ratio between 1.7 and 1.9 were subjected to further analysis for molecular and quantitative data.

A 25 µL PCR mixture was prepared for amplification containing 200 µM of each dNTP, 10X PCR buffer, 2U Taq DNA polymerase, 5 pM of each forward and reverse primer for each gene[8,9] (**Table 1)** 2mM of $Mgcl_2$ and 60 ng of extracted genomic DNA. PCR amplification of both the NPY and IGF-1 gene was done using the following thermo-cycles: Pre Denaturation at 95ºC for 5 min, followed by 35 cycles of denaturation at 95ºC for 45 sec, annealing at 60ºC for 45 sec, elongation at 72ºC for 45 sec and post extension at 72ºC.

The obtained amplicon size obtained for the NPY gene was 240 bp in size. These were then subjected to restriction endonuclease (RE) digestion using *Dra*I enzyme by incubating at 37ºC overnight. The digested products were then separated in 2% agarose gel for visualising the bands in 0.5 X TAE containing 1.0 µM ethidium bromide. Observation of the separated bands was done under a UV trans-illuminator and photographs of the gel were taken using the Gel Doc system for further interpretation.

For the IGF-1 gene, the size of the PCR product obtained was 621 bp which were subjected to RE digestion with *Pst*I restriction enzyme overnight at 37 ºC. The separations of the digested bands were done in 2.5% agarose gel in 0.5 X TAE containing 1.0 µM ethidium bromide. For visualization and interpretation of the fragments UV trans-illuminator and Gel Doc system were used. The primers and restriction enzymes used for PCR-RFLP analysis have been mentioned in **Table 1.**

Table 1: Gene, PCR amplicon size, primer sequence, annealing temperature for PCR and restriction endonuclease (RE) enzyme used for RFLP analysis:

Gene		Primer sequence (5´ - 3´)	Reference	Product size (bp)	RE	Incubation
NPY	F	TCTCAGAGCTCAACCGTATGA	8	240	*Dra*I	37ºC overnight
	R	ATATTTCTGTGCTGAACAACA				
IGF-1	F	GACTATACAGAAAGAACCAC	9	621	*Pst*I	37ºC overnight
	R	TATCACTCAAGTGGCTCAAGT				

Statistical Analysis

Gene and Genotypic frequencies

Allelic and genotypic frequencies were obtained using the POPGENE 32 software[15] and the mentioned formulae for further analysis:

$$Genotype\ frequency = \frac{D}{N} \qquad Gene\ frequency = \frac{2D + H}{2N}$$

Where,
D = Number of individuals having the particular genotype in the population
H = Number of the heterozygous genotype in the population
N = Total number of individuals in the population

Hardy-Weinberg equilibrium

The obtained genotype frequencies were then used for testing the Hardy-Weinberg equilibrium by using exact probability (P-values) tests provided in POPGENE 32 version.

Observed and expected heterozygosity

The observed and expected heterozygosities were also calculated using the POPGENE 32 software following the mentioned formulae.

The observed heterozygosity (H_o) has been calculated as the actual percentage of heterozygosity occurring in the sample population.

$$Ho = \frac{number\ of\ heterozygotes}{total\ number\ of\ samples} X100$$

The expected heterozygosity or genetic diversity was measured[16] by the formula mentioned below,

$He=1-\sum P^2_i$ (Where P_i is the frequency of i^{th} allele).

Polymorphism Information Content (PIC) value

The polymorphism information content (PIC) is used to measure the informativeness of a particular genetic marker for the purpose of linkage analysis. Its range is from 0-1 and higher heterozygosity gives rise to a higher PIC value. PIC values were obtained in this study by using the formula mentioned below[17]:

$$PIC = 2\sum_{i=1}^{k-1}\sum_{j=i+1}^{k} x_i x_j (1 - x_i x_j)$$

Where,

k= number of alleles

x_i= allele frequency at the homozygous loci

$x_i x_j$= allele frequency at the heterozygous loci

Ethical approval

All the techniques applied in this study have been performed following standard protocols specified under animal ethics regulations in the country. The protocols have also been approved by the Institutional Animal Ethics Committee (IAEC) of India. The reference number of the approval is as follows: CVSC/CAU/IAEC/19-20/P-21.

Results

Neuropeptide Y (NPY) gene

The digestion of 240 bp PCR amplification product with the restriction endonuclease enzyme *Dra*I resulted in three different genotypes for the NPY locus, AA (240 bp); Aa (79, 161 and 240 bp) and aa (79 and 240 bp) in all the samples studied. All three genotypes AA, Aa and aa are shown in **Figure 1.**

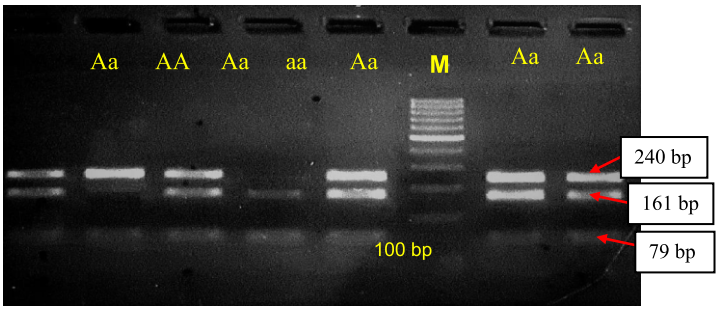

Figure 1: Genotypes of NPY gene digested with RE DraI in 2% agarose gel Lane M: 100 bp gene ruler

A perusal of the **Table 2** showed that the genotype aa was observed to be predominant among the local chicken of Mizoram with a frequency of 0.44. The genotype AA was the least, with a frequency of 0.20 in the population. The heterozygote Aa frequency was found to be 0.36. As revealed by the RFLP analysis, allele a was seen to be predominantly prevalent with a frequency of 0.62 in the local chicken population of Mizoram. On the other hand, it was recorded that the A allele (0.38) was rarely distributed in the population. The calculated Chi-square value revealed that the population of local chicken 'Zo-ar' of Mizoram was under the Hardy-Weinberg equilibrium (HWE) with respect to the NPY locus genotypes.

The unbiased estimate of the observed and expected heterozygosity parameter was calculated by taking the number of alleles into account. The observed heterozygosity value of the NPY locus (0.36) was found to be less than the expected heterozygosity value (0.48). The PIC value of the NPY gene was found to be 0.36 in the studied 'Zo-ar' chicken population (**Table 3**).

Insulin like growth factor-1 (IGF-1) gene

The digestion of IGF-1 gene PCR amplified fragment (621 bp) with the restriction endonuclease enzyme *Pst*I showed polymorphism yielding three different genotypes *i.e.* bb (257 and 364 bp), Bb (257, 364 and 621 bp) and BB (621 bp) genotype yielded an uncut fragment. All three genotypes BB, Bb and bb are shown in **Figure 2.**

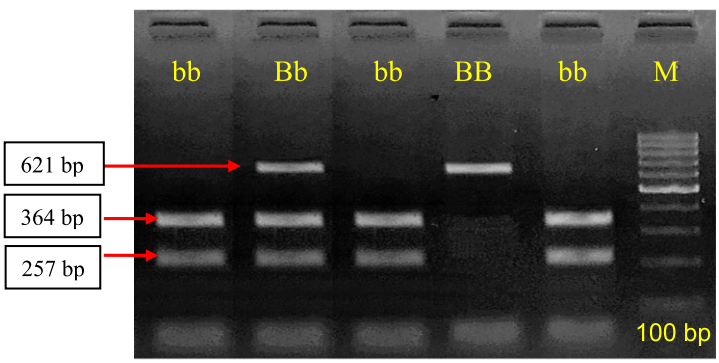

Figure 2: Genotypes of IGF-1 gene digested with RE PstI in 2.5% agarose gel Lane M: 100 bp gene ruler

The findings revealed (**Table 2)** that the genotype BB was found to be least with a frequency of 0.16 among the local chicken population of Mizoram. The Bb (0.44) and bb (0.40) genotypes were almost equally distributed among the population. The allele b has been seen to be predominantly prevalent with a frequency of 0.62 in the local chicken population of Mizoram. The calculated Chi-square value revealed that the local chicken population of Mizoram was conforming to the Hardy-Weinberg equilibrium for the IGF-1 locus.

Table 2: Allelic and Genotypic frequency along with Chi-square (χ^2) value of NPY and IGF-1 gene in the 'Zo-ar' chicken population of Mizoram, India:

Genes	Genotypic frequencies			Allelic frequencies		χ^2 value
NPY	AA (0.20)	Aa (0.36)	aa (0.44)	A (0.38)	a (0.62)	3.04^{NS}
IGF-1	BB (0.16)	Bb (0.44)	bb (0.40)	B (0.38)	b (0.62)	0.29^{NS}

NS = Not significant

The observed and expected heterozygosity values for the IGF-1 locus were found to be 0.44 and 0.48, respectively. The PIC value was found to be 0.36 for the IGF-1 locus too which was similar to the NPY locus in the studied 'Zo-ar' chicken population (**Table 3**).

Table 3: PIC value, Observed heterozygosity and expected heterozygosity values of the NPY and IGF-1 loci in the 'Zo-ar' chicken population of Mizoram, India:

Genes	Observed heterozygosity	Expected heterozygosity	PIC value
NPY	0.36	0.48	0.36
IGF-1	0.44	0.48	0.36

Discussion

Similar to the present findings, the NPY gene was found to be polymorphic in West-Azarbaijan native chickens by using restriction enzyme *Dra*I[8], in Noi chicken of Vietnam[18], in the Lien Minh chicken population[19] and in Mazandaran native chickens[20]. The present findings of higher frequency of aa genotype (0.44) in the native chicken 'Zo-ar' of Mizoram is in close agreement with that in West-Azarbaijan native chicken (0.538)[8]. Whereas, the heterozygote Aa was observed to be the most predominant genotype (0.50) in the Chinese Wengchang chicken breed[9]. In the Lien Minh chicken population[19], the AA homozygote was found to have the highest frequency (0.76) compared to the other two genotypes, Aa (0.20) and aa (0.04). Moreover, the higher frequency of an allele of the NPY gene as found in the present study is in close agreement with the earlier reports in a commercial broiler population (0.78)[7] and in the Chinese Wengchang chicken breed (0.54)[9]. On the contrary, a lower frequency of an allele compared to the A allele was noticed in the Noi chicken of Vietnam (0.43)[18] and in the Lien Minh chicken population (0.14)[19].

The Aa genotype individuals for NPY locus were seen to be having an early age at first egg[7]. Whereas, the AA genotype showed better results for total egg numbers at 400 days of age (NE 400d) and average days at continual egg-laying in Chinese Wengchang chicken[9]. Moreover, higher egg numbers and egg mass in Mazandaran native chicken[20] and higher egg production until 300 days of age in West-Azarbaijan native chicken[8] were found to be associated. Significant association with growth traits like body weight at sexual maturity with the heterozygote Aa genotype was also observed[20]. However, in the present study, these two genotypes (AA and Aa) were in lower frequencies compared to the aa genotype, which may be the possible result of poor performance in most of the economic traits in these chickens of Mizoram.

The lower frequency of the BB (0.16) genotype for the IGF-1 locus, in the present study was in accordance with that of Noi chicken population (0.06) of Vietnam[18]. Whereas, in Chinese Wengchang chicken[9,21] for IGF-1 locus a lower frequency of bb genotype (0.27) was observed. The present findings of higher b allele frequency were found to be similar to that in the Noi chicken population of Vietnam[18] where the b allele frequency was reported to be 0.79. However, a lower frequency of the b allele (0.47) as compared to that of the B allele (0.53) was observed in the Chinese Wengchang chicken population[9].

In Chinese Wengchang chicken[9, 21] significant associations were observed between the IGF-1 genotypes and some egg production traits like NE 300d, NE 400d and ADCE where better results were associated with bb genotype individuals for all the traits. Besides, the bb individuals were also reported to have a better DYE result but the difference was not found to be significant with the other genotypes. The present findings of moderate frequency of bb genotype indicated that there is scope for genetic improvement for these economic traits in the Mizoram native chicken population.

The observed heterozygosity values were calculated as 0.36 and 0.44 for NPY and IGF-1 loci respectively in the studied population. The low values of observed heterozygosity in both loci revealed the presence of a low level of heterozygosity. The calculation of PIC values of the NPY and IGF-1 loci was done and the value was found to be 0.36 for both the loci which can be stated as a moderate PIC value of the used marker in the study (*i.e.* >0.25 and <0.50) with respect to the PIC value classification.

Conclusions

The present finding of moderate presence of A allele (0.37) is indicative of the fact that the Mizo native chicken has the genetic potential for disease resistance and antiviral activity, which may be exploited for further improvement by genetic selection. The population conforming to the Hardy-Weinberg equilibrium suggests the absence of selection with respect to disease resistance traits and also the absence of migration in the breeding tract. Thus, the knowledge generated on the allelic and genotypic distribution pattern in the native 'Zo-ar' chicken population of Mizoram, India can be useful for future genetic improvement of the population.

Conflict of Interest: The a*uthors declare that there is no Conflict of Interest regarding the publication of this article*

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References

1. Mayengbam P, Tolenkhomba T C & Ali M A A Brief Biological Note on Semi-wild Indigenous Chicken 'Sikhar' of Mizoram, India. *Int J Livest Res*, 7 (2017) 146-152.

2. Lalhlimpuia C, *Phenotypic characterization of Local Chicken of Mizoram in its Home tract.* M.V.Sc. Thesis Central Agricultural University, Imphal, Manipur, 2020.

3. Haunshi S & Doley S Performance of Native Chickens of Mizoram under Intensive System of Rearing. *Indian Vet J*, 88 (2011) 45-47.

4. Ruscica M, Dozio E & Magni P NPY (neuropeptide Y). *Atlas Genet Cytogenet Oncol Haematol*, 14 (2010) 404 – 408.

5. Kuenzel W J & Fraley G S Neuropeptide Y: its role in the neural regulation of reproductive function and food intake in avian and mammalian species. *Poult Avian Biol Rev*, 6 (1995) 185-209.

6. Wang C, Liang Z, Yu W, Feng Y, Peng X, Gong Y & Li S Polymorphism of the prolactin gene and its association with egg production traits in native Chinese ducks. *S Afr J Anim Sci*, 41 (2011) 64 – 69.

7. Dunn I C, Miao Y W, Morris A, Romanov M N, Wilson P W & Waddington D A study of association between genetic markers in candidate genes and reproductive traits in one generation of a commercial breeder hen population. *Heredity*, 92 (2004) 128-134.

8. Abdi M, Sayedabadi H & Gorbani A Prolactin and NPY Gene Polymorphism and its Associations with Production and Reproductive traits in West-Azarbaijan Native chicken. *Bull Env Pharmacol Life Sci*, 3 (2014) 39-45.

9. Li H F, Zhu W Q, Chen K W, Wu X, Tang Q P, Gao Y S, Song W T & Xu H L Polymorphism in NPY and IGF-1 genes associated with reproductive traits in Wenchang chicken. *Afr J Biotechnol*, 8 (2009) 4744 – 4748.

10. Zhou H, Mitchell A D, McMurtry J P, Ashwell C M & Lamont S J Insulin like Growth Factor 1 gene Polymorphism Association with Growth, Body composition, Skeleton integrity and metabolic traits in Chickens. *Poult Sci*, 84 (2005) 212-219.

11. Kajimoto Y & Rotwein P. Structure of the chicken insulin-like growth factor 1 gene reveals conserved promoter elements. *J Biol Chem,* 266 (1991) 9724-9731.

12. Klein S, Morrice D R, Sang H, Crittenden L B & Burt D W Genetic and physical mapping of the chicken IGF1 gene to chromosome 1 and conservation of synteny with other vertebrate genomes. *J Hered*, 87 (1996) 10 – 14.

13. Amillis M, Jimenez N, Villalba D, Molina E, Cubilo D, Marcos C, Francesch A, Sanchez A & Estany J Identification of three single nucleotide polymorphisms in the chicken insulin-like growth factor 1 and 2 genes and their associations with growth and feeding traits. *Poult Sci*, 82 (2003) 1485 – 1493.

14. Kim M H, Seo D S & Ko Y Relationship between egg productivity and insulin like factor 1 genotypes in Korean native Ogol chickens. *Poult Sci*, 83 (2004) 1203 – 1208.

15. Yeh F C, Yang R C, Boyle T B J, Ye Z H & Mao J X POPGENE the user friendly shareware for population genetic analysis. University of Alberta, Canada. 1997.

16. Nei M Genetic distance between populations. *Am Nat*, 106 (1972) 283-291.

17. Botstein D, White R L, Skolnick M & Davis R W Construction of a genetic linkage map in man using restriction fragment length polymorphism. *Am J Hum Genet,* 32 (1980) 324-331.

18. Vu C T & Ngu N T Single nucleotide polymorphisms in candidate genes associated with egg production traits in native Noi chicken of Vietnam. *Int J Plant Anim Environ Sci*, 6 (2016) 162 – 169.

19. Nguyen T T, Duc N H, Quy V D, Giang P T, Linh N M, Thuy D T N & Thuy N T D Polymorphism candidate genes of Indigenous Lien Minh chicken. *Vietnam J Agri Sci*, 1 (2018) 174 – 181.

20. Fatemi S A, Mehrabani-Yeganesh H, Nejati-Javaremi A & Niknafs S Association of neuropeptide Y and gonadotropin-releasing hormone receptor gene SNPs with breeding value for growth and egg production traits in Mazandaran native chickens. *Gen Mol Res,* 11 (2012) 2539-2547.

21. Li H F, Zhu W Q, Chen K W, Wu X, Tang Q P and Gao, Y.S.. Association between GHR and IGF-1 gene polymorphisms, and reproductive traits in Wenchang chicken. *Turk J Vet Anim Sci*, 32 (2008) 281 – 285.