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GENETIC ANALYSIS OF SNPS OF CGH (GROWTH GENE) IN LOCAL BREED OF KASHMORE,SINDH,PAKISTAN

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Article Info

Abstract

Present study revealed different single nucleotides mutations in GH (growth) hormone gene were found in Desi, (Local breed) varieties of chickens. The Desi chicken is a hardy breed, well suited to cold as well as hot climates. Desi chickens are domesticated for hundreds of years ago, the common desi breed is a best mother for hatching and possess a unique appearance but distinguish from other cross breeds. The chicken growth hormone (cGH) gene plays a crucial role in controlling growth and metabolism, leading to potential correlations between cGH polymorphisms and economic traits. Mutations were identified through PCR sequencing technique based on Genetic codons, these mutation were classified and explained. In Desi parent chickens on 45Bp a Non sense mutation was found that result in termination of protein formation. Such type of mutations is always considered to be a nonproductive for species growth. In F1 generation of Desi chickens a mis sense mutation was identified that denotes replacement of valine < Glycine at 118Bp and the second one mutation was found at 182 Bp as frame shift mutation results in productive amino acid from deleted codon. F2 generation Desi chicken had single mutation at 81 Bp in replacement of A<T results as mis sense mutation. However, the mutation percentage in all breeds was not greater than 1%, it means there was no any polymorphism obtained from cross breeding of both varieties but there were different mutations that resulted in replacement of deleted codons and also formation of essential and non-essential amino acids. Statistically it was proved to be more beneficial for in reduction of their mortality rates and a viable improvement, in their growth as well as different morpho-genic traits.

Keywords: *PCR, Mutation, Chicken, Sequencing.GH gene*

Introduction

Most of developing countries are facing fierce scarcity of animal protein including Pakistan with gap of approximately (10 g) in per capita availability. This scarcity is high in denser rural areas which covers major part of the population. In this context very rapid source of the protein is only poultry chicken's production. The production capability of Desi (domestic) chicken, which is used as an important and cheap supply of required protein diet in rural areas, due to its slow growth may not fulfill the demands of local consumers (Zhu *et al* .,2019). The increasing demand for production of Desi (domestic) chickens is due to self-caring, least investment and also useful for degradation of environmental hazards and harmful wastes. Most of local consumers have positive perception that the meat and desi chicken's products are much better than the poultry chickens. In spite of that the poultry industries of chickens has immerged rapidly during the last three decades. The supply of commercial poultry yields in rural areas is much low as compare to urban areas. Pakistan still lying below in list of per capita production in poultry food stuffs and chicken meat as compared to other countries on globe is showing national health status (Ojeda *et al* .,2009).

The use of first generation of crossing offers a mean of rapid improvement that could be achieved in desirable characters (growth, productivity, fitness and appearance), but these superiorities lowered in advanced generations due to reassortment of genes. Theoretically, the level of heterosis is inversely related to the degree of genetic resemblance between purebred populations. Indigenous or native poultry plays very important role in the strengthening of economy of backyard peoples, it is source of food and employment for small poultry keepers without investing a penny on the management, disease control and nourishment. Native poultry survives well in their local environment and can be reared on kitchen waste and may be as free range on open lands. Although commercial poultry has taken its place but native poultry is still playing very crucial role in the economy of third world countries like Pakistan and extensive work is required to improve the economic traits of native chicken through modern techniques that helps in selection(Guo *et al* .,2022).

The chicken growth hormone (cGH) gene plays a crucial role in controlling growth and metabolism, leading to potential correlations between cGH polymorphisms and economic traits. The (cGH) gene coordinates postnatal growth of multiple tissues, including skeletal muscles. The growth-promoting actions of (cGH) are mediated by circulating or locally produced IGF-1, which is a critical myogenic agent promoting muscle growth. Genetically modified animals have significantly contributed to our understanding of different aspects related to immunity, infectious diseases, neurology, behavior, and developmental biology. The generation of genetically modified chickens has wide applications in agricultural and biomedical research. Benefiting from gene editing, cross technologies and germ line transmission of PGCs, new knowledge was brought to light about specific gene functions (Padhi *et al*., 2016).

Materials and Method

Rearing of chickens

A total 100 chickens as un-sexed about 90 days old both male and female with the ratio of 1:4 were collected from local peoples and hatchery of Kashmore. These were placed under intensive care and free range environment. A deep litter system was maintained for required period. Feeding system and water availability was arranged according to NRC and ISA guidelines (Taha *et al*., 2013).

DNA Extraction from Blood samples

About 100µl of whole blood was added into 2.0 ml tube, 200µl of S1 solution followed by vertex and 20µl of Proteinase K into solution..Incubated sample tube into water bath for about 30 minutes at the temperature of 56°C. After that 200µl of (96-100%) ethanol was added and solution was transferred into spin column with collection tube and centrifuge at 10,000 rpm for about 2 minutes. Discarded the flow through

and 500µl of S3 was added into tubes, then centrifuge at 10,000 rpm for 1 minute. Added 500µl of S4 solution into collection tubes and again centrifuge at 10,000 rpm for about 3 minute .Again discarded the flow and transferred the column into clean sterilized 1.5 ml Eppendorf tube. Added about 30-50µl of EB Buffer into the central part of membrane of column to elute the genomic DNA. (Schiebelhut *et al*.,2017).

Gel electrophoresis and Nano drop for DNA quantification

Agarose powder (1.5gram) was weighted by using of electronic balance machine, transferred into conical flask with 100ml of TBE buffer. The mixture was heated into microwave oven until the formation of homogenous solution, added 110ul of (dilute) Ethidium Bromide into conical flask and mixed well. Transferred the Agarose gel into sealed gel tray carefully allowed the mixture to cool sufficiently and added comb for formation of wells. After gel had polymerized, removed the comb and sealed from gel tray. The PCR product samples were mixed with 6x loading dye if the Master Mix was without dye. The gel was electrophored into 1x TBE buffer at 60-70 volts at room temperature for 60 minutes (Xin *et al*., 2003).

Nucleic acids were quantified to check the concentration and purity of DNA within the sample. Two different methods of Nucleic acid quantification were performed. It was performed from Jamil-ur- Rehman Center for genomic Research Institute Karachi.

PCR and DNA Sequencing

Polymerases chain reaction was performed through thermo cycler machine and PCR products were sent to Macrogen Company of Korea for Purification as well as sequencing. The sequencing results were obtained through the email having secret ID. The results of sequencing were downloaded, and blast analysis was performed by Using the Ensemble genome browser database (http: /www.ensemble.org.) and subsequently mutations were detected.

Data Analysis

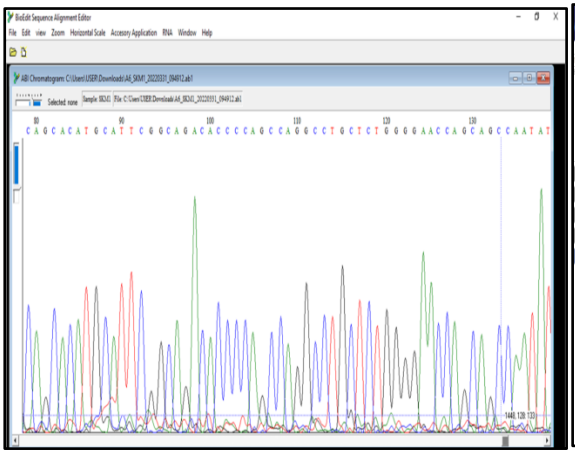

Figure:01 electrophorogram

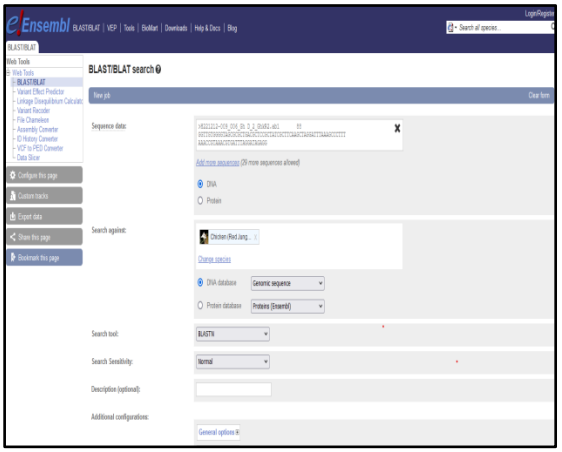

Figure:02 BLAST by Enseml 109

Data were analyzed on e!Ensembl genome browser by Blast the query sequence of cast gene of selected breeds with the genomic sequence.

Results

Nano Drop

Table 1: Nano drop of Desi chickens breeds

Ecology	Desi Parents (Male)	Desi Parents (Female)	Desi F1 (Male)	Desi F1 (Female)	Desi F2 (Male)	Desi F2 (Female)
Intensive Management Conditions	1.42 ng/ul	1.42 ng/ul	1.42 ng/ul	1.42 ng/ul	1.42 ng/ul	1.42 ng/ul
	1.35 ng/ul	1.35 ng/ul	1.35 ng/ul	1.35 ng/ul	1.35 ng/ul	1.35 ng/ul
Mean Values	ng/ul	ng/ul	ng/ul	ng/ul	ng/ul	ng/ul
Free Range Conditions	1.38 ng/ul	1.38 ng/ul	1.38 ng/ul	1.38 ng/ul	1.38 ng/ul	1.38 ng/ul
	1.41 ng/ul	1.41 ng/ul	1.41 ng/ul	1.41 ng/ul	1.41 ng/ul	1.41 ng/ul
Mean Values	ng/ul	ng/ul	ng/ul	ng/ul	ng/ul	ng/ul

BLAST from Ensemble 109

Figure: 04 Desi parents BLAST

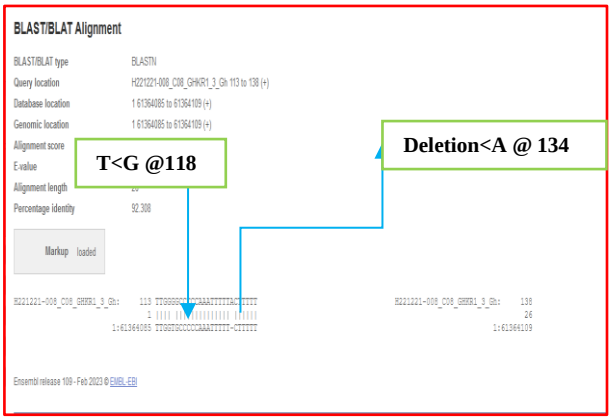

Figure: 05 Desi F1 generations BLAST

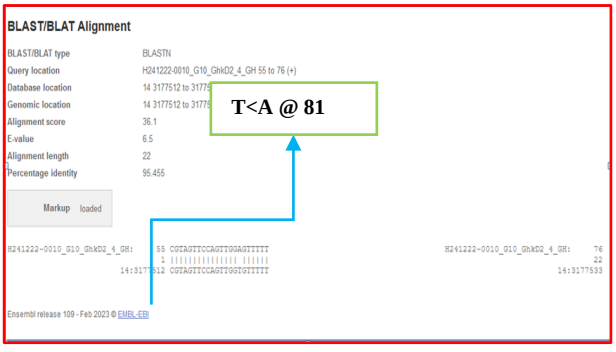

Figure: 06 Desi F2 generation BLAST

Table 2: Different types of mutations found in Desi parents, Desi F1 and Desi F2 generation

Name of Gene	Name of goat breeds	Found SNPs/Mutations	Percentage method	Total percentage %
GH gene	Desi Chickens	01	1x100/200	**0.5**
	Desi 1st generation chickens	02	2x100/200	**1.0**
	Desi 2nd generation chickens	01	1x100/200	**0.5**

Table 3: Shows amino acids substitution during mutations

Sample Name	Position of changing nitrogenous base	Original Codon	Change Codon	Original Amino Acids	Change Amino Acids	Types of Point Mutation
Desi parents	45	G	A	TGG (Tryptophane)	TAG (Stop codon)	Non sense mutation
	118	T	G	GTG (Valine)	GGG (Glycine)	Missense mutation
Desi F1	134	Deletion	A	T-C (Deletion)	TAC (Tyrosine)	Frame shift mutation
Desi F2	81	T	A	TGT (Cysteine)	AGT (Serine)	Missense mutation

Discussion

Four SNP of the chicken GHc gene were identified in the present study. Most of these SNP were found in non-coding regions, even though the amplified 600 bp fragments almost covered the 1/8 cDNA of the chicken GH gene. Most SNP were bi-allelic with one tri-allelic SNP (T/C/A), result was similar to that reported for chicken insulin and leptin receptor gene (Nie *et al*., 2005). The 55 SNP, 14 PCR-RFLP markers were developed, which would improve the efficiency of SNP genotyping for further study. In addition, the nucleotide diversity of the chicken cGH gene ($\theta = 1{\cdot}45 \times 10^{-3}$) was quite a lot lower than the one obtained for GHc gene ($\theta = 2{\cdot}72 \times 10^{-3}$). This proved that the chicken cGH gene was much more conservative than its ligand.

Results showed that Desi 2nd generation chickens had highest weight on final calculations. After PCR samples were preceded for sequencing from Microgen company Korea and results were interpreted through the ensemble.9 software BLAST were performed. Desi chickens had a nonsense mutation at 45 bp through the replacement of G<A which resulted in the formation of the stop codon. Desi F1 generation chicken samples showed two different types of mutations, like frameshift mutation at 134bp as deletion mutation and missense mutation at 118bp T<Gas Glycine amino acid. A missense mutation was observed in Desi F2 generation chicken samples at 81bp with T<A, forms Serine amino acid.

Desi chickens showed greater immunity and recovery against many seasonal diseases both in summer as well as winter. The chicken GHc gene seemed to have much more effect on males than on females. As shown by both single SNP and haplotypes, they were significantly associated with most growth traits in males, and with very few in females. In contrast to mammals, the dosage compensation of the homology sex chromosome was absent in female birds Because the chicken cGH gene is located on the Z chromosome, males and females have different gene copies of two and one each The co-expression of two alleles in Z chromosome, as shown by similar studies, would give rise to more cGH in males than in females. This could probably explain most, if not all, of the different effects of cGH on males and females in this study.

Acknowledgment

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Conflicts

The authors have not declared any conflict of interests

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