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DETECTION THE POLYMORPHISMS OF INSULIN GENE HORMONE INSG ON HYBRID BROILER ROSS 308

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Abstract – In order to detect the polymorphisms of Insulin gene Hormone INSg in hybrid broiler Ross 308 blood samples of a 200 chicks - one day old (wings numbered) and reared in closed system in order to identify the single nucleotide polymorphisms of INSg using PCR-RFLP. Results indicate that only two genotypes were found (the wild and the heterozygous SNP at T3737C in the flanking region of (intron 2)) only. This can be used to achieve a basic continues foundation studies for this gene beside the effect of genetic polymorphisms on performance, meat quality and economic treats in commercial hybrids and breed with their crossing over to be used in selection programs too.

* Part of Master Degree thesis of this author

I. INTRODUCTION

Chickens that belong to *Gallus Gallus* domestics were found to be the very old and important organism protein source in human food as eggs or meat production so it generally played important roles on health welfare life for humans for more than 6000 years, and in order to achieve their important traits, researchers tried to use chickens in many fields in scientific researches to be a good living model can be studied from the first hours of incubation (Miller et al 1971), and not only because of their short life cycle (Burt, 2002) but also because that it can be a good tool to be used in detecting mutation during embryonic development at any stage of incubation period (Al-Anbari, 1999) or to be used in studying physiological traits (Bloom et al, 1981) or for breeding programs when the scales of bird body as size or weight of growth used (Goliomytis et al 2003, Monica et al 2011), more over there are enormous amount of genomic information resources available recently for the chicken genome (ICPNC, 2004 & Burt, 2005) also when the first Avian Karyotype completed (Masabanda et al 2004). This is why poultry became a useful tool in modern researches that depend on implication of using Polymerase Chain Reaction (PCR) technique method and using molecular genetic markers with selection programs between and within breeds to create the highly economic traits performance lines (Emara & Kim, 2003). There are various kinds of genetic markers can be used to improve poultry performance depending on methods of genomic selection to evaluate DNA polymorphism (Tanaka et al, 1995). Using SNPs in poultry studies are the most important markers used in this field, beside the Restriction Fragment Length Polymorphisms (RFLP) because these markers are correlated with more than one of genetic loci of active economic trait (Dekkers & Hospital, 2002), so it became very important to use selection programs that depend on these genetic markers (Beuzen et al 2000). Important traits such as growth or other economic traits the somatotropic axis genes play a direct effect on these treats as Zhao et al, 2004 said. These axis consist of the essential components hormones such as growth hormone (GH), Insulin (INSg), Insulin – like growth factors bought (IGF-1, 1GF-2), Leptin, Thyroid hormones, etc.. (Kadiec et al, 2011). Most of these hormones effect on roles in animal development and growth (Zhou et al. 2007). It took place on effecting on stimulating amino acid uptake, DNA synthesis, glucose metabolism or proliferation of different cell types and then affecting and regulating growth (Mc Murtry. 1998) while the function of these genes affects and exists a variation which evaluate its effects on important traits, growth performance and muscle fiber in chicken (Lei et al, 2007). It improves health simultaneously while improving production traits too, and recently many researches revealed that it is very interesting to use SNPs of one or some of these somatotropic axis genes that affecting growth and many other development quantitative traits in chicken. One of them is the Insulin Hormone which belongs to the family of the polypeptide hormones differs from human insulin in six and seven amino acids and it seems normal in liver but differ in muscle fiber of chicken (Dupont et al, 2009). Insulin secreted from of Langerhanse islands pancreatic β cells and affect on Glucose uptake or oxidation and Glycogen synthesis and protein and the most important one of its effect is the DNA synthesis or gene expression and fatty acid synthesis also (Sonia, 2001). Gene Insulin consists of three introne with four deferent SNPs (Qui et al, 2006), the purpose of this study is to detect one of these SNPs fragment the T3737C in one of important commercial hybrid broiler Ross 308 in Iraq.

II. MATERIALS AND MEATHOD

This study was conducted at the poultry farms of Animal production Department, University of Baghdad, College of Agriculture Abu Graib for the period from 27\9\2015 to 31\2\2016 for farming breeding period and laboratory works percolated at Al-Musyeb Bridge Com. In order to detect the polymorphisms of Insulin gene Hormone on one of a common commercial broiler breed in Iraq. A total of 200 Ross308 one day - old broiler chicks were used. Birds were

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marked individually with wing ringed markers and placed in a deep litter with crushed straw in a closed system in order to control nutrition and other environment conditions. Birds dieted with commercially pellet mixture from Sulaymaniya sechuled as follows.. the starter from (1-14) days, the grower (14-27) days and the finisher from (27-35) days respectively to the end of experiment (35 days), food and water were given ad libitum. After the 4th week, blood samples for all birds were collected in EDTA –treated tubes and then Genomic DNA was extracted by using Geneaid Gsync™ DNA EXTRACTION KIT protocol for nucleated blood (bird). The work was as follow 10 µl of blood were translated to 2ml ependrof tubes then 190 µl of PBS Buffer and 20 µl of proteins K were added and mixed in puppet with shaking then incubated in water bath 60^0C \5m., 200 µl of GSB Buffer for 10 sec. shaking vigorously and incubated at 60^0C \5m with flipping the tube every 2 minutes, 200 µl absolute ethanol to the sample lysate were added with shake in puppet immediately for 10 sec. Then the GS Column placed in a new 2ml collection Tube and transfer all the mixture to the GS column centrifuged at 14-16 .000 xg \1 min, discard the 2ml collection tube containing the flow – through and transfer to new 2ml Collection Tube, washed with 400 µl of W1 buffer, centrifuged for 30 sec as the previous step then discarding the flow – through, and place the GS column in 2ml collection tube and adding 600 µl of wash Buffer to the GS column, centrifuged as before and discard the flow- through, and place the GS column in 2ml collection tube, centrifuged for 3 min at 14-16000xg to dry the column matrix genomic DNA. To measure the quality and quantity of extracted DNA NanoDrop Spectrophotometer was used. The polymerase chain reaction (PCR) assay was carried out in a total volume of 20 µl, containing 1U Taq DNA polymerase, 250mM, dNTP, 10 mM Tris, 30mM KCL, $Mgcl_2$ 1.5 mM, 3µl of DNA and 2µl of INSg specific FW primer with sequence 5` ctccatgtggcttccctgta and RF 5`ggcttcttggctagttgcagt. The PCR conditions were 95^0 C for 5 min to activate DNA polymerase, followed by 35 cycles for all of Denaturation stage at 95^0 C for 30 sec 62^0 C for 30 sec Annealing stage and 72^0 C for 1 min for Extension stage also and 5 min for the last final Extension cycle. By using electrophoresis the PCR product were amplified with 2% agarose gel, 7µl of PCR product, 5µl DNA ladder and 5µl athedum bromide dye at 45v followed with 100v for 2 hours. The banding pattern was observed with UV light, and by using 0.5µl of MSPI restriction Enzyme the product was digested in an incubator at 37^0 C for 3 hours. The PCR-RFLP product was electrophoresis with 3% agarose gel and 50v followed by 100v for 2 hours for whole time. The fragments PCR-RFLP size was determined by photo document system.

III. RESULTS AND DISCUSSION

Figure 1 shows the product of extracted DNA, while the second was for the PCR product of INSg with size of 372 bp with primers, While the third is the PCR-RFLP product with MSPI digestive enzyme. This study was subjected to screen SNPs of INSg at loci T3737C intron 2 in broiler Ross 308, so various genotype and allele frequency were identified at this nucleotide loci for the insulin gene as bands size differ which resulting from digestive with MSPI enzyme (Table1).

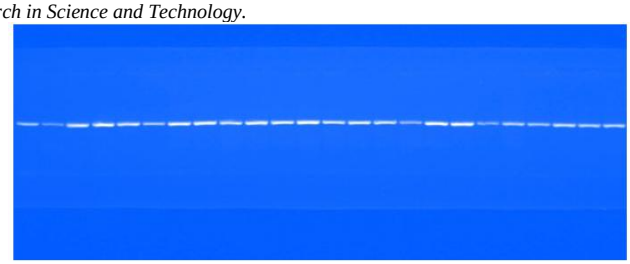

Figure 1: band of product extracted DNA samples.

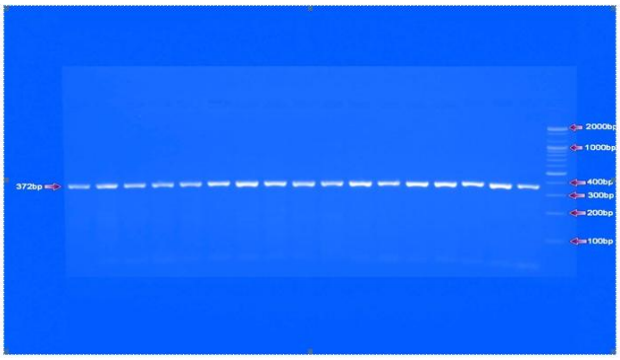

Figure 2: the PCR product of Insulin gene hormone INSg with 372bp and ladder of 100-2000 bp in Ross308.

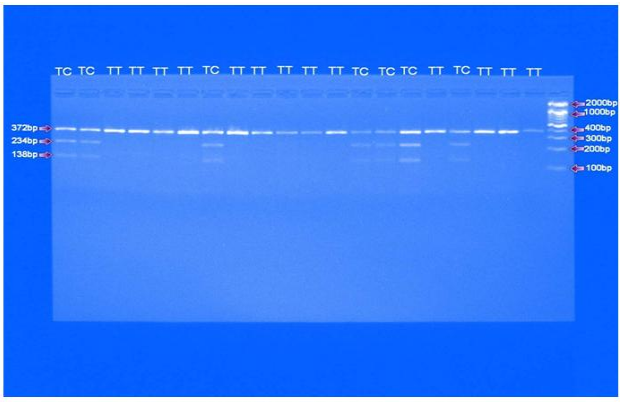

Figure 3: Gel picture of chicken INSg product digested with MSPI restriction enzyme. Ladder (100-2000 bp).

A Statistical Analysis System SAS (2012) was used to analyzed the polymorphism of INSg on Ross308. Hardy-Weinberg Equilibrium (HWE) was estimated too. The genotypic frequency and alleles frequency of INSg in broiler Ross308 breed were analyzed . The single nucleotide polymorphisms in insulin 2 gene were identified at nucleotides T3737C in broiler Ross308. Digested with MSP1 restriction enzyme produce two loci and fragments with deferent band size 372bp, 138bp and 234bp. The first loci the T of 372bp, while the second was C with length of 138Pb, 234Pb size of both bands. Results indicated that there were only Tow genotypes for INSg in Ross 308 breed that was the TT that seemed as one bond of identical allele that was the homozygous the Wild, while the second was the heterozygous TC. Table 1 shows the genotypic frequencies for insulin gene in Ross308. It seems that was a high

frequency P ≤0.01 between both genotypes with 66%, 34% as a co-dominance for TT when compared with TC genotype respectively.

Table 1 Genotype frequency for Insulin gen in commercial breed Ross 308

(%)	N.	(Genotype)
66.00	**132**	**TT**
34.00	**68**	**TC**
0.00	**0**	**CC**
%100	**200**	**Total**
**** 10.227**	**---**	**(χ^2)**
(P<0.01)**		

Table 2: Allele frequency for Insulin gene in a commercial breed broiler Ross 308

Frequency	Allele
0.83	**T**
0.17	**C**

Table 2 shows low frequency for C allele with (0.17). While there was a high frequency for allele TT with (0.83). These results agreed with result of Khoa et al (2013) study for INSg on Cobb 500 breed too on 32 individual when PCR- RFLP technique used with MCPI restriction enzyme also, two genotypes where found only they were TT and TC with frequency of 76% & 24% respectively, while CC genotype shows nil frequency 0%. These results were the same as with local Vietnam breeds and other commercial ones, the CC genotype was ranged between 4% as the highest percentage and 1% as the lowest one on all other studied breeds (Khoa et al, 2013). Qui et al, 2006 found that this T3737C SNP affect and associated significantly with length of small intestine, while Lei et al, 2007 found that this SNIP affect the muscle fiber density of broiler. In conclusion, single nucleotide polymorphisms of insulin gene we detected in this sample of broiler Ross308 can be used in selection programs to create breed or hybrids with this SNP, which affect body weight according to its effect on the more important part of digestive system the length of small intestine that response on the absorption of the diet, to improve body weight, growth rate, meat quantity or other quality traits also, by the way.. because chicken contain a great genetic potential recourses for improving performance treaties, so we need to study this SNP in various broiler commercial chicken breed or local one in Iraq too in order to be improved.

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