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Detection the Polymorphisms of IGFBP-3 gene in Awassi Lambs.

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ABSTRACT

IGFBP-3 is one of the structural proteins whose response has many effects on the growth factors ,also it is responsible for the different effects of Insulin-like Growth Factors (IGFs) In most protein, it is spatial President carrier growth factors .This study aimed to identify the IGFBP3 gene (Exon 2) polymorphism in Awassi Lambs . Blood DNA was extracted from 66 lambs to determine the genetic polymorphism of IGFBP3 .Restricted Fragment Lengths Polymorphism (RFLP) was don with HaeIII restriction enzyme and then the desired regions were amplified using PCR and Sequenced . Results showed that no genetic variance within the studied samples were seen and all individuals had the wild genotype (AA).This result may be due to the low sample size studied and select against low weight trait associated with the mutant genotype

Keywords: Protein associated with insulin-like growth factor, insulin-like growth factors, genetic markers, polymerase, deportation

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INTRODUCTION

Awassi is widespread breed of sheep in many countries of middle east and contribute of 58.2% of Iraqi sheep (Ishaq and Ageel, 2013). Using of genetic strategies is one of the efficient methods to increase the animal production through active selection for the important and desire genetic factors and increase its potential ability (Gutirrez and Goyche, 2007).

IGFBP-3 gene is a member of the family that is responsible for coding to produce a structural protein called Insulin like Growth factors (IGFs)(AL-Hanafy and Salem, 2009). The gene is located at the 4th chromosome in goat, sheep and cattle (Kappes et.al., 1997).Length mRNA of this gene is about 1.65 kb and it contains five exons (Kim et.al.2007 ; AL-Hanafy and Salem, 2009). The nucleotide frequency of this gene is 90% similar in sheep, goat, cows and buffalo and the similarity rate in amino acids among them is about 93% (Kumar, 2006). IGFBP-3 is the most important member of insulin like growth factors family because it is responsible for multiple effects of insulin like growth factors in most of the mammalian species and considered the major transfer protein of growth factors (Zhan et.al.2015). Polymerase Chain Reaction (PCR) is one of the many techniques used to determine the genotypes and the genetic biodiversity among animal breeds especially to determine the quantitative traits loci (QTL) (Lorenz, 2012; Varoni et.al.2016). In the last decades, scientists exploit this technique to study and tracing the mutations which occur in genome especially the single nucleotide polymorphism (SNPs) and used it as genetic markers (Williams,2005).

Because of the rare studies about this gene especially in Iraq, the current study aimed to highlight this gene polymorphism and the allele frequency to use it as guideline in the selection for producing generations with high fecundity rate in Awassi sheep breed.

MATERIALS AND METHODS

Experimental animals: 66 Turkish Awassi lambs in 2-4 months of age and17-26 kg weight were selected from the commercial flock reared in Diyala governorate (57km east of Baghdad) . Flock was housed under half open sheds. Feeding lambs depends on free grazing as well as concentrated feed(about 400 gm / head / day).

DNA extraction : Blood samples from each ewe were collected at the same time (10 ml from jugular vein) to determine the studied region (333bp) (Figure 1) of IGFBP-3 gene (partial intron 1, complete exon 2 and partial intron 2).

Primers: The following primers were used for the amplification of the gene .

F: 5' TATCAATGACCGTCAAGTCTGTG -3' and R: 5' - GTGATCTCTGGATACCCAGGC -3'(Sharma, 2011)

For amplification, 25 µl of PCR reaction was prepared by adding profi taq PCR PreMix KIT (12 µl), Template DNA (5 µl), Primers (0.5 µL F,0.5 µL R) and DNase Free Water(6 µl)(BioNeer.2018).The amplification programmer used was comprised of an initial denaturation step at 94 °C for 1 min followed by 40 cycles of denaturation at 94 °C for 1min, annealing at 56°C for 1min and extension at 72°C for 1 min, and a final extension of 72°C for 5 min(Sharma, 2011). the PCR-RFLP technique and *HealII* restriction enzyme(An aliquot of 20 µl of PCR product was digested 3 hour at 37 0 C with 1 units of HaeIII Enzyme (Bioneer .2018)) and DNA sequencing (Sanger sequencing using ABI3730XL, automated DNA sequencer , by Macrogen Corporation – Korea(macrogen2018). The results are received by email then analyzed using genious software (Geneious 2018)) were used to determine polymorphism of IGFBP-3 gene (partial intron 1, complete exon 2 and partial intron 2) .

TATCAATGACCGTCAAGTCTGTGCCTCCTTGTGTCCTTCAA**GGAAATGGCAGTGAGTCGGAAGAAGACCACAGCATGGG**
GAGCACGGAGAACCAGGCTCTCCCCACCACACGCCGGGTGCCCGACTCCAAATCCCACCTCGCCCACACCAAGATGGAT
GTCATCAAAAAAGGTCATGCCAAGGACAGCCAGCGCTACAAGGTTGACTACGAGTCTCAGAGCACAGACACCCAGAAC
TTCTCCTCCGAGTCCAAGCATGAGACAGAATACGTGAGAGCTTTTCCTCTTGTTGATGTGGGGGTGGGGCCACCTGGCCT
GGGTATCCAGAGATCAC

Figure1 : The studied region (333bp) of IGFBP-3 gene

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RESULTS

PCR-RFLP technique showed existence one band (305bp), this means that one or more invisible fragments were produced by the restriction enzyme (Figure 2).DNA sequencing and webcutter site detected that the length of invisible fragments were 20 and 8bp.(figure 3 and 4)

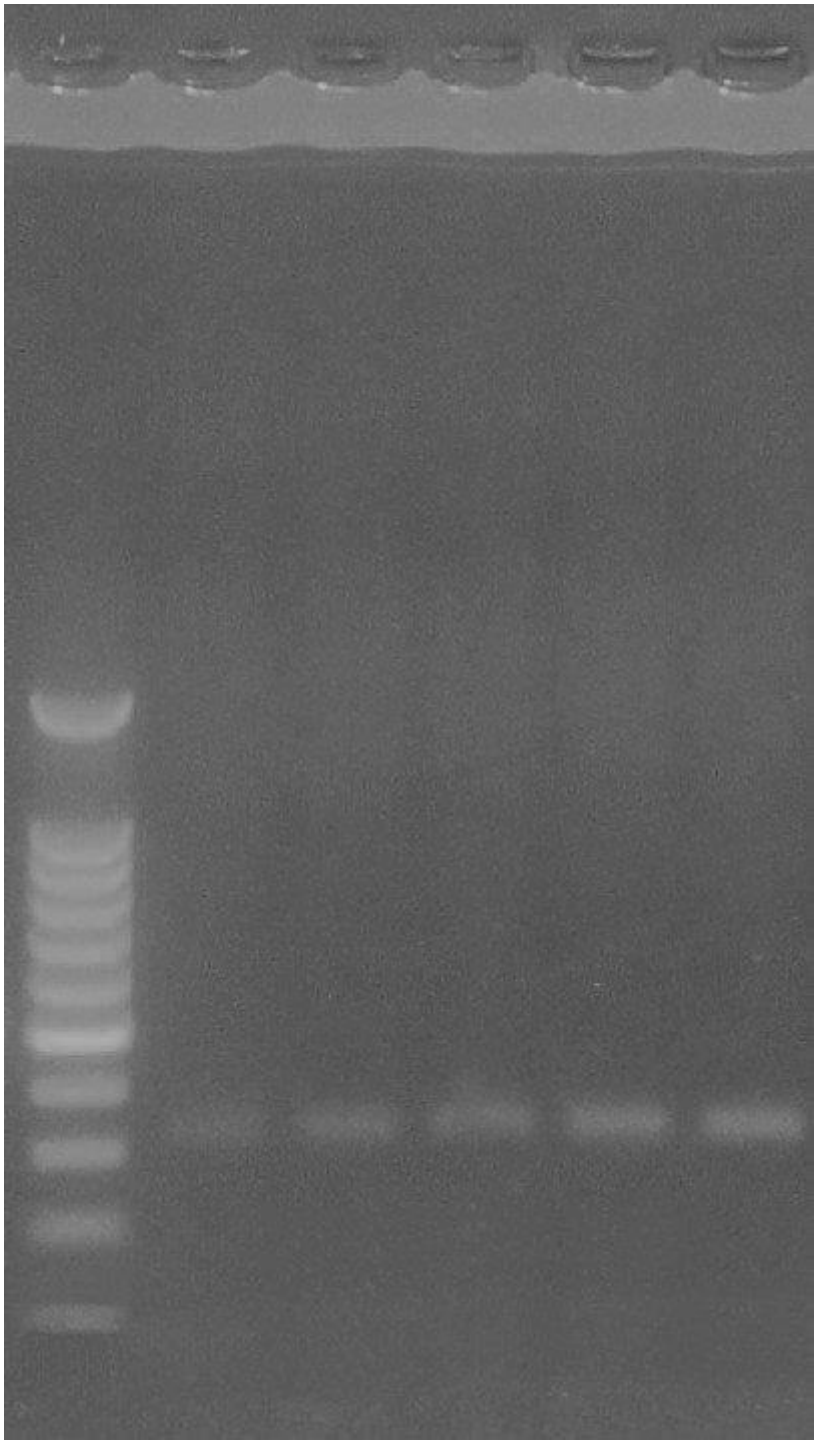

Fig. 2 *HaeIII* PCR-RFLP of 333 bp IGFBP3 fragment. 1%agrouse and 100 bp ladder

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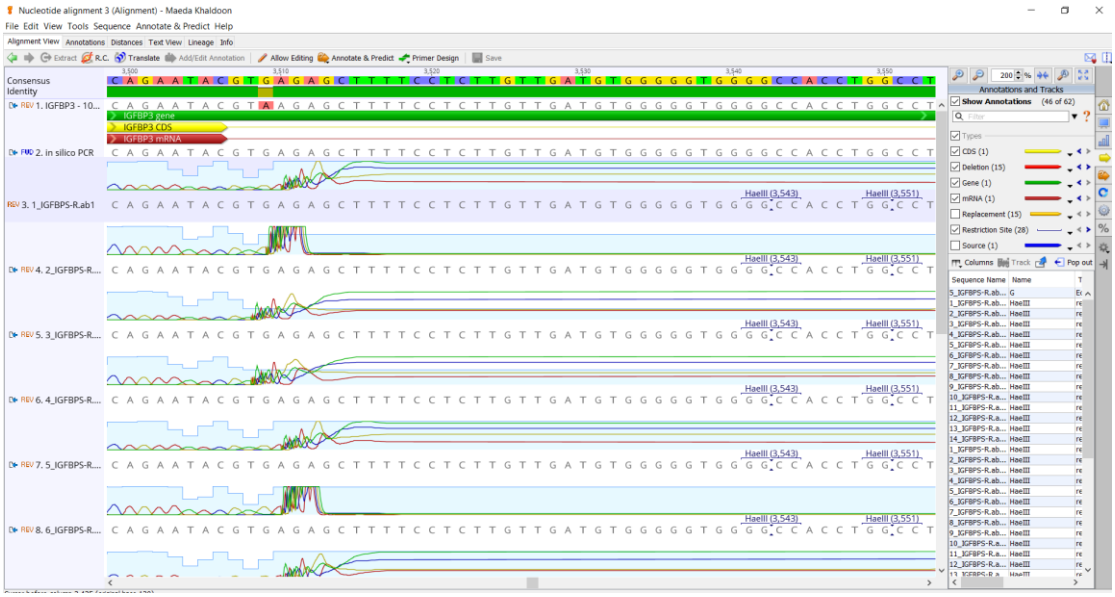

Figure3 DNA sequencing analyzed(genious software)

tatcaatgaccgtcaagtctgtgcctccttgtgtccttcaaggaaatggcagtgagtcggaagaagaccacagca base pairs
atagttactggcagttcagacacggaggaacacaggaagttcctttaccgtcactcagccttcttctggtgtcgt 1 to 75
tggggagcacggagaaccaggctctccccaccacacgccgggtgcccgactccaaatcccacctcgcccacacca base pairs
acccctcgtgcctcttggtccgagaggggtggtgtgcggcccacgggctgaggtttagggtggagcgggtgtggt 76 to 150
agatggatgtcatcaaaaaaggtcatgccaaggacagccagcgctacaaggttgactacgagtctcagagcacag base pairs
tctacctacagtagtttttccagtacggttcctgtcggtcgcgatgttccaactgatgctcagagtctcgtgtc 151 to 225
acacccagaacttctcctccgagtccaagcatgagacagaatacgtgagagcttttcctcttgttgatgtggggg base pairs
tgtgggtcttgaagaggaggctcaggttcgtactctgtcttatgcactctcgaaaaggagaacaactacaccccc 226 to 300
HaeIII HaeIII
tgggg ccacctgg cctgggtatccagagatcac base pairs
acccc ggtggacc ggacccataggtctctagtg 301 to 333

Figure4: *Hae*III restriction enzyme site (webcutter software(Webcutter 2018))

DISCUSSION

The current results were consistent with many others previous studies those reported monomorphic restriction *Hae* III enzyme profile of 333 or 654 bp fragment (partial of exon 2, complete intron 2, complete exon 3 and partial of intron3) involving the current studied region in many sheep breeds (Mahrous,2015. Kumar atel,2016. Sharma, 2011) that showed the prevalence of the wild genotype. Ali (2009) and Padma (2004) suggested that this region of IGFBP3 gene have very high sequence homology within sheep. On the other hand, this may be caused by the selection against the low weight as in the studied herd. Because the mutant form of IGFBP-3 gene is not link with the IGF factor or corresponding forms such as wild genotype. It also leads to product an inhibitor protein reducing the (IGF) receptor inside cells (Yan atel,2014). AL-Khuzai (2018) found that the mutant form of Awassi sheep is associated with the low body weight, while the wild form was associated with high and moderate body weight.

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