WORLD JOURNAL OF PHARMACEUTICAL RESEARCH
SJIF Impact Factor 5.990
Volume 5, Issue 1, 87-93. Research Article ISSN 2277– 7105

GROWTH HORMONE GENE IN IRAQI AND TURKISH AWASSI SHEEP USING PCR-RFLP

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Article Received on
08 Nov 2015,

Revised on 29 Nov 2015,
Accepted on 19 Dec 2015

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ABSTRACT

The objective of the study was to examine the polymorphic *GH/HaeIII* gene in Awassi sheep breed (Iraqi and Turkish) Eighty genomic DNAs that consisted from 40 Iraqi and 40 Turkish Awassi sheep were isolated from blood samples of those animals. A fragment of *GH/HaeIII* gene, comprising of a part of intron 2, complete exon 3, complete intron 3, complete exon 4 and a part of intron 4, was amplified. The amplified product with the length of 934 bp was digested with *Hae*III restriction enzymes and in Turkish Awassi showed the presence of *GH HaeIII* homozygotes AA genotype, with fragments 277, 202, 110, 100, 94, 68, 49 and 22,8,4 did not appeare on agarose gel under Uv light, Homo And heterozygous genotype with fragments 277,256,202,110,100,94,68,49bp showed in Iraqi Awassi sheep. The present study concluded that *GH/HaeIII* could be a genetic marker in sheep for improvement the purity of the Turkish breed. And diversity in the Iraqi Awassi sheep.

KEYWORDS: Awassi sheep, *HaeIII* enzyme, homozygosity, hetrozygosity, growth hormone.

INTRODUCTION

Awassi is the most common breed of sheep in the east of Mediterranean. It is the main sheep breed in Iraq and Syria, the only native breed in Jordan and Palestine (Hailat, 2005) and represents an important contribution to sheep breeds in Turkey 3.5% of total sheep population (Gürsoy, 2005).

Awassi sheep is a highly productive indigenous dairy breed as well as producing wool and meat (Azawi and Al-Mola, 2010).

Growth hormone (GH) is an anabolic hormone synthesized and secreted by the somatotroph cells of the anterior lobe of the pituitary in a circadian and pulsatile manner, it is a polypeptide hormone of about 22kDa molecular weight, composed of 190 or 191 amino acids (Ayuk and Sheppard, 2006).

It plays an important role in body growth and metabolism through protein synthesis, protein deposition and fat catabolism in tissues and organs and leads to increased nitrogen retention (Hart and Johnson, 1986; Gluckman *etal*., 1987), decreased energy retained as fat, gluconeogenesis and cell division (Eisemann *etal*., 1986; Neathery *etal*., 1991).

Growth hormone gene is encoded by 1800 base pairs (bp), consisting of five exons, separated by four intervening sequences (Gordon *etal*., 1983).

In the ovine, two alleles of the GH gene have been described. The Gh1 allele contains a single gene copy (GH1), whereas in the Gh2 allele the gene is duplicated (copies GH2-N and GH2-Z) with the two copies being located 3.5 kb apart (Valinsky *etal*., 1990).

Sequence differences between the GH2-N and GH2-Z copies have been demonstrated and polymorphisms have been found in oGH coding and non-coding regions (Ofir and Gootwine, 1997).

This study shed light on the polymorphic of the GH/HaeIII gene in Awassi sheep breed (Iraqi and Turkish).

MATERIALS AND METHODS

Blood collection

Eighty blood samples collected from (40) Iraqi and (40) Turkish Awassi sheep of different ages (male and female) took from ministry of science and technology/agricultural researches (Al-Zaafarania,20 km south of Baghdad) and from ministry of agriculture/research station of sheep and goat (Abu-Ghraib,25km west of Baghdad), from November 2014 to April 2015 at university of Baghdad/Institute of genetic engineering and biotechnology for post graduate studies.

Blood samples were collected from jugular vein by vacutainer needle into 6 ml tubes containing 1 ml of 10% ethylene diamine tetra acetic acid (EDTA). The samples were stored in cool box containing ice jelly pack during collection process then kept at (-20^{o}C) until ready for DNA isolation.

DNA isolation and amplification

The genomic DNAs were successfully isolated from the blood samples using DNA extraction kit (Bioneer, genomic DNA isolation kit, korea).

Polymerase Chain Reaction (PCR) was carried out using Eppendorf thermocycler (Biometra, Germany) and PCR Green Master Mix (Promega Corporation, USA) containing bacterially derived Taq DNA polymerase, dNTPs, MgCl2 and reaction buffers at optimal concentrations for efficient amplification of DNA templates by PCR. Each reaction mixture consisted of 12.5 µl of taq green PCR master mix, 5 µl of the DNA solution (50 to 100 ng/µl), 1 µl of each primer (10 pmol/µl) and 5.5 µl free nuclease water .Amplification for a 934 bp fragment from the intron II to the intron IV of the oGH gene was carried out using primer according to Kuulasma (2002) as follows.

GH-F: 5'-GGAGGCAGGAAGGGATGAA-3' and

GH-R: 5'-CCAAGGGAGGGAGAGACAGA-3'.

The amplification was carried out using thermolcycler (Eppendorf Mastercycler) with the following conditions: initial denaturation at 95°C for 5 min, by 33 cycles of denaturation at 95°C for 45s; annealing at 60°C for 45s and extension at 72°C for 45s followed by a final extension at 72°C for 10 min. Each amplified product was analyzed by electrophoresis on a 2% (w/v) agarose gel, using ethidium bromide.

RFLP analysis and electrophoresis

Restriction fragment length polymorphism (RFLP) analysis was conducted to detect polymorphism sites. The PCR amplicons were digested with *Hae*III restriction endonuclease (Bio Labs Inc, New England) The amount of digestion mixture was 5.7 µl free nuclease water, 3 µl Digest buffer, 1 µl *Hae*III restriction enzyme, 0.3 µl from bovine serum albumine (BSA) with 20 µl PCR DNA product. The reaction mixture was digested in a thermocycler at 37^{o}C for 4 h., then twenty µl of samples and then detected by 3.5% agarose gel electrophoresis. The electrophoresis product restriction by *Hae*III enzyme was interpreted by comparing them with 50bp *marker* band.

RESULTS AND DISCUSSION

Amplification of growth hormone gene

This study throw light on the awassi sheep that consider the most important breed in Iraq and high light on the importance of the gene that coding for growth hormone which in turn responsible for the meat and milk production in the sheep.

The DNA that extracted from blood sample of Iraqi sheep and also the Fourty Turkish awassi sheep were used for comparising showed clear sharp bands that presented on agarose gel in electrophoresis and then examined under Uv light, the purity of these DNAs were perfect ranged from 1.8-1.9 and the concentration were ranged from 16-20ng/µl according to Sambrook and Russel (2001).

The PCR product or amplicon of awassi sheep (Iraqi and Turkish) was also sharp and match to the marker 934 bp and that come in agreement with the *oGH* gene of lohi breed that located from intron II to the intron IV (accession no Genbank GQ452268, Gul, *etal*., 2009) And Indonesia fat tailed sheep (Donggala and East java breed, Malewa, *etal*., 2014).

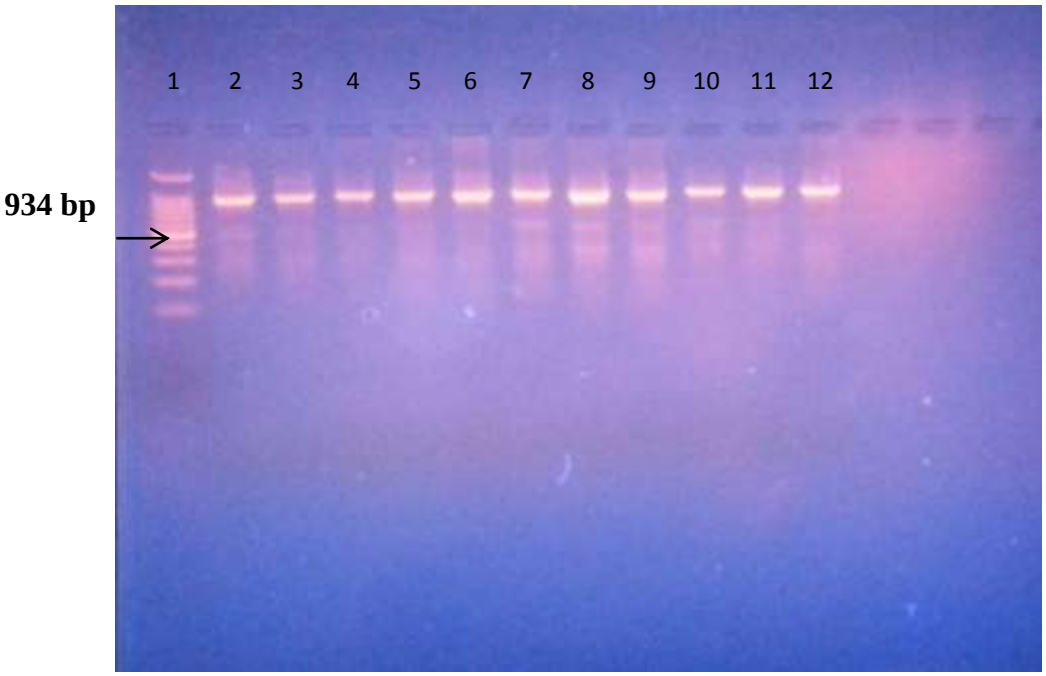

Figure (1): PCR products of growth hormone gene with size of 934 bp. The product was electrophoresis on 2% agarose gel at 5 volt/cm^2 for 1hour. Lane 1 DNA ladder (100-1000), Lane (2-12) PCR products of the growth hormone gene from Awassi sheep with size 934bp.visulized under U.V light after stain with Ethidium Bromide.

The result of restriction enzyme were revealed AA allele (homozygous) that had seven fragments namely 277,202,110,100,94,68,49bp in Turkish Awassi sheep found and in Iraqi Awassi AB allele (heterozygous) namely 277,256,202,110,100,94,68,49bp and homozygous also found this showed the range of purity of the Turkish herd that had been sampling of it, and it is pure breed and there is inbreeding between them, the probability of homozygous presence may be due to the adaptation of these breed to live in this region. the diversity in Iraqi Awassi sheep is marking there is cross breeding between them.

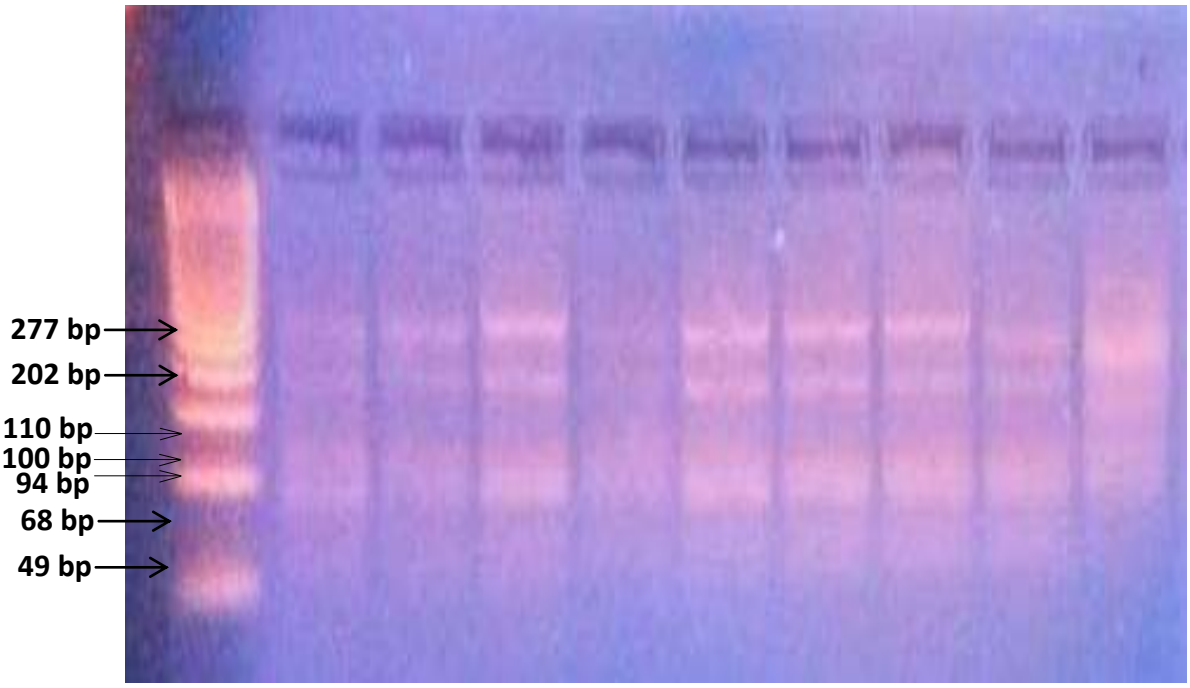

Figure (2): PCR product digested with *HaeIII* restriction enzyme electrophoresis on 3.5%. Lane1: DNA ladder (50 bp). Lane 2, 10: AA allele (homozyguse) of growth hormone gene. The (RFLP) products at agarose gel at 5 volt/cm^2 for 1hour. visulized under U.V light after stain with Ethidium Bromide.

The result of this study showed that GH/HaeIII had the different in both Iraqi and Turkish awassi sheep in homo and hetrozygosity and this came in agreement with study of, Malewa (2014) revealed there was both homo and heterozygots in Indonesia fat tailed sheep (Donggala and East Java breed) Which had allele AB (277, 256, 202, 110, 100, 94, 68, 49,22,21,8,4) that notes transition in the exon 3 (AG-CC) to (GG-CC) of the gene at 227 base IV (accession no Genbank GQ452268, Gul, *etal* .,2009) or at 255 base of PCR product.

Cobra, *etal.* (2013) suggested that there were association between different growth hormone genotypes and growth triats including birth weight (BW), weaning weight (WW), six month

weight (SW), nine month weight (NW) and w12weight (yearling weight) of makooei sheep by using PCR-SSCP. Gupta *etal*. (2007) used the PCR-SSCP analysis to investigate growth hormone polymorphism in Indian black banegal goats, while Bastos *etal*. (2001) detected two and five SSCP patterns for exon4 and 5 respectively.

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