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Identification of GH gene Polymorphism using PCR-RFLP of Iraq and Belarus Population sheep breeds

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ABSTRACT

The Growth Hormone gene is confederated to be a major active gene in mass gaining for the improvement of meat industry production, Therefore, our study aims to identify the GH gene polymorphism that correlated with meat gain parameters and traits in the Belarus and Iraq sheep with the application of PCR-RFLP method and the use of the HaeIII enzyme for the digestion of the PCR product. As result, three genotypes were spotted (n= 150), two homozygous genotypes GH AA, GH GG and heterozygous genotype GH AG, with an actual frequency of 0.153, O.247 and 0.6, respectively. The obtained results aid meat production amelioration by controlling the expression of the identified genotypes of the GH gene.

Keywords
Growth Hormone gene GHG , Sheep, meat, PCR-RFLP, genotype, HaeIII.

Introduction

Meat, one of the most important proteins source which counts its nutritional quality, meat can also characterize by its eating quality that can be decided by the consumers that can be very critical (Bohrer, 2017). Meat industry split in two big categories red and white meat, where the first category consists mainly of beef, pig and sheep that is known to be the modest sector comparing to the other meat category in several countries based on the consumption and demand (Kearney, 2010), along with the distribution and availability of this animal where the high percentage of the sheep population found to be in Asia with more than 44% followed by Africa with 29% which made sheep meat very consumed in those countries, the low amount of the sheep heads in Europe and America made it less demanding in contrast to the situation in the Asian countries (Cloete, 2012), for this reason in recent years significant progress has been achieved in animal husbandry in one of those countries that is the Republic of Belarus in order to jump with this meat to a higher level on consumption, which is not the case in the republic of Iraq where meat is the main purpose for sheep breeds raising this require improving sheep's meat quality to reach the consumer's desires.

In order to control meat characteristics, improve its quality and nutritive value molecular genetic works on identifying and localization the genes that control meat traits, where identifying each gene that can open the path to control and increasing or decreasing its expression can help to control the meat quality by increasing or decreasing some mechanisms that can change meat parameters and by that reaching the consumers desire, yet to identify any gene an effective method is needed, one of the most used techniques (Casas and Kehrli, 2016, Gao et al., 2007, Williams, 2008).

Several genes are nominated as the best target for meat quality improvement related to the most demanded traits, one of those important genes that is responsible for animal growing, The growth hormone (GH) gene that is expressed in the cells of pituitary gland which produce and secrete the Growth hormone (GH), that attaches and interact with its extracellular receptors to effect by that

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cell growth and metabolism (Mayo et al., 2000, Xia et al., 2016). The GH gene is the main player in postnatal growth, where it acts on the development of bones and muscles by participating in cell proliferation and multiplication that leads at the end in weight gain which also one of the desirable and wanted traits in the meat industry (Jeay et al., 2002, Ohlsson et al., 1992).
To reach molecule genetic goals for identification of meat traits genes such as the GH gene, a good and a direct technique must be applied such as the polymerase chain reaction and restriction fragment length polymorphism (PCR-RFLP) technic that is the main assay in this domain for been fast, specific, practical and economic that demand few consumables and steps (Guan et al., 2018, Horvat and Bünger, 1999).
Therefore, our study aims to identify the GH gene polymorphism that correlated with meat gain parameters and traits in the Belarus and Iraq sheep using the PCR-RFLP method.

Materials and methods

Animal samples

A total of 150 (n=150) animals from three sheep breeds were used for this work as following:

- 50 sheep of the Texel breed from Hvineviehy farm Hrodno region.
- 75 sheep of Ile de France breed from Istern farm Minsk region Belarus
- 25 sheep of Awassi breed from Agriculture research centre from the Baghdad-Iraq.

PCR-RFLP assay

A PCR amplification reaction was performed using specific primer that was designed on the basis of DNA sequence of the GH gene
GH F: CTC TGC CTG CCC TGG ACT
GH R: GGA GAA GCA GAA GGC AAC
A PCR cocktail consisted of 1.0 μM of upper and lower primer, 0.2 mM dNTPs, 10x of PCR reaction buffer (200 mM (NH4)2SO4, 0.1 Mm Tween 20, 750 mM Tris-HCl (pH 8.8) and 1.25 units of Taq polymerase. The cocktail was aliquot into PCR tubes with 100 ng of sheep or goat DNA.
As a start, a primary denaturation was exhibited by running the reaction at 94°C for 5 min, after that 35 heating cycles was effectuated at 95°C for 30 sec for each for a total denaturation, then a touchdown annealing from 65-52°C for 30 sec, 72°C for 45 sec and a final extension at 72°C for 7 min.
As for the Restriction Fragment Length Polymorphism (RFLP), the 422 bp fragment produced by the PCR were digested using restriction enzyme; HaeIII, where Ten microliter of PCR product was digested with 1 μL of FastDigest restriction enzyme for 5 min at a temperature of 37°C. Finally, the restriction fragments were subjected to electrophoresis in 2% agarose ethidium bromide gel in 1x TBE buffer (0.09 M Tris-boric acid and 0.002 M EDTA). Gels were visualized under UV light and documented in FX Molecular Imager apparatus.

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Results and discussions

Table 1. Restriction fragment length in the tasted sheep breeds

Gene	Genotypes	Restriction enzyme	Number of fragments (bp)
GH	AA	*Hae III*	1 (422)
	GG		2 (366, 56)
	AG		3 (422, 366, 56)

Table 2. The frequency of genotypes and alleles of GH genes of 150 sheep;

Gene	Genotypes	Frequency percentage of genotypes %		Alleles	Frequency of allele %	X^2
		Expected	Actuel			
GH	AA	7.84	15.33	A	28	21.78
	GG	40.32	24.67	G	72	
	AG	51.84	60.00			

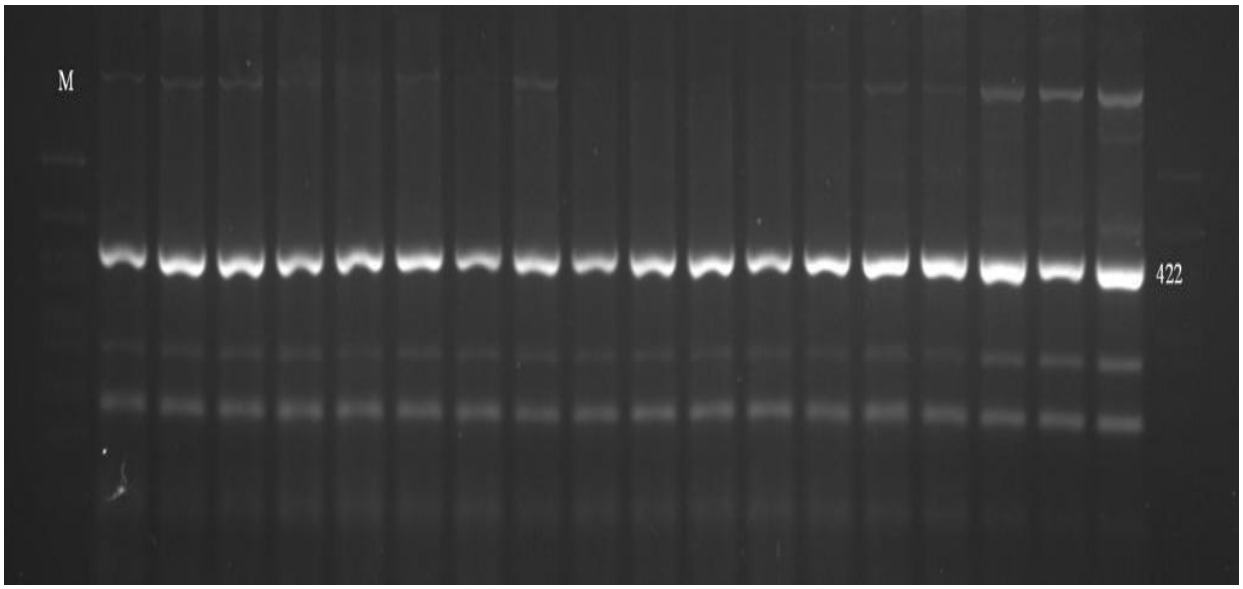

Figure 1. Electophoregram showing the amplification product. M – DNA size marker, 1-18 – our sample.

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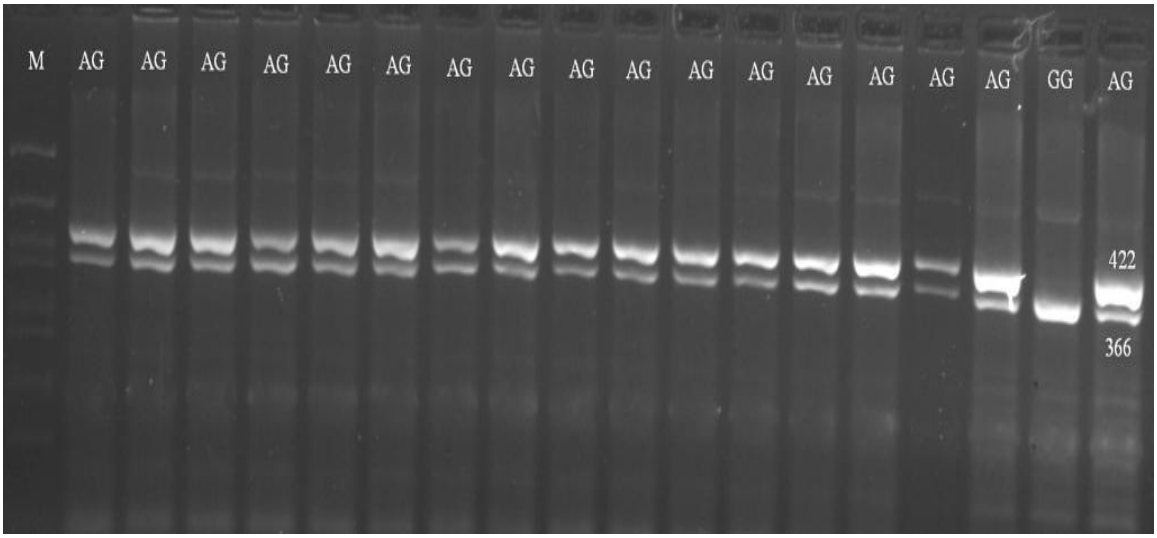

Figure 2. DNA electrophoric pattern of GH gene amplicons after digestion with HaeIII enzyme.

In this work piece, the polymerase chain reaction and restriction fragment length polymorphism (PCR-RFLP) technic was used for being known as fest practical and specific and demands only the presence of primer in order to detect and identify a very important gene polymorphism in meat quantity trait and mass gaining, GH gene. Where 150 sheep was conducted from three different breeds and regions.

422 was the length of the amplification product of the PCR that was subjected to the digestion with the use of the HaeIIIrestriction enzyme, where five fragments were obtained as following: two digested fragments for the G GG homozygous genotype at 366 bp and 56 bp, as for the allele A AG heterozygous genotype three fragments at 422 bp, 366 bp and 56bp were resulted for the restriction step as demonstrated in figure 2 and intable 1.

The frequency and alleles genotypes of the GH gene are shown in table 2 where two alleles represent this gene A and G which was the more frequent with a rate of 72% comparing to the A allele that showed a frequency of 28%.

The actual and expected frequency percentage of the genotype in the selected sheep breeds is presented in table 2, a high deferent was noted between the actual and expected frequency genotype for the AA homozygous genotype, where the actual rate was two times higher than the expected with a percentage of 15.33 and 7.84, respectively; while the opposite was recorded regarding the GG homozygous genotype frequency with a 40.32% for the expected rate against an actual rate of 24.67%. a deference of 8.16% was remarked between the actual and the expected frequency of the AG heterozygous genotype that was 60% for the anticipated frequency and 51.84 for the real rate.

The heterozygous genotype AG was the more frequent in the GH gene of the studied sheep breeds comparing to the homozygous genes which due to the combination of the two alleles A and G (Table 2). From the conducted study on the one hundred and fifty sheep, the obtained results segues that the sheep's population is monomorphic for the GH gene, where the 422p 366 bp and 56 bp amplificated fragments obtained from the HaeIII digestion enzyme are displayed for the AG genotype.

Growth hormone is an essential pituitary hormone whose secretion is modulated differently by sex in many animal species. It is a growth factor for bones and muscles, a differentiation factor and a metabolic regulator for the liver, adipose tissue and muscle (Devesa et al., 2016, Hemmings et al., 2015, Rosales Nieto et al., 2019). Therefore, Recognizing and putting a finger on one of a key player gene in meat quantity traits that is the Growth Hormone phenotype open the path to supervise and regulate its expression in the cells of pituitary gland of sheep, this means the capacity to control the secretion of the growth hormone and by that controlling cells metabolism, division, multiplication and proliferation so the yield of animal production can be improved by increasing the growth rate of animals using growth hormones that bind to specific receptors in target tissues

Conclusion

Three sheep breeds were chosen from the population of Iraq and Belarus in order to localize and identify a majorly important gene in meat production traits “the growth hormone gene” with the application of the RPC-RFLP technique, two homozygous genotypes GH AA and GH GG were identified with addition with one heterozygous genotype GH AG, where the obtained results help to control expression this gene and by that of meat gaining mass for meat industrial production.

Conflict of interests

None.

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