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# Estimate of the Association of IGF-I and IGFALS Genes with Growth Traits in Hamdani Sheep

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**HIGHLIGHTS**

- Effect of IGF-I and IGFALS on growth traits.
- Genotype and allele frequency.
- Use IGF-I and IGFALS genes as molecular marker.

**Abstract:** IGF-I and IGFALS play a vital stimulator role in skeletal growth, cell differentiation, metabolism, and other physiological processes. A total of 65 (male and female) animals were used in the study. Animals were measured for growth traits at birth weight, weaning weight, and weights at 6 months. The average daily gain (ADG) was calculated from birth to weaning (ADG1) and from birth to 6th month (ADG2). PCR-RFLP analysis was used to detect IGF-I polymorphism at the 5' regulatory region and IGFALS at Exon 1. Three genotypes (AA, AB and BB) were observed for IGF-I/*BfoI* locus with allele and genotype frequency 0.79(A) and 0.21(B); 0.71(AA), 0.15(AB) and 0.14(BB). Also, three genotypes (AA, AB and BB) were found for IGFALS/*HinfI* site with allele and genotype frequency as 0.22(A) and 0.78(B); 0.11(AA), 0.23(AB) and 0.66(BB). The genes were in agreement with Hardy-Weinberg equilibrium ($p>0.05$). Association analysis suggested that IGF-I and IGFALS significantly affected the growth traits ($P<0.05$). In terms of birth weight, The AA genotypes of IGF-I were higher than AB and BB. The AB genotype in terms of IGF-I had higher ADG2 compared with other genotypes. The AA genotype of the IGFALS gene was higher in terms of birth weight than other genotypes. In addition, the BB genotype was higher ADG1 than AA and BB. It is suggested that polymorphism of the IGF-I and IGFALS genes may be a potential molecular marker for growth traits in Hamdani sheep.

**Keywords:** IGF-I; IGFALS; Hamdani sheep; growth traits; candidate gene.

## INTRODUCTION

Developments in molecular technologies have facilitated many things, including identifying genetic variations in the genome and detecting an association between genes and production traits. The marker-assisted selection (MAS) technique has shown great effectiveness in the genetic improvement of quantitative traits with significant economic returns, such as the sheep's growth and development traits [1-4]. Growth and development are considered one of the most important economic traits. These traits are an essential criterion for selection programs in sheep [5-7]. The Hamdani sheep are native Iraqi breeds distinguished by their large size, with adult rams weighing up to 80 kg and ewes up to 65 kg. It also has the advantage of yielding milk and heavyweight wool [8-10]. It requires making the genetic improvement of Hamdani sheep by identifying essential genes that affect the production traits. Many candidate genes affect the growth and development of sheep breeds, including IGF-I and IGFALS. IGF-I, also called somatomedin C, is a signaling system consisting of two receptors IGF-1 and IGF-II, and six binding proteins (IGFPB-1-IGFPB-6) [11-13]. It has an essential role in growth and development metabolism in various mammals [14-16]. Therefore, the IGF-I gene is considered a candidate gene associated with growth traits in livestock. Ovine IGF-I gene is located on chromosome 3 and contains six exons [17,18]. ALS (The acid-labile subunit) protein binds to IGF-I and IGFBP-3 and plays an essential role in maintaining the IGF/IGFBP systems integrity. ALS stabilizes the 150 kDa ternary complex and regulates the growth, development, and other physiological/pathophysiological processes [19,20]. Consequently, ALS is essential to keep normal circulating IGF-I and IGFBP-3 levels. The ALS protein is synthesized in the liver and encoded by the IGFALS gene. Ovine IGFALS gene is located on chromosome 24 and contains three exons [19]. Previous studies reported the possibility of using IGFALS as a candidate gene for its association with growth traits in cattle and sheep [19,21]. Therefore, this study aimed to determine the IGF-I and IGFALS genes polymorphism and their association with Hamdani sheep's growth traits.

## MATERIAL AND METHODS

A total of 65 (41 male, 24 female) Hamdani sheep were used in the study. Males and females weights were taken at birth, weaning, and at 6 months. The animals were on a commercial farm in Kirkuk city/Iraq. The ewe diets contained; barley %65, wheat bran %25, soybean meal %8, salt %1, limestone %1. The chemical composition were; protein 15%, energy (MJ / kg) 12%, dry matter %73. Up to the age of 90 days, newborns were dependent on their mother's milk.

### Body weight measurement

The body weight of lambs was recorded at birth within 24 hrs. of lambing. Also, the lambs were weighed at weaning age in three months and six months.

### Calculate average daily gain

The ADG1 (Average daily gains from birth to weaning) and ADG2 (Average daily gains from birth to 6th month) was calculated by using the following formula below:

ADWG1=(Weaning weight-Brith weight)/( 90 day)

ADWG2=(6th month weight-Weaning weight)/(180 day)

### Sample collection and DNA extraction

The blood was collected from the jugular vein using tubes containing EDTA and stored at -20°C. Genomic DNA was extracted from whole blood by using the phenol-chloroform methods. The primer sequence and PCR conditions of the IGF-I and IGFALS gene locus were given in (Table 1). The PCR was done in a reaction volume of 20 µL, contains 5 µL (50 ng) DNA, 5 µL of PCR Master Mix (GoTaq® G2 Green Master Mix, Promega, USA), 0.5 µL for each primer (10 µ mol) and 9 µL distilled water.

**Table 1.** The primer sequences of IGF-I and IGFALS gene locus

| Gene | Position | Primer Sequence | FL | RE | PCR conditions | Reference |
|---|---|---|---|---|---|---|
| IGF-I | 5' regulatory region | 5'-TGAGGGGAGCCAATTACAAAGC-3'<br>5'-CCGGGCATGAAGACACACACAT-3' | 294bp | *BfoI* | 94°C 6m,<br>94°C 30s,<br>55°C 30s,<br>72°C 30s,<br>30 cycles<br>72°C 10m | He and coauthors (2012) [23]. |
| IGFALS | Exon 1 | 5'-GTGAAAGCAAACAGAGCAG-3'<br>5'- CATTGACCACTGGAGACTG -3' | 1113 bp | *HinfI* | 95°C 5m,<br>95°C 45s,<br>57°C 45s,<br>72°C 45s,<br>35 cycles<br>72°C 10m | Alizadeh and coauthors (2020) [19]. |

FL: fragment length

### Genotyping by RFLP method

The mix consisted of 8 µL PCR product, 4 µL distilled water, 2 µL 10X buffer and 1 µL restriction enzyme (Total of 15 µL). Digestion products were separated at 3% agarose gel in 100 V for 60 min. The gel stained by ethidium bromide and used a 3000 bp DNA marker (Vivantis, Malaysia). The results were checked under ultraviolet light.

### Statistical analysis

The allele and genotype frequency of the genes and the Chi-square test $\chi^2$ were calculated by popgen32 (ver.1.32). Association analyses were done by using the General Linear Model (GLM) of Minitab 16. The least-squares means were compared using Tukey, the least significant difference test.

The general linear model was:

$Yijk = \mu + \alpha i + \beta j + eijk$

Yijk: traits measured

µ: overall mean for each trait

αi: genotypes effect

βj: sex effect

eijk: random error

## RESULTS and DISCUSSION

### IGF-I locus polymorphism

294bp of PCR product was amplified. Three genotypes (AA, AB, and BB) were obtained after digestion by enzyme; AA (194 and 100bp), AB (294, 194 and 100bp), and BB (294bp) (Figure 1). Chi-square $\chi^2$ test showed agreement to Hardy-Weinberg equilibrium ($p>0.05$) (Table 2). The allele and genotype frequency was 0.79(A) and 0.21(B); 0.71(AA), 0.15(AB) and 0.14(BB). Mutations in the 5' regulatory region of the IGF-I gene have also been found in other livestock species. Ge and coauthors [22] showed that a polymorphism in the IGF-I regulatory region significantly affects the growth traits in the Angus breed cattle. They hypothesized a direct action of the found mutation on gene transcription and, subsequently, on phenotypic traits [22]. He and coauthors [23] found three genotypes (AA, AB, and BB) in Small Tail Han and Hu sheep, two genotypes (AA and AB) in Texel, and only one genotype (AA) in Dorset with A and B allele frequencies 0.809, 0.638, 0.969 and 1.000; 0.191, 0.362, 0.031 and 0.000, respectively. The allele frequency was 0.94 for the G and 0.06 for the C allele in Sarda sheep [24]. Bakhtiar and coauthors [25] estimated A and B allele frequencies in the Sanjabi sheep as 0.52 and 0.48, respectively. The allele frequency of the A and B Kivircik sheep was 0.915 and 0.085, respectively [26]. Umego and coauthors [27] reported 0.72 and 0.28 for A and B alleles in Yankasa sheep. 5' regulatory region were monomorphic in Akkaraman sheep [28]. Sebastiano and coauthors [29] determined allele frequency of G and C in the 5' regulatory region for Sarda sheep as 0.90 and 0.10, respectively. It is evident from the current study and previous studies that the A allele frequency is higher than the B allele. This might indicate that the B allele may be negatively related to a trait or traits historically selected against it.

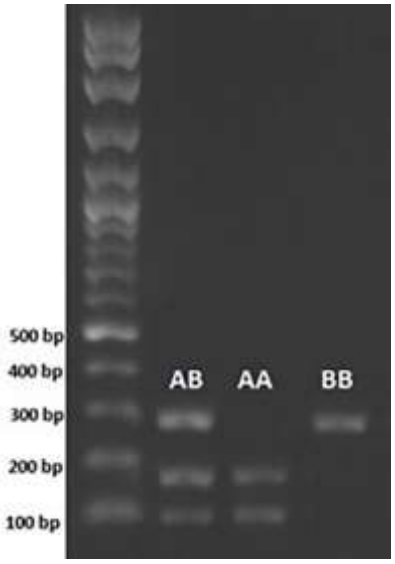


**Figure 1.** PCR-RFLP results of IGF-I gene, AB (294, 194 and 100 bp); AA (194 and 100 bp); BB (294 bp)

**Table 2.** Genotype and allele frequency of the IGF-I and IGFALS gene locus.

| Gene | N | Genotype Frequencies | Allele Frequencies | $\chi^2$ |
|---|---|---|---|---|
| IGF-I | 46 | 0.71(AA) | 0.79(A)<br>0.21(B) | 19.29 |
| | 10 | 0.15(AB) | | |
| | 9 | 0.14(BB) | | |
| IGFALS | 7 | 0.11(AA) | 0.22(A)<br>0.78(B) | 7.26 |
| | 15 | 0.23(AB) | | |
| | 43 | 0.66(BB) | | |

## Association between IGF-I locus and growth traits

Association results confirmed a significant association between IGF-I locus and growth traits ($p<0.05$) (Table 3). The animal with the AA genotype showed a higher weight at birth and weaning. In contrast, animals with the AB genotype showed the highest ADG2 compared to AA and BB genotypes. Tahmoorespur and coauthors [30] confirmed the association between IGF-I gene and average daily gain from birth to weaning in Baluchi sheep. Umego and coauthors [27] indicated IGF-I locus association with growth traits and suggested that IGF-I is a molecular marker in Yankasa sheep. He and coauthors [23] revealed that polymorphism of the 5' regulatory region of the IGF-I gene affects litter size in Small Tail Han sheep. In Iranian Makuisheepof sheep, reported association of the polymorphism in the 5’ flanking region of the ovine IGF-I gene with growth and development traits [31]. Sebastiano and coauthors [29] showed an association between the IGF-I gene and reproductive traits in Sarda dairy sheep. Bakhtiar and coauthors [25] reported the effect of the IGF-I gene on semen quality in Sanjabi sheep. In contrast, no significant association could be detected between IGF-I genotypes and growth traits in Gaddi goats [12]. Also, no significant effect was found of the IGF-I gene on growth traits [15]. Darwish and coauthors [32] found a significant association between the 5' regulatory region of the IGF-I gene and wool traits. It is evident from the current study and previous studies that the 5' regulatory region of the IGF-I gene is a candidate gene that significantly affects production traits in sheep.

**Table 3.** Association analysis between IGF-I and IGFALS gene locus gene locus and milk composition

| Traits | Genotypes (mean ± standard error) | | | p-value |
|---|---|---|---|---|
| | IGF-I | | | |
| | AA | AB | BB | |
| Birth | 4.582±0.111[a] | 4.111±0.100[b] | 4.274±0.102[ab] | **0.009*** |
| Weaning 90 days | 23.71±0.192[a] | 22.98±0.174[b] | 23.59±0.177[a] | **0.014*** |
| 6.month weight | 35.98±0.193[ab] | 36.22±0.175[a] | 35.52±0.178[b] | **0.025*** |
| ADG1 | 212.4±2.404 | 209.6±2.176 | 214.7±2.223 | 0.289 |
| ADG2 | 174.4±1.159[b] | 178.4±1.049[a] | 173.7±1.071[b] | **0.007*** |
| | IGFALS | | | |
| | AA | AB | BB | |
| Birth | 4.522±0.102[a] | 4.153±0.094[b] | 4.291±0.115[ab] | **0.034*** |
| Weaning 90 days | 23.16±0.177 | 23.43±0.163 | 23.69±0.199 | 0.145 |
| 6.month weight | 35.97±0.178 | 35.88±0.164 | 35.87±0.200 | 0.913 |
| ADG1 | 207.0±2.214[b] | 214.2±2.047[ab] | 215.4±2.492[a] | **0.021*** |
| ADG2 | 174.7±1.067 | 176.3±0.987 | 175.5±1.202 | 0.574 |

* $p<0.05$; ADG1: Average daily gains from birth to weaning, ADG2: Average daily gains from birth to 6th month

## IGFALS locus polymorphism

1113bp of PCR product was amplified. Three genotypes (AA, AB and BB) were obtained after digestion by enzyme; AA (1113bp), AB (1113, 869 and 264bp) and BB (869 and 264bp) (Figure 2). Chi-square $\chi^2$ test showed agreement to Hardy-Weinberg equilibrium ($p>0.05$) (Table 2). The allele and genotype frequency was 0.22(A) and 0.78(B); 0.11(AA), 0.23(AB) and 0.66(BB). Because being IGFALS gene is a novel gene in livestock, there are not many studies that show the genetic distribution of sheep. Alizadeh and Farhadi [18] two AB and BB genotypes of the IGFLAS-*HinfI* site with the frequency of 0.34 and 0.66 were observed in Makouei sheep and AA, AB, and BB genotypes with the frequency of 0.09, 0.21, and 0.70 were observed in Ghezel sheep. While the allele frequencies A and B in Makouei and Ghezel sheep was 0.17, 0.83 and 0.20, 0.80 respectively. The results of the Hamdani sheep agree with Makouei and Ghezel sheep. As it is evident from the results, the B allele frequency is higher than the A allele.

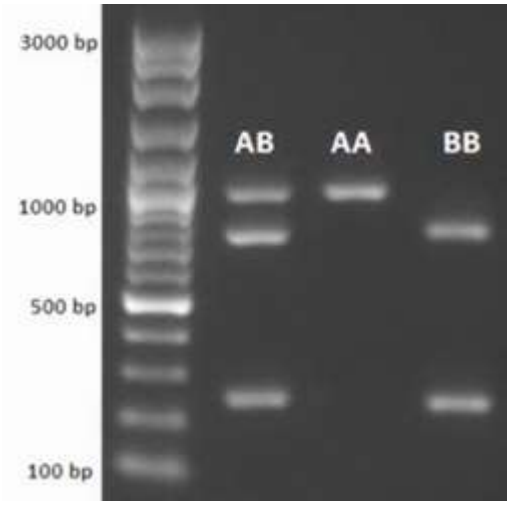


**Figure 2.** PCR-RFLP results of IGFALS gene, AB (1113, 869 and 264 bp); AA (1113 bp); BB (869 and 264 bp).

## Association between IGFLAS locus and growth traits

Association analyses showed a significant association between IGFALS gene locus and birth weight and ADG1. Animals with BB genotype had higher ADG1 compared with AA and AB genotypes. In terms of birth traits, the AA animals showed higher weights than other genotypes. A significant association was determined between the IGFLAS polymorphisms with birth weight in Ghezel and birth weight, weaning weight, and chest girth in Makouei sheep [19]. Liu and coauthors [21] reported that SNP g1219: T>C and SNP g2696: A>G of IGFLAS locus affected growth traits in Chinese beef cattle and suggested that IGFLAS can be used as a genetic marker for the selection of beef cattle for growth traits. Högler and coauthors [33] indicated that IGFALS gene dosage effects on serum IGF-I and glucose metabolism, body composition, bone growth in length and width, and the pharmacokinetics of recombinant human IGF-I administration. A significant

association was identified in the current study between the polymorphism of the IGFALS gene and growth traits. It can be used as a genetic marker to improve the traits in Hamdani sheep. However, many studies are required in this regard.

## CONCLUSION

In conclusion, this study reported the genetic distribution of genes and investigated their associations with growth traits in Hamdani sheep. The results showed an association between genes and growth traits. Our study provided evidence that IGF-I and IGFALS polymorphisms may be used as genetic markers for improving sheep growth traits. However, we conclude that further research and validation of the various allelic effects, functional mechanisms, and bioactivity are needed in a larger population to explore the usage of IGF-I and IGFALS genes in sheep breeding.

**Conflicts of Interest:** The authors declare no conflict of interest. Authors must identify and declare any personal circumstances or interest that may be perceived as inappropriately influencing the representation or interpretation of reported research results.

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