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Effect of IGF-1 and GH Genes Polymorphism on Weights and Body Measurements of Awassi Lambs in Different Ages

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Abstract: The study was carried on 68 ewes of local Awassi sheep in the Al-Kafeel sheep station Karbala governorate, Iraq for the period from 1/10/2017 to 1/8/2018. Genetic analysis was carried out in the molecular genetics laboratory at the College of Agriculture / Basrah University in order to extract (DNA) and determine the genotype of the IGF-1(Insulin-like growth factor 1) and GH (Growth Hormone) genes. This study aimed to know the association between the genotype of the ewes of IGF-1 and GH genes and their relationship to the productive traits of the lambs, which included the weights of lambs from birth to weaning and body measurements, Three of the genotypes for IGF-1 were revealed AG, GC, CC and two for GH CT, TC . The effect of IGF-1 genotypes was significant ($P<0.05$) on weights of lambs at weaning, age 6 months and some body measurements with superior genotype GC. The genotypes of GH had a significant effect on the weight of the lambs at birth by CT superiority. While the weights of weaning, six months and body measurements were not significantly affected by different genotypes.

Keywords: Sheep, Polymorphism IGF-1**,** GH, Body Weight, Body Measurements.

Introduction

The number of sheep in Iraq were 7,722,375 head and its percentage was 63.86 % of the total an animals compared with the cows, buffalos, goats and camels were 21.11 %,2.36 %,12.20 % and 0.48 % respectively (Iraq statistical book, 2011). Awassi fat tail sheep consisted of 58.2% of local sheep in Iraq and characterized by good meat quality and the ability for milk production (Al-Barazinji & Othman, 2013) Many studies indicated that to improving animals production need new breeding plans including many performance traits (Trukhachev *et al*., 2016). IGF-1 is an important metabolic hormone in the regulation of metabolism and cellular growth. It works to absorb glucose, decompose glycogen and increase the absorption of amino acids needed to

synthesize protein and reduce protein degradation (Mordian *et al*., 2013; Sun *et al*., 2014). Growth hormone stimulates the growth of muscle cells by stimulating amino acid and protein synthesis. The most important function of this hormone is to stimulate overall growth in the body bones and muscles (Gadelha *et al*., 2012; Othman *et al*., 2015). This study aimed to know the relationship between the genotype of IGF-1 and GH genes in the economic traits such as birth, weaning and at age of six months weights and body measurements as well as the growth rate from birth to weaning period.

Materials and Methods

The study was carried on 68 ewes of local Awassi sheep in the Al-Kafeel sheep station, Karbala for the period of ten months from 1/10/2017 to 1/8/2018 Genetic analysis were carried out in the Molecular Genetics Laboratory at the College of Agriculture. University of Basrah in order to extract (DNA) and determine the genotype of the IGF-1and GH genes and finding their relationship to the weight and body measurements. The weights of the lambs were taken at different ages using suspended electronic balance which includes, the birth body weight, weaning (weight at 3 months) and weight at six months of age. Animal's body measurements were taken directly after each weighting processes using the measuring tape included body length and wither height, rump height, chest circumference, abdominal circumference. The daily growth rate was calculated from birth to weaning and from weaning to six months as well as from birth to six months.

DNA extraction and electrophoresis

Blood samples were taken from the jugular vein of ewes using a 10 ml syringe after cleaning and sterilizing the jugular vein area, by alcohol. The DNA was extracted by using Genaid extraction kit (Korea). PCR was done to amplified a 265 bp fragment of IGF-1 gene using pair of primers: Forward, 5'–ATTACAGCTGCCTGCCCCTT-3'and Reveres, 5' CACATCTGCTAATACACCTTAC CCG -3' (Mordian *et al* . (2013). PCR mixture was 20 µl, containing 10µl Master Mix , 1µl for each primer forward & reverse, 2µl DNA template 20-50 ng and dd water was added to 20µl. The PCR program was : 95°C for 4 min, followed by 31 cycles of 95°C for 30 s, 60°C for 30 sec, 72°C for 30 s and a final extension at 72°C for 10 min.

The fragment of GH gene was 370 bp as amplified using a pair of primers gene with the following nucleotide sequences: Forward, 5'GTGATGATGCGCTGCTCAAG-3'and Reveres, 5' CTTCTTTCTGCCCCAGGAGG -3' (Rothschild & Soller 1997). PCR mixture was 20 µl, containing 10µl Master Mix , 1µl for each primer forward & reverse, 2µl DNA template 20-50 ng and dd water was added to 20µl. The PCR program was : 95°C for 4min, followed by 31 cycles of 95°C for 30 s, 58°C for 30 sec, 72°C for 30 s and a final extension at 72°C for 10 min.

The resulting PCR products were separated by electrophoresis on a 2% agarose gel in parallel with a 100-bp DNA marker. PCR products were purified and sequenced by Yang Ling Biotechnology Co; Itd China. The sequences were analyzed by Clustal O. (2018) program to determine the gene polymorphism.

Statistical Analysis

SPSS (2013) was used to find the significant differences between the means of the studied traits mathematical model and the age of dams and sex lambs were adjusted by Statistical model**:**

$Yijk = \mu + Ai + Sj + + eijk$

Where is:

μ = Overall mean

Yijk = The value of k observation of each trait.

Ai = Effect of genotype of IGF1 gene (i = 3).

Sj = effect of genotype of GH gene (j = 2).

eijk = The effect of the experimental error which is distributed randomly and naturally and with an average of zero and variation. $\sigma^2 e$.

Results & Discussion

Molecular characterization of genes studied

The DNA was extracted successfully and obtained the required 265 IGF-1 is shown in Fig. (1), and 370 GH shown in Fig. (2). The sequencing of IGF-1 gene revealed identical sequences for all ewes except the bases of 61, 62, 63 of the studied piece. These bases produced three different genotypes, The first was AG(AGC), CC(AGC<CCA) and lastly GC (AGC<GCA). While the sequencing of GH gene revealed an identical sequences for all ewes except the bases of 19, 20. These bases produced two different genotypes TC and CT.

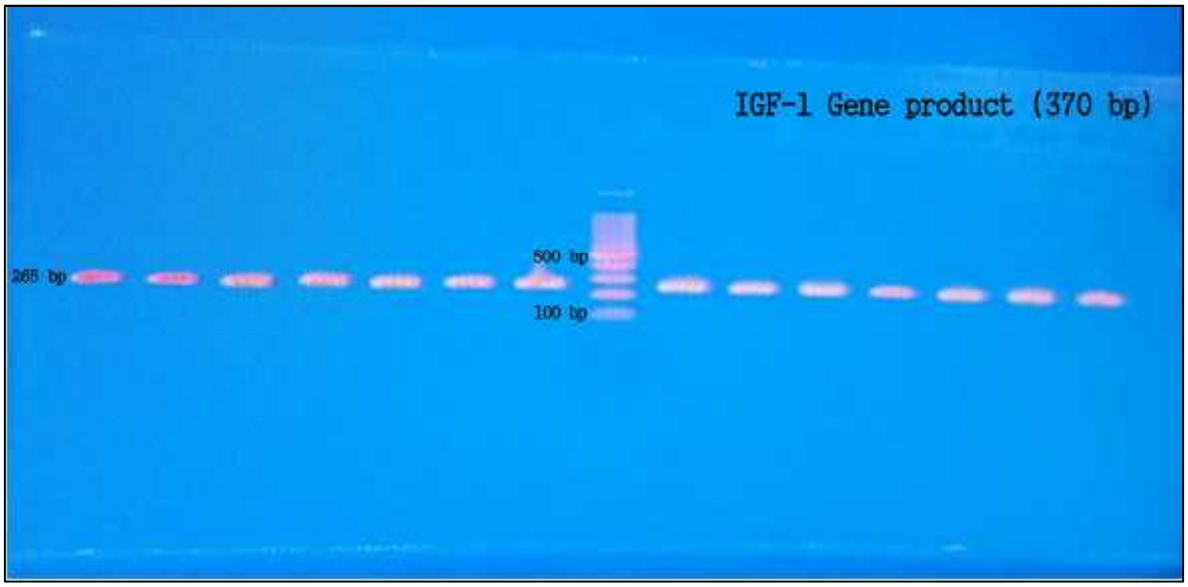

Fig. (1): PCR product for the IGF-1 gene.

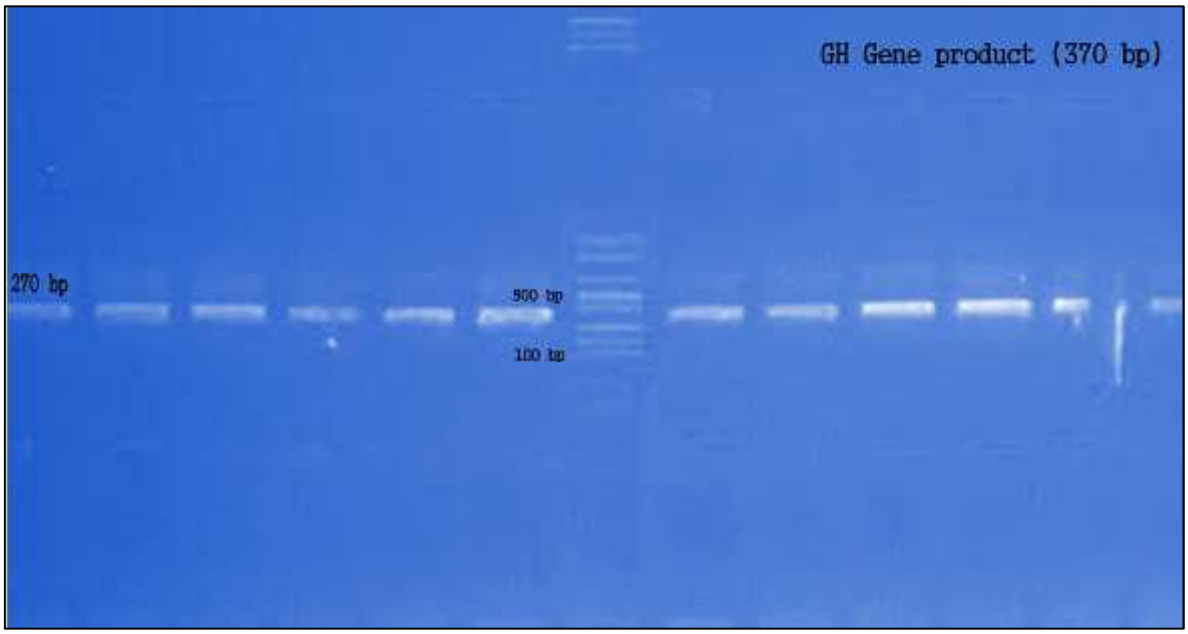

Fig. (2): PCR product for the GH.

Genotypes effect of IGF-1 and GH genes on birth weight and its measurement

Table (1) showed no significant differences in birth body weight at and lambs body measurement among different genotypes. The average weight of lambs at birth was 3.75, 3.72 and 3.81 kg for genotypes AG, CC and GC respectively. `These results were in agreement with the results of Masoud *et al*. (2013), however, it was not consistent with many other studies indicating that there was a significant effect of genotype IGF-1 gene in lambs weight at birth (Mojtaba *et al*., 2009; Senegal *et al*., 2014). The genotypes of GH gene had a significant effect at ($p<0.05$) in lambs weight at birth. The lambs produced by dams with the CT, and TC has birth weight 4.18 kg and 3.34 kg respectively with differ 0.84 kg. This result was agreed with the result of Jit *et al*. (2014) in his study on the Chinese XW sheep breed and the study results of Al-Salhi (2016) on the Turkish Awassi sheep. The CT genotype superior in body measurement (wither height, rump height, chest circumference), which were 39.37, 39.52 and 37.39 cm respectively, while the average of TC genotype were 37.74, 37.68 and 36.03 cm.

Table (1): Effect of genotype IGF-1 and GH genes in birth weight and body measurements ± Standard error.

Genotypes / Traits	genotypes of IGF-1			genotypes of GH	
Number of animals	AG	CC	GC	CT	TC
	24	30	14	41	27
Birth weight (kg)	3.75 ± 0.94 a	3.72 ± 0.88 a	3.81 ± 0.12 a	4.18 ± 0.08 a	3.34 ± 0.09 b
Body length (cm)	35.26 ± 0.69 a	36.40 ± 0.64 a	36.31 ± 0.91 a	36.81 ± 0.58 a	35.17 ± 0.66 a
Wither height(cm)	38.66 ± 0.45 a	38.12 ± 0.42 a	38.88 ± 0.59 a	39.37 ± 0.38 a	37.74 ± 0.43 b
rump height (cm)	38.84 ± 0.47 a	38.29 ± 0.44 a	38.66 ± 0.63 a	39.52 ± 0.40 a	37.68 ± 0.45 b
Chest circumference (cm)	36.98 ± 0.49 a	36.93 ± 0.46 a	37.05 ± 0.65 a	37.39 ± 0.41 a	36.03 ± 0.47 b
Abdominal circumference (cm)	36.24 ± 0.24 a	35.74 ± 0.39 a	36.79 ± 0.55 a	36.77 ± 0.35 a	35.75 ± 0.40 a

The means with different letters within the same row differ significantly ($P<0.05$).

Genotypes effect of IGF-1 gene and GH gene on weaning weight and its measurement

Table (2) showed the significant differences Significant ($p<0.05$) in average weaning weight of lambs when among different genotypes of IGF-. The highest average weaning of 15.94 kg was recorded in GC genotype, whereas the lowest average weight of 14.08 kg was recorded in AG genotype. The reason for this superiority among genotypes may due to the high abundance of milk yield and good mothers care with GC genotype. Which, increases the rate of growth of lambs before weaning or may be due to the increase in birth weight of this genotype. This facts mentioned by Mousad *et al*. (2013) who informed that there is a positive correlation

between birth weight and weight at weaning. A significant effect was also observed on different genotypes in some body measurements such as the wither height, rump height and the abdominal circumference. The GC genotype was superior to AG genotype, with an average of 54.89, 57.30, 72.82, 52.82, 55.22 and 68.08 cm respectively. The reason behind the increase in lambs growth for the GC genotype might due to the high growth rate from birth to weaning which recorded an average of 0.134 kg/day. These results in agreement with the result of Chunxiang *et al*. (2008) and did not match the results of Gholibeikifard *et al*. (2013) and Ewa *et al*. (2017) in their study on different breeds of sheep.

The genotype of GH gene had no significant effect on the weaning weight, body measurement and lambs growth rate from birth to weaning. While slight increases were observed in weaning weight and some body measurements for the CT genotype compared with TC genotype. This result agreed with Jit, *et al*. (2014) in his study on XY Chinese sheep ,but not with indicated by Al-Salhi, (2016) and Cauveri *et al*. (2016) in different breeds of sheep.

Effect of genotypes of IGF-1 and GH genes on body weight and its measurement in six months

Table (3) showed the significant effect of different genotypes on lamb weight at age of 6 months. GC was superior to AG genotype by 2.68 kg and mean weights were 30.65, and 27.97 kg, respectively. This superiority may due to the increase in weaning weight of GC genotypes.

Table (2): The effect of genotypes of IGF-1 and GH genes in weaning weight and body measurement ± standard error.

Genotypes Number animals / Traits	genotypes of IGF-1			genotypes of GH	
	AG	CC	GC	CT	TC
	24	30	14	41	27
Weaning weight (kg)	14.08 ± 0.64 b	15.02± 0.60 ab	15.94 ± 0.84 a	15.03 ± 0.54 a	14.59 ± 0.61 a
Body length (cm)	51.22 ± 1.03 a	52.35 ± 0.96 a	52.78 ± 1.36 a	52.77 ± 0.89 a	51.46 ± 0.99 a
Wither height (cm)	52.82 ± 0.85 b	53.47± 0.70 ab	54.89 ± 1.19 a	54.14 ± 0.71 a	53.18 ± 0.81 a
Rump height (cm)	55.22 ± 0.88 b	0.82 ± 56.76 ab	57.30 ± 1.16 a	56.85 ± 0.74 a	56.01± 0.84 a
Chest circumference (cm)	63.49 ± 1.18 a	66.64 ± 0.10 a	64.03 ± 1.56 a	65.35 ± 1.00 a	64.35 ± 1.13 a
Abdominal circumference (cm)	68.08 ± 1.33 b	70.91 ± 1.24 ab	72.82 ± 1.76 a	70.92 ± 1.12 a	70.29 ± 1.27 a
Growth rate from birth to weaning (kg/d)	0.37 ± 0.114 b	0.74 ± 0.125 ab	0.94 ± 0.134 a	0.31± 0.121 a	0.35 ± 0.125 a

The means with different letters within the same row differ significantly (P<0.05).

This view one of the most important for the economic characteristics in sheep which determined marketing weight at the age of six month (Abu-Rahef, 2013). The results showed that the genotypes of GC were significantly higher ($p<0.05$) in the chest circumference and abdominal circumference, growth rate form weaning to six months and from birth to six months with an average of 87.64, 90.18 cm,0.94 and 0.79kg/d respectively. This confirms the positive correlation between body weight and its measurements (Al-Mahdawi, 2011).

Table (3): Effect of genotypes of IGF1- and GH genes in weight at age of six months and body measurements ± Standard error.

Genotypes / Number animals / Traits	genotypes of IGF-1			genotypes of GH	
	AG	**CC**	**GC**	**CT**	**TC**
	24	30	14	41	27
6 months weight (kg)	27.97 ± 1.08 b	29.68 ± 1.06 ab	30.65 ± 1.45 a	28.57 ± 0.93 a	28.73 ± 1.07 a
Body length (cm)	63.19 ± 0.63 a	0.58 ± 63.10 a	63.19 ± 0.83 a	63.29 ± 0.53 a	63.03 ± 0.60 a
Wither height(cm)	64.01 ± 0.68 a	64.34 ± 0.64 a	64.34 ± 0.43 a	64.05 ± 0.58 a	64.41 ± 0.65 a
Rump height (cm)	66.15 ± 0.82 a	66.92 ± 0.76 a	66.50 ± 1.08 a	66.30 ± 0.69 a	66.75 ± 0.79 a
Chest circumference (cm)	83.43 ± 1.40 b	87.14 ± 0.31 ab	87.64± 1.85 a	84.63 ± 1.18 a	87.51 ± 1.34 a
Abdominal circumference (cm)	86.75 ± 1.62 b	89.58 ± 1.52 ab	90.18± 1.95 a	87.68 ± 1.37	89.10 ± 1.56
(kg/d) Growth rate from weaning to 6 months	0.87 ± 0.154 b	0.83 ± 0.16 ab	0.94 ± 0.164 a	0.73 ± 0.150 ± a	0.84 ± 0.16 a
Growth rate from birth to 6 months (kg/d)	0.60 ± 0.13 b	0.98 ± 0.14 ab	0.79 ± 0.149 a	0.50 ± 0.14 a	0.58 ± 0.14 a

Table (3) did not showed a significant effect of the GH genotypes in all studied traits. This result is in line with the findings of Naicy *et al*. (2017), Jit *et al*. (2014), Cauveri *et al*. (2016) on the other hand, Moradian *et al*. (2013) found a significant effect on different genotypes at the weight of six months in the Iranian Makooei sheep and Ivan *et al.* (2017) in the Russian Salsk sheep.

Conclusion

Three genotypes of IGF-1 gene and two genotypes of GH gene were detected in Awassi sheep. A significant relationship between the genotypes of dams IGF-1 gene and growth of their lambs maybe use as Genetic Marker to improve growth traits. While there is no significant relationship between the genotypes of dams GH and growth of their lambs.

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