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Estimation of Breeding Values and Genetic Variances at First Exon-region of *IGF1* Gene and its Association of Growth Traits in Awassi Sheep which are Breeding in Iraq

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Abstract

The study aimed to determine the breeding values and genetic. Variation based on the information of the genetic polymorphism of the first exon region of *IGF1*geneusing by *RFLP* andits relationship to growth traits (birth weight, weaning weight, and average daily gain). The study was conducted at the Ruminant Research Station of the General Authority for Agricultural Research / Ministry of Agriculture a sample of 55 Awassi Turkey ewes in the animal breeding station in Baghdad and the Center for Biotechnology / University of Al-Nahrain. The breeding value (BV) of genotype BB was higher (4.04) compared with AA and AB (3.72 and 3.88) for the birth weight (BWT) respectively, while BV was higher for AA genotype in weaning weight (WWT) and average daily gain (ADG) traits (20.47 and 16.34), respectively. It is found that the value of dominance variance (VD) within the genetic variation is high as compared with the additive variance (VA) in WWT and ADG it reached 0.037 and 0.018 which indicates the effect of the dominance interaction on thesis traits. The average of the allele effect and the effect of substitution of alleles were a favor for the allele A compared to mutant allele (B), which gives evidence about the direction for the selection of the dominant allele, as the frequency of allele A was more distributed (0.70) in the studied sample, so it is necessary to maintain the frequency of this allele and increase its frequency with the help of selection with genetic markers.

Key words : Breeding values, genetic variations, IGF1 gene, Awassi sheep.

Insulin-like growth hormone IGF1 is a polypeptide hormone with a molecular weight of 7.5 kDa made of 70 amino acids (Daughaday and Rotwein, 1989 & Kajimoto and Rotwein, 1991) that plays an important role in mammary gland development and cell differentiation (Mam-Ghali *et al*. 1991). As well as it interferes with the synthesis of DNA, RAN, protein, and cell proliferation (Etherton, 2004), it is a member of the IGF family, which are structurally related proteins and hold the key in cell differentiation, embryonic development, growth, and metabolism regulation (Siadkowska *et al*., 2006) also found that the IGF1 gene is linked to the growth rate Meat production and thus its implications for birth weight and weaning weight of newborns (Cobro *et al*., 2013). The IGF1 gene is located on the third chromosome of sheep and is made up of 6 expression regions and is approximately 90 kg in length (Steenbergh *et al*., 1991 and Nixon *et al*., 1999). Growth traits, growth hormone (GH), growth hormone receptor (GHR), insulin-like growth factor I(IGF-I), leptin (LEP), caprine-pituitary-specific transcription factor-1 (POU1F1), caprinemyostatin (MSTN), and bonemorphogenetic protein (BMP) genes are necessary for bone formation, birth weight, weaning weight, body condi-

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tion, and muscle growth (Supakorn,,2009). Genetic improvement is based on the extent of the inheritance of additive variation and its transmission from one generation to another using assisted selection by genetic markers that play a role in the physiological and production processes in farm animals (Cloete *et al*., 2004). Dominance effects are an important factor in influencing genetic variation, which affects the share of pooled variance when studying trait variances, especially complex production traits (Eila and Colleagues, 2005 and Falconer, 1992). Therefore, the study of genetic maps has a great light power on combative variances, and many studies have endeavored to demonstrate the distribution of non-clustering variances and their effect on the cumulative effect on quantitative trait sites (QTL) (Hermiz and Colleagues, 2005). The different genotypes are the main focus of different aspects due to the purity or hybridization of alleles images, meaning that the distribution of all alleles, whether negative or positive, contributes to the appearance of the trait and genetic selection in generations, but redistributesthe proportions of these alleles between individuals or in societies in order to increase their frequency and thus an attempt Replace the unwanted allele with another and in the same location has a significant effect which is referred to as allele substitution pathway (Falconer & Mackay, 1996). MAS may increase theannual rate Of genetic gain in livestock by 15 to 30 percent % without increasing the risk involved in breeding schemes (Ge *et al*.,2001). Therefore, the objective of this study was to determine associations of *IGF1* gene genotypes with estimated breeding values of growth traits in Awassi sheep.

Materials and Methods

The study was conducted at the Ruminant Research Station of the General Authority for Agricultural Research / Ministry of Agriculture on a sample of 55 Turkish Awassi ewes. The genetic analysis were conducted in the laboratories of the Biotechnology Research Center / Al-Nahrain University. The blood sample (5 ml) was extract from the jugular vein of each animal in test tubes containing K2EDTA coagulation inhibitor and placed in a container ofRefrigerated tubes and transferred to the laboratory for freezing at -18C^0 until the time of extraction of DNA from them. Two primer pairs5'-ATTACAAAGCTGCCTGCCCC-3 (forward) and 3'-ACATCTGCTAATACACCTTACCCG-5' (reverse) were employed targeting a fragment of 265bp as described by (Yilmas *et al*., 2005) to find out the A and B alleles of *IGF1* gene. The PCR reactions were performed on an ABIVeritithermo cycler and the PCR cycling condition was a preliminary denaturizing at 97 C^0 for 2 min., then, followed by 1 cycle of denaturing at 94 C^0 for 45 sec., annealing at 58 C^0 for 45 sec. and extension at 72 $C^0$for 45 sec. by 31 cycles and finally followed by 5 min. at 72 C^0asend extension. The amplified fragment of *IGF1* gene was digested by the restriction endonuclease *HeaIII* (BIOLAB CO.). The next step was the separation of digested products by electrophoresis on 2% (v/w) agarose gel stained with Safe View (NBS Biological, UK) Electrophoresis was performed in a 1X TBE buffer atroom temperature and constant 70 V and 40 Am for 90 min.

Statistical analysis

The equations for each calculated value were applied as follows (FalconerandMackay,1996)

1. The average effect of allele A:$\alpha 1 = q[a+d(q-p)]$
2. The average effect of allele B:$\alpha 2 = - p[a+d(q-p)]$
3. The effect of substitution of alleles:$\alpha = \alpha 1 - \alpha 2$
4. The breeding values : AA=2 $\alpha 1$, AB= $\alpha 1+\alpha 2$, BB=2$\alpha 2$
5. The dominant deviations : AA=2 q^2 d, AB= 2pqd, BB= 2 p^2 d
6. The different variances : VA=2pq α^2, $VD=4p^2q^2d^2$, VG=VA+VD

Results and Discussion

After determining the genotypes using the RFLP-PCR conducting using the restriction *enzyme HeaIII*, three genotypes of the first exon region of the IGF1 gene were found in the studied ewes sample: AA, AB and BB with allelic frequency, p_A = 0.70 and q_B= 0.30. AA genotype and the mutant AB as a result of the change of the allele A to B, as ewes with the BB genotype the carriers of the mutant alleles at BWT were

Table I. Breeding value, dominance deviation, and genetics variations of genotypes in the *IGF1* gene for birth weight (BWT), weaning weight (WWT), and Average daily gain (ADG) traits.

			BWT			
Genotype	**Mean**	**BV**	**DD**	**VA**	**VD**	**VG**
AA	3.75	3.72	3.84			
AB	3.83	3.88	3.76	0.0017	0.003	0.005
BB	4.20	4.04	3.95			
WWT						
AA	20.17	20.47	20.45			
AB	19.72	20.30	20.17	0.0019	0.037	0.039
BB	20.20	20.12	20.82			
ADG						
AA	16.41	16.34	16.22			
AB	15.93	16.05	16.03	0.0055	0.018	0.023
BB	16.10	15.76	16.48			

BV: Breeding value, DD: dominance deviation, VA: Additive variance, VD: Dominance variance, VG: Genetic variance.

highest (4.20 kg) compared to the ewes carrying the wild genotype (AA) and the hybrid (AB) (3.75 and (3.83) Kg of their offspring, respectively. This result was consistent with the breeding value oftheBB genotypewas highest (4.04), while the ewes carrying the wild genotype (AA) weresuperior tothe WWT and ADG traits (20.47 and 16.34), respectively toAB and BB. The result of the study indicates an increase in the genotype variance of WWT and ADG of the wild genotype (AA) (0.0019 and 0.0055) were higher than BB and AB (Table I). As for the dominance variance, which is the interaction of the alleles of the same site, it shows us the mechanism of variation of the hybrid individuals and the extent of its contribution to the genetic variation. (Visscher *et al*., 2008) .Findthe dominance varianceof AB was higher (0.037) compared toAA and BB genotypes (Table II) which reflectsthe level of allelic interaction betweenthem alleles in the*IGF1* gene and its high contribution to total genetic variation.The average effect of theB allele was positive (0.114) on BWT, while the case differed for WWT and ADG as the mutant allele had a negative effect (- 0.121 and - 0.201) respectively, as we find that theAverage effect of gene substitution to mutant allele (B) was positive (0.065) for BWTand this ratio was reversed to have a positive effect (0.069 and 0.115) when replacing the allele A with the mutant (B) in WWT and ADG, respectively (Table II).

The results showed that selection in favor of the wild genotype (AA) was better in both the WWT and ADG traits, as the presence of the mutant allele (B) with one copy led to a decrease in the breeding value, which represents the amount of additive variation in the trait from its absence, and the breeding value increased when there were two copies of Mutant allele in BWT trait. The reason for the superiority of dove ewes to the mutant structure in its two copies (BB) may be that this gene (IGF1) affects the development of the mammary gland and its effect on cell differentiation, embryonic development, growth and metabolism regulation (Siadkowska and Colleagues, 2006), which was reflected in the BWT of their newborns resulting from the illiterate Maternal effects and this is in agreement with Mojtaba (2009) study on Iranian Baluchi sheep. The departure of the newborn from the mother and his dependence on himself resulted in the superiority of the land composition, which led to the increase in the breeding value of WWT and ADG of the lambs of wild genotype (AA). However, the high breeding value of AA was more likely than BB in the selection programs, especially the high frequency of the A allele in the experimental. Samples compared to the B allele, so the frequency of the A allele must be increased at the expense of the B allele, since

Table II : The average gene effect and the Average effect of thegene substitution of alleles for birth weight (BWT), weaning weight (WWT), and Average daily gain (ADG) traits.

Traits	Alleles	Average gene effect	Average effect of gene substitution
WBT	A	0.049	0.065
	B	0.114	0.065
WWT	A	0.052	0.069
	B	0.121	0.069
ADG	A	0.086	0.115
	B	0.201	0.115

the WWT trait is the most important economically. Despite the convergence of WWT and ADG between the AA and BB genotypes, this convergence came as a result of the birth selection programs at the breeding station in which the research was conducted, in addition to the fact that most lambs of mothers carrying this combination are single, which provides them with greater quantities of milk. On the tendency of lambs of less weight to compensatory feeding at this stage (Al-Anbari, 1998 and Abu Raheef, 2013), and the approximation of the weights of the AA genotype with BB as a result of interest in the bornlamb in the breeding station and thus there is a compensatory increase due to an environmental impact that worked on a convergence in the WWT and ADG, and this is consistent with the results of Al-Mahdawi (2011).

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