Effect of age of Dam and genotypes of IGF-1 gene and GH gene on wool production and some of its physical traits in local Awassi sheep

Rahman Hussein Hamza Al Qasimi [1] Amad Falah Hassan [2] Bassam Y. Khudair3[3]

[1]College of Agriculture / Al-Qasim Green University

[2]College of Agriculture / University of Basrah

[3]College of Veterinary Medicine / University of Basrah

Abstract:

The study was conducted on a random sample consisting of 68 local Awassi ewes in the fields of Al-Kafeel Station in Karbala Governorate, and their productive performance was followed up (genotype) of the IGF-1 gene as well as the GH gene, which were 24, 30, 14 in the studied ewes sample for the AG, GC, and CC genotypes, respectively, for the IGF-1 gene, and 41, 27 for the CT, TC genotypes, respectively, for the GH gene. The purpose of this study was to determine the effect of the age of the dam and the genotypes of these genes on the traits of wool production and certain of its physical traits. The results showed the effect of the dam's age significantly on some of the productive and physical traits of wool. On the contrary, it was noted that the genotypes of the GH gene had a significant effect on some products and physical traits of wool, as the ewes of the genotype TC recorded the highest averages in of greasy and clean fleece weight, staple length and fiber length. As for the percentage of clean wool and fiber diameter, they were not significantly affected by the difference in genotypes.

key words: Awassi sheep, wool traits, genotypes IGF-1, GH.

Introduction

Sheep are one of the most important sources of livestock in Iraq and represent a large part of the agricultural national income, and sheep are one of the important pillars in the field of animal production (2 , 5). Local sheep are among the wide-tailed breeds with greasy buttocks, and Awassi sheep account for 58.2% of all Iraqi sheep. (1 , 14) Agricultural animal breeders resorted to following programs that would raise the animal’s productive efficiency by improving its genotypes using methods However, the period of time (generation period) required for this is often long in animals such as sheep, which may reach 4-4.5 years. While educators can follow other methods to reach the goal in the shortest possible time, and one of those methods is the use of early selection after conducting an evaluation of animals at early ages through genes that can be used as electives guides for important economic traits (15). Recent studies indicated that improving the productive traits of sheep requires studying the genetic makeup of these animals and choosing the best one, by

ISSN 2072-3857

studying the genes that affect the productive traits, and the most important of these genes is the insulin-like growth factor (IGF-1) and the growth hormone gene (3 , 4 , 16 , 11 , 23).

The importance of these two genes and their relationship to the productive traits of ewes, this study aimed to find out the effect of the genotypes of the insulin-like growth factor (IGF-1) gene, the growth hormone (GH) gene, and the age of the dam of Awassi ewes on the production of wool and some of its physical traits.

Materals and Methods

The study was conducted in the fields of Al-Kafeel station in Karbala governorate, with a sample of 68 local Awassi sheep. Genetic analyses were conducted at the Molecular Genetics Laboratory of the College of Agriculture/University of Basrah in order to separate the genetic material (DNA) and determine the genotype. For the IGF-1 gene, as well as the GH gene, blood samples were drawn from the jugular vein of ewes, and blood samples were placed in tubes containing EDTA (anticoagulant), and then preserved at a freezing point. The laboratories of the Faculty of Veterinary Medicine at Al-Qasim Green University were also used to measure the traits of wool.

Measurement of wool production and its physical traits

The sheep were sheared in early April and weighted immediately. Before shearing, a sample of 10 x 10 cm of wool was taken from the center of the right side of the animal as the best sampling area according to (21). The samples were weighed with a sensitive balance and then washed. In three troughs to remove dust and dirt with stirring and squeezing, then they were dried at room temperature for 48 hours, then the clean wool samples were weighed to extract the percentage of clean wool and the weight of clean fleece as in the following equation:

Clean wool percentage = (clean sample weight / greasy sample weight) x 100

Clean weight of the fleece = percent of clean wool x greasy weight of the fleece

Staple length was measured after taking three staples randomly from each sample, and their lengths were measured using the usual ruler without pulling or pulling, from the base of the tuft to its end. The length of the fiber was measured manually using the ruler and for a number of up to 50 fibers random from the sample, and the general average was extracted after fixing its two ends by Special clamps to make it tight and straight to adjust its true length. The diameter of the fiber was measured using a microscope containing an Axio cam in the Histology and Anatomy Laboratory of the College of Veterinary Medicine / Al-Qasim Green University, where ten fibers were taken from each sample and the general average was extracted. The fiber is placed under the microscope and appears on the computer screen connected to the microscope, showing the fiber diameter in microns.

ISSN 2072-3857

DNA extraction and electrophoresis

DNA was extracted from the blood, according to the instructions of the kit (Kit) provided by the Korean company Geneaid, and the success of the extraction was confirmed by using electrophoresis technology on agaros gel (18)

Selection of primers and polymerase chain reaction (PCR)

Primers were selected to find out the genotype of the IGF-1 gene (13) and the GH gene (17) :

5`- ATTACAGCTGCCTGCCCCTT -3` f

5`- CACATCTGCTAATACACCTTACCCG -3`

5`- GTGATGATGCGCTGCTCAAG -3` and

5`- CTTCTTTCTGCCCCAGGAGG -3`

Then, they were placed in special tubes with the PCR reaction mixture into a 100 microliter tube, and the tubes were placed in a centrifuge for 30 seconds. After that, the tubes were placed in a PCR (Thermo cycler), then the device's work program was adjusted according to the initiator used and for each gene separately.

statistical analysis

The SPSS 22 statistical program (20) was used, and the significance of the differences between the means studied was selected at a probability level (0.05). The following Mathematical model was used:

$Yijklm = \mu + Ai + Sj + Gm + eijkmn$

Where is :

μ= overall mean

Ai= the effect of age of dam (i=3)

Sj= the effect of IGF-1 gene (j=3)

Gm= the effect of GH gene (m=2)

eijkmn= the effect of the experimental error which is distributed randomly and naturally with on averge of zero and variation σ^2 e.

Results and discussion

Amplification of IGF-1 and GH gene packages using PCR technology

The results of DNA extraction showed that the DNA concentration ranged between 25.1 - 60.8 ng / microliter, and the purity 260/280 ranged between 1.7-1.9 nanometers, which is within the normal range (8), which was detected by the Nano drop device.

The results of electrophoresis of the PCR product showed the success of the amplification process, as clear bands were formed with a size of 265 base pairs representing the IGF-1 gene and 370 base pairs representing the GH gene. Figures 2 and 3 show the results of amplifying the gene bands on the agarose gel using electrophoresis technique

ISSN 2072-3857

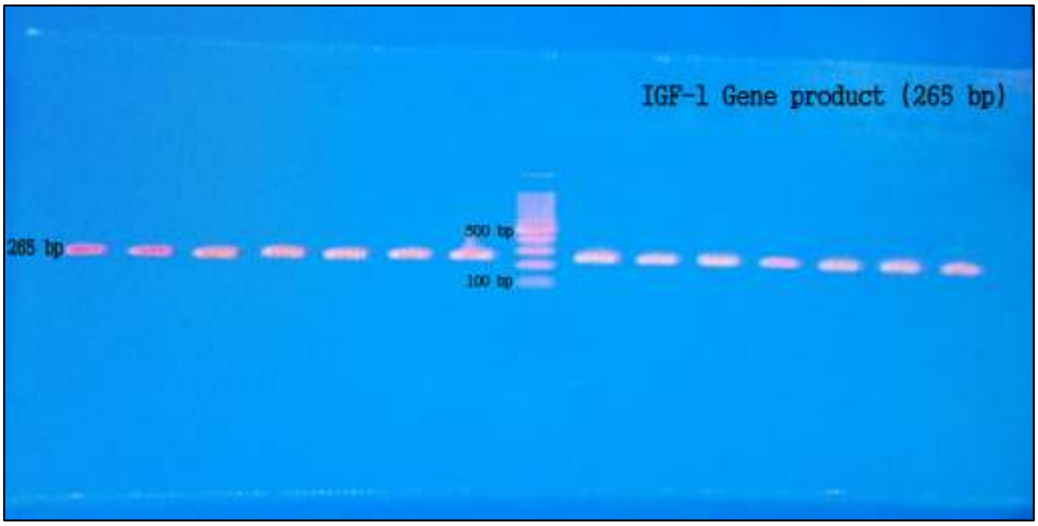

Figure 2: PCR product of IGF-1 gene carried over agarose gel with electrophoresis apparatus

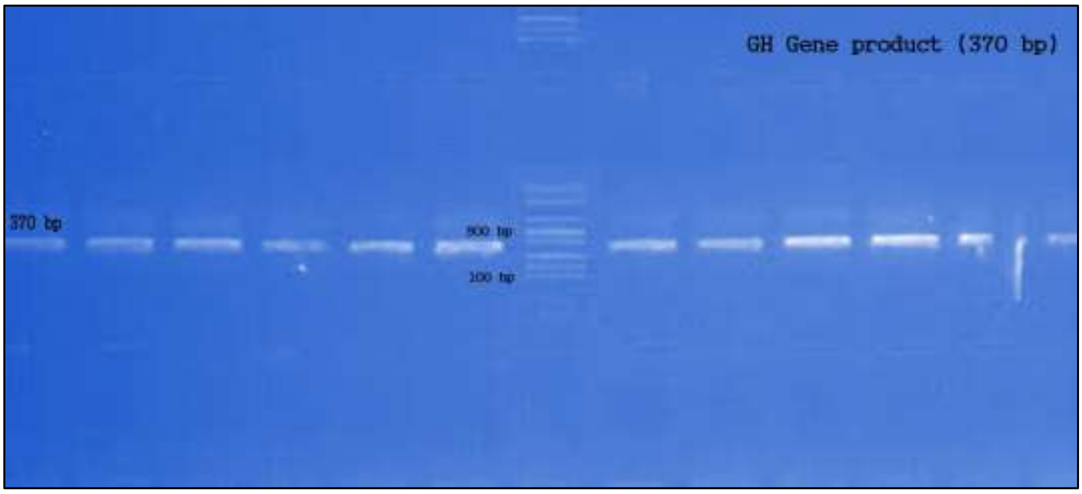

Figure 3: PCR product of GH gene carried over on agarose gel with electrophoresis apparatus

Genotypes of IGF-1 and GH genes using MSA

The results of the detection of the sequence of nitrogenous bases by multiple sequence alignment (MSA) method showed CLUSTAL Omega 1.2.4. 2017) .Obtaining three genotypes for the IGF-1 gene and two genotypes for the GH gene, as follows:

The AG genotype carries the AGC nitrogenous bases at positions 61, 62, and 63

*CC genotype AGC bases have been replaced by CCA bases (AGC > CCA)

*GC genotype AGC bases have been replaced by GCA bases (AGC > GCA)

AG

GTGGACAGCATGAGATCCATTCCCTCAC
TTGGCACCACCAGGACGAGGAGGTCAT
CCCAC 60

CC

GTGGACAGCATGAGATCCATTCCCTCAC
TTGGCACCACCAGGACGAGGAGGTCAT
CCCAC 60

GC

GTGGACAGCATGAGATCCATTCCCTCAC

ISSN 2072-3857

TTGGCACCACCAGGACGAGGAGGTCAT
CCCAC 60

****
****

AG
AGCGCCGTCTTCCAGTCTAGTTTACCCC
AGTCGTTTGAGGGTTAAAATCATAGAGT
ATGC 120
CC
CCAGCCGTCTTCCAGTCTAGTTTACCCC
AGTCGTTTGAGGGTTAAAATCATAGAGT
ATGC 120
GC
GCAGCCGTCTTCCAGTCTAGTTTACCCC
AGTCGTTTGAGGGTTAAAATCATAGAGT
ATGC 120

****
****

AG
TTGAGATGGTCTTTTTTTCATTTCTTGTT
TTTTAAATTTTGTGTTGGCTCTGGAATAT
AA 180
CC
TTGAGATGGTCTTTTTTTCATTTCTTGTT
TTTTAAATTTTGTGTTGGCTCTGGAATAT
AA 180
GC
TTGAGATGGTCTTTTTTTCATTTCTTGTT
TTTTAAATTTTGTGTTGGCTCTGGAATAT
AA 180

****
****

AG
AATTGCTCGCCCATCCTCCACGAATATT
CCTTTCTACGGGTAAGGTGATTAGCAGA
TGTG 240
CC
AATTGCTCGCCCATCCTCCACGAATATT
CCTTTCTACGGGTAAGGTGATTAGCAGA
TGTG 240
GC
AATTGCTCGCCCATCCTCCACGAATATT
CCTTTCTACGGGTAAGGTGATTAGCAGA
TGTG 240

****
****

AG GAAGGGGGGGGACA 265
CC GAAGGGGGGGGACA 265
GC GAAGGGGGGGGACA 265

Figure 3: Genotypes of IGF-1

As for the GH gene, a silent mutation was found in the nitrogenous bases of the piece used in the study, as follows:

*The genotype that carries the TC nitrogenous bases at positions 81, 19

*CT genotype TC bases replaced by CT bases (CT > TC)

TC
CCGTCATGTCTTCAATCTCCCCCTCGCT
GTCCTGCCTGACCCCACCCCCTAGAATA
GAAT 60

ISSN 2072-3857

CT
CCGTCATGTCTTCAATCCTCCCCTCGCT
GTCCTGCCTGACCCCACCCCCTAGAATA
GAAT 60
****
****
***

TC
CTGGAGTGGCACCTTCCAGGGTCTAGGA
AGGCACGGGGAAGGGGAACCACCGAAG
GGTGG 120
CT
CTGGAGTGGCACCTTCCAGGGTCTAGGA
AGGCACGGGGAAGGGGAACCACCAAAG
GGTGG 120

****
****

TC
GAACTAAAAAGGGCCACTTGGCCTCCC
CAAACCGCCAACCTTTCTGGACCTTCAG
GACCT 180
CT
GAACTAAAAAGGGCCACTGGGCTTCCC
CAAACCGCCAACCTTTCTGAACCTTCAG
GACCT 180

****
****

TC
GAACTAAAAAGGGCCACTTGGCCTCCC
CAAACCGCCAACCTTTCTGGACCTTCAG
GACCT 240
CT
GAACTAAAAAGGGCCACTTGGCCTCCC
CAAACCGCCAACCTTTCTGAACCTTCAG
GACCT 240

****
****

TC
TCTCCGTCTTGTGCAGGTCCTTCCGGAA
GCAGGAAAGCAGACCGTAGTTCTTGAG
CAGCG 300
CT
TCTCCGTCTTGTGCAGGTCCTTCCGGAA
GCAGGAAAGCAGACCGTAGTTCTTGAG
CAGCG 300

****
****

TC
GCAACTAGAAGGCGCAGCTGGCCTCCC
CGAAGCGGCGACACTTCATGACCCTCA
GGTACG 360
CT
GCAACTAGAAGGCGCAGCTGGCCTCCC
CGAAGCGGCGACACTTCATGACCCTCA
GGTACG 360

****
****

46 CTCCTTCCAA 370
50 CTCCTTCCAA 370
****

Figure 4: Genotypes of GH

ISSN 2072-3857

Effect of dam's age and genotypes of IGF-1 gene and GH gene on greasy and clean fleece weight and percentage of clean wool

It is clear from Table (1) that the age of the dam had a significant effect ($P<0.05$) on the weight of raw and clean fleece, as the ewes at the age of four years gave the highest weight of greasy and clean fleece with averages of 1.45 and 0.917 kg, respectively, then the ewes came at the age of three years, with averages of 1.41 , 0.882 kg, while ewes at the age of two years recorded the lowest weight of greasy and clean fleece with averages of 1.13 and 0.725 kg, respectively. The increase in the weight of the fleece with the advancing age of the ewes is attributed to the increase in the surface area of the skin, the size of the animal, the efficiency of feed conversion, and the maximum benefit from the feed provided to the old ewes compared to the young ewes. The percentage of clean wool was not significantly different depending on the age of the ewes. This is consistent with the findings of (22) in the Arabi sheep breed bred in Iran.

Different genotypes of the IGF-1 gene did not affect greasy and clean fleece weight and wool percentage. This result is not consistent with the result of (12), which showed a relationship between the genotypes of the IGF-1 gene and the fleece weight in the Iranian Makooei sheep breed, as it found a significant superiority of the genotype AG in the weight of wool at the age of one year over the other genotypes. Other ages did not notice a significant effect of the genotypes of the IGF-1 gene on the weight of wool, which indicates that the weight of wool at ages more than one year is affected by the environment more than the genetic effect. (7) also found a significant effect of the genotypes in the IGF-1 gene on the weight of greasy and clean fleece in the Egyptian Barqi sheep breed.

The genotypes of the GH gene had a significant effect on the weight of the greasy and clean fleece, as the ewes of the genotype TC were significantly ($P<0.05$) with averages of 1.40 and 0.889 kg, respectively, compared to the ewes of the genotype GT, which recorded averages of 1.26 and 0.791 kg, respectively. It was not significantly influenced by the various genotypes. This result is consistent with the result of (17) who noted a significant superiority of the G4 genotype over other genotypes in fleece weight of the Iranian Makooei strain. Although, (9) did not observe in their study a significant effect of GH gene genotypes on the percentage of clean wool.

ISSN 2072-3857

Table (1) Effect of dams age and genotypes of IGF-1 gene and GH gene on the mean weight of greasy and clean fleece and the percentage of clean wool ± standard error

influencing factors		number of animals	Greasy fleece weight (kg)	Clean fleece weight (kg)	clean wool percentage (%)
Dam age (years)	2	18	1.13 ±0.06 b	0.725 ±0.04 b	64.15 ±0.76
	3	27	1.41 ±0.04 a	0.882 ±0.03 a	62.55 ±0.59
	4	23	1.45 ±0.05 a	0.917 ±0.04 a	63.24 ±0.68
The genotype of the IGF-1 gene	AG	24	1.37 ±0.05	0.855 ±0.03	64.59 ±0.64
	CC	30	1.36 ±0.05	0.861 ±0.03	63.30 ±0.59
	GC	14	1.27 ±0.06	0.803 ±0.05	63.32 ±0.84
The genotype of the GH gene	CT	41	1.26 ±0.04 b	0.791 ±0.03 b	62.77 ±0.54
	TC	27	1.40 ±0.05 a	0.889 ±0.03 a	63.50 ±0.61

The averages with different letters within the same column are significantly different ($P<0.05$).

Effect of dam`s age and genotypes of IGF-1 gene and GH gene on staple length and fiber length and diameter

The age of the dam had a significant effect on all the studied physical characteristics of wool, where ewes at the age of four years were superior to ewes at the age of two years in the characteristic of staple length, fiber length, and fiber diameter, as averages of 12.10, 14.91 cm, and 30.34 microns were recorded, respectively. For ewes at two years old, they reached 10.53 and 13.08 cm. , 28.19 µm, respectively (Table 2). This may be because the efficiency of wool production increases with age. (4) indicated that there is a significant positive correlation between the weight of the greasy fleece and the physical characteristics of the wool of two breeds of local sheep. This finding is consistent with that of (22).

The genotypes of the IGF-1 gene did not have a significant effect on the physical characteristics of the wool. This result was confirmed by the result of (7) as it did not find a significant effect of the genotypes of the IGF-1 gene on the fiber diameter in the Egyptian Barqi sheep breed, while (19) noted a highly significant effect of the genotypes of this gene on the physical characteristics of the wool of merino sheep in China. As well as (12) in the Iranian Makooei sheep breed.

The genotype of the GH gene had a significant effect on some physical wool traits, where the ewes carrying the genotype TC excelled in staple length and fiber length and recorded averages of 11.71 and 14.89 cm,

ISSN 2072-3857

respectively, over the ewes bearing the genotype CT, which gave averages of 10.19 and 14.89 cm. The traits of the fibre diameter was not impacted by the different genotypes. This result was confirmed by the result of (9) in their study on three breeds of Egyptian sheep, Al-Barky, Al-Rahmani, Madarib Al-Rahmani and Al-Awassi in some physical wool traits such as the length of the staple and the diameter of the fiber.

Table (2) Effect of dams age and genotypes of IGF-1 gene and GH gene on staple length, fiber length, and fiber diameter ± standard error

influencing factors		number of animals	Staple length (cm)	fiber length (cm)	fiber diameter (micron)
Dam age (years)	2	18	10.53 ± 0.33 b	13.08 ± 0.33 b	28.19 ± 0.63 b
	3	27	11.57 ± 0.26 a	14.29 ± 0.25 a	28.07 ± 0.59 b
	4	23	12.10 ±0.30 a	14.91 ± 0.29 a	30.34 ± 0.64 a
The genotype of the IGF-1 gene	AG	24	11.46 ± 0.29	14.19 ± 0.28	29.38 ± 0.45
	CC	30	11.56 ± 0.26	14.22 ± 0.26	28.73 ± 0.54
	GC	14	11.18 ± 0.37	13.87 ± 0.36	29.81 ± 0.44
The genotype of the GH gene	CT	41	10.19 ±0.23 b	13.09 ± 0.23 b	29.12 ± 0.47
	TC	27	11.71 ± 0.27 a	14.89 ± 0.26 a	30.10 ± 0.53

The averages with different letters within the same column are significantly different (P<0.05).

Conclusions:

We conclude from this study that the production of wool and some of its physical traits increase with the advancing age of the ewes, as well as the existence of a relationship between the GH gene and the production of wool. Consequently, it is considered a genetically it is possible to improve wool production and its physical traits in local Awassi sheep.

References:

1. Al-Barzinji YMS, Othman GU (2013) Genetic Polymorphism in *FecB* Gene in Iraqi Sheep Breeds Using RFLP-PCR Technique. IOSR J. of Agri. and Vet. Sci., 21 (2): 46 – 48.
2. Al-Qasimi; Rahman HH, Jaffar AA, ALKhauzai ALD (2021) Relationship *GDF9B* (*BMP-15*) Gene Polymorphism with litter size in Iraqi Awassi Sheep. Euphrates Journal of Agriculture Science-13 (4): 187-194.

ISSN 2072-3857

3. Al-Qasimi; Rahman HH, Amad F. H, Bassam Y. Khudair (2020). Effect of IGF-1 gene polymorphism and some non-genetic factors on milk yield and chemical composition in Awassi sheep. Euphrates Jour of Agri Sci-12 (2):265-273.
4. Al-Qasimi; Rahman HH, Amad F. H, Bassam Y. Khudair (2019). Effect of IGF-1 and GH Genes Polymorphism on Weights and Body Measurements of Awassi Lambs in Different Ages. Basrah J. Agric. Sci., 32(1): 39-46.
5. Al-Rawi A (2011) The relationship between the formal measurements of udder Turkish Awassi ewes produce milk and some of its components and their impact on the growth of lambs to weaning, MSC. Thesis, agriculture collage, Al-Anbar University.
6. Clustalw Omega (2017) http://www.ebi.ac.uk/ /Tools/msa/clustalo/.
7. Darwish HR, El-Shorbagy HM, Abou-Eisha AM (2017) New polymorphism in the 5′ flanking region of *IGF-1* gene and its association with wool traits in Egyptian Barki sheep. Genetic Engineering and Biotechnology.15(2), 437-441.
8. Dauphin LA, Walker RE, Petersen JM, Bowen MD (2011) Comparative evaluation of automated and manual commercial DNA extraction methods for detection of Francisella tularensis DNA from suspensions and spiked swabs by real-time polymerase chain reaction. Diagn Microbiol Infect Dis., 70(3): 299– 306.
9. Farag IM, Ahmed MD, Hassan RD, AbdelAziz KB, Ramadan WA, Mohamed MI, Othman EO (2016) Polymorphism of growth hormone gene and its association with wool traits in Egyptian sheep breeds. Afr. J. Biotechnol., 15 (14): 549 - 556.
10. Hajihosseinlo A, Semsarnejad A, Abollow E, Hasbrafi F, Negahdary M (2013) Effect of GH gene polymorphisms on biometric traits in Makooei sheep. Ann. Biol. Res., 4 (6): 351 - 355.
11. Ivan, F, Gorlov YA, Kolosov NV, Shirokova LV, Getmantseva (2017) Association of the growth hormone gene polymorphism with growth traits in Salsk sheep breed Ivan. Small Rumin Rese 150 11–14.
12. Masoud N, Sahar M, Abbas H (2014) Genetic Effect of IGF1, PIT1 and Leptin Genes on Wool Weights in Makooei Sheep. Electronic J. Bio., 10(2) : 46-51 .
13. Moradian C, Giti E, Abbas H (2013) polymorphismat of IGF-1 in Makooei sheep using PCR-SSCP . European Journal of Exper Bio, 3(2):490-494.

ISSN 2072-3857

14. Muhana, Kareem H , Azhar A. Jaffar and Rahman H. Hamza Al Qasimi (2021). Relationship between Calpastatin Gene Polymorphism and Production Traits in Awassi Sheep in Iraq. Indian Journal of Ecology 48 Special Issue (17): 251-255.
15. Okonkwo JC, Omeje IS, Egu UN (2010) Identification of X - chromatin and determination of its incidence in Nigerian goat breeds. Livestock Rese for Rural Deve, 22, (12).
16. Roman N, Grzegorz C, Jerzy K, Mariola N, Marsin S (2014) Polymorphism of insulin-like growth factor (*IGF-1*) gene in Polish Lowland Sheep from Podlaskie Voivodeship. Animal Sci 53: 43-46.
17. Rothschild MF, Soller M (1997) Candidate gene analysis to detect genes controlling traits of economic importance in domestic livestock. Probe 8: 13 - 20.
18. Sambrook S, Russell DW (2002) Molecular Cloning: A Manual (in Chinese), Science. Laboratory Press, Beijing 463–470.
19. Shen M (2008) A novel polymorphism of IGFBP-3 gene and its relationship with several wool traits in Chinese Merino sheep Zhongguo yi chuan xue hui bian ji, 30 (9) pp. 1182-1186.
20. Spss. (2013) Statistical Package of Social Science .Ver . 22. Application Guide .Copy right by Spss Inc.USA.
21. Taddeo HR , L Duga, P Willemas, R Samlo (2000) Variation of mohair quality over the body in Angora goats. Small.Run.Reas. 36 (3) :285 – 291.
22. Taherpour N, Mahnaz S, Farhad M (2012) Evaluation certain environment factors on wool characteristics of Arabi sheep breeds. Open J. Anim. Scie 2(1): 11-14.
23. Trukhachev V, Valentin S, Andrey K, Marina S, Arslan O (2016) Polymorphisms of the IGF-1 gene in Russian sheep breed and Their influence on some meat production parameters. Slov. Vet. Res., 53(2): 77-83.

ISSN 2072-3857