Journal of Genetic Engineering and Biotechnology 15 (2017) 437–441

HOSTED BY

Contents lists available at ScienceDirect

Journal of Genetic Engineering and Biotechnology

journal homepage: www.elsevier.com/locate/jgeb

Original Article

New polymorphism in the 5′ flanking region of *IGF-1* gene and its association with wool traits in Egyptian Barki sheep

H.R. Darwish [a], H.M. El-Shorbagy [b,*], A.M. Abou-Eisha [a], A.E. El-Din [a], I.M. Farag [a]

[a] Cell Biology Department, National Research Centre, Dokki, Giza, P.O. Box 12622, Egypt
[b] Zoology Department, Faculty of Science, Cairo University, Giza, P.O. Box 12613, Egypt

ARTICLE INFO

Article history:
Received 31 December 2016
Received in revised form 6 May 2017
Accepted 1 August 2017
Available online 10 August 2017

Keywords:
Clean fleece weight
Fiber diameter
IGF-1 gene
Ovis aries
Restriction fragment length polymorphism
Single-strand conformation polymorphism

ABSTRACT

Insulin-like growth factor-1 gene (*IGF-1*) is considered as a major candidate gene for the economic traits of animal production. Polymorphism of 5′ flanking region of *IGF-1* gene in Barki sheep (n = 91) and its association with wool traits were studied using the polymerase chain reaction coupled with single-strand conformation polymorphism technique (PCR-SSCP), PCR-restriction fragment length polymorphism (PCR-RFLP), sequence analysis and different measurements of wool traits (clean fleece weight and fiber diameter). PCR-SSCP analysis revealed three different banding patterns corresponding with three genotypes frequencies GG (0.25), GA (0.58), AA (0.17). PCR-RFLP and corresponding sequence analysis revealed nucleotide transversion from Guanine (G) to Cytosine (C) at nucleotide position 85 and transition from (G) to Adenine (A) at position 87. This is the first study that recorded two SNPs within the 5′ flanking region of *IGF-1* gene in Egyptian Barki sheep, which were submitted to DNA Data Bank OF Japan (DDBJ) with Accession No. LC151463.1. The genotype GG showed positive significant association ($P < 0.001$) with clean fleece weight (CFW) trait (Odd Ratio = 2.83). By contrast, genotype AA had negative significant association ($P < 0.05$) with such trait (Odd Ratio = 0.15). On the other hand, fiber diameter (FD) measurements showed no significant association ($P > 0.05$) with different *IGF-1* genotypes. This study adds evidence of the association between *IGF-1* gene polymorphism and CFW of wool in Egyptian Barki sheep. Therefore; it is important to consider *IGF-1* gene as a candidate gene marker for wool weight traits and it should be identified before using successful breeding program.

1. Introduction

Insulin-like growth factor-1 gene (*IGF-1*) encodes a 70 amino acids polypeptide hormone, which has a notable role in cell differentiation, embryogenesis, metabolism and growth [1,2]. Moreover, most of growth hormones are mediated by *IGF-1* gene [3,4]. Thus, it is considered as a major candidate gene for economic traits of animal production [2,4]. The ovine *IGF-1* gene is located on chromosome 3 and has 1–6 exons [5]. Detection of single nucleotide polymorphism in the transcription factor binding sites is an important point of view, because nucleotide substitutions may change the level of gene expression [6]. Negahdary et al. demonstrated that the *IGF-1* gene polymorphism in Makooei sheep plays a great role in wool production [7]. In goats, authors found that intron 4/Hae III polymorphism (G/C) had significantly associated with first shearing fleece weight [8]. In the Nanjiang Cashmere goat, authors found that *IGF-1* gene polymorphism had significantly associated with cashmere production and body weight traits [9]. Moreover, Shen et al. observed that the genetic polymorphism of *IGFBP-3* gene had affected many wool characteristics in Chinese Merino sheep [10]. In the North African regions, although sheep genetic resources play a substantial role, most of the local breeds have not been adequately characterized [11]. However, gene markers associated with wool traits need to be substantiated in a large number of sheep and in other breeds to build molecular tools that facilitate genetic programs for improvement of wool sheep. However, previous studies had some limitations, such as small sample size [12], insufficient analyzed loci and deviation from Hardy-Weinberg equilibrium [13]. To our knowledge, no reports have been published concerning the polymorphism of the 5′ flanking region of the ovine *IGF-1* gene or its putative association on wool characteristics in Egyptian sheep breed. although a single nucleotide substitution in regions such as transcription factor binding sites may alter gene expression level. Additional polymorphisms

Peer review under responsibility of National Research Center, Egypt.
* Corresponding author.
E-mail address: haidan@sci.cu.edu.eg (H.M. El-Shorbagy).

http://dx.doi.org/10.1016/j.jgeb.2017.08.001
1687-157X/

in growth hormone axis genes were found to be associated with production traits in ruminants. However, wool traits are polygenic, and many genes have been documented to be associated with wool quality traits; beside the nutrition and environmental conditions that may influence wool yielding. Also, it is worthy to investigate whether and how SNPs in 5′ flanking region of *IGF-I* gene affect its expression and lead to variations in wool fleece quality in the future studies. Therefore, the present study was designed to identify the potential novel SNPs in the sheep *IGF-1 gene*, and to figure out their possible association with wool production and quality traits that can be used as useful marker-assisted for high quality wool production and breeding selection in Egyptian Barki sheep.

2. Material and methods

2.1. Chemicals

All chemicals were of analytical grade and were purchased from Sigma-Aldrich (St. Louis, MO). Restriction Enzyme (Bst H_2I E171) was purchased from SibEnzyme Ltd. Russia.

2.2. Animals and ethical considerations

Animals used in this study were 91 Fat-tailed Egyptian male sheep, Barki breed (*Ovis aries*) two years old, which originated in North Africa in the coastal Mediterranean zone extending from west of Alexandria to the eastern provinces in Libya. The breed is named according to the Libyan province Barka (Cyrenaica). This study was performed according to authorization and granted by the National Research Center. All procedures involving animals were approved by the Institutional Animal Care and Use Committee (IACUC), Faculty of Science, Cairo University, Egypt with Permit Number: CUFS F Mol. Biol. 50 15.

2.3. DNA extraction and PCR amplification of ovine IGF-1 gene

From each sheep, 3 ml of total blood were collected from the left jugular vein using vacuum tubes containing 7.5 mg of EDTA. Genomic DNA was extracted from blood samples using DNA extraction kit (Thermo Scientific™ genomic extraction kit), and quantified using Nanodrop 2000 spectrophotometer, (Thermo Fisher Scientific, USA 02451). PCR reaction was performed using thermal cycler PCR (Techne TC-3000, Burlington, NJ, USA) by the following condition: denaturation for 5 min at 95 °C, followed by 31 cycles of 30 s at 95 °C, 35 s at 61 °C and 35 s at 72 °C, with a final extension of 7 min at 72 °C; using the following primers' sequences designed by (NCBI/ Primer-BLAST): F: 5′-ATTACAAAGCTGCCTGCCCCTT-3′ and R: 5′- CACATCTGCTAATACACCTTAC CCG-3′. PCR amplicons were electrophoresed in 2% agarose gels, using 0.5X TBE buffer (89 mM Tris, 89 mM boric acid and 2 mM Na_2EDTA) containing 200 ng/ml of ethidium bromide and was visualized under UV light and photographed by Bio-Rad Laboratories, Hercules, CA, USA.

2.4. Single-strand conformational polymorphism

7 μl aliquot of each amplicon was mixed with 12 μl of loading dye (98% formamide, 0.05% bromophenol blue and 0.05% xylene cyanol). After denaturation at 95 °C for 10 min, samples were rapidly cooled on wet ice for 10 min., then loaded on 20 cm × 15 cm, 10% acrylamide:bisacrylamide (29:1) gels [14]. Electrophoresis was carried out at a constant voltage of 200 V at 4 °C for 12 h. Gels were ethidium bromide-stained for 5 min, washed with distilled water, and visualized under UV light and photographed by Bio-Rad Laboratories, Hercules, CA, USA.

2.5. PCR-RFLP analysis

RFLP analysis was performed by incubating a mixture of 4 μl of PCR product, 1 μl of enzyme buffer, 4.6 μl of distilled water, and 0.3 μl of Bst H_2I E171enzyme (SibEnzyme Ltd. Russia), at 65 °C for 3 h. 10 μl of samples were loaded into 3% agarose gel containing 200 ng/ml of ethidium bromide and were electrophoresed at a constant 80 V and 60 mA using 0.5X TBE buffer (89 mM Tris, 89 mM boric acid and 2 mM Na_2EDTA) [15]. Electrophoretic bands were photographed by BioRad Documentation System (USA). Photographs were analyzed using LabImage Platform 3.2, Kapelan Bio-Imaging, Germany.

2.6. Sequence analysis

PCR products representing different SSCP patterns of *IGF-1* gene (2 for wild type and 5 for variants) were purified and sequenced by Macrogen Corporation, South Korea. The nucleotide sequences were compared against the corresponding sheep gene sequence (GenBank Accession Number: X17229) for *IGF-1* gene and analyzed by cluster wide analysis using CodonCode Aligner software, CodonCode Corporation, USA. The nucleotide sequences of new *IGF-1* gene variants were submitted to DDBJ.

2.7. Wool shearing and measurements

Wool samples were collected from the mid-side region of the animals for analysis. Ten staples were taken from each greasy sample and used for measuring the percentages of clean fleece weight (CFW) and fiber diameter (FD) [16]. Measurements were as follow:

- Fiber diameter (FD): Short sections of at least 200 fibers were prepared and mounted in paraffin oil on glass slides and covered with glass cover using the method adopted by [17]. Fiber diameter was estimated using light microscope and image captured by image analysis software (Video Pro, Leading Edge Ltd, S. Aust.) and device (LEICAQ 500 MC) with lens 4/0.12. The average fiber diameter (FD) and standard error (SE) of each sample were calculated.
- Mean fiber diameter (MFD) was divided into comfort factor or fiber diameter less than 30 nm (F < 30), prickle factor or fiber diameter more than 30 nm (F > 30).
- Clean Fleece Weight (CFW%): Determination of clean wool weight for each sample was carried out using the method suggested by [18] as follow:

$$\text{Clean scoured yield} = \frac{\text{Weights of clean scoured and dry wool}}{\text{Weights of greasy wool}} \times 100$$

2.8. Statistical analysis

Genetic equilibrium for Egyptian sheep breeds were assessed according to Hardy-Weinberg equilibrium (HWE) and chi-square test by using SAS Genetics (v9.3, SAS Institute Inc., Cary, NC, USA). The effect of *IGF-1* alleles on wool traits of Egyptian sheep was estimated by using general linear model (GLM) procedure and the least squares means of the genotypes were compared by the Tukey-Kramer test. The odds ratios were processed to evaluate the power of the relationship between *IGF-1* gene polymorphism and wool characteristics of Egyptian sheep. Significance was set at $P \leq 0.05$, $P \leq 0.01$ and $P \leq 0.001$ [19–24]. All statistical analyses were performed by applying SAS program (v9.3, SAS Institute Inc., Cary, NC, USA).

3. Results

A total of 91 sheep were genotyped for polymorphism in the 5′ flanking region of *IGF-1* gene (Fig. 1). The PCR product successfully amplified 265 bp fragment of *IGF-1* gene, flanking region 467–732 bp upstream from the 5′ end of exon 1 (GenBank Accession Number: X17229).

3.1. PCR-SSCP analysis

PCR-SSCP analysis revealed three different banding patterns GG, AA, GA (Fig. 2) with frequencies of (0.25), (0.17) and (0.58), respectively. Genetic equilibrium was estimated based upon the Hardy-Weinberg equilibrium and Chi-square test using allele procedure. Allelic frequencies were 0.54 for G allele and 0.46 for A allele. This showed that the number of individuals with G allele is approximately equal to that of A allele. Results showed that genotype distributions of *IGF-1* are in agreement with Hardy-Weinberg equilibrium ($P > 0.05$) for Egyptian sheep breed, (Table 1).

3.2. PCR-RFLP analysis

The digestion of *IGF-1* gene PCR product (265 bp) with Bst H_2I E171 (Sib™ restriction Enzyme), resulted in two fragments lengths of 85 and 180 bp for genotype GG; 265, 85 and 180 bp for genotype GA, and only 265 for genotype AA (Fig. 3).

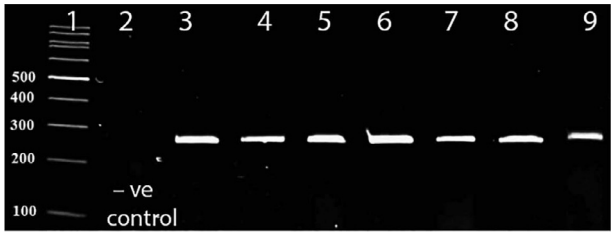

Fig. 1. Ethidium bromide-stained 8% acrylamide gel of PCR products representing: lanes 3–9: amplicon of *IGF-1* gene in Egyptian sheep (265 bp). Lane 1: 100 bp ladder marker. Lane 2: negative control.

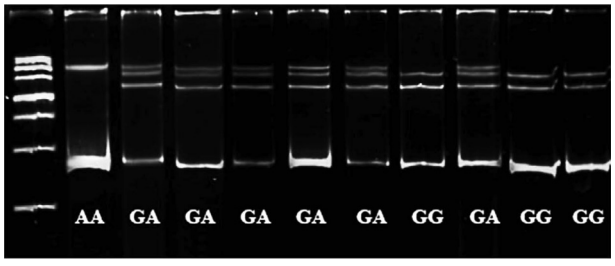

Fig. 2. PCR-single-strand conformational polymorphism of the ovine *IGF-1* gene. Amplicons were electrophoresed on a 12% non-denaturing acrylamide/bis-acrylamide gel; 200 V, 4 °C for 12 h. Three SSCP patterns, GG, AA and GA were detected.

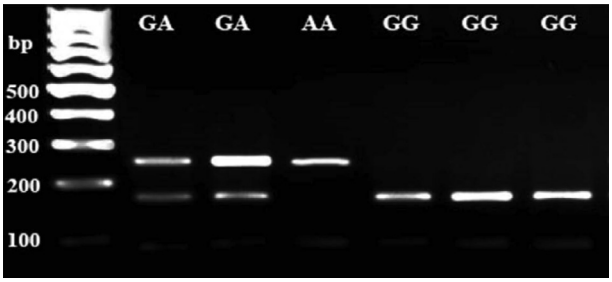

Fig. 3. The electrophoretic pattern obtained after digestion of PCR amplified fragment of *IGF-1* gene with Bst H_2I restriction enzyme showing three different bands (265, 85 and 180 bp). Lane 1: 100 bp ladder marker.

3.3. Sequencing analysis

Sequencing analysis revealed two point mutations at positions 85 and 87 of the sequence when compared with *IGF-1* gene (accession number AY803775.1); both mutations were not located at the encoding sequence of the interested gene (Fig. 4). A nucleotide transversion from Guanine (G) to Cytosine (C) at position 85 and a transition from Guanine (G) to Adenine (A) at positions 87 were found. The nucleotide sequence of the new *IGF-1* gene variant was submitted to DDBJ. DNA Data Bank of Japan (DDBJ) with Accession No. LC151463.1. The A allele was defined as the nucleotide sequence with variant, and the G allele as the sequence without variant.

3.4. Effect of IGF-1 genotypes on wool traits

The effects of *IGF-1* genotypes on wool characteristics of Egyptian Barki sheep, like fiber diameter (FD) and clean fleece weight (CFW), were chosen to be studied in this research work. The least square means and standard error for the effect of *IGF-1* genotypes on wool traits are presented in Table 2. According to the statistical analysis, no significant effect ($P > 0.05$) of FD was found between different IGF-*1* genotypes. On the other hand, the analysis of CFW measurements showed that GG pattern of *IGF-1* gene had high significant effect on CFW ($P < 0.001$). In order to identify which genotype has the greatest grade of association with wool characteristics, the binary logistic regression had applied to obtain the odds ratio (OR) of each genotype. The results indicated that the individuals of genotype GG, GA and AA showed no significant association with FD as displayed in Table 3. Conversely, GG genotype exhibited the highest degree of association with CFW ($P < 0.05$), whereas AA genotype showed the lowest odds ratio value (Table 3).

4. Discussion

The system of marker-assisted selection in animal populations is considered as the main procedure that gives opportunities to enhance genetic improvement in livestock programs [25]. Thus, genetic improvement studies of the Egyptian sheep breeds were important over the last two decades. Some of these studies

Table 1
Genotype distribution and allelic frequencies at the *IGF-1* locus of the Barki Egyptian sheep.

Breed	Observed genotypes and frequencies				Allelic frequencies			
	GG	GA	AA	Total	G	A	χ^2	P-value
Egyptian sheep (Barki)	0.25 (23)	0.58 (53)	0.17 (15)	91	0.544	0.456	2.75	0.097

GG, GA and AA genotype frequencies was at the *IGF-1* locus; n = 91 male sheep; genotypes and alleles frequencies were assessed according to Hardy-Weinberg equilibrium (HWE) and χ^2, chi-square value. The number of animals per genotype is indicated in parentheses.

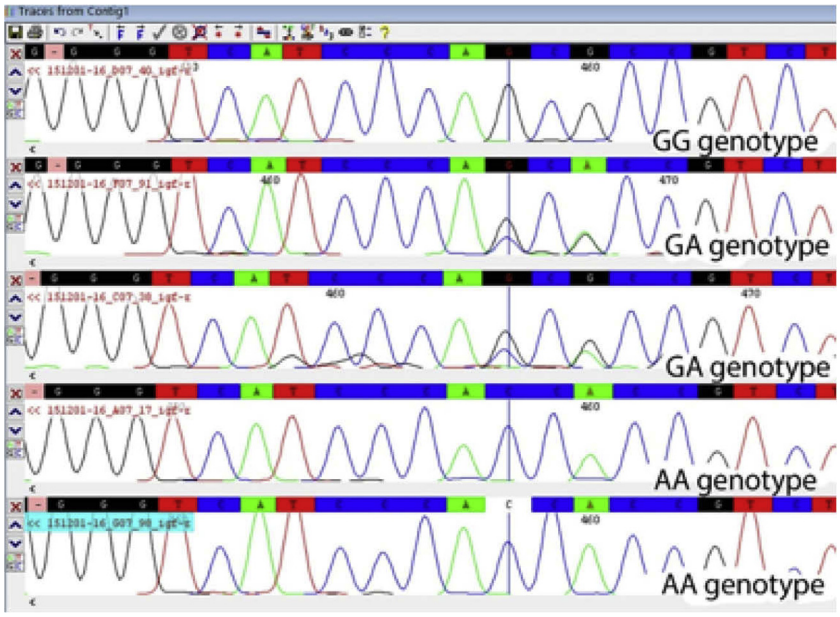

Fig. 4. Chromatogram alignment of GG, GA and AA genotypes of sheep IGF-1 gene.

Table 2
The effect of *IGF-1* genotypes on fiber diameter (FD) and clean fleece weight (CFW).

IGF-1 genotypes	Number of animals/genotype	FD		CFW	
		LSM ± SE	P	LSM ± SE	P
GG	23	30.16 ± 1.11		74.14 ± 1.55	
GA	53	29.69 ± 0.57		67.20 ± 1.08	
AA	15	30.42 ± 1.02		63.11 ± 1.47	
GG-GA		0.48 ± 1.11	0.67	6.93 ± 1.86[a]	<0.01
GG-AA		−0.26 ± 1.47	0.86	11.03 ± 2.48[b]	<0.001
GA-AA		−0.73 ± 1.29	0.57	4.097 ± 2.18	>0.05

Results were expressed as least-squares means (LSM) ± standard error (SE); statistically significantly different comparable to GA at a $P \leq 0.01$; comparable to AA at $P \leq 0.001$; not significant ($P > 0.05$); results were compared by the Tukey-Kramer test; n = 91 male sheep.
Mean values marked with the different letter are different (significant, P < 0.05).

Table 3
Association between *IGF-1* genotypes, fiber diameter (FD) and clean fleece weight (CFW) traits of Egyptian sheep using binary logistic regression analysis.

Genotype	FD					CFW				
	High%	Low%	OR	Coefficient	P-value	High%	Low%	OR	Coefficient	P-value
GG	52.17	47.83	1.09	0.087	0.84	73.91	26.09	2.83	1.042	0.03[a]
GA	45.28	54.72	0.83	−0.189	0.49	47.17	52.83	0.89	−0.113	0.68
AA	53.33	46.67	1.14	0.134	1.14	13.33	86.67	0.15	−1.872	0.014[a]

FD, fiber diameter; CFW, clean fleece weight; OR, odds ratio and [a] $P \leq 0.05$ considered statistically significant; Chi-square for (FD) = 0.48, P = 0.79 and for (CFW) = 13.41 $P = 0.001$; R^2 for (FD) = 0.005 and for (CFW) = 0.20; n = 91 male sheep.

included reports on breed characteristics, genetic distances among breeds, growth rate and reproduction [26,27]. However, in Egypt, the genetic improvement of such breeds for wool production is missing or moderate. The successful wool program must identify genes involved in the improvement of wool production and quality. Wool characteristics like clean fleece weight (CFW) and fiber diameter (FD) are a very notable goal in sheep breeding methods for favorable wool market and industry. In wool follicle of transgenic sheep, Damak et al. showed that heritable and stable expression of *IGF-1* gene was capable of increasing fleece weight at yearling shearing, while there was no significant effect of *IGF-1* gene on the fiber diameter [28]. Also, Harris et al. found that local IGF-1 infusion increased blood flow, oxygen utilization and amino acid uptake in sheep skin [29]. Philpott et al. reported that IGF-1 stimulated hair growth in cultured human follicles [30]. In addition, polymorphism in the transcription factor binding sites is important, because nucleotide substitutions may change the level of gene expression [6]. Thus, the current study was focused on the 5′ flanking region of *IGF-1* gene in Barki sheep.

In the current study, PCR-SSCP analysis revealed three genotype patterns GG, AA and GA in ovine *IGF-1* gene. GG genotype of *IGF-1* gene had high significant effect on CFW compared to other genotypes, and the association analysis revealed a strong relationship between GG genotype and wool yield, where GG genotype resulted in higher wool yield (about three folds) than that of AA genotype. GA genotype had a significant effect on CFW in compare to AA genotype. On the other hand, statistical analysis showed no association between different genotypes and fiber diameter. Taken

together, the present results indicate that different *IGF-1* genotypes affect the wool yield regardless of the fiber diameter measurements which may be an indication to their effects on the transcriptional level. Our findings are in harmony with that reached by Negahdary et al.; who stated that Makooei sheep with the wild type GG genotype of *IGF-1* gene had an excellent wool weight as compared with other genotypes [7]. Also, *IGF-1* gene polymorphism had a dominant effect on the goat fleece weight. This was reported by Kurdistani et al.; who revealed a relationship between caprine *IGF-1* gene polymorphism in intron 4 and exon 4; and in yearling fleece weight in Markhoz goats. Shen et al. observed significant association between polymorphism in *IGFBP-3*, a member of IGFs family, [10] and some wool traits in Chinese Merino sheep, whereas greasy fleece weight (GFW) and follicle density were significantly elevated in individuals with genotype GA ($P < 0.01$) or variant genotype than that in individuals with wild genotype. Moreover, Shen and colleagues found that the average fiber diameter was significantly reduced in animals that have wild and heterozygous genotypes as compared to animals with variant genotype. In Nanjiang Cashmere goat, Qiong et al. reported a correlation between genetic polymorphism in caprine *IGF-1* gene and wool fineness trait [9]. However, such trait in GA genotype animals was significantly higher than that in GG genotype, and the body weight of GC genotype individual was significantly ($P < 0.05$) higher than that of individuals with AA genotype. However, uncontrolled feeding program is considered as a limitation for the present study.

Based on the above mentioned considerations, the observed SNPs in the present study may affect promoter activity and transcriptional level of ovine *IGF-1* gene [8], giving a performance of the "G" allele over the "A" allele on the tested trait and showing that animals with genotype GG have higher clean fleece weight compared to the other genotypes.

5. Conclusion

The present study adds evidence on the association between *IGF-1* gene polymorphism and Clean Fleece Weight (CFW) of wool in Egyptian sheep breeds; therefore, it is important to make *IGF-1* gene a candidate gene marker for wool weight traits and could be identified before using successful breeding program.

Acknowledgements

This work was supported by National Research Center and Cairo University – Egypt.

Conflict of interest

There is no potential conflict of interest or competing interest.

References

[1] Daughaday J, Rotwein P. Insulin-like growth factors I and II. Peptide, messenger ribonucleic acid and gene structures, serum, and tissue concentrations. Endocrine Rev 1989;10(1):68–91.
[2] Estany J et al. Association of CA repeat polymorphism at intron 1 of insulin-like growth factor (IGF-I) gene with circulating IGF-I concentration, growth, and fatness in swine. Physiological Genomics 2007;31(2):236–43.
[3] Froesch ER et al. Insulin-like growth factor I: physiology, metabolic effects and clinical uses. Diabetes/Metab Rev 1996;12(3):195–215.
[4] Honarvar M et al. Study of polymorphisms in the 5 flanking region of the ovine IGF-I gene in zel sheep. World Appl Sci J 2012;16(5):726–8.
[5] Hajihosseinlo A et al. Effect of GH gene polymorphisms on biometric traits in Makooeisheep. Ann Biol Res 2013;4(6):351–5.
[6] Ge W et al. Association of a genetic marker with blood serum insulin-like growth factor-I concentration and growth traits in Angus cattle. J Anim Sci 2001;79(7):1757–62.
[7] Negahdary M, Majdi S, Hajihosseinlo A. Genetic effect of IGF1, PIT1 and Leptin genes on wool weights in Makooei sheep. Electron J Biol 2014;10(2):46–51.
[8] Kurdistani ZK et al. Evaluation of insulin-like growth factor-I gene polymorphism on growth traits and yearling fleece weight in goats. Small Ruminant Res 2013;111(1):10–5.
[9] Qiong W et al. A novel mutation at exon 4 of IGF-1 gene in three indigenous goat breeds in China. Asian J Anim Vet Adv 2011;6(6):627–35.
[10] Shen M et al. A novel polymorphism of IGFBP-3 gene and its relationship with several wool traits in Chinese Merino sheep. Yi chuan = Hereditas/Zhongguo yi chuan xue hui bian ji 2008;30(9):1182–6.
[11] Galal S. Animal genetic resources in Near East and North Africa region with special reference to Egypt and small ruminants. Egypt J Sheep Goat Sci 2010;5 (1):3–16.
[12] El-Hanafy AA, Salem HH. PCR-RFLP of IGFBP-3 gene in some Egyptian sheep breeds. Am-Eurasian J Agric Environ Sci 2009;5:82–5.
[13] Zhang C et al. A new single nucleotide polymorphism in the IGF-I gene and its association with growth traits in the Nanjiang Huang goat. Asian-Aust J Anim Sci 2008;21(8):1073–9.
[14] Gasser RB et al. Single-strand conformation polymorphism (SSCP) for the analysis of genetic variation. Nat Protoc 2006;1(6):3121–8.
[15] Yilmaz A et al. Detection of two nucleotide substitutions and putative promoters in the 5′ flanking region of the ovine IGF-I gene. J Appl Genet 2005;46(3):307–9.
[16] El-Gabbas H. Wool fiber diameter and its distribution in Barki sheep with regard to the sampling positions and seasons. Egypt J Anim Prod 1998;35 (2):123–41.
[17] Farag IM et al. Polymorphism of growth hormone gene and its association with wool traits in Egyptian sheep breeds. Afr J Biotechnol 2016;15(14):549–56.
[18] Chapman R. The biology of the fleece. Animal Research Laboratories Technical Paper 1960;(3).
[19] Valipour M, Banihabib ME, Behbahani SMR. Comparison of the ARMA, ARIMA, and the autoregressive artificial neural network models in forecasting the monthly inflow of Dez dam reservoir. J Hydrol 2013;476:433–41.
[20] Valipour M. How much meteorological information is necessary to achieve reliable accuracy for rainfall estimations? Agriculture 2016;6(4):53.
[21] Valipour M. Variations of land use and irrigation for next decades under different scenarios. Irriga 2016;1(01):262–88.
[22] Valipour M. Analysis of potential evapotranspiration using limited weather data. Appl Water Sci 2014:1–11.
[23] Valipour M, Ma Gholami Sefidkouhi. Temporal analysis of reference evapotranspiration to detect variation factors. Int J Global Warming; 2017. http://dx.doi.org/10.1016/j.wse.2015.01.004 [in press, 2018].
[24] Valipour M, Sefidkouhi MAG, Raeini M. Selecting the best model to estimate potential evapotranspiration with respect to climate change and magnitudes of extreme events. Agric Water Manage 2017;180:50–60.
[25] Rong E et al. Association of methionine synthase gene polymorphisms with wool production and quality traits in Chinese Merino population. J Anim Sci 2015;93(10):4601–9.
[26] Mahfouz ER et al. Genetic variation between some Egyptian sheep breeds using RAPD-PCR. Res J Cell Mol Biol 2008;2:46–52.
[27] Othman OE et al. Genotyping of growth hormone gene in Egyptian small ruminant breeds. Biotechnology 2015;14(3):136.
[28] Damak S et al. Improved wool production in transgenic sheep expressing insulin-like growth factor 1. Nat Biotechnol 1996;14(2):185–8 [Nature Publishing Company].
[29] Harris P et al. Direct infusion of a variant of insulin-like growth factor-I into the skin of sheep and effects on local blood flow, amino acid utilization and cell replication. J Endocrinol 1993;139(3):463–72.
[30] Philpott M, Sanders D, Kealey T. Effects of insulin and insulin-like growth factors on cultured human hair follicles: IGF-I at physiologic concentrations is an important regulator of hair follicle growth in vitro. J Invest Dermatol 1994;102(6):857–61.