Molecular Biology Reports (2023) 50:913–917
https://doi.org/10.1007/s11033-022-07975-0

SHORT COMMUNICATION

# Polymorphism of the *ADIPOQ* gene and its association with productive traits in Awassi Ewes

**Wisam Salim Al-Jumaili[1] · Alaa Hasan Kadhim[1] · Tahreer Mohammed AL-Thuwaini[1]**

Received: 21 July 2022 / Accepted: 20 September 2022 / Published online: 4 November 2022


## Abstract

**Background** Adiponectin (ADIPOQ) plays a critical role in energy and lipid metabolism, indicating that adiponectin could affect livestock productivity. There is an association between polymorphisms in the *ADIPOQ* gene and variations in livestock productive traits. Therefore, this study investigated the relationship between *ADIPOQ* polymorphism and productive traits in Awassi ewes.

**Methods and results** This study included 200 sexually mature ewes, aged 2.5 to 5 years, non-pregnant and not lactating. A phenotypic measurement consisting of live weight and body dimensions, was taken. Samples of blood were taken for extraction of genomic DNA and PCR-SSCP based genotyping, followed by sequencing to confirm variants in amplified fragments. A novel c.198,473,337 C > A SNP was found in exon 1 of the *ADIPOQ* gene confirming heterogeneity with genotypes AA, CC, and CA. The AA genotype differed significantly ($P < 0.05$) by comparison with the CA and CC genotypes concerning live body weight and body measurements. An association between productive traits and the c.198,473,337 C > A SNP revealed a significant association of the A allele (odds ratio: 2.22 (95% CI: 0.94–5.30) in the additive genetic model.

**Conclusion** Sheep with the AA genotype were heavier and had larger body dimensions, implying superior production and reproduction. Further studies are required in other breeds to prove the results.

**Keywords** *ADIPOQ* polymorphism · Body morphometric · Sheep

## Introduction

Many agricultural traits are complex and highly influenced by environmental and genetic factors [1]. For domestic animals to be more productive, it is imperative to understand the genetic basis of these traits [2]. Moreover, genetic studies on sheep breeds revealed gene candidates associated with productivity [3]. Among candidates for productive traits is the adiponectin gene (*ADIPOQ*) [4]. This gene is positioned on the bovine chromosome (BTA) 1 [5] and chromosome 1q27 in sheep. It consists of three exons and two introns (NCBI Reference Sequence NC_019480.2). It encodes adiponectin (MW 30 kDa and 244 amino acids), one of several biologically active adipokines secreted from white adipose tissue [6]. Adiponectin plays a central role in maintaining energy homeostasis, and it has a newly identified role as a "starvation gene" [7]. Additionally, studies suggest that adiponectin improves insulin sensitivity and glucose tolerance through crosstalk with insulin [8, 9]. It appears that adiponectin is involved in a variety of tissue-specific signaling pathways. Muscle fatty acid oxidation and glucose uptake are enhanced by adiponectin through AMP-activated protein kinase (AMPK) (9). Adiponectin stimulates the proliferation of skeletal muscle via the p38-AMPK pathway, which can influence carcass characteristics [10]. Furthermore, adiponectin inhibits the accumulation of ectopic lipids in adipocytes and is negatively correlated with body fat, which affects meat quality traits [11].

Using these markers effectively requires an investigation of polymorphisms in candidate genes that affect traits of economic importance. Several polymorphisms within the adiponectin gene have been reported in domestic animals concerning body weight and productivity. It has been shown that the ovine *ADIPOQ* gene has been implicated in low birth weight and metabolic disorders in Merino rams

✉ Tahreer Mohammed AL-Thuwaini
tahrearmohammed@agre.uoqasim.edu.iq;
tahreermohammed@ymail.com

[1] Department of Animal Production, College of Agriculture, Al-Qasim Green University, Babil, Iraq

[12]. In addition, the *ADIPOQ* gene polymorphism affects muscle area and fat thickness in Angus cattle [13], indicating that it can be beneficial for animal production. There is also evidence that this gene affects goat growth traits, where a novel BC140488:m.832T > A polymorphism in the caprine *ADIPOQ* gene showed significant associations with chest circumference [14]. Choi et al. [15] found that three genetic variants (g.81966235CNT, g.81,966,377 TNC, and g.81966364DNI) in Hanwoo cattle are associated with marbling score and carcass traits. Moreover, *ADIPOQ* polymorphisms have been linked to the sheep fatness trait [16]. Although these findings are based on polymorphisms in an *ADIPOQ* gene, no reports have identified concerning Awassi sheep productivity.

## Materials and methods

### Animals and experimental design

The study was approved by the research committee at Al-Qasim Green University and carried out according to international animal care and use standards (Agri no. 020,7,18) from July 2020 to March 2021. Two hundred sexually mature ewes were included, aged 2.5 to 5 years, not pregnant, and not lactating. In this study, ewes were randomly selected from two stations (Babylon and Karbala). All of the ewes were fed seasonal grass, concentrated food, and fresh water *ad libitum*. The measurements of live body weight and body measurements were taken phenotypically.

## DNA extraction, genotyping, and sequencing reaction

A salting-out technique was employed to isolate genomic DNA from whole blood [17]. To determine the quality of the DNA samples and as a template for polymerase chain reaction (PCR), a NanodropμLITE spectrophotometer (Biodrop, UK) was used. A PCR reaction containing 10 pmol of each primer, 50 ng genomic DNA, 50 μM dNTPs, 10mM Tris-HCl (pH 9.0), 30mM KCl, 1.5mM $MgCl_2$, and 1U Top DNA polymerase was performed. The *ADIPOQ* gene (exon 1) primer was designed using the ovine sequence in GenBank (NC_019480.2) and Primer-BLAST. A forward primer and a reverse primer were designed to be 5´- CCTGTATCTCT CCCCACCCT – 3´ and 5´- GTGTGATGCCTGCAGCTCTA – 3´ respectively. A PCR reaction involving four minutes of denaturation at 94 °C, followed by 35 cycles of 30 s denaturation at 94 °C, annealing for 30 s at 57 °C, extension for 30 s at 72 °C, and extension for 10 min at 72 °C. An agarose gel (2%) was used for the visualization of the PCR products [18].

In the following step, PCR-SSCP (single strand conformation polymorphisms) was performed to genotype the samples [19]. For each PCR amplicon, SSCP denaturing-loading buffer was added in equal quantities. After being denatured for seven minutes, samples were cooled on frozen ice for 10 min, then transferred to polyacrylamide gels (10%) in a 0.5X TBE buffer. Electrophoresis was conducted for four hours at room temperature using 200 mA and 100 V. The gels were stained using the rapid protocol according to Byun et al. [20]. All samples were sequenced by Macrogen (Geumchen, Korea), then edited and visualized using BioEdit 7.1 (DNASTAR, Madison) and SnapGene Viewer ver. 4.0.4(https://www.snapgene.com). To verify the novelty of the observed variants of *ADIPOQ*, Ensemble genome browser 96 was used (https://asia.ensembl.org/index.html).

### Statistical analysis

Analyses of the *ADIPOQ* gene's genetic diversity were conducted utilizing the PopGen32 software, v. 1.31. A Chi ($\chi 2$) test was employed to verify deviations from the Hardy-Weinberg Equilibrium (HWE). The ssociation between variants of the *ADIPOQ* genotype and traits of interest was calculated using the following SPSS statistical model (version 23.0):

$$Y_{ijk} = \mu + G_i + P_j + e_{ijk}$$

Where: $Y_{ijk}$ = phenotype characteristics, μ = the mean of all traits, $G_i$ = fixed effect of $i^{th}$ genotypes ($i$ = CC, CA, AA) $P_j$ = fixed effect of $j^{th}$ parity ($j$ = 1, 2, 3, 4), and $e_{ijk}$ = random error. The Tukey-Kramer test was used to compare means. The correlation coefficient was calculated using a Pearson correlation coefficient, and ($P < 0.05$) was set as the significance level. Preliminary statistical analysis concluded that factor interaction, seasonality, station, and nutrition did not significantly influence the traits studied, so these variables were excluded from the model.

## Results

### Genotyping, sequencing, and genetic diversity analysis of *ADIPOQ* gene

An *ADIPOQ* gene fragment of 368 bp along with its flanking regions was screened using PCR design (Fig. 1, A). Within the 368 bp amplicon of exon 1, three distinct PCR-SSCP were detected (Fig. 1, B). One of the SSCP variants

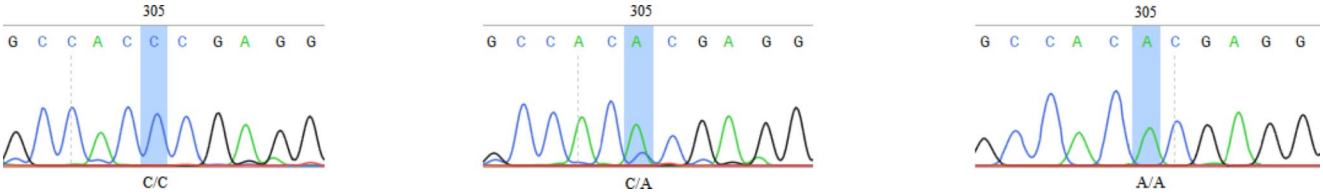


**Fig. 1** Analysis of the *ADIPOQ* gene in the Awassi sheep. (A) PCR products amplifications of the *ADIPOQ* gene electrophoresed on agarose gels. B) SSCP non-denaturing polyacrylamide gel electrophoresis of PCR products of the *ADIPOQ* gene (exon 1) determined CC, CA, and AA genotypes in the Awassi ewes. C) DNA sequencing revealed a mutation at the c.198,473,337 C > A SNP.

**Table 1** Genetic diversity of the *ADIPOQ* gene in Awassi ewes detected by PCR- SSCP

| Variable | CC | CA | AA | $\chi^2$ |
|---|---|---|---|---|
| **Genotype distribution** | | | | |
| Observed genotypes | *n* = 97 | *n* = 66 | *n* = 37 | 15.38 |
| Genotype frequencies | 0.48 | 0.33 | 0.19 | |
| **Allele distribution** | **C** | **A** | | |
| Allele frequencies | 0.65 | 0.35 | | |

Abbreviations: *n* – number of individuals, $\chi^2$ – Chi-square. All Chi-square tests had two degree of freedom and within the significance level P < 0.05

**Table 2** The association of the *ADIPOQ* genotypes with the live body weight, age and body measurement of Awassi ewes

| Indices | *ADIPOQ*Genotype (LSM ± SE) | | | *P*-value |
|---|---|---|---|---|
| | CC (97) | CA (66) | AA(37) | |
| **Live body weight (Kg)** | **44.11 ± 1.01$^{b}$** | **47.51 ± 0.92$^{ab}$** | **51.01 ± 1.31$^{a}$** | **0.03** |
| **Age (year)** | 3.53 ± 0.14 $^{a}$ | 4.04 ± 0.13 $^{a}$ | 4.24 ± 0.22 $^{a}$ | 0.32 |
| **Body length (cm)** | **66.64 ± 1.34$^{b}$** | **69.83 ± 1.44$^{ab}$** | **73.54 ± 1.25$^{a}$** | **0.02** |
| **Head length (cm)** | 23.53 ± 0.04 $^{a}$ | 23.64 ± 0.12 $^{a}$ | 24.74 ± 0.25 $^{a}$ | 0.28 |
| **Neck length (cm)** | 24.42 ± 0.42 $^{a}$ | 24.92 ± 0.04 $^{a}$ | 25.51 ± 0.10 $^{a}$ | 0.37 |
| **Height at shoulder (cm)** | 71.24 ± 1.63 $^{a}$ | 72.15 ± 1.11$^{a}$ | 72.66 ± 1.82 $^{a}$ | 0.23 |
| **Height at hip (cm)** | **67.57 ± 1.73$^{b}$** | **70.17 ± 1.19$^{ab}$** | **73.33 ± 1.14$^{a}$** | **0.02** |
| **Chest girth (cm)** | 85.62 ± 1.61 $^{a}$ | 86.36 ± 1.33 $^{a}$ | 86.97 ± 1.53 $^{a}$ | 0.14 |
| **Chest width (cm)** | **22.47 ± 0.18$^{b}$** | **24.75 ± 0.04$^{ab}$** | **25.15 ± 0.41$^{a}$** | **0.02** |

LSM ± SE, Least square means ± Standard error. Different superscript in the same raw within each classification indicated significant differences (P < 0.05)

contained the c.198,473,337 C > A SNP, verifying the heterogeneity in exon 1 based on the sequencing reactions. Using the c.198,473,337 C > A substitutions, this variant was assigned the CC, CA, and AA genotypes, for homozygous C/C and A/A and heterozygous C/A patterns (Fig. 1, C). Analyses of the *ADIPOQ* gene diversity in Awassi ewes demonstrated that genotype CC had the highest prevalence of 0.48 (n = 97) over genotypes CA (n = 66) and AA (n = 37) (Table 1). Furthermore, the Chi-square test indicated a significant deviation from HWE for polymorphisms of the *ADIPOQ* gene at the c.198,473,337 C > A SNP locus (P < 0.05).

## Association analysis of *ADIPOQ* genotypes with productive traits of Awassi ewes

Live body weights were significantly different (P ≤ 0.05) between the three *ADIPOQ* genotypes (Table 2). AA genotypes were typically heavier (51.01 ± 1.31) (Kg) than CC and CA genotypes. In terms of morphometric traits, the AA genotype in ewes was associated with a longer body length (73.54 ± 1.25) (cm), hip height (73.33 ± 1.14) (cm), and chest width (25.15 ± 0.41) (cm) compared to those with the CC and CA genotypes (Table 2). Furthermore, the AA genotype correlated strongly with body weight and measurements shown in Table 3.

## Discussion

The CC genotype was most prevalent in the *ADIPOQ* gene population (0.48, n = 97), and the AA genotype was less prevalent (0.19, n = 37). The Chi-square test showed significant deviations from the HWE for polymorphisms at the c.198473337 C > A SNP within the *ADIPOQ* gene (P ≤ 0.05). In livestock, several authors reported genetic polymorphisms in various fragments of the *ADIPOQ* gene. A study by Pandy et al. [21] demonstrated three genotypes in the promoter region of the bovine *ADIPOQ* gene using PCR-RFLP for Indian Sahiwal Cattle. Shin and Chung [22] also determined three genotypes – CC, CT, and TT – within the *ADIPOQ* promoter region in the Hanwoo cattle population using TsaI/PCR-RFLP. According to An et al. [4], the *ADIPOQ* gene is polymorphic in two exon 1 and 3 and intron 2 in Romney lambs from New Zealand. Furthermore, An et al.

**Table 3** Correlation between *ADIPOQ* genotypes and other variables in Awassi ewes

| Variables | *ADIPOQ* genotypes | | | | | |
|---|---|---|---|---|---|---|
| | **CC** | | **CA** | | **AA** | |
| | **r** | ***P*-value** | **r** | ***P*-value** | **r** | ***P*-value** |
| **Live body weight** | **0.36** | **0.04** | **0.46** | **0.02** | **0.68** | **0.01** |
| **Age** | 0.18 | 0.21 | 0.22 | 0.12 | 0.24 | 0.11 |
| **Body length** | **0.32** | **0.01** | **0.43** | **0.02** | **0.57** | **0.02** |
| **Head length** | -0.12 | 0.39 | 0.14 | 0.22 | 0.09 | 0.14 |
| **Neck length** | 0.08 | 0.11 | -0.12 | 0.42 | 0.13 | 0.21 |
| **Height at shoulder** | **0.36** | **0.04** | **0.52** | **0.03** | **0.67** | **0.01** |
| **Height at hip** | **0.42** | **0.04** | **0.46** | **0.03** | **0.53** | **0.02** |
| **Chest girth** | **0.13** | **0.04** | **0.24** | **0.03** | **0.34** | **0.02** |
| **Chest width** | **0.24** | **0.05** | **0.46** | **0.03** | **0.52** | **0.01** |

$P < 0.05$: Significant, $P > 0.05$: Not significant

[23] explored 13 SNPs identified in the *ADIPOQ* exon 1 and exon 2 regions in the Romney lambs. The A1 and B1 alleles dominated in exon 1, whereas A2 and D2 dominated in exon 2, and their frequencies differed. Recently, Pandey et al. [5] explored the SNPs located in the *ADIPOQ* gene promoter in Indian dairy cattle and determined three genotypes, the most common genotype being CT (62.32%), second the CC (24.64%), and finally TT (13.04%) genotypes. Despite this, very limited information has been found about *ADIPOQ* polymorphism in Awassi sheep.

Regarding the association analysis, the three genotypes showed significant differences ($P \leq 0.05$) in live body weight and morphometric traits. Ewes with AA genotypes have higher live body weight, body length, hip height, and chest width than those with CC and CA genotypes. Physiologically, adiponectin is necessary to maintain energy homeostasis [7]. Moreover, adiponectin synergizes with insulin signaling in order to improve insulin sensitivity and glucose tolerance in different tissues [9]. This protein stimulates AMPK to increase the oxidation of fatty acids and glucose in skeletal muscle and stimulates the proliferation of skeletal muscle tissue through the p38-MAPK pathway, which may have effect on these productive traits of sheep [10]. In livestock, *ADIPOQ* gene variability determines the expression of productive traits. Genetic variability in this gene could also affect productivity and reproductive traits [4]. Polymorphism analysis of the *ADIPOQ* gene is conducted concerning its association to the thickness of carcass fat, meat marbling, and muscle area of the ribeye in cattle [21], with the growth and carcass traits in New Zealand (NZ) Romney lambs [4], and with productivity and reproduction in Indian dairy cattle [5]. The *ADIPOQ* polymorphisms in Chinese cattle are associated with growth traits (body weight and measurements) as reported in a previous study by Zhang et al. [24]. The AA genotype also correlates significantly with body weight and dimensions. The morphometric indices and live weight of animals are good indicators of animal utility. This study is consistent with the study conducted by Zhang et al. [25], which showed a significant correlation between *ADIPOQ* polymorphism and body measurements in seven Chinese cattle breeds.

## Conclusions

The *ADIPOQ* gene regulates several productive traits. Sheep with the AA genotype have heavier and larger body dimensions, thereby improving their productivity and reproducibility. Further studies are required in other breeds to prove the results.

**Authors contribution** All authors contributed equally. In addition, all authors reviewed and approved the final manuscript.

**Funding** This study was not supported by any specific grant from funding.

## Declarations

**Conflict of interest** None.

**Ethical approval** This research was approved by Al-Qasim Green University's research committee and was conducted according to the international guidelines on animal care and use (Agri no. 020,7,18).

**Informed consent** This manuscript has been read and approved by all named authors and that are no other persons who satisfied the criteria for authorship but are not listed.

**Consent to publish** Not applicable.

## References

1. Al-Thuwaini TM (2021) The relationship of hematological parameters with adaptation and reproduction in sheep; A review study. Iraqi J Vet Sci 35(3): 575–580. DOI: https://doi.org/10.33899/ijvs.2020.127253.1490
2. Al-Thuwaini TM (2021) Novel single nucleotide polymorphism in the prolactin gene of Awassi ewes and its role in the

reproductive traits. Iraqi J Vet Sci 35(3): 429–435. DOI: https://doi.org/10.33899/ijvs.2020.126973.1423
3. Gebreselassie G, Berihulay H, Jiang L, Ma Y (2020) Review on genomic regions and candidate genes associated with economically important production and reproduction traits in sheep (*Ovies aries*). Animals 10(1): 33. https://doi.org/10.3390/ani10010033
4. An Q, Zhou H, Hu J, Luo Y, Hickford JG (2017) Haplotypes of the ovine Adiponectin gene and their association with growth and carcass traits in New Zealand Romney lambs. Genes 8(6): 160. https://doi.org/10.3390/genes8060160
5. Pandey V, Nigam R, Singh SP, Sharma D, Tiwari M, Garg SK (2020) Genetic polymorphism in the adiponectin (*ADIPOQ*) gene and its association with the production and reproduction traits of Indian dairy cattle. Veterinarski arhiv 90(1): 16–26. https://doi.org/10.24099/vet.arhiv.0411
6. Jayanthi R, Srinivasan A, Gopal N (2018) Gene polymorphism of adiponectin SNP + 45 with reference to thyroid status-a comparative study among non-obese, overweight and obese type 2 diabetics. J Obese Weight-Loss Medic 4: 020.
7. Choi HM, Doss HM, Kim KS (2020) Multifaceted physiological roles of adiponectin in inflammation and diseases. Int J Mol Sci 21(4): 1219. https://doi.org/10.3390/ijms21041219
8. Natah TM (2014) Serum adiponectin levels in pre-postmenopausal metabolic syndrome women and the correlation with some physio-biochemical parameters. Aust J Basic Appl Sci 8(17): 360–366.
9. Ruan H, Dong LQ (2016) Adiponectin signaling and function in insulin target tissues. J Mol Cell Biol 8(2): 101–109. https://doi.org/10.1093/jmcb/mjw014
10. Frankenberg ADV, Reis AF, Gerchman F (2017) Relationships between adiponectin levels, the metabolic syndrome, and type 2 diabetes: a literature review. Archives of Endocrinology and Metabolism 61: 614–622. DOI: https://doi.org/10.1590/2359-3997000000316
11. Palin MF, Jafarikia M, Gariépy C, Fortin F, Maignel L, Wyss S, Sullivan B (2014) Association of SNPs and haplotypes in adiponectin and adiponectin receptors with pig meat quality traits. In Proceeding, 10th World Congress on Genetics Applied to Livestock Production 10 (5): 22–26.
12. Muhlhausler BS, Ritorto V, Schultz C, Chatterton BE, Duffield JA, McMillen IC (2008) Birth weight and gender determine expression of adipogenic, lipogenic and adipokine genes in perirenal adipose tissue in the young adult sheep. Domest Anim Endocrinol 35(1): 46–57. https://doi.org/10.1016/j.domaniend.2008.01.007
13. Zhang L, Chen H, Lan X, Zhang C, Zhang L, Zhang A, Zhang Q, Lei C (2009) The novel 5 bp deletion polymorphism in the promoter region of bovine *ACRP30* gene. Mol Biol Rep 36(5): 895–899. https://doi.org/10.1007/s11033-008-9260-1
14. Lan X, Wei T, Zhang L, Chen H, Hu S, Lei C, Fang XT (2009) Novel Polymorphism at the 3′ UTR of the Caprine Adiponectin Gene. Biochem Genet 47(3): 251–256. https://doi.org/10.1007/s10528-009-9223-9
15. Choi Y, Davis ME, Chung H (2015) Effects of genetic variants in the promoter region of the bovine adiponectin (*ADIPOQ*) gene on marbling of Hanwoo beef cattle. Meat Science 105: 57–62. https://doi.org/10.1016/j.meatsci.2015.02.014
16. Li B, Qiao L, An L, Wang W, Liu J, Ren Y, Yangyang Pan Y, Jing J, Liu W (2018) Transcriptome analysis of adipose tissues from two fat-tailed sheep breeds reveals key genes involved in fat deposition. BMC Genomics 19(1): 1–13. https://doi.org/10.1186/s12864-018-4747-1
17. Al-Shuhaib MBSA (2017) A universal, rapid, and inexpensive method for genomic DNA isolation from the whole blood of mammals and birds. J Genet 96(1): 171–176. https://www.ias.ac.in/article/fulltext/jgen/096/01/0171-0176
18. Al-Thuwaini T (2020) Association between polymorphism in *BMP15* and *GDF9* genes and impairing female fecundity in diabetes type 2. Middle East Fertil Soc J 25(1): 1–10. https://doi.org/10.1186/s43043-020-00032-5
19. Imran FS, Al-Thuwaini TM, Al-Shuhaib MBS, Lepretre F (2020) A Novel Missense Single Nucleotide Polymorphism in the *GREM1* Gene is Highly Associated with Higher Reproductive Traits in Awassi Sheep. Biochem Genet 1–15. https://doi.org/10.1007/s10528-020-10006-x
20. Byun SO, Fang Q, Zhou H, Hickford JGH (2009) An effective method for silver-staining DNA in large numbers of polyacrylamide gels. Anal Biochem 385: 174–175. https://doi.org/10.1016/j.ab.2008.10.024
21. Pandey V, Nigam R, Sing P, Singh SP, Sharma D, Tiwari M (2019) Identification of polymorphism in intron 2 of adiponectin (*ADIPOQ*) gene in Indian Sahiwal cattle. J Anim Res 9(2): 265–268. https://doi.org/10.30954/2277-940X.02.2019.8
22. Shin S, Chung E (2013) Novel SNPs in the bovine *ADIPOQ* and *PPARGC1A* genes are associated with carcass traits in Hanwoo (Korean cattle). Mol Biol Rep 40(7): 4651–4660. https://doi.org/10.1007/s11033-013-2560-0
23. An Q, Zhou H, Liu X, Li S, Luo Y, Hickford JG (2018) Effect of haplotypes of *ADIPOQ* on growth traits in ovine with different genders. Acta Veterinaria et Zootechnica Sinica 49(4): 825–832.
24. Zhang L, Li M, Lai X, Yang M, Xu Y, Hua L, Lan X, Zhang C, Chen H (2013) Haplotype combination of polymorphisms in the *ADIPOQ* gene promoter is associated with growth traits in Qinchuan cattle. Genome 56(7): 389–394. https://doi.org/10.1139/gen-2013-0054
25. Zhang L, Yang M, Li C, Xu Y, Sun J, Lei C, Lan X, Zhang C, Chen H (2014) Identification and genetic effect of a variable duplication in the promoter region of the cattle *ADIPOQ* gene. Anim Genet 45(2): 171–179. https://doi.org/10.1111/age.12112