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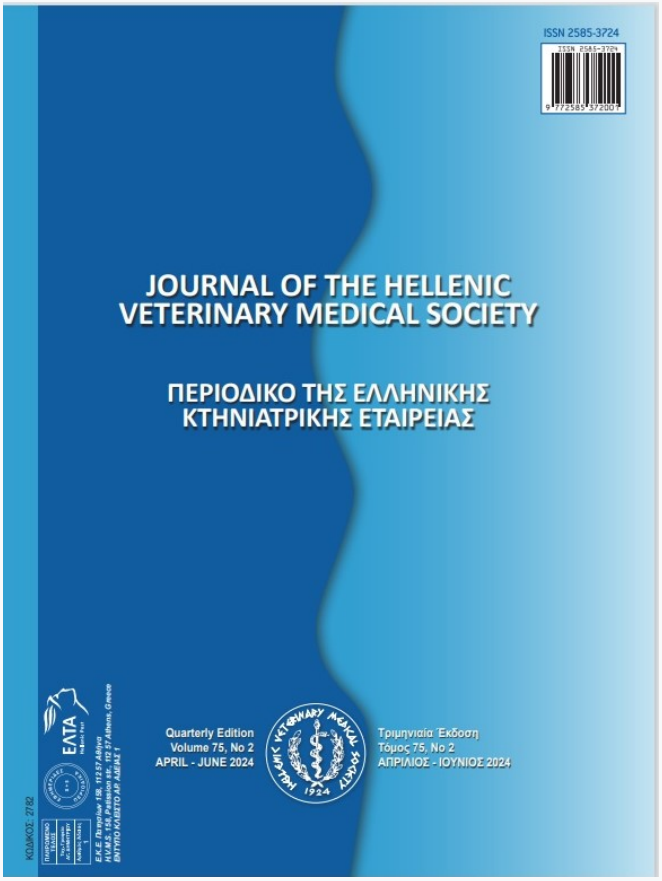

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Effect of *PITX2* gene polymorphism on growth traits in Awassi ewes

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ABSTRACT: Genetics and environment influence quantitative traits. Several genes contribute to sheep's growth traits, including the paired-like homeodomain transcription factor 2 (PITX2). Therefore, the research investigated whether the growth traits of Awassi ewes are associated with PITX2 gene variability. Two hundred and thirty-two ewes aged three to four years were studied. Phenotypic analysis was conducted by measuring body weight and body dimensions. Sheep genomic DNA was extracted and subjected to genotyping and sequencing to confirm the presence of variants derived from the amplification of exon 5 of PITX2. A 382-bp amplicon displayed three genotypes, CC, CT, and TT. A comparison of *PITX2* genotypes with growth traits showed that CC-type ewes had significantly heavier live body weights, birth weight, and higher body dimensions compared to CT-type and TT-type ewes. The CC genotypes were recorded strongly positive correlation with live body weight, height at back, width at shoulder, width at pelvic, chest width, and abdomen width ((r=0.77, *P*=0.04), (r=0.97, *P*=0.02), (r=0.98, *P*=0.02), (r=0.96, *P*=0.03), (r=0.92, *P*=0.03), and (r=0.98, *P*=0.01) respectively. Sheep of the CC genotype had higher live body weights and body measurements, making them more productive. These findings provide valuable insight into the characteristics and functions of the PITX2 gene for the future of sheep breeding.

Keywords**:** B*ody* dimensions*;* lamb weight; *PITX2* polymorphism; sheep.

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INTRODUCTION

Sheep body weight and body dimensions are crucial selection criteria for enhancing livestock production. The body weight and body condition of livestock represent vital economic characteristics for livestock breeding and production, which require an accurate estimation. Farmers also benefit from knowing the live body weight of animals to determine their feed requirements to sustain growth, maintenance, and production (Putra et al., 2019). Research and management activities, such as growth rate assessment, are based on the live body weight of the animal. Moreover, modern sheep farming depends on litter size as an economic factor that depends on the ewe's body condition and genetic factors (Wang et al., 2021; Al-Thuwaini and Kareem, 2022). Genetics and environment also influence sheep's growth traits. Identifying genetic factors determining the phenotypic variation in domestic sheep (*Ovis aries*) will accelerate genetic improvement efforts. Additionally, identifying the genes contributing to sheep growth traits would contribute to the worldwide effort to increase mutton production (Ghanem et al., 2022). The results of a study by Abbasi and Ghafouri-Kesbi (2011) show that a genetic link exists between body weight and dimensions in Makooei sheep. Booujenane and Diallo (2017) found a strong genetic correlation between maternal body weights and lambs' birth weights. Kumar et al. (2018) also found a high genetic correlation between linear body measurements of dams and lambs' birth and weaning weights. Recently, advances in molecular markers have contributed to identifying DNA markers linked to quantitative traits to improve phenotypic traits (Ajafar et al., 2022a). Therefore, improving these traits should be a priority in sheep production.

Several genes, including the *PITX2* gene, influence growth traits, as do environmental factors (Zhang et al., 2018). Homeodomain transcription factor 2 (*PITX2*) *also called RIEG* belongs to the bicoid-like homeobox transcription factor family (Yan et al., 2018; Cao et al., 2019). A *PITX2* gene is located on chromosome 6 of sheep with six exons (NCBI Reference Sequence NC_056059.1), and on chromosome 6 of cows with seven exons (Zhang et al., 2018). Several tissues express this gene and are conserved within and across species, indicating its importance to organisms and numerous physiological functions (Cao et al., 2019). *PITX2* pathways are closely related to POU1F1 pathways, crucial for mammalian development and growth (Zhang et al., 2018). The *PITX2* gene in animals also regulates asymmetric organ development (Yan et al., 2018). In addition, *PITX2* regulates myogenic differentiation (MyoD) during development and controls redox conditions during myogenesis and muscle formation (Zhang et al., 2018). *The PITX2 gene controls hematopoiesis, differentiation, and organogenesis* (Cao et al., 2019; Zhao et al., 2013). Several studies have examined variations in *PITX2* and its relationship to phenotypic characteristics in livestock. In native Chinese cattle, a deletion of 24 bp within the *PITX2* gene remarkably affects growth parameters (Zhang et al., 2018). The *PITX2* gene has an indel mutation of 22 bp, affecting goats' growth traits (body length and chest width) (Yan et al., 2018). In the intron of the *PITX2* gene, two novel indel loci are identified (NC_019463:g.14890658-14890667: g.14885723:14885734), and four significant differences are found between four Chinese sheep breeds in body dimensions (Zhao et al., 2018).). The mutations g.18161C>G, g.18117T>C, and g.18353T>C are strongly linked to growth traits in Hainan black goats and Guanzhong dairy goats (Zhang et al., 2020). The studies mentioned above have provided limited research on the connection between birth type and *PITX2* polymorphisms and the growth traits of sheep. Furthermore, no previous investigation has explored their impact on sheep growth traits.

MATERIALS AND METHODS

Animal

Research and ethics committees at Al-Qasim Green University approved this study conducted between July 2021 and April 2022 according to international animal care guidelines (Agri, No. 015, 7, 20). Study participants included 232 sexually mature ewes aged three to four years, weighing 40-60 kg. This group of ewes was randomly assigned to two stations - Babylon and Karbala. Animals consumed grain concentrate with 59% barley, 40% bran, and 1% salt, proportionally to 2.5% of their body weight. The animal also consumed a kilogram of straw and three kilograms of alfalfa. Fresh water was always available to all animals. Measurements of phenotypic characteristics were taken, including body weight and body dimensions.

DNA extraction, genotyping, and sequencing reaction

Genetic testing was conducted using blood collected from the sheep's jugular vein, then DNA was extracted from the blood samples using rapid salt-

ing-out (Al-Shuhaib, 2017). Amplification of all 232 *PITX2* genetic sequences was performed using NCBI Primer-BLAST. Primers for exon 5 (382 bp) of the *PITX2* gene were designed using an ovine sequence from GenBank (NC_056059.1) and Primer-BLAST. A forward primer and a reverse primer were designed to be 5´- GCCCAATTCCATCTCGTCCA -3´ and 5´- CCCAGTCTTTCAAGGGCAGA -3´ respectively. The amplification was accomplished by subjecting the sample to a denaturation period of four minutes at a high temperature of 94°C. This was followed by a precise sequence of 30 cycles, each consisting of 30 seconds of denaturation at the same temperature, 45 seconds of annealing at 59.8°C, and 30 seconds of elongation at 72°C. PCR products were electrophoresed on agarose gels (2%), and agarose gel images were visualized using a Chemidoc Gel Imager (Bio-Rad, USA). A genotype was determined for each of the PCR products according to Mohammed et al. (2022). A denaturing-loading buffer containing 95% formamide, 20 mM EDTA, and 0.05% xylene cyanol at pH 8 was added to each PCR product equally. After seven minutes of denaturation, wet ice was applied to amplicons for ten minutes. Loading of polyacrylamide gels with neutral denaturants into 0.5 TBE buffers was performed. Four-hour electrophoresis was performed at room temperature with 200 mA and 100V. Byun et al. (2009) provided a protocol for staining the gels. The Sanger sequencing reaction (Macrogen, Geum Chen, Korea) was performed immediately following SSCP bands detection on polyacrylamide gels. NCBI provided the sequence for the *PITX2* gene (https://www.ncbi.nlm.nih.gov). For viewing and editing polymorphisms within genotypes, SnapGene Viewer 4.0.4 and BioEdit 7.1 were used. A genomic browser (https://asia.ensembl.org/index.html) was used to check for novelty.

Data analysis

Genotypes and allele frequencies were calculated using PopGen32, version 1.31. After determining Hardy-Weinberg equilibrium (HWE), polymorphism information content (PIC) was detected according to Botstein et al. (1980). For the analysis of the association between birth type and *PITX2* genotypes with growth traits, IBM SPSS 23.0 (NY, USA) was used as follows:

$$Y_{ijk} = \mu + \alpha_i + \beta_j + \epsilon_{ijk}$$

Where Y_{ijk} = value for studied traits; μ = overall mean; α_i = effect of i genotype; β_j = parity effect (*j* = 1, 2, 3); Єijk = random errors. Tukey-Kramer test was used to compare means. The data were tested for normality with a Kolmogorov-Smirnov test. Pearson correlation coefficients with a significance threshold of 0.05 were used to analyze the correlation. In an initial statistical analysis, factor interactions and age had no statistical significance, and were excluded.

RESULTS

Analyses of genetic diversity, genotyping, and sequencing of *PITX2* genes

The CT genotype of the *PITX2* gene showed a novel 319C>T substitution in exon 5. According to genetic diversity findings for 319C>T, 113 genotypes of the CC genotype were identified, with a total frequency of 0.42. This was followed by the TT and the CT genotypes with a frequency of 0.35 and 0.23. Significant genetic variation was observed at the 319C>T SNP locus as *He* values were higher than *Ho* values (Table 1). The present study reveals a moderate level of polymorphism at the 319C>T SNP locus, as indicated by the classification of polymorphism information content (PIC) ($0.25 < PIC < 0.50$). A chi-square analysis of the 319C>T SNP locus of *PITX2* revealed a significant deviation from HWE ($P \leq 0.05$).

Association Analysis

The CC genotype at the 319C>T locus was associated significantly ($P \leq 0.01$) with more lamb weight at birth and lamb weight at weaning compared to the CT and TT genotypes (Figure 1). In the association analysis of *PITX2* genotypes with growth traits, in-

Table 1. Genotypic and allelic frequencies and population indexes for two single nucleotide polymorphisms (SNPs) of the PITX2 gene in Awassi ewes.

Genotypes (n)	Genotype frequencies	Allele	Allele frequencies	*Ho*	*He*	*Ne*	PIC	χ^2 (*P*-value)
CC (113)	0.42	C	0.57	0.17	0.49	1.96	0.37	101.26
CT (40)	0.23	T	0.43					(***P*=0.001**)
TT (79)	0.35							

Abbreviations: *n* - number of individuals, *Ho* - observed heterozygosity, *He* - expected heterozygosity, *Ne* - effective allele frequency, PIC, polymorphism information content, χ^2 - Chi-square, the *P* value with statistical significance are indicated in bold numbers.

dividuals with CC genotypes differed significantly from those with CT and TT genotypes in terms of live body weight, chest girth, shoulder width, neck length, chest width, and abdomen girth. Individuals with the CC genotype had significantly ($P \leq 0.05$) higher live body weight (57.00 ± 4.26) (kg), chest girth (107.50 ± 7.38) (cm), width at shoulder (29.00 ± 2.07) (cm), neck length (13.50 ± 0.66) (cm), chest width (33.00 ± 2.75) (cm), and abdomen girth (112.50 ± 7.36) (cm) than individuals with CT and TT genotypes (Table 2).

Statistical correlation between *PITX2* genotypes with growth traits of the Awassi ewes is shown in (Table 3). The CC genotypes were recorded strongly positive correlation with live body weight, height at back, width at shoulder, width at pelvic, chest width, and abdomen width ((r=0.77, P=0.04), (r=0.97, P=0.02), (r=0.98, P=0.02), (r=0.96, P=0.03), (r=0.92, P=0.03), and (r=0.98, P=0.01) respectively, whereas it was non-significant ($P \geq 0.05$) for the other variables.

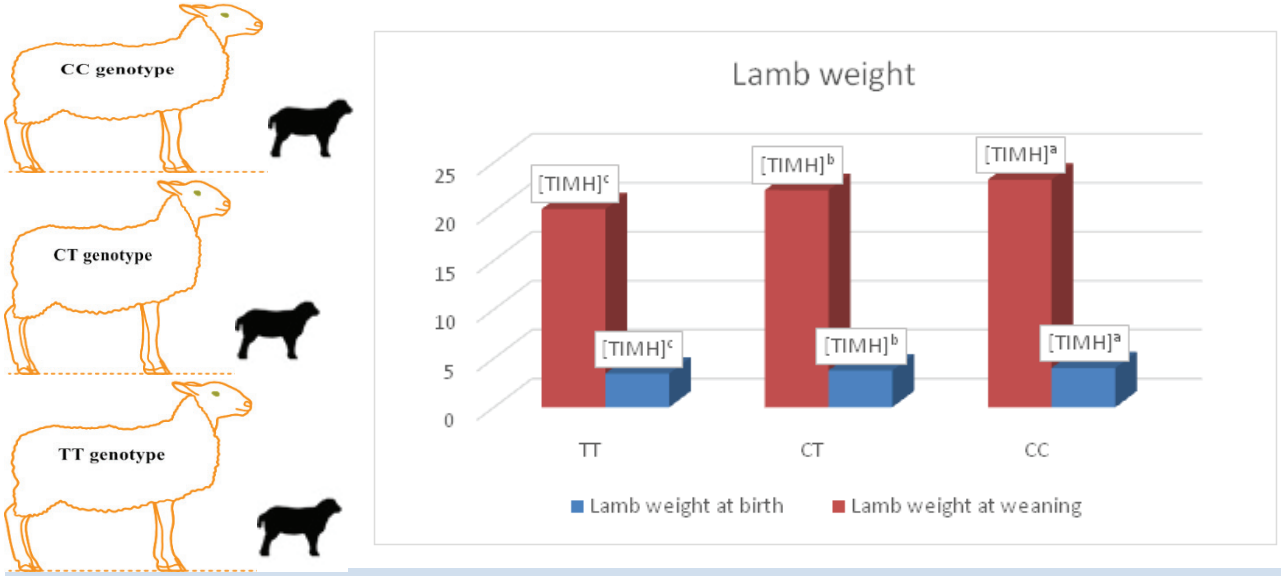

Figure 1. Association of lamb weight with the *PITX2* gene polymorphism in Awassi ewes.

Table 2. Association of *PITX2* genotypes with live body weight and body measurement in Awassi ewes

Indices	*PITX2* genotypes (Least square Mean ± SE)			*P*-value
	TT	CT	CC	
Live body weight (Kg)	46.25 ± 4.77 [c]	50.25 ± 3.97 [b]	54.00 ± 4.25 [a]	**0.04**
Body length (cm)	75.00 ± 5.68	76.00 ± 6.18	75.50 ± 6.10	0.61
Head length (cm)	22.75 ± 1.42	23.25 ± 1.64	23.50 ± 1.84	0.57
Chest girth (cm)	100.50 ± 8.08 [c]	104.25 ± 7.68 [b]	107.75 ± 6.91 [a]	**0.03**
Height at front (cm)	67.75 ± 4.41	68.25 ± 4.70	70.75 ± 5.41	0.09
Height at back (cm)	61.25 ± 6.76	62.50 ± 6.06	61.75 ± 5.88	0.24
Width at shoulder (cm)	22.25 ± 1.82 [c]	25.00 ± 1.30 [b]	28.25 ± 1.08 [a]	**0.03**
Width at pelvic (cm)	35.25 ± 1.93	36.75 ± 2.13	37.50 ± 1.78	0.11
Forelimb length (cm)	36.25 ± 1.84	37.50 ± 2.21	37.00 ± 2.91	0.31
Hind limb length (cm)	35.25 ± 1.50	35.75 ± 1.93	35.25 ± 2.00	0.42
Neck length (cm)	10.00 ± 0.71 [b]	11.75 ± 0.54 [a]	12.25 ± 0.67 [a]	**0.05**
Neck width (cm)	19.50 ± 2.59	19.50 ± 1.31	20.25 ± 1.93	0.34
Chest width (cm)	29.50 ± 1.23 [b]	30.25 ± 1.67 [b]	32.50 ± 1.53 [a]	**0.02**
Abdomen width (cm)	35.00 ± 2.37	35.50 ± 2.14	37.00 ± 2.09	0.22
Abdomen girth (cm)	105.50 ± 8.90 [c]	108.50 ± 7.24 [b]	112.00 ± 8.14 [a]	**0.03**
Tail length (cm)	33.50 ± 1.03	32.75 ± 1.35	33.00 ± 2.09	0.25
Tail width (cm)	28.25 ± 0.85	29.25 ± 1.51	30.25 ± 1.68	0.08

SE, standard error. [a,b,c] different lowercase letters indicate a significant difference within each genotype ($P \leq 0.05$).

Table 3. Correlation between *PITX2* genotypes and other variables in Awassi ewes

Variables	*PITX2* genotypes					
	CC		CT		TT	
	r	*P*-value	r	*P*-value	r	*P*-value
Live body weight (Kg)	0.77	0.04	0.54	0.04	0.39	0.03
Body length (cm)	0.70	0.29	0.20	0.79	0.33	0.66
Head length (cm)	0.30	0.69	0.32	0.19	-0.19	0.32
Chest girth (cm)	0.66	0.33	0.78	0.21	0.50	0.15
Height at front (cm)	0.30	0.69	-0.45	0.54	0.41	0.56
Height at back (cm)	0.97	0.02	0.34	0.05	0.22	0.03
Width at shoulder (cm)	0.98	0.02	0.80	0.01	0.45	0.03
Width at pelvic (cm)	0.96	0.03	0.18	0.04	0.24	0.03
Forelimb length (cm)	0.57	0.42	-0.40	0.59	-0.40	0.53
Hind limb length (cm)	0.76	0.23	0.57	0.42	0.11	0.88
Neck length (cm)	0.70	0.29	0.57	0.23	0.42	0.29
Neck width (cm)	0.89	0.10	0.23	0.14	0.22	0.77
Chest width (cm)	0.92	0.03	0.70	0.03	0.55	0.04
Abdomen width (cm)	0.98	0.01	0.42	0.05	0.16	0.03
Abdomen girth (cm)	0.87	0.12	0.65	0.34	0.60	0.39
Tail length (cm)	0.33	0.66	0.36	0.63	0.32	0.16
Tail width (cm)	0.19	0.80	0.14	0.86	0.57	0.42

r: Correlation coefficient, $P \leq 0.05$: Significant, $P \geq 0.05$: Not significant

DISCUSSION

Sheep contribute significantly to the economy and industrial development due to their high production and reproduction abilities (Haldar et al., 2014; Asaduzzaman et al., 2020). To develop livestock breeding programs, it is necessary to identify the genetic variation contributing to their economic value (Ajafar et al., 2022b; Al-Thuwaini and Al-Hadi, 2022). Numerous studies have conclusively shown that genetic variations in *PITX2* significantly impact various desirable traits in livestock. For instance, Zhao et al. (2013) highlighted the direct connection between these variations and milk characteristics in dairy goats. In addition, the relevance of *PITX2* is further demonstrated in Guanzhong dairy goats. This study by Zhang et al. (2020) highlights its correlation with essential growth characteristics. *PITX2* is crucial for goats, and plays a critical role in determining carcass characteristics in chickens, as indicated in the research by Cao et al. (2019). These findings underscore the immense potential for targeted breeding and genetic improvement in livestock by manipulating *PITX2*. In Awassi sheep, however, there needs to be more information available regarding the *PITX2* variation.

A comprehensive analysis of *PITX2* genotypes and their impact on growth traits revealed that ewes with CC genotypes exhibit remarkable variations in body composition and live weights compared to those with CT and TT genotypes. A positive correlation was also found between genotype CC and phenotypic measurements. Genetic variations can be identified with their phenotypic manifestations and a database that tracks economic traits (AL-Thuwaini, 2022). In this regard, *PITX2* gene polymorphisms and their relation to livestock productivity have been examined in several studies. The *PITX2* gene polymorphism in native Chinese cattle, Chinese sheep, Hainan black goats, and Guanzhong dairy goats has been extensively studied (Zhao et al., 2018; Zhang et al., 2018; Yan et al., 2018; Zhang et al., 2020). This effect may be due to *PITX2* being a candidate gene related to back fat thickness and growth and playing a role in skeletal muscle development (Zhang et al., 2020). *PITX2* regulates several growth and development pathways, including Wnt/Dvl/beta-catenin signaling between the hypothalamus and pituitary and the hypothalamus and adrenal (Zhang et al., 2018). As these pathways are essential for growth, development, and reproduction, the *PITX2* gene could significantly impact livestock reproduction and production. Additionally, *PITX2* is involved in activating and stabilizing the Wnt/β-catenin pathway and mRNA (Briata et al., 2003). This signaling pathway is critical for embryonic development and for maintaining adult tissue homeostasis (Yan et al., 2018). Accordingly, the present study identified an ovine *PITX2* gene polymorphism (319C>T) that correlated significantly with

live weight and some body measurements. A sheep marker-assisted selection breeding program will be developed based on these results.

CONCLUSION

The *PITX2* gene has a vital function within the hypothalamic-pituitary-adrenal system, influencing the growth and development of animals. Sheep with the CC genotype exhibited higher live weights in ewes and lambs and increased body measurements, enhancing productivity. This finding highlights the substantial impact of genetic variations in ovine *PITX2* on growth traits, offering valuable insights for upcoming advancements in sheep breeding practices.

CONFLICT OF INTEREST

None.

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REFERENCES

Abbasi MA, Ghafouri-Kesbi F (2011) Genetic (co) variance components for body weight and body measurements in Makooei sheep. Asian-Australas J Anim Sci 24(6): 739-743.

Ajafar MH, Al-Thuwaini TM, Dakhel HH (2022a) Association of OLR1 gene polymorphism with live body weight and body morphometric traits in Awassi ewes. Mol Biol Rep 1-5.

Ajafar MH, Kadhim AH, AL-Thuwaini TM (2022b) The Reproductive Traits of Sheep and Their Influencing Factors. Rev Agric Sci 10: 82-89.

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.

AL-Thuwaini TM (2022) Adiponectin and Its Physiological Function in Ruminant Livestock. Rev Agric Sci 10: 115-122.

Al-Thuwaini TM, Al-Hadi ABA (2022) Association of lamb sex with body measurements in single and twin on the Awassi ewes. Adv Anim Vet Sci 10(8): 1849-1853.

Al-Thuwaini TM, Kareem ZA (2022) Novel missense variant L46Q of fatty acid synthase gene and fatty acids content in Awassi sheep. Acta Sci - Anim Sci 44.

Asaduzzaman M, Shahi PKJMG, Bari AFY (2020) Multi-farm evaluation of morphometric, reproductive and productive traits of Jamuna basin indigenous and Muzaffarnagari cross breed sheep of Bangladesh. J Appl Anim Res 13(1): 31-50.

Botstein D, White RL, Skolnick M, Davis RW (1980) Construction of a genetic linkage map in man using restriction fragment length polymorphisms. Am J Hum Genet 32(3): 314.

Boujenane I, Diallo IT (2017) Estimates of genetic parameters and genetic trends for pre-weaning growth traits in Sardi sheep. Small Rumin Res 146: 61-68.

Briata P, Ilengo C, Corte G, Moroni C, Rosenfeld MG, Chen CY, Gherzi R (2003) The Wnt/β-catenin→ Pitx2 pathway controls the turnover of Pitx2 and other unstable mRNAs. Mol Cell 12(5): 1201-1211.

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(1): 174-175.

Cao H, Dong X, Mao H, Xu N, Yin Z (2019) Expression analysis of the *PITX2* gene and associations between its polymorphisms and body size and carcass traits in chickens. Animals 9(12): 1001.

Ghanem N, Zayed M, Mohamed I, Mohammady M, Shehata MF (2022) Co-expression of candidate genes regulating growth performance and carcass traits of Barki lambs in Egypt. Trop Anim Health Prod 54(5): 1-13.

Haldar A, Pal P, Rajesh MD, Pal SK, Majumdar D, Biswas CK, Pan S (2014) Prolificacy and its relationship with age, body weight, parity, previous litter size and body linear type traits in meat-type goats. Asian-Australas J Anim Sci 27(5): 628-634.

Kumar S, Dahiya SP, Malik ZS, Patil CS (2018) Prediction of body weight from linear body measurements in sheep. Indian J Anim Res 52(9).

Mohammed MH, Al-Thuwaini TM, Al-Shuhaib MBS (2022) High association of a novel variant in the adiponectin gene with the litter size in Awassi ewes. J Saudi Soc Agric Sci 21(5): 296-301.

Putra WPB, Agung PP, Anwar S, Said S (2019) Polymorphism of bovine growth hormone receptor gene (g. 3338A> G) and its association with body measurements and body weight in Pasundan cows. Trop Anim Sci J 42(2): 90-96.

Wang F, Chu M, Pan L, Wang X, He X, Zhang R, Tao L, La Y, Ma L, Di R (2021) Polymorphism detection of *GDF9* gene and its association with litter size in Luzhong mutton sheep (Ovis aries). Animals 11(2): 571.

Yan H, Jiang E, Zhu H, Hu L, Liu J, Qu L (2018) The novel 22 bp insertion mutation in a promoter region of the *PITX2* gene is associated with litter size and growth traits in goats. Arch Anim Breed 61(3): 329-336.

Zhang S, Xu H, Kang Z, Cai H, Dang R, Lei C, Chen H, Guo X, Lan X (2018) Bovine pituitary homeobox 2 (PITX2): mRNA expression profiles of different alternatively spliced variants and association analyses with growth traits. Gene 669: 1-7.

Zhang S, Zhao H, Lei C, Pan C, Chen H, Lin Q, Lan X (2020) Effects of genetic variations within goat *PITX2* gene on growth traits and mRNA expression. Anim Biotechnol 31(2): 107-114.

Zhao H, Wu X, Cai H, Pan C, Lei C, Chen H, Lan X (2013) Genetic variants and effects on milk traits of the caprine paired-like homeodomain transcription factor 2 (*PITX2*) gene in dairy goats. Gene 532(2): 203-210.

Zhao H, He S, Wang S, Zhu Y, Xu H, Luo R, Lan X, Cai Y, Sun X (2018) Two new insertion/deletion variants of the *PITX2* gene and their effects on growth traits in sheep. Anim Biotechnol 29(4): 276-282.