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The Impact of Genetic Polymorphism of THRSP Gene on Body Dimensions and Growth Traits in Local and Shami Goats

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Abstract: This study was conducted at the Ruminant Research Station of the Agricultural Research Department, Ministry of Agriculture, located west of Baghdad in the Akerkouf region, from October 1, 2023, to April 1, 2024. It aimed to investigate the genetic polymorphism of the THRSP gene through detecting SNPs in a specific gene fragment and studying its association with body measurements (chest circumference, body length, height at withers and height at rump) and growth traits (birth weight, weaning weight, weight gain from birth to weaning, and current weight) in local and Shami goats. Sequencing of the targeted gene fragment revealed a size of 486 bp in both goat breeds, with a single nucleotide change at position 322 within exon 1, changing from G to C. This missense mutation produced three genotypes: GG, GC, and CC. In local goats, the highest frequency was for GG, followed by CC, then GC. Similarly, in Shami goats, GG was most frequent, followed by GC, then CC. These differences were statistically significant ($P \leq 0.01$). In local goats, the GC genotype outperformed the other two genotypes in weaning weight and weight gain, while the genotypes of the gene had no significant effect on the other studied traits. In Shami goats, no significant differences were observed between the three genotypes regarding growth traits and body dimensions. Genotype GG outperformed the others in observed and expected numbers. Biodiversity indices showed that the effective allele number was lower than the actual allele count, and the Shannon-Weaver index values were low in both breeds.

Keywords: THRSP gene, local and Shami goats, growth traits, body measurements.

INTRODUCTION

Livestock is a crucial component of Iraq's national economy due to its role in achieving food security. Studying the genetic characteristics of local breeds is vital for preserving genetic diversity, also, animal performance traits significantly influence livestock production economics (1). Genetically-based selection has proven essential in breeding, especially for economically important quantitative traits controlled by multiple genes. Traditional phenotype-based selection is often inaccurate, and while quantitative methods (e.g., breeding value estimations) offer better precision, they are time-consuming and labor-intensive(2).Recent advances in molecular genetics allow the identification of genetic markers strongly associated with economic traits(3). One such gene is the Thyroid Hormone Responsive Spot 14 (THRSP) gene, first identified in liver tissue mRNA expression studies in mice exposed to thyroid hormone(4). It is actively expressed in liver and adipose tissues and is notably induced by high-carbohydrate diets, particularly in mammary glands (5). In goats, the THRSP gene is located on chromosome 29 and contains two exons. Therefore, the aim of this study was to discover the possibility of the effect of the polymorphism of the THRSP gene on some growth traits and body dimensions in the local Iraqi and Shami goat breeds.

Materials and methods

The study was conducted from October 1, 2023, to April 1, 2024, involving 62 female goats (31 local and 31 Shami), aged between 2 to 5 years, housed at the Ruminant Research Station in Akerkouf, 23 km west of Baghdad.This research involved the analysis of growth and body conformation traits in local and Shami goat breeds based on data obtained from station records. Blood samples (3 ml) were collected from the jugular vein into EDTA K3 tubes and transported chilled to the laboratory for DNA extraction using the Geneaid DNA extraction kit.PCR amplification targeted a 486 bp fragment of the THRSP gene using specific primers:

Forward Primer: 5'-AGTCTGCGGGACTCCATATG-3'

Reverse Primer: 5'-AAAATGGGACAGGCCATGT-3'(Ghasemietal.,2022) PCR conditions were as follows: Initial denaturation was 95°C for 5 min. and 1 cycle, denaturation was 95°C for 0.5 min. and 38 cycles, annealing was 60°C for 0.5 min. and 38 cycles, elongation was 72°C for 0.75 min. and 38 cycles, and last step was final elongation with 72°C for 10 min. and 1 cycle. PCR products were electrophoresed on 1.5% agarose gel at 70V and 85mA for 45 minutes, and visualized using UV Gel Documentation. Sequencing was performed by Macrogen (Korea), and results were analyzed using BLAST (NCBI)and Bio Edit software. Data were statistically analyzed using the SAS software (6), examining the genetic polymorphism of the THRSP gene and its association with body and growth traits. Significant differences between means were compared using Duncan's test (7)

RESULTS AND DISCUSSION

The DNA was successfully extracted using the Geneaid kit. PCR amplification of the THRSP gene fragment was successful, producing a 486 bp product as visualized through agarose gel electrophoresis (Fig.1).Sequencing analysis using BLAST and BioEdit revealed a SNP at position 322 (G>C) in exon 1 of the THRSP gene. This missense mutation resulted in three genotypes: GG (wild), GC (heterozygous), and CC (mutant). The mutation changed the codon from GTG (valine) to CTG (leucine), altering the final amino acid in the THRSP peptide sequence.

https://www.ncbi.nlm.nih.gov/nuccore/LC811270.1(/

The results of Tables 1 and 2 indicated the number of animals, the percentage of genotypes, alleles, allele frequency, and the Chi-square (χ^2) value, which were distributed across the studied sample for the THRSP gene in both breeds. It was found that the frequency of the wild-type allele G for both breeds (Shami and Local) were 0.71 and 0.677, respectively, while the frequency of the mutant allele C for the same breeds were 0.29 and 0.323, respectively.This confirms the dominance of the wild-type G allele over the mutant C allele in the studied samples of both Local and Shami goats. This result is consistent with what was reported by (8) in a study on the Markhoz goat breed in western Kurdistan, Iran, where the wild allele G was more frequent than the mutant allele A for the first gene fragment (148 bp).However, our results differed from those of (8) in the second gene fragment (173 bp), where the wild genotype GG had the highest percentage at 58%, followed by the homozygous mutant genotype CC at 23%, and the

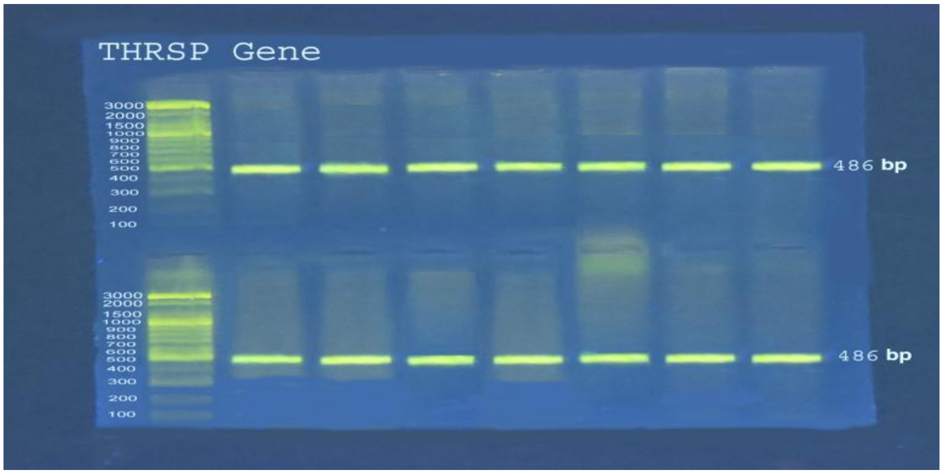
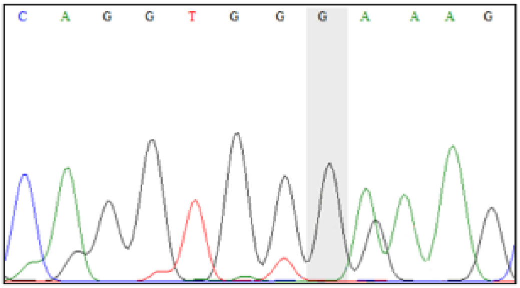
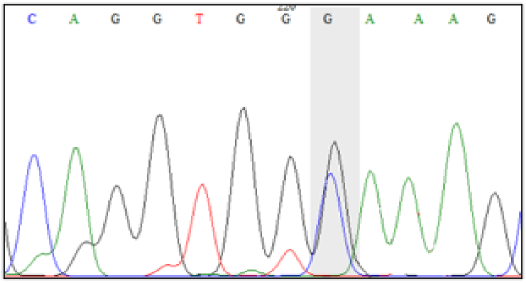


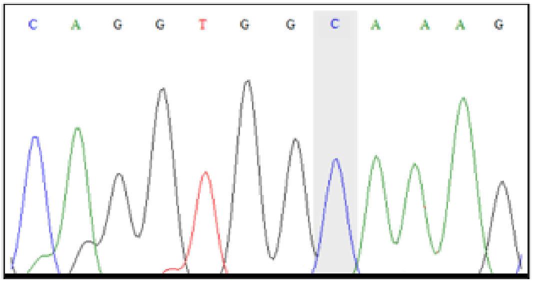
Fig.1: Electrophoresis of the THRSP gene amplification product (486 base pairs) was performed on 1.5% agarose gel at 70 volts and 85 mA for 45 minutes.



GC



GG



CC

Fig.2: The 322 T>C mutation in the THRSP gene in two goat breeds (Local and Shami).

Table 1. Genotype and allele frequencies of the THRSP gene in local goats

Genotype	Count	Percentage	Allele	Allele Frequency	Chi-square
GG	18	58%	G	0.677	19.451**
GC	6	19%	C	0.323	
CC	7	23%	-	1.00	

heterozygous genotype GC at 19%.The Chi-square value was 19.451, confirming statistically significant differences among the three genotypic frequencies (wild GG, heterozygous GC, and mutant CC).As for the Shami goats, the wild genotype GG also had the highest percentage at 58%, followed by the heterozygous genotype GC at 26%, and the mutant genotype CC at 16%. The Chi-square value was 18.161, also indicating significant differences among the three genotypic frequencies in the Shami goats.These findings align with what was reported by (8) in the Markhoz goats in western Kurdistan, Iran, where the distribution of THRSP gene genotypes in the exon 1 region showed the wild genotype AA had a frequency of 0.743 in the 148 bp fragment, while the heterozygous AG genotype for the 173 bp fragment had a frequency of 0.257.

Table 2. Genotype and allele frequencies of the THRSP gene in Shami goats

Genotype	Count	Percentage	Allele	Allele Frequency	Chi-square
GG	18	58%	G	0.71	18.161**
GC	8	26%	C	0.29	
CC	5	16%	-	1.00	

The statistical analysis results in Table 3 showed no significant effect of the THRSP gene genotypes (GG, GC, CC) on birth weight and current weight in local goats.However, for the weaning weight trait in local goats, there were highly significant differences ($P \leq 0.01$) among the three THRSP gene genotypes. The individuals carrying the heterozygous GC genotype had the highest weaning weight value (16.66 kg), outperforming the wild-type GG and the mutant CC genotypes, which recorded 14.571 and 12.861 kg, respectively.Regarding the average daily weight gain, there were also highly significant differences (P≤0.01) between the genotypes. Again, the heterozygous GC genotype performed best with an average of 14.050 kg, compared to GG (10.372 kg) and CC (12.100 kg).This suggests that individuals with the heterozygous genotype (as observed in the Shami goats) may possess advantages such as faster growth, better disease resistance, or higher productivity, compared to individuals with the wild-type genotype (GG), which may not have been genetically improved, and the mutant genotype (CC), which may have random or unstable genetic alterations (8).

Table3 . Effect of the 322 T>C Genotype on Growth Traits in Local Goats

Genotype	No. of Animals	Birth Weight (kg)	Weaning Weight (kg)	Average Gain (kg)	Current Weight (kg)
GG	18	2.488 ± 0.167	12.861 ± 0.407 B	10.372 ± 0.352 B	40.666 ± 1.612
GC	6	2.616 ± 0.183	16.666 ± 1.801 A	14.050 ± 1.887 A	40.666 ± 2.216
CC	7	2.471 ± 0.275	14.571 ± 0.841 AB	12.100 ± 0.840 AB	47.000 ± 3.380
Significance	-	NS	**	**	NS

NS: Not Significant P ≤ 0.01: Highly Significant , Different letters (A, B, AB) in the same column indicate significant differences.

In Shami goats, statistical analysis results from Table 4 showed no significant differences among the three genotypes for all growth traits.The lack of significant differences in these traits may be due to the limited and weak role of the THRSP gene in regulating growth and weight traits in goats, especially when compared to other more influential genes such as IGF1, GH, and GHR. Environmental factors may also play a stronger role, possibly masking the minor genetic effects of THRSP, additionally, small sample sizes might have limited the detection of real differences among the genotypes, supporting the idea that further research is needed on the role of THRSP variation in different goat breeds.This result contrasts with the findings of (10), which indicated that the THRSP gene may have a significant influence on growth traits in goats.

Table 4. Effect of the 322 T>C Genotype on Growth Traits(kg) in Shami Goats

Genotype	Number of Animals	Birth Weight	Weaning Weight	Average Weight Gain	Current Weight
GG	18	2.372 ± 0.129	15.277 ± 0.850	12.905 ± 0.812	46.388 ± 2.560

Genotype	Number of Animals	Birth Weight	Weaning Weight	Average Weight Gain	Current Weight
GC	8	2.462 ± 0.299	14.375 ± 1.100	11.912 ± 1.120	41.250 ± 2.657
CC	5	2.100 ± 0.100	15.300 ± 0.624	13.200 ± 0.583	50.800 ± 5.499
significance	31	NS	NS	NS	NS

NS = Not Significant

From Tables 5 and 6, it is evident that no clear distinction was observed in the average values of individuals carrying the three genotypes for body measurements traits in both local and Shami goat breeds. These findings contradict the study by (10), which investigated THRSP gene polymorphisms in 610 goats from three breeds: *Xinong Saanen (SN), Guanzhong (GZ), and Boer*, and reported a significant effect ($P < 0.05$) on chest width. In that study, the mutant genotype AA showed the highest average (56.00 cm) compared to GG (54.63 cm) and GA (54.49 cm).For body length, no significant differences were observed between the three genotypes in either breed, although a slight numerical advantage was found for the CC genotype in Shami goats, CC individuals averaged 87.400 cm, compared to GG (81.750 cm) and GC (82.000 cm), while in local goats, CC individuals averaged 85.000 cm, compared to GG (83.17 cm) and GC (82.611 cm). Also results indicated no significant differences among the genotypes for front and rear body height in local and Shami goats.These results suggest that the lack of significant effect of the THRSP gene on body dimensions may be due to the gene's stronger association with energy and fat metabolism rather than with bone or muscle growth. Alternatively, environmental factors such as nutrition and management may have had a greater impact on body measurements, potentially masking any subtle genetic effects. Additionally, the sample size may have been too small to detect real differences between genotypes, even if the gene has a minor actual effect.

Table 5. Effect of the 322 T>C Genotype on Body Measurement Traits in Local Goats

Genotype	Chest Width (cm)	Body Length (cm)	Front Body Height (cm)	Rear Body Height (cm)
GG	83.111 ± 1.342	82.611 ± 1.255	75.833 ± 0.848	77.777 ± 0.951
GC	83.166 ± 2.056	83.167 ± 1.869	77.667 ± 0.614	79.833 ± 0.654
CC	85.142 ± 2.005	85.000 ± 1.091	77.714 ± 1.768	79.285 ± 2.168
Significant	NS	NS	NS	NS

NS = Not Significant

Table 6. Effect of the 322 T>C Genotype on Body Measurement Traits in Shami Goats

Genotype	Number of Animals	Chest Width (cm)	Body Length (cm)	Front Body Height (cm)	Rear Body Height (cm)
GG	18	85.333 ± 1.793	82.000 ± 1.602	76.666 ± 1.072	78.888 ± 1.081
GC	8	81.750 ± 2.050	81.750 ± 2.250	76.125 ± 1.440	78.375 ± 1.413
CC	5	88.200 ± 3.277	87.400 ± 2.501	78.400 ± 2.315	79.800 ± 2.727
Significant	31	NS	NS	NS	NS

NS = Not Significant

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