Research Article

Polymorphisms of Somatostatin Receptor (*SSTR-1*) Gene and its Relationship with Carcass Traits in Iraqi Awassi Sheep

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Abstract | The current study was conducted on 100 lambs of Iraqi Awassi sheep, slaughtered at the age of 10 months. DNA extraction and PCR reactions were carried out at the biology sciences laboratory of the Marshes Research Center/Thi-Qar University. Sequencing technology was used to detect mutations in *SSTR1* gene. The results of the multiple alignment analysis of the sequence of *SSTR1* gene indicated the presence of one mutation 264 A>G. The obtained mutation produced three genotypes, namely AA, AG, and GG. The frequency of the genotypes AA, AG and GG were 0.50, 0.17 and 0.33, respectively. While the frequency of the A and G alleles were 0.58 and 0.42, respectively. There were significant impacts of *SSTR1* gene polymorphisms and the weights of carcass characteristics including heart and liver weight alongside recovery rate. Meanwhile, there was no significant effects between polymorphisms of *SSTR1* and the remaining carcass traits including live weight, carcass weight, clearance percentage, and spleen and kidneys weights. This study highlights the possible efficiency of using *SSTR1* as candidate marker for carcass traits in sheep.

Keywords | *SSTR1* gene, Polymorphisms, Sheep, Awassi, Carcass traits

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INTRODUCTION

Sheep are one of the most significant livestock breeds in Iraq and contribute significantly to the country's agricultural income. Sheep are one of the key components of animal production, and 58.2% of all sheep in Iraq originate from the Awassi region (Al-Qasimi et al., 2021; Muhana et al., 2021). Because it includes necessary elements that no other diet can match, animal meat plays a significant role in human nutrition and health (Bhat et al., 2015).

Sheep characteristics that affect growth are highly valuable economically (Marai et al., 2000). In terms of growth, animals frequently exhibit phenotypic variance (Ocak and Guney, 2010). By examining this variation at the DNA level, the genes/alleles underlying these qualities can be discovered. The best animals can then be selected using this knowledge (Salces-Ortiz et al., 2013). Utilizing genomic technology has enhanced molecular genetics and opened up intriguing possibilities for the identification of useful genes. Numerous single nucleotide polymorphisms (SNPs) have been discovered in the genomes of several livestock animals thanks to genome sequencing programs. These genetic markers, or SNPs, can be used to identify the genetic variation that underlies traits in livestock animals that are economically significant and to better understand

how genetic variants connect to various phenotypes (Daw et al., 2005). In order to make it easier to choose breeding animals that will actually improve traits connected to growth, genetically based improvement programs should be created (Ibrahim et al., 2023).

Numerous genes have been employed as genetic markers to choose animals with the finest growth characteristics and premium carcasses. *SSTR1* is one of these genes (Jin et al., 2010). The fundamental control of metabolic systems that affect growth, such as obesity and dietary absorption, depends on somatostatin proteins (SST) and its receptors (SSTR1, SSTR2, SSTR3, SSTR4, and SSTR5). According to Zhao et al. (2018), somatostatin receptors (SSTRs) are hypothesized to control the growth-inhibitory effects of somatostatin as well as the production of growth hormone. According to ovine genome sequence (NC_019475.2), *SSTR1* gene located on chromosome 18 and contains one large coding exon of 1173 base pair (nt 47172694-47173866).

According to Maecke and Reubi (2011), there are five somatostatin receptor subtypes in mammals (SSTR1–5). Widely present in the placenta, kidney, liver, pancreas, lungs, and central nervous system, SSTRs are engaged in a variety of biological functions (Quan et al., 2020), but they only function when they bind to G-protein-coupled SSTRs (Anzola et al., 2019). In the first place, SSTRs inhibit the release of prolactin, thyroid-stimulating hormone (TSH), gastric secretory hormone, growth hormone releasing hormone (GHRH), secretin, glucagon, insulin, and SST itself in the pancreas (Lloyd et al., 1997). Second, by preventing the production of digestive enzymes and gastrointestinal hormones, SSTRs reduce the rate of food absorption in the gastrointestinal tract (Tulassay, 1998). Additionally, they reduce blood flow, gallbladder contraction, and gastrointestinal motility, which in turn affects feed conversion and growth traits (Strowski et al., 2000). This controls the rate of digestion and absorption.

Mammals, particularly goats and sheep, have similarities in the structure and function of the *SSTR1* gene, however there are some changes between sheep and goats in the sequence of the 3′-UTR region (Debus et al., 2001). A study on the *SSTR1* gene in goats using the SSCP-PCR technique, it was revealed presence of four mutations, namely GU014693: g.647T>C, 844A>C, 970T>C and 1039T>A (Jin, et al., 2011). These SNPs resulted in two the two haplotypes, TATT and CCCA, were significantly associated with animal body length and body height. The results of a study on Romney sheep in New Zealand using the (PCR-SSCP) approach to identify polymorphisms in the 3′UTR of the *SSTR1* gene revealed that three polymorphisms were identified (Zhaoa et al., 2018). It was discovered that the presence of A allele is linked to a reduction in the weight of hot carcasses. Meanwhile the presence of C allele is linked to an increase in subcutaneous deep fat as well as a reduction in birth weights.

The objective of the current study was to examine the *SSTR1* gene's sequence and identify any connections between potential mutations and growth and carcass traits of Iraqi Awassi sheep.

MATERIALS AND METHODS

Animals And Experimental Samples

The current study, which employed one hundred lambs of the Awassi sheep breed, was carried out at one of the breeders' fields in the city of Al-Qasim, about 35 km away from it in the province of Babylon. Lambs had 37.81 kg average body weight. Animals were slaughtered when they were 10 months old. Broad spectrum anthelmintic was administered to the animals as directed as a prophylactic dose. All animals were housed in identical housing circumstances throughout the duration of the trial and had no prior history of metabolic or concomitant illnesses. The Composition of the concentrate feed mixture (CFM) fed to growing in kilograms (Kg) was as follows: 400 Corn, 300 wheat bran, 250 soya beans, 10 sodium chloride, 20 Ca carbonate, and 1 premix.

Lambs' jugular veins were used to obtain blood samples for DNA extraction, which was done using a kit made by the Geneaid Taiwanese Company. One milliliter of blood was taken from the jugular vein of each lamb for DNA extraction.

Dna Extraction And Polymerase Chain Reaction

The Marshes Research Center/Thi-Qar University's biology sciences lab performed DNA extraction and PCR procedures. DNA was extracted using the Geneaid/Korea extraction kit. A 718-bp fragment from the coding region of the *SSTR1* gene was amplified using the following two primers F-5′ ACCATCAGGG-TGATCTTGCG -3′ and R-5′-CTGCAGACGGGATGGAAGAA -3′. The used primers were designed based on the reference transcript recorded in the GenBank under code XM_012098844.4. Table (1) shows the amplification program for the selected fragment of the *SSTR1* gene. Table (2) shows the materials used in the amplification reaction and their quantities.

Dna Sequencing And Polymorphism Detection

Prior to the development of DNA sequencing, Jena Bioscience # pp-201s/Munich, based in Hamburg, Germany, developed techniques for purifying PCR and removing primer dimmers, nonspecific bands, and other impurities to

yield the intended amplified product of the expected scope (Boom et al., 1990). By using a Nanodrop (Waltham, MA, USA, UV-Vis spectrometer Q5000) to assess PCR output, adequate quality and good concentrations were attained (Boesenberg-Smith et al., 2012). All of the examined samples' PCR products were transferred to the Korean business Macrogen for nucleotide sequencing examination. The alignment of all sequences was carried out using the BioEdit software version 7.2.5 after receiving the results of the *SSTR1* gene sequencing (Hall 1999).

Table 1: Amplification PCR protocol for *SSTR1* gene.

Stage	Temperature	Time	Cycle
Initial denaturation	96 ° C	5 min	1
Denaturation	96 ° C	30 sec	35 cycle
Annealing	56° C	30 sec	
Extension	72° C	1 min	
Final extension	72° C	7 min	1
Store	4°C		

Table 2: PCR reaction components for *SSTR1* gene.

Reagents	Volume µl
Master Mix	13 µl
Primer forward	1 µl
Primer reverse	1 µl
DNA template (30-100ng)	1-3 µl
Free water	7-9 µl
Total	25 µl

Statistical Analysis

Allelic and genotypic frequencies and Hardy–Weinberg equilibrium were estimated using by POPGENE software v1.32. Chi-square- test was used to compare the percentages of alleles for each genotype in the studied sheep sample. Associations of the *SSTR1* gene polymorphisms with growth and carcass traits were calculated by the SPSS statistical program (Version 22.0, 2013).

To test association between *SSTR1* gene polymorphisms and growth traits, the following model was used:

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

Where, Y_{ijk} is the phenotypic value of the trait, µ is overall population mean, G_i is the effect of *SSTR1* genotype, and e_{ijk} is the random error effect.

RESULTS AND DISCUSSION

DNA extraction showed that the DNA concentration ranged between 34 - 98 ng /Î¼l and that the purity using wavelengths 260/280, ranged between 2.03 - 1.71 nm. The results of agarose gel electrophoresis showed the successful amplification of the *SSTR* gene, where the size of the PCR product was 718-bp.

The sequencing of the amplified area of the *SSTR1* gene underwent repeated alignment analysis, and the findings revealed one mutation at location 264 A>G (Figure 1). Three genotypes AA, AG and GG were noticed. The valine amino acid code AGT was used to create the mutation, which resulted in the code changing to GGT. Of the 390 amino acids that make up the SSTR1 peptide chain, 141 remained in their original location in relation to the whole protein.

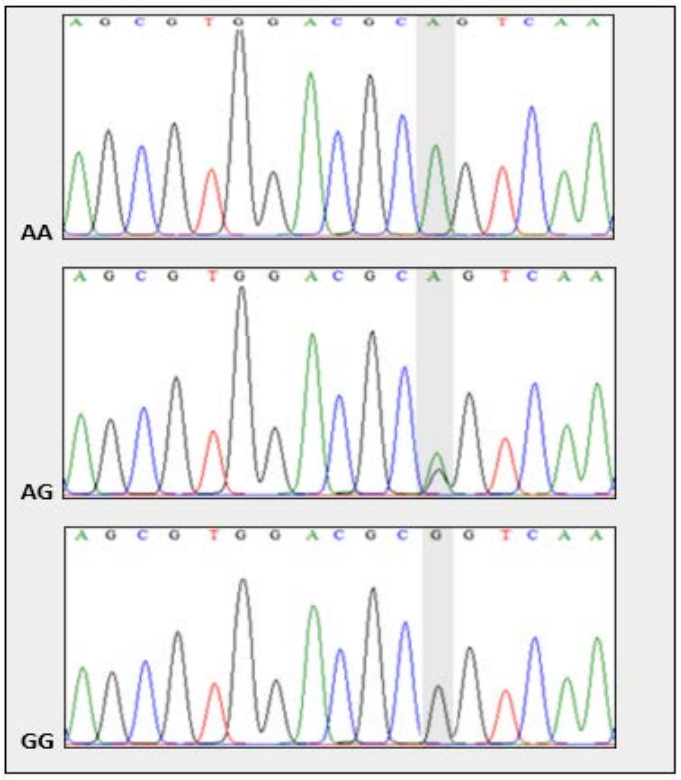

Figure 1: Chromatograph of DNA sequence for *SSTR1* gene indicating AA, AG, and GG genotypes.

Table (3) shows genotypes and allele frequencies for *SSTR1* gene polymorphisms. Results indicated that the frequency of the genotypes AA, AG and GG were 0.50, 0.17, and 0.33, respectively. While the frequency of the A and G alleles were 0.58 and 0.42, respectively. The polymorphisms of the *SSTR1* gene did not have any significant effect on live weight, carcass weight, and clearance percentage of Awassi lambs (Table 4). This is consistent with (Zhao, et al., 2018). As for the percentage of recovery, the GG genotype was superior to the AA and AG.

Table 3: Genotype and allele frequencies for *SSTR1* gene

Genotype	Genotype frequency		Allele	Allele Frequency	X^2
AA	50	0.50	A	0.58	10.36
AG	17	0.17	G	0.42	
GG	33	0.33			
Total	100				

Table 4: Effect of *SSTR1* gene polymorphisms on carcass traits

Traits	Genotype	N	Mean	Std. Error	P value
Live Weight	AA	50	37.18	1.093	0.70 N.S
	AG	17	35.31	0.616	
	GG	33	40.01	1.154	
	Total	100	37.81	0.743	
Carcass Weight	AA	50	17.27	0.552	0.32 N.S
	AG	17	16.12	0.229	
	GG	33	18.79	0.501	
	Total	100	17.59	0.371	
Clearance percentage	AA	50	46.42	0.271	0.64 N.S
	AG	17	45.77	0.284	
	GG	33	46.98	0.226	
	Total	100	46.50	0.178	
Recovery percentage	AA	50	73.86 b	0.301	0.00 **
	AG	17	70.52 c	0.501	
	GG	33	75.25 a	0.302	
	Total	100	73.77	0.380	

- A= Adenine; G= Guanine; N= number; and *STRR1*=Somatostatin receptor

Table 5: Effect of *SSTR1* gene polymorphisms on giblet weight traits.

Traits	Genotype	N	Mean	Std. Error	P value
Heart	AA	50	0.1467 b	0.005	0.001 **
	AG	17	0.1475 b	0.004	
	GG	33	0.1950 a	0.012	
	Total	100	.1629	0.006	
Liver	AA	50	0.6817 b	0.023	0.005 **
	AG	17	0.5800 c	0.034	
	GG	33	0.7712 a	0.034	
	Total	100	0.6946	0.0215	
Spleen	AA	50	0.0917	0.005	0.579 N.S
	AG	17	0.0825	0.004	
	GG	33	0.0937	0.007	
	Total	100	.0908	0.003	
Kidneys	AA	50	0.1433	0.005	0.21 N.S
	AG	17	0.1250	0.013	
	GG	33	0.1462	0.006	

-A= Adenine; G= Guanine; N= number; and *STRR1*=Somatostatin receptor

Table (5) shows the effect of polymorphisms of the *SSTR1* gene on giblets weight of Awassi lamb carcasses. A highly significant effect of polymorphisms on the weight of the heart and liver, as lambs bearing the polymorphism GG excelled on lambs bearing AA and AG genotypes. As for the spleen and kidneys, polymorphisms did not have any significant effect. The fundamental goal of the majority of genetic-selection programs is growth, which is one of the most significant economic variables measured in domestic animals (Koller et al., 2020). Body weight and body measurements, which serve as the primary indicators of growth features, have a significant impact on the production of meat and wool (Luo et al., 2021).

Depending on whether they are found in regulatory sequences or coding regions, single nucleotide polymorphisms (SNPs), which are characterized as a substitution, insertion, or deletion of a single nucleotide, are significant genetic sources for animal breeding (Stevenson, 2015). As it is well known, there are many elements that affect gene expression (Singh et al., 2018). One of these elements is SNPs. SNPs regulate how genes are expressed and how proteins work. According to Taboada et al. (2007), SSTR1 is a significant peptide hormone that modulates a variety of processes, including neurotransmission, cell proliferation, and, specifically, growth hormone production. The relatedness between *SSTR1* gene polymorphism and growth traits of sheep is scarcely reported. Li et al. (2021) referred to his study on Chinese Hulun Buir sheep and revealed the relationship of the polymorphisms of the *SSTR1* gene to the characteristics of growth and carcasses. The study revealed four SNPs mutations in the second exon of the gene. A significant effect of the CC genotype was noticed compared to CT resulting from the third mutation of the studied region of the gene, where the CC genotype was superior in the body weight, body height, body length, chest circumference, chest depth, chest width, hip width, and cannon circumference

Jin et al. (2011) found also two SNPs (GU014695:g.801 C>T, GU014695:g.948 C>T) in the *SSTR1* gene that affect growth traits in caprines. Significant relationships between the three GU014695:801 C>T genotypes and chest circumference, body height, and length. These findings imply that goat development features may be influenced by the caprine *SSTR1* genes. In the same respect, Zhao et al. (2017) screened for variation in the *SSTR1* gene in New Zealand Romney sheep using polymerase chain reaction-single-strand conformational polymorphism (PCR-SSCP) analysis. DNA sequencing identified three distinct nucleotide sequences (A-C) and three distinct PCR-SSCP banding patterns. It was discovered that the presence of A was linked to a reduction in the weight of the heated carcass, whilst the presence of C was linked to higher subcutaneous fat depth and lower birth weights.

Depending on their chromosomal position, mutations have the capacity to change every step of gene expression. They have the ability to influence mRNA expression when found within transcriptional regulatory elements (Mendell and Dietz, 2001). SNPs can affect mRNA splicing, nucleo-cytoplasmic export, stability, and translation when they occur in genes. They can alter the function of a protein when they are found in a coding sequence and result in an amino acid change (also known as a non-synonymous SNP or mutation; Nicholson et al., 2010). A non-synonymous SNP was elaborated by our findings. The mutation was made using the valine amino acid code AGT, which led to the code altering to GGT. In regard to the entire protein, 141 of the 390 amino acids that make up the SSTR1 peptide chain were left in their original positions. The aforementioned justifications led us to more successfully discover SNPs linked to growth traits using exon sequencing technologies.

CONCLUSION

PCR-DNA sequencing genetic assessment of *SSTR1* gene revealed nucleotide sequence variants among Awassi sheep. There is a relationship between the *SSTR1* gene polymorphisms and carcass characteristics. *SSTR1* is therefore seen as a genetic marker that could help to improve the native Awassi sheep carcass features.

INSTITUTIONAL REVIEW BOARD STATEMENT

All animal management procedures, tested trial gathering, and sample discarding were carried out underneath the supervision of the University of Sumer, Iraq.

RECOMMENDATIONS

Future studies might focus on investigating the *SSTR1* gene polymorphism on other sheep breeds. Large number of animals should be acknowledged during this investigation.

NOVELTY STATEMENT

The relatedness between *SSTR1* gene polymorphism and growth traits of sheep is scarcely reported. Finding this polymorphism by SNPs could regulate how genes are expressed and how proteins work.

INFORMED CONSENT STATEMENT

All dairy farmers provided their knowledgeable agreement to contribute to the investigation.

DATA AVAILABILITY STATEMENT

Upon reasonable request, the supporting information for the study's findings will be provided by the corresponding author.

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CONFLICT OF INTEREST

The authors declare no conflict of interest.

AUTHOR CONTRIBUTIONS

The experimentation was developed, the PCR completed, and the paper written by Dhurgham K. Seger. Rahman H. H. Al Qasimi, Azhar A. Jaffar, and Salah H. Faraj performed DNA sequencing and contributed to the writing of the paper. Contributions were made to the planning of the manuscript and data analyses by Ahmed I. Ateya. All authors have read and agreed to the published version of the manuscript.

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