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The Relationship Between CYP19 Gene Polymorphism and Pregnancy Hormone in Awassi Ewes

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Abstract. Leuconostoc mesenteriodes is an important probiotic bacteria with impact characteristics and benefits for human health. Last year, probiotic bacteria were used as an alternative to traditional antibiotics in treat of different diseases especially in the broad distribution of MDR bacteria. L. mesenteriodes isolate was purchased from (Al-Amin Center for Research and Biotechnology\ Najaf, Iraq) and re-diagnosis using MRS media in anaerobic condition then diagnosis by vitek 2 system. The antibacterial activity of L. mesenteriodes was assessed using the agar well diffusion method against MDR pathogenic bacteria isolated from different clinical cases. The L. mesenteriodes crude was obtained by centrifuging the bacterial isolates. Five different concentrations of probiotic bacteria crude were tested against MDR pathogenic bacteria revealing the antibacterial activity of L. mesenteriodes crude concentrations (stock, 90%, 75%, 50% and 25%) against MDR Staphylococcus aureus (22, 20, 20, 17, 14 mm), MDR Micrococcu. leuts (24, 23, 18, 16, 11 mm), MDR Proteus. mirabillis (22, 22, 18, 17, 14 mm), MDR Escherichia. coli (22, 20, 18, 12, 11 mm) and MDR Klebsiella pneumoniae were (20, 18, 14, 12, 11mm) an respectively. L. mesenteriodes probiotics improve antibacterial activity especially when used against multi-drug resistant bacteria

Highlights:

1. Study CYP19 exon 8 polymorphisms and pregnancy hormones in Awasi sheep.
2. Genotype distribution: TT (20%), TC (30%), CC (50%); TC highest ($P<0.05$).
3. TC genotype linked to higher pregnancy hormone levels than TT, CC..

Keywords: Exon8. CYP19 Gene . Awassi ewes . pregnancy hormone.

Introduction

Quantitative traits are among the most important traits of economic importance, as they are controlled by dozens to hundreds of genes. Therefore, the need arose to use additional indicators to detect these traits, such as the use of DNA markers (1).The primary objective of utilizing genetic markers is to pinpoint the locations of significant quantitative traits in genetic selection programs, thereby enhancing the productive

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qualities of agricultural animals. (1, 2). Molecular biology has revolutionized practical applications and the advancement of modern technologies, with one of the most notable breakthroughs being the polymerase chain reaction (PCR) technique. The method can analyze any DNA fragment.)3), The primary purpose of utilizing DNA markers is to identify quantitative traits critical for genetic selection programs aimed at enhancing productivity traits in farm animals. (4)Notably, the CYP19 gene, commonly referred to as the "cure" gene, holds significant importance due to its role in producing aromatase. (4)This enzyme facilitates the conversion of androgens to estrogens, playing a crucial role in regulating reproductive functions and fat storage in both males and females. (5,6) and in growth (7). The enzyme belongs to the family of iron-containing proteins and shows a peak absorption wavelength of 450 nm. (8,6). The enzyme generated by granulosa cells plays a vital role in the development of follicles and the quality of eggs. (9,10) It also promotes estrus and produces mammary odor by transforming androgens into estrogens. When estrogen production is inadequate, androgens can accumulate in ovarian follicular cells, potentially hindering follicle development and ultimately resulting in follicular degeneration. (11,12). This study aimed to identify genetic polymorphisms in the CYP19 gene within the exon 8 region of the Awasi sheep breed. The alleles were analyzed to examine the impact of these genetic variants on pregnancy hormone levels

Methods

Animals used in experiments

In this experiment, fifty of Awassi ewes were used. Each ewe gave birth to a single lamb and was in a healthy, milk-producing state. The breeding system is managed in a closed environment, with the sheep being kept indoors for feeding Take it out to graze every morning.

celetion Blood samples.

Obtain 6 ml of blood from the jugular vein of each animal using a serum-specific blood collection tube. Furthermore, collect 3 ml of blood in a tube containing K3 EDTA. Transport the samples in a chilled container to the laboratory and store them at -20°C until DNA extraction is performed.

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Using an ELISA device to measure estrogen and progesterone levels:

Isolation of DNA

DNA was extracted from the blood samples of ewes for the molecular analysis of the targeted CYP19 Exon 8 gene, using the procedure outlined below.

DNA Extraction .

DNA was extracted from blood following the protocols provided by Geneaid kit.(USA). 6 ml of sheep blood sample was placed in a tube containing an anticoagulant to separate the serum. The tubes were centrifuged at 14,000 rpm for 5 minutes to isolate the necessary components for DNA extraction. Following centrifugation, the serum was carefully removed from the cellular components using a micropipette and transferred to an Eppendorf tube. The concentration of hormones and pregnancy markers was subsequently evaluated utilizing the enzyme-linked immunoassay (ELISA) in accordance with the instructions provided by the Elabscience Detection Kit manufactured in China.

Assessing DNA purity using the NanoDrop device:

Genomic DNA screening utilizing the Nano Drop (Thermo Fisher Scientific, United States of America)

* Electrophoresis*

Electrophoresis is a technique used to examine DNA segments following extraction for identification of DNA and offering insights into the sizes of the resulting fragments. The detection of specific genes, such as the CYP19 gene, is carried out using Polymerase Chain Reaction (PCR).

Select the Initiator:

Selecting the right primer is crucial for molecular detection and for understanding the Genotype linked to exon 8 of CYP19 gene.

Table 1 lists the primer sequences provided by IDT (Integrated DNA Technologies) in

abbreviated gene	Sequence of primers	Length(bp)
EXONE8 OF *CYP19*	(F) ACC AGT GCA TAT TGG AAA TGCTG	23
	(R) CTC TTC AAC CTG GGG ATG CT	20

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Analysis of the PCR Sequence's Interaction with the Target Gene:

The samples were organized in the reaction apparatus based on the specific conditions designated for each duplicate gene segment. Once the reaction was complete, the polymerization results were examined to verify the successful amplification of the target segment. The materials were subsequently combined using a vortex mixer. The tubes were then transferred to the polymerization reaction apparatus, where the conditions for the (PCR) were set as detailed in the table below.

Table 2: Materials Used in the CYP19 Polymerase Enzyme Chain Reaction.

(volume) (ml)	Reagent
5	Master Mix
2	DNA
F : 1 R: 1	Primer
16	Nuclease free Water
25	Total volume

The parameters established for identifying the CYP19 gene in the PCR apparatus are outlined in

Table 3, which outlines the necessary conditions for amplifying the CYP19 gene during the PCR procedure.

cycles	Time	temperatures	Stage	ت
1	5 min	94°C	Initial denaturation	1
35	30 sec	94°C	denaturation	2
	30 sec	58°C	Annealing	3
	30 sec	72°C	Extension	4
1	5 min	72°C	Extension	5

To prepare for the (PCR) and perform Electrophoresis:

10 µL of a DNA ladder was loaded alongside 10 µL Loading PCR products into a 2% agarose gel.. Electrophoresis was carried out at a voltage of 100 volts per centimeter and a current of 65 milliamps for one hour. After the run, the bands were visualized using a UV trans illuminator and captured with a photographic system.

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To sequence the nitrogenous bases of the target region:

The genetic material was extracted and the target region was amplified using PCR technology. Resulting in a segment of 101 bp. This segment was then sent to the Korean company Microgen for sequencing the nitrogenous bases of each experimental sample. The sequencing results were subsequently analyzed.

The nitrogenous base sequence of the CYP19 gene:

The sequencing results were analyzed with NCBI, while BioEdit and Mega7 software were utilized for sequence alignment to identify SNPs and examine the evolution of exon 8 of the CYP19 gene Polymorphism. (2).

Statistical analysis for the study:

was performed using SPSS version 27. This included calculating the average hormonal concentrations during pregnancy to investigate the relationship between the genotypes in the Exon 8 region of the CYP19 gene and the hormonal levels.

Result and Discussion

DNA Extraction:

Purification was performed using the gel electrophoresis method, as illustrated in Figure 1.

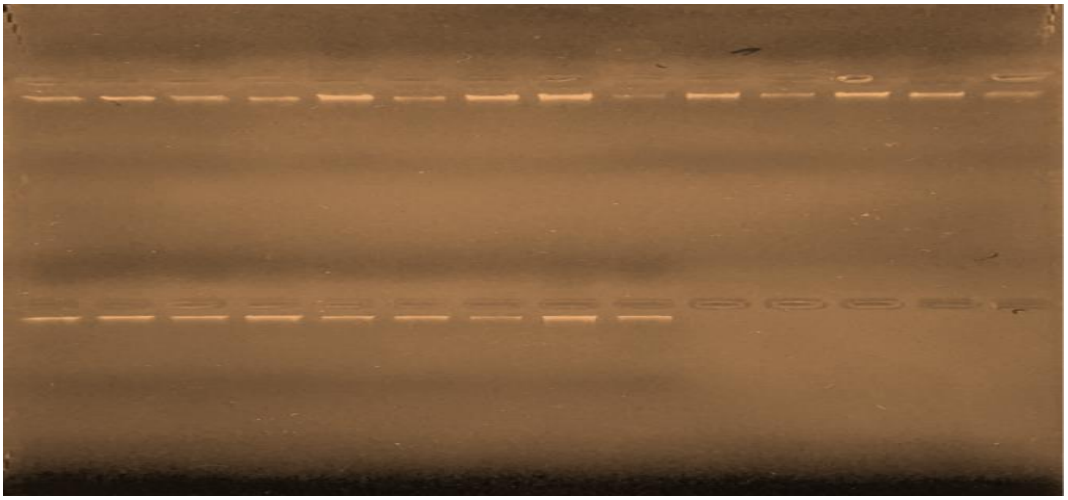

Figure 1 Illustrates DNA fragments obtained from blood samples of Awassi sheep through the electrophoresis method carried out on a 1% agarose gel.

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Detection of the CYP19 gene using the PCR technique.:

The samples analyzed in this study included this exon8, and the electrophoresis results confirmed its presence, showing a band size of 101 base pairs, as illustrated in Fig. 2.

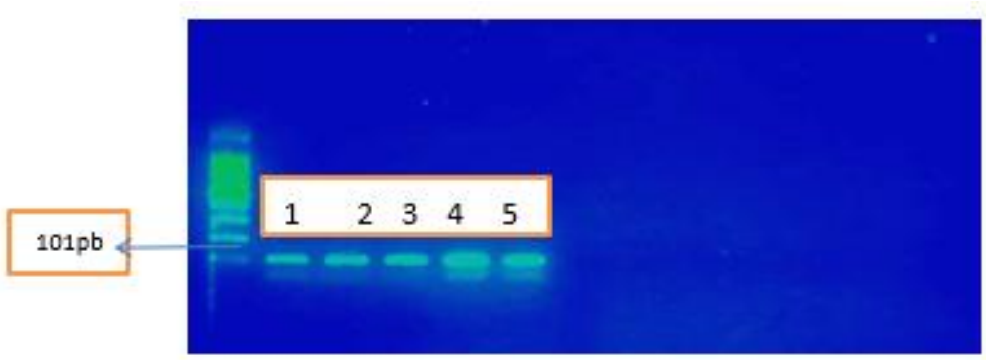

Figure 2 presents The electrophoresis results for the exon 8 region of the CYP19 gene, carried out on a 2% agarose gel. The standard DNA bands represent the amplified gene products obtained through PCR. L:Ladder (100-1200) samples of Awassi.

CYP19 Sequence:

A nucleotide sequence was analyzed to identify the specific arrangement of nitrogenous bases within the 101 base pair segment of the CYP19 gene located in exon 8. The analysis revealed the presence of genetic variants (TT, TC, CC) in the sample. These genotypes were determined through genetic testing results.

Proportions and counts of CYP19 gene genotypes:

The results of the current study reveal that the CC genotype was the most prevalent, occurring in 50% of cases, while the TT genotype was the least common at 20%, and the TC genotype represented 30%.) 13) examined the genotypes of the CYP19 gene in the third exon region and found that the AA genotype was the least frequent, present in only 8.75% of cases. This aligns with the findings of the current study, which also reported a low occurrence of the TT genotype. Conversely, the AB genotype was identified in 58.75% of cases, and the BB genotype was found in 32.50%. Furthermore, a study conducted in Brazil noted the absence of the AB and BB genotypes in the 1/2 Dorper, Poll Dorset, Santa Inês, and Brazilian Somali strains, with frequencies of 0.64 and 0.36, respectively. This absence was associated with a decrease in the frequency of the A allele, as all rams in that study were classified as AB. (14).

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Table 4: Percentage Distribution of Awassi Sheep Samples for the CYP19 Gene in Exon 8.

Genotype h	The u number	Percentage
TT	10	**0.20**
TC	15	**0.30**
CC	25	**0.50**
Total	50	**100%**
Chi square n	----------	**7.0758***
Allele		
T		**0.35**
C		**0.65**
**(P<0.05)		

Genotype

The arrangement of nitrogenous bases within the 101 base pair segment of the Exon8 (CYP19) gene exhibited variation, as demonstrated in Fig(. 3)

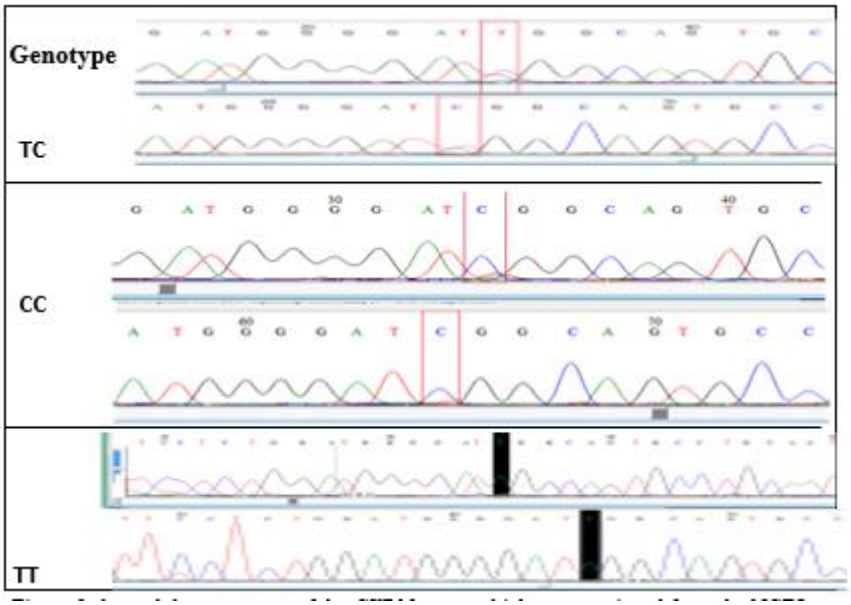

Figure 3 showed the genotypes of the CYP19 gene, which were retrieved from the NCBI database for sequencing alignment and subsequently analyzed with BioEdit software.

the association between genotypes and pregnancy hormone levels.

The TC hybrid model, exhibiting a concentration of 0.398 ng/ml, outperformed the pure genotypes TT and CC, which had concentrations of 0.300 ng/ml and 0.298

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ng/ml, respectively. This hybrid model influences reproductive traits and modifies the variability of responses to nighttime changes, especially when night conditions surpass certain thresholds for specific traits. In contrast to other breeds, this effect was not seen in the sheep breed examined. (15). Additionally, the genetic mutation significantly impacts LH concentration and pregnancy hormones in the TC genotype. This mutation alters the organism's genetic structure, leading to the development of distinct phenotypes. Although certain mutations can be harmful, others may provide benefits. An organism's genetic makeup influences its development, leading to phenotypic traits that improve its ability to adjust to environmental conditions. (11) The differences in results indicate a missense genetic mutation, which alters the amino acid sequence. As a result, this genetic variation affects the gene's function, especially in the secretion of pregnancy hormones, leading to marked increases in concentrations in the mutant pattern. This phenomenon has attracted considerable interest from researchers, underscoring the significance of genetics and the progress of contemporary genetic enhancement methods. Grasping the impact Understanding the genes, genetic traits, and genotypes of sheep breeds is crucial in this field. (16).

Table 6 show the average levels of pregnancy hormones in the study samples, organized by the genotypes of the CYP19 gene in exon 8 of Awassi sheep.

Genotype	**pregnancy hormone Level (ng/ml)**
TT	0.300 ± 0.011 A
TC	0.398 ± 0.010 B
CC	0.298 ± 0.022 A

Conclusion

In Awassi sheep, the CYP19 gene exone8 exhibits a genotype distribution of 50% CC, 20% TT, and 30% TC. The polymorphism of CYP19 gene significantly influences hormonal levels related to reproduction and pregnancy. In the current study, individuals with the TC mutant genotype consistently outperformed other genotypes in all assessments, including the measurement of hormonal concentrations associated with

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modulation and pregnancy. Those with the CC genotype ranked second in significance, after the TC genotype, regarding hormonal levels and pregnancy metrics. Furthermore, the TT and TC genotypes demonstrated better performance than the CC genotype in hormone measurements

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