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Genetic Variation of Myostatin Gene and Its Relationship with Carcass Parameters and Body Measurements in Local Iraqi Sheep

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Abstract **- myostatin (MSTN) gene's genetic relationships in local Iraqi sheep were the main focus of this investgation. 50 samples were collected. Information on dimensions of the body, live weight before slaughter, carcass weight for each animal. 2 milliliters of blood was drawn using a sterile syringe for DNA extraction. The HaeIII restriction enzyme was used to amplify and digest target fragment of MSTN gene, located in exon 3 with size 337 base pairs. To identify genotypes and allele frequencies, the PCR-RFLP technique was employed. According to the results there are two genotypes for the MSTN gene: mm-Mm, with the mm genotype representing 86% of the population, and 14% for the Mm genotype. A frequency of m allele was 0.931, while it was 0.069 for the M allele. With a highly significant difference ($P \leq 0.01$) to chi-square (χ^2) value of 22.349, suggesting that natural selection, genetic drift, or other evolutionary factors may be influencing allele distribution. Phenotypic analysis revealed no statistically significant differences in live body weight before slaughter or carcass weight between the genotypes. However, A significant difference $P \leq 0.05$ was noticed in the weight of viscera, with the mean of mm genotype being 1.400 kg and the mean of the Mm genotype being 1.058 kg. No significant differences were noticed between genotypes with regard to body length, height at withers, height at the rump, chest circumference, or abdominal circumference.**

Keywords- Myostatin gene (MSTN), Genetic polymorphism, Local Iraqi sheep, PCR-RFLP, Body measurement.

INTRODUCTION

The main sheep breeds in Iraq are Awassi, Karadi, and Arabi, with Awassi known for its tolerance to harsh conditions(1).Genetic improvement programs are important for improving sheep by studying and understanding the gene that affect increasing production, breeding and improving economic returns by associated genes related to milk production, growth, meat quality, and others traits (2).

Among these genes, MSTN (Myostatin) is considered one of the most common genes studied regarding meat quality and characteristics (3). Reduced MSTN gene expression increases muscle and meat production, but there is a question as to whether it may affect meat quality (5). MSTN is a member of transforming growth factor-beta (TGF-β) superfamily and is sometimes referred to as growth differentiation factor 8 (GDF-8)(6). In sheep, MSTN gene is found on chromosome two. MSTN gene influences fat deposition and acts as a regulatory element for genes involved in adipose tissue formation during increased muscle growth. It also plays a significant role in shaping carcass traits by promoting muscle hypertrophy and accelerating muscle fiber contraction through glycogen breakdown. This, in turn, enhances both the quality and efficiency of meat production (7). This study examines how MSTN polymorphisms correlate with body measurements and carcass weight across different age groups.

MATERIALS AND METHOD EXPERIMENTAL ANIMALS:

The city of Babylon was the site of the experiment in the Musayyib project area for the period from September 2024 to February 2025. As samples were collected from the private sector. 50 samples were taken from Iraqi local sheep, from both sexes (28males and 22 females) with varying weights and ages. Blood samples were also collected, with 3 mL drawn from the jugular vein of each animal and transferred into a tube containing EDTA as an anticoagulant. The samples were immediately placed in a cooling container and transported to the laboratory for molecular analysis. Live body weight, carcass weight, and the weight of edible internal organs were measured using a digital scale. In addition, body measurements including body length, height at the front (wither), height at the rear (rump), chest circumference, and abdominal circumference were taken using a graduated measuring tape. These measurements were recorded for all animals included in the

study to examine the association between MSTN gene polymorphism and selected morphological traits. RFLP technique was used to determine the polymorphisms in the DNA extracted from the blood samples.

Ethical approval:

The laboratory work was conducted at the Al-Musaib Technical College, Al-Furat Al-Awsat Technical University, with approval from the Animal Welfare Committee (Approval No. 7/37/5574, dated 20/10/2024).

The DNA Extraction:

A pair of primers was designed based on sequences reported by (8). were used to amp up the targeted area of the MSTN (Myostatin) gene. the reverse (R:TCA TGA GCA CCC ACA GCG GTC). and forward (F:CCG GAG AGA CTT TGG GCT TGA) primers.

PCR amplification was performed in a final volume of 25 µl. reaction mixture consisted of 5 µL of genomic DNA, 1 µL of forward primer, 1 µL of reverse primer, 5 µL of a commercially available master mix (Premix), and 13 µL of deionized water. All components were mixed gently and briefly centrifuged before being placed in the thermal cycler.

PCR This was done using the AccuPower® PCR PreMix kit amplification was performed using a thermal cycler under following conditions: initial denaturation at (95°C) for five minutes, followed by 35 cycles of denaturation at (94°C) for 30 seconds, annealing at (62°C) for 30 seconds, and extension at (72 °C) for one minute. To ensure that all of the PCR products had undergone complete extension, a final extension step was performed at (72 °C) for five minutes.

Electrophoresis of PCR Products:

The PCR products were analyzed by electrophoresis using a 2% agarose gel containing ethidium bromide. The gel was run at a constant voltage, and DNA fragments were visualized using a gel documentation system under UV transillumination.

Analysis of RFLP (Restriction Fragment Length Polymorphism):

To analyse genetic variation within MSTN gene, 10 µL of an amplified PCR product was treated with 1 µL of a specific restriction enzyme. Digestion reaction was carried out by incubating the mixture at (60 °C) for 30 minutes. The resulting DNA fragments were then separated by electrophoresis on a 3% agarose gel. A 100 base pair DNA ladder served as a molecular size marker to estimate the lengths of the digested fragments. The gels were stained with ethidium bromide, and the resulting band patterns were visualized and documented under ultraviolet light using a gel imaging system.

Statistical Analysis:

For data analysis , Spss has been used version 29.0. A chi-square test was performed to study relationship between the genetic constructs, and to compare biochemical parameters, a t-test was used, and the results were in the form of mean ± standard error (mean ± standard error), The results were expressed as the mean accompanied by the standard error (mean ± SE), and differences were considered statistically significant when the P-value was equal to or less than 0.05.

RESULTS AND DISCUSSION:

Figure (1): illustrates quantity and quality of DNA that was extracted from the samples.. After amplification by PCR, MSTN gene was successfully obtained with a length of 337 base pairs. PCR product was then digested using the restriction enzyme HaeIII, which resulted in two genetic patterns: the homozygous dominant genotype mm, producing fragments of 83, 123, and 131 base pairs, and the heterozygous genotype Mm, producing fragments of 121 and 123 base pairs, as illustrated in Figure (2). The results presented in Table (1) indicate that the mm genotype appeared in 86% of the samples, representing 37 individuals, while the Mm genotype was observed in 14% of the samples, representing 6 individuals. A frequency of m allele was calculated at 0.931, whereas M allele showed a frequency of 0.069.

This indicates presence of more than one genotype, as noted by (9) in their study on Awassi and Naeemi sheep, where they examined MSTN gene with a length of (337 base pairs). In accordance with findings of research, All three genotypes (MM, Mm, and mm) were identified in both breeds. These findings align with previous reports presented by (10) in their study on the Mehraban breed, in which they employed the same methodology used in the current research. Similarly, they identified two genotypes, mm and Mm, with mm genotype appearing in 94.7% of the samples and the Mm genotype in 5.3%.

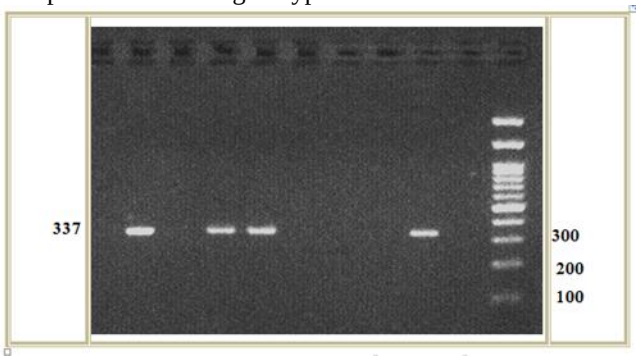

Figure 1. The DNA extraction

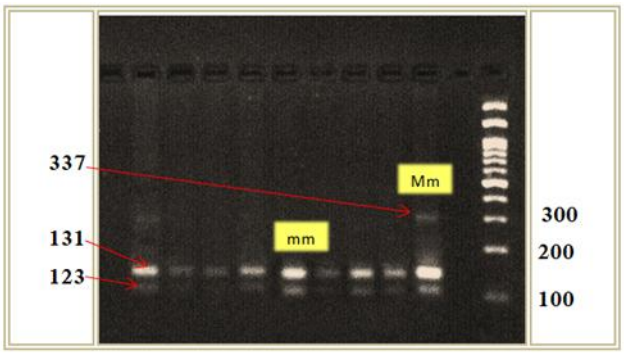

Figure 2. The products of restriction enzyme HaeIII digestion of the myostatin gene segment and the M100 DNA ladder were used.

Table 1. Allele and genotype frequencies for MSTN loci

Locus	Genotype		Allele		χ^2
	mm	Mm	m	M	
MSTN	%.86	%13	0.931	0.069	22.394 **
(P≤ 0.01) **					

The Effect of Myostatin Gene Genotypes on Carcass Weight:

Table (2): demonstrates that there were no significant between MSTN gene genotypes (mm and Mm) in terms of body weight before and after slaughter. The average body weight for animals with the mm genotype was 35.32 kg before slaughter and 17.3649 kg after slaughter, with a dressing percentage of 49.17%. before slaughter, average body weight for Mm genotype was (31.50 kg) and(15.5000 kg) after, with a dressing percentage of 49.2%. These results suggest that body weights were nearly identical between the two genotypes, which may indicate that the animals were raised under similar environmental and management conditions, or that this genetic variation at The MSTN gene variants showed no significant impact on growth-related traits in the local Iraqi sheep population.

The results of (11) showed that prevalence and effect of MSTN structures and alleles varies depending on the breed of sheep. Therefore, in some strains the Myostatin gene may represent a genetic indicator of developmental traits. This was confirmed in the study conducted by (12), who studied two breeds, Madras Red and Mecheri. Their research identified two genotypes—MM and Mm—in both breeds. However, in the Madras Red breed, significant differences were observed among two genotypes in terms of body weight at 9 and 12 months of age. No significant effects were found for genotype on birth and weaning weight (at three months), or six-month weight within the same breed. In contrast, for the Mecheri breed, there were no significant in average weights at birth, weaning, six, nine, or twelve months among the MM and Mm genotypes.

Results presented in Table (2) reveal a statistically significant difference $p \leq 0.05$ in internal organ weight between the two genotypes. average weight for the mm genotype was 1.4000 ± 0.063, whereas it was 1.0583 ± 0.6635 for the Mm genotype. This finding contrasts with previous studies and may be attributed to the imbalance in sample sizes(37) samples for the dominant mm genotype compared to only 6 for the heterozygous Mm genotype. Such a disparity in group size could affect the accuracy and reliability of the observed differences, particularly given the very limited number of samples in the Mm group. (13)in their study on Ramlıç breed investigated its crossbreeding with the BC1 line (a cross between Ramlıç and Texel), resulting in the BC2 line with 87.5% Ramlıç and 12.5% Texel genetic composition. Significant variations ($p \leq 0.05$) in liver weight were found, favouring Ramlıç breed. This may reflect a higher metabolic activity or a more efficient nutrient processing capability compared to the BC2 hybrid or Texel lines.

Table 2. Influence of myostatin gene constructs on carcass weights

Traits	Live Weight (kg)	Carcass Weight (kg)	Dressing percentage (%)	Viscera weight(kg)
Mm (mean±SE)	1,49±35.32	0.78±17.36	1.08±49.26	0.63±1.400
Mm (mean±SE)	3,16±31.50	2.05±15.00	2.24±48.56	0.66±1.058
Significance	N.S.	N.S.	N.S.	*
N.S. No- Significant , (P ≤ 0.05) *				

Influence of myostatin gene genotypes on carcass dimensions:

Results in Table (3) indicate that there were no significant among two genotypes in body length, height at withers, height at rump, chest circumference, or abdominal circumference. These traits have been investigated in several previous studies aimed at evaluating body dimensions, and while some studies reported differences, they were typically limited to specific traits.

For instance, (14) found no significant in body length among the different phenotypes. However, the effects of the myostatin gene appeared to be more pronounced in animals with the BB genotype compared to the AB genotype, apart of chest circumference, which was more strongly influenced by heterozygous AB genotype ($p \leq 0.05$). Similarly, (15), in their study on the Nanyang, Qinchuan, and Jishan breeds, reported significant differences ($p \leq 0.05$) in height at the withers at 18 months of age in the Jishan breed. Animals with AA genotype had significantly greater height compared to those with the AB genotype.

Table 3. Effect of Myostatin Gene Genotypes on Carcass Dimensions

Traits	Body length (cm)	Height at withers (cm)	Height at rump (cm)	Chest girth (cm)	abdominal girth (cm)
Mm (mean±SE)	1.1±66.519	0.8±67.240	0.8±68.626	1.4±84.273	1.9±92.242
Mm(mean±SE)	2.6±65.833	1.5±66.173	1.9±67.839	4.3±82.830	5.0±90.171
Significance	N.S.	N.S.	N.S.	N.S.	N.S.
N.S. No- Significant					

CONCLUSION:

No statistically significant differences were observed among the MSTN genotypes regarding body measurements such as body length, height at the withers and rump, chest circumference, abdominal circumference, live weight, or carcass weight. Although some numerical differences appeared among genotypes, these fell within the normal biological variation and did not reach statistical significance. Therefore, under the conditions of this study, the MSTN gene does not appear to have a significant direct effect on the evaluated production traits.

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