Study of the effect of different genetic patterns of the fifth expression region of the growth hormone gene on biochemical characteristics in local sheep

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Abstract

In this study, 50 Iraqi local sheep, males and females, were used and were selected at different ages ranging from 5 months to one and a half years for the period from November 2023 to December 2024 with the aim of determining genotype of the fifth expression region of the growth hormone and its relationship to growth hormone, thyroxine hormone, insulin-like growth hormone 1 and biochemical traits. Samples were taken from a local slaughterhouse outside the technical college and DNA was separated from frozen blood samples and the growth hormone gene was amplified using specific primers and then genetic profiling was performed using PCR-SSCP technology. The percentages of distribution of genotype in the sheep varied, as the two genotype of the growth hormone gene GG, AG appeared at an average of (62.22%, 37.78%) respectively, while no percentage of genotype of the growth hormone gene AA appeared. The genetic makeup difference did not significantly affect the traits measured in the study.

Introduction

Sheep are one of the important sources of livestock in Iraq and represent a large part of the national agricultural income. The number of sheep and goats, according to the Food and Agriculture Organization of the United Nations, is (9.350 million) (7). Sheep are known for their tolerance to harsh climatic conditions, their resistance to diseases, their tolerance to heat, and their meat quality, in addition to the consumer's preference for them. Therefore, animal breeders follow programs that aim to increase the animal's productive capacity by improving its genotype. However, the required time period is often long in animals such as sheep and may reach 4.5 years, while breeders can follow other methods to reach the goal in the shortest possible time. Among these methods is the use of early (indirect) selection. In order to continue improving local Iraqi sheep, it is necessary to update the methods of genetic improvement and study the genetic composition of these animals and choose the best of them, by studying the genes that affect growth and production traits and knowing the genetic mutations and linking them to the phenotypic composition using the polymerase chain reaction (PCR), technology SSCP (Single strand conformation polymorphism), which together help in studying the required genes and multiplying them in the laboratory and determining genotype of the fifth expression region of the growth hormone gene, as the pituitary gland secretes growth hormone, which shares with other hormones associated with general metabolism in the

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rapid activation of cell division and body growth and construction. It is a protein hormone that activates the growth of muscle cells by activating the intake of amino acids and protein synthesis. One of the most important biological functions of this hormone is to activate growth in general in the body. However, the main goal of this hormone is the growth of bones and muscles [1]. The GH gene is classified as a candidate for selection based on markers in various field animals [8]. Accordingly, the aim of the study is to know which genotypes of one of the growth hormone gene segments of the fifth exon region are more influential in the growth and biochemical characteristics of different ages of Iraqi local sheep using the SSCP technique. Single-strand Conformation polymorphism((

Materials and methods

This study was conducted in the laboratory of the Animal Production Department / Al-Furat Al-Awsat University / Al-Musayyab Technical College on a sample of 50 Iraqi local sheep, 25 for each season, where the first season was 12 males and 13 females, and the second season was 15 males and 10 females. Samples were collected from the slaughterhouse in two periods, the first in November 2023 and the second in December 2024. 5 ml of blood was collected from the jugular vein of sheep using a sterile medical syringe with a capacity of 5 ml and placed in a plastic test tube containing an anticoagulant (K2EDTA). To prevent blood clotting, the tube was rotated immediately after blood collection for one minute to mix the blood with the anticoagulant. After that, the animal several number was fixed on the tube and transferred to a laboratory cooler box to be frozen at (-20 C) until serum extraction and biochemical analyses were performed (laboratory side.(

DNA extraction and genetic measurements

.DNA extraction was conducted from blood samples for molecular testing of the GH gene. A Nanodrop device was used to measure the concentration and purity of genetic material in a volume of 1-2 microliters. Purity was measured at a wavelength of 260\280 and the results for the study samples ranged from 1.8 to 2.00, while the compositions ranged from 6.00 ng/microliter to 25.00 ng/microliter. The primers were forward CTGCCAGCAGGACTTGGAGC-3′-5′ and reverse GGAAGGGACCCAACAATGCCA -3′-5′ targeting amplification of a 200 base pair band within the fifth expression region of the growth hormone gene (GH). The gel was prepared at a concentration of 1%) to investigate the DNA after the extraction process and at a concentration of 2%) to detect the PCR reaction product and to determine the PCR-SSCP bands. where the bands appear, the PCR reaction of the studied gene

The samples were placed in the polymerase chain reaction device according to the reaction conditions for each duplicated gene fragment. After

The reaction was completed, the polymerase chain reaction product was transferred to ensure that the desired fragment was duplicated. The polymerase chain reaction was performed

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Table (1) Optimal conditions for growth hormone gene duplication

NO	Phase	Tm(C)	Time	NO.OF CYCLE
1-	Initial Denaturation	94 c	5 min	1cycle
2-	Denaturation-2	94c	30sec	35 cycle
3-	Annealing	60c	30sec	35 cycle
4-	Extension-1	72c	30sec	35 cycle
5-	Extension-2	72c	7 Min	1 cycle

Statistical Analysis

The data were analyzed statistically using the Statistical Analysis System –SAS (2018) program to study the effect of the GH gene genotypes on productive and growth traits, some hormones and blood traits. The significant differences between the means were compared using the [2] multinomial test by applying the least squares method.

Frequency of the first allele:

PA=(2 × No.of Homozygous + 1 × No.of Heterozygous)/(Total number of sample) × 2

Since: P + q = 1

Then the second allele replication is:

qB = 1 – PA

Results and discussion:

Duplicate a piece of the growth hormone gene

The required piece of the growth hormone gene Figure (1) was doubled using PCR technology according to the SSCP method and using the PCR kit, primers, and total DNA samples and adjusting the thermal cycler device, then the sample of 5 microliters from each sample was transferred in agarose gel at a concentration of 1% and the voltage was adjusted and the transfer result was photographed to ensure the success of the multiplication process and obtain the required piece with a size of 200 base pairs. The resulting genetic packages differed according to the different ratios of genotype of the fifth coding region Exon5 of the GH gene

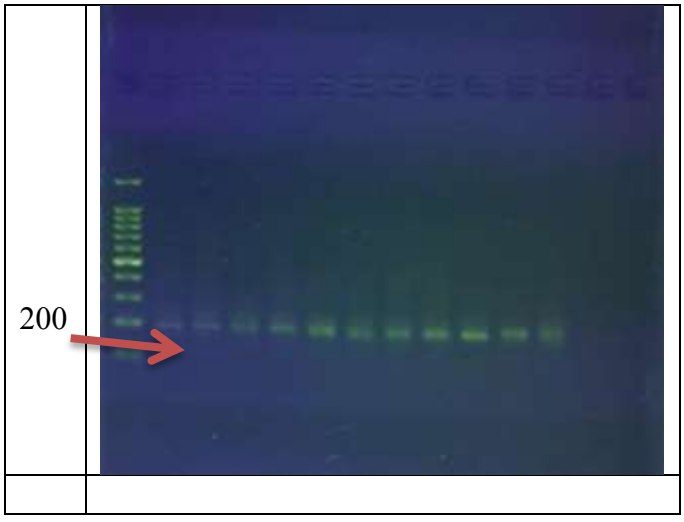

Figure (1): represents the targeted package with a size of 200 base pairs

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The results of Table (1) showed that there was no significant effect of genotype of the fifth expression region of the growth hormone gene on the content of insulin-like growth hormone. This result indicates Although this site may not clearly affect the levels of these hormones in local sheep, in fact, this result is consistent with the absence of a significant effect of the genetic makeup on weight traits, since the genetic makeup of the growth hormone gene must be reflected in the level of this hormone. The absence of a significant difference also indicates the presence of other influences that affect the level of these hormones, or perhaps other genetic sites other than this site. Among these influences is the weight of the individual at birth, and this trait clearly affects the level of thyroxine [4]Accordingly, it is believed that the birth weights and individuals in this study are close, which contributed to eliminating the differences between them and led to the absence of significance. Also, separating mothers at birth leads to a decrease in thyroxine in newborns [4]. This leads us to think about the effect of upbringing on the level of hormones, which may be a decisive factor, especially since the research samples return to one type of upbringing, which is the semi-intensive type.

Table 1: Relationship of the genotype of the growth hormone gene to the level of the studied hormones in local sheep

Mean ± Standard Error			numbers	genotype)Genotype
Insulin-like growth hormone 1) ng/ml(	Thyroxine hormone)ug/dl(	Growth hormone)ng/ml(		
3.67± 23.71	0.78± 12.57	0.04± 3.16	28	GG
4.68± 24.44	1.07± 12.06	0.07± 3.19	17	AG
N.S.	N.S.	N.S.	---	significance level
NS :nonsignificant.				

Relationship between genotype and biochemical traits in local sheep

The results in Table (2) indicate that there are no significant differences between the averages of cholesterol, glucose and total protein traits for the two genotype AG, GG, while the differences were significant at the level (P≤0.05) for triglycerides between the two compositions and the genetic composition AG. This indicates possible effects of the genotype of this site for the growth hormone gene on fat metabolism. The decrease in cholesterol (not significant) seems logical as it is associated with a decrease in growth hormone (not significant). However, the increase in the level of tissue fat with this logic, since studies have indicated a decrease in the level of fat, if the increase in growth hormone leads to an increase in metabolic

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rates and energy consumption, which leads to a reduction in the body and enhances its decomposition [5] and this will lead to a decrease in the level of fat from the body due to its use to provide energy [9]. The results also showed that the increase in protein in the AG genetic structure (not significant) is associated with a decrease in growth hormone, and this relationship is expected because the increase in growth hormone leads to an increase in the breakdown and formation of new proteins [7]. Accordingly, the insignificant decrease in protein in the blood is consistent with the insignificant decrease in growth hormone, as the increase in glucose (not significant) also came associated with the increase in growth hormone, as it was higher in the GG genetic structure than it is in the AG genetic structure, as a result of the decomposition of glycogen in the liver and kidneys. [4]stated that Patients who take high doses of growth hormone have a high increase in the activity of gluconeogenesis in the liver and kidneys. The results in Table () showed a significant effect on the creatinine trait, as it increased significantly in the AG (1.132) structure than in the GG (0.925) genetic structure. It is noted that growth hormone also increased in the GG genetic structure, and although this increase was not significant, it seems that it clearly affected the level of creatinine. If the increase in growth hormone increases muscle mass and thus affects creatinine indirectly, leading to its increase [8[The results in Table () also showed no significant differences between the average compressed cell volume trait for both genotype, while there were significant differences ($P \leq 0.05$) between the averages of the hemoglobin trait in favor of the AG genetic structure compared to the GG genetic structure. The presence of significant differences in the hemoglobin trait indicates its importance in Supplying the body with the necessary oxygen for growth, so the relationship between hemoglobin and growth hormone is close, and accordingly we find that an increase in hemoglobin is accompanied by an increase in growth hormone (not significant), as growth hormone stimulates bone growth and stimulates it to produce red blood cells inside the bone marrow due to the body's need for oxygen, which leads to an increase in their size and numbers [6]. The size of the packed cells is also affected by the same thing as hemoglobin, as the relationship between them is positive, and hemoglobin represents a third of the size of the packed cells [10] In general, the biochemical characteristics were a logical reflection of the hormone levels in this study.

Table (2) Relationship between genotype and biochemical traits in local sheep

Mean ± Standard Error								
Hemoglobin) g/dl(	compressed cell volume (%)	Creatinine)mg/dl(	Total protein)g/dl(	Glucose)mg/dl(	Triglycerides) mg/dl(	Cholesterol) mg/dl(	numbers	genotype
13.78	44.03	0.925	6.25	88.31	18.51	39.52	28	GG

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0.29±	1.20±	0.06±	0.26±	6.22±	1.24±	2.47±		
14.38 0.46±	44.70 1.79±	1.132 0.08±	6.47 0.32±	94.02 6.72±	22.43 1.69±	37.48 6.52±	17	AG
*	N.S.	*	N.S.	N.S.	*	N.S.	---	significance level
) *P≤0.05 ،(NS :non significance								

Conclusions

The GG genotype in the fifth expression region of the growth hormone gene was the most common among the studied local Iraqi sheep, while the lowest was the AG genotype, and the absence of the AA genotype. The biochemical characteristics were a logical reflection of the hormone levels in this study, as well as the effect of breeding on the hormone level, which may be a decisive factor, especially since the research samples belong to one breeding pattern, which is the semi-intensive pattern.

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