Impact of growth hormone receptor gene on measurements of dimensions and body weights of local Iraqi sheep

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Abstract

This research involved a sample of 50 local Iraqi sheep, consisting of 27 males and 23 females, aged between 5 and 17 months. Data collection occurred during two distinct seasonal periods: October 2022 and January 2023. The primary objective was to investigate the variations in the expression of the growth hormone receptor (GHR) and its influence on the physical attributes of local Iraqi sheep within this age range. The results showed that the GHR gene exhibited a marked increase in expression during the second seasonal timeframe with levels of 23.8 ± 0.2 and 29.7 ±1.4 for first period. This increase in gene expression was paralleled by substantial differences in the sheep's body weights, with values of 33.7376±0.732(kg) for the first seasonal period and 35.77±1.111(kg) for the second seasonal period. Similarly, the dressing percentage showed significant differences, with values of 47.71±0.49 (kg) for the first period and 50.11±0.59 (kg) for the second period. Furthermore, carcass weight demonstrated highly statistically significant variations, measuring 16.037±0.440 (kg) during the first seasonal period and 17.978±0.655 (kg) during the second seasonal period. Gender-based analyses revealed significant disparities, with males exhibiting greater weight measurements (kg) (36.764±1.002) compared to females (32.122±806.0). The same trend was observed in carcass weight (18.362±0.606 for males and 15.242±0.369 for females), dressing percentage (49.8±0.0062 for males and 47.8±0.0047 for females), and viscera weight (1.382±0.029 for males and 1.258±0.032 for females). In addition to these findings, body dimension measurements (cm) showed significant differences in lump height, with measurements of 67.81±0.891 in the first period and 64.88±0.717 in the second period. However, non-significant differences were observed in other body dimension measurements. These findings have implications for understanding the genetic factors affecting the growth and development of sheep which can inform breeding and livestock management practices.

Keywords: Growth hormone receptor gene, gene regulation, sheep growth, body weight

Introduction

The productive performance of Iraqi sheep faces many difficulties due to the lack of natural pastures and their exposure to heat stress resulting from high and low temperatures in the region, as well as due to the lack of use of genetic improvement methods [1]. Modern genetic improvement methods increase the productive capacity of animals, including early (indirect) selection after evaluating animals at early ages for some traits that can be used as indirect indicators of selection for economic traits [2]. The increase in weight reflects the production of meat according to the animal's genetic characteristics, and the body's weight and dimensions at different ages may indicate good growth, the ability to fatten, and the possibility of benefiting from it in breeding processes and genetic improvement of animals. Growth hormone receptors (GHR) have an important role in regulating growth and development in sheep, and variations in the GHR gene have been linked to differences in body dimensions and weights. Research has shown that some genetic variants of GHR are associated with increased body weight and increased body dimensions in sheep, The relationship between GHR genetic variants and body dimensions and weights is influenced by a range of factors such as breed, age, and gender [3]. In addition, the GHR gene is one of several genes that contribute to the growth and development of sheep. In general, variations in the GHR gene can be associated with differences in body dimensions and weights in sheep, but more research is needed to understand the mechanisms behind these associations and their practical applications for sheep breeding and production. The study aimed to determine the effect of season and gender on the gene expression of the growth hormone receptor gene and various growth traits in local Iraqi sheep.

Materials and Methods

The study was conducted in the Physiology Laboratory of the Department of Animal Production Technologies/College of Technology/Al-Mussaib. In cooperation with a private company for biological studies / Baghdad. The study involved the collection of 50 samples of local Iraqi sheep belonging to the same region, which were collected from the Mahmoudiya slaughterhouse/Baghdad with ages ranging between (5-17 months) samples were divided into two seasons timeframes of 25 for each one (1/10/2022 to 10/10/2022 and 1/1/2023 until 10/1/2023). The sex and age of the animal were recorded, and the animal’s live weight was measured along with the carcass weight and the weight of the eaten viscera using a digital scale(kg). The body dimensions represented by body length, height at withers, lump height, heart girth, and loin girth were measured using a graduated tape(cm).

Genetic analysis, a 1 mm biopsy of liver tissue was taken and placed in a test tube containing a preserving fluid (DNA/RNA Shield™, etc.). Next, samples were transferred to the laboratory to conduct the extraction process and determine the level of gene expression by applying real-time PCR (RT-PCR).

Extracting ribonucleic acid (RNA) and studying the gene. by Using the RNA Mini Prip™ RNA extraction kit (Direct-zol) from ZYMO / USA, and following the instructions in the leaflet accompanying the kit to extract RNA from liver tissue samples. Using the NanoDrop device, the concentration and purity of ribonucleic acid (RNA) were measured by adding 2 microliters of the extracted sample to the device. The concentration and purity were determined at 260/280 nm wavelength. The acceptable ratio for the concentration and purity of RNA is between 1.8 and 2.0 ng/microliter [4]. Using a special preparation kit (Prime Script TM mix), RNA was converted into complementary DNA (cDNA). Then RT-PCR technology was applied. and Using primers to directly measure the expression of the growth hormone receptor (GHR) gene.
F: TTCTGGGAATCCTAAATTCACCAA

R: CTGTAAACTGTGATTAGCCCATC

Statistical analysis

Statistical analysis tool was performed using SAS to examine and interpret the data. The quantitative data were presented as mean ± SE, with confidence intervals of 95% or less were considered significant. Independent sample t-test was used to compare the mean values among the different studied groups.

Results and Discussion

Gene expression

The results of the study revealed a highly significant difference ($P \leq 0.01$) in the direct expression of the growth hormone receptor gene as the level of expression in the first period was lower (higher number of cycles threshold CT than in the second period) 29.7 ± 1.4, and 23.8 ± 0.2 respectively. This was accompanied by a highly significant increase ($P \leq 0.01$) in the direct expression of the comparison protein gene (RPL19, housekeeping gene), as the expression level was higher in the second period (21.3±0.3) than in the first period (24.524±0.9). For the relative expression, the results showed a significant increase in the second period compared to the first period (6.896 , 1) as shown in (table 1). The decline in gene expression noted during the initial seasonal period, which correlated with increased temperatures, indicates a potential suppressive impact of heat stress on gene expression. It is well-known that elevated temperatures can have adverse effects on gene expression, which may subsequently affect the body weights of the local sheep [5]. Additionally, the reduced gene expression during the first period might also be linked to limited feed availability, as this period is known for its dry conditions

Table (1): The effect of gender and period on gene expression, weights, and body dimensions

Effect	Seasonal timeframes		p-valu	Gender		p-value
	First	second		male	female	
Target gene (GHR)	29.7 ±1.4	23.8 ±0.2	**	26.3 ±1.02	26.5 ±1.2	NS
Reference gene (RPL19)	24.5 ±0.9	21.3 ±0.3	**	22.2 ±0.5	23.4 ±1.08	NS
$2^{\Delta ct}$	0.025	0.172		0.0581	0.119	
Relative expression	1	6.896		1	2.052	
Live animal weight (kg)	33.7376 ±0.732	35.77 ±1.111	*	36.764 ±1.002	32.122 ±806.0	**
Carcass weight (kg)	16.037 ±0.440	17.978 ±0.655	**	18.362 ±606.0	15.242 ±369.0	**
dressing percentage (kg	47.71 ±0.49	50.11 ±0.59	*	49.8 ±0062.0	47.8 ±0047.0	**
viscera weight (kg)	1.351 ±0.035	1.322 ±0.031	NS	1.382 ±029.0	1.258 ±032.0	**
Body length (cm)	58.86 ±1.306	59.44 ±0.638	NS	59.22 ±699.0	58.39 ±1.208	NS
Height at withers (cm)	64.81 ±0.830	63.28 ±655.0	NS	64.56 ±548.0	62.72 ±949.0	NS
Lump height (cm)	67.81 ±891.0	64.88 ±717.0	*	66.48 ±590.0	65.44 ±1.194	NS
Heart girth (cm)	84.38 ±1.720	83.96 ±1.051	NS	84.11 ±1.183	83.67 ±1.641	NS
Loin girth (cm)	90.38 ±2.341	88.80 ±1.237	NS	89.04 ±1.769	89.83 ±1.796	NS
(p≤0.01)** (p≤0.05)* NS (Non-significant)						

Regarding to gender analyses, the obtained results indicated that there were non-significant differences in the level of direct expression of GHR gene and (RPL19) (the housekeeping gene), while the relative expression of the GHR was higher in males than in males (Table 1). The increase in GHR expression is attributed to the direct stimulation of elevated growth hormone levels in females. This stimulation is influenced by the presence of progesterone and estrogen, which are known to promote the growth of follicles [6,7]. As a result, it's expected that the number of growth hormone receptors within these follicles will also increase to facilitate the growth process. Notably, the regulation of GHR expression is not only influenced by steroid hormones but also by factors such as nutritional status and physical growth stages, reflecting how it governs cell sensitivity to growth hormones [8].

Body weights

The results of the analysis revealed that there was a significant difference ($p \leq 0.05$) during the first and second periods for both the live animal weight (33.7363±0.732 for the first period and 35.77±1.111 for the second period) and for the dressing percentage (47.71±0.49 for the first period and 50.11±0.59 for the second period). As well, there was a height significant difference ($p \leq 0.01$) for carcass weight of 16.037±0.440 and 17.978±0.655 for the first and second periods respectively (table 1). Moreover, there were non-significant differences observed in the viscera weight. This result is consistent with the clear difference in gene expression during the second period, which affected weight traits. Although nutrition depends mainly on agricultural waste, and grazing on crop residues, Studies also indicate that the months of birth have an impact on the weight of the animal, as those born in the cold months (December and January) outnumber those born in the months (February to October). The reason may be due to the environmental conditions and heat stress in this period and the lack of nutrition in this period [9].

The gender analysis revealed a significant difference ($P \leq 0.01$) for the animal weight, carcass weight, and viscera weight for males compared to females (table 1). The reason may be due to the intermittent secretion of the male growth hormone and the Stat5 factor. In general, IGF-1 secretion is controlled by the Stat5 compound under the influence of growth hormone. In rodents, male-pattern (intermittent secretion) growth hormone secretion is more potent in promoting body growth and IGF-I expression, STAT5b activity in the liver, compared to the continuous secretion characteristic of females. When Stat5B activity decreases, it leads to a reduction in male body weight, while females are less affected by this decrease. a simultaneous reduction in both Stat5a and Stat5B in females significantly impairs growth compared to males. Stat5B plays a crucial role in male growth, whereas Stat5a regulates growth in both sexes [10]. It is worth noting that there are many factors that affect the STAT5a and STAT5b, including the factors of glucocorticoid that act as an effective agent (CO ACTIVTAR) with Stat5b in the liver [11]. Estrogen and its receptors overlap with Stat5a [12]. Also, the decrease in the SOCS2 factor leads to the support of the Stat5B, which plays an important role in organizing the growth hormone for the growth of physical cells [13].

Body measurements

The results of the study revealed non-significant differences in the length of the body, heart girth, lion girth, and the height at withers for the first and second periods(Table1). while for the lump height, the result showed significant difference (67.81 ± 891.0) for the first period and (64.88 ± 7170) for the second period. this may be attributed to the difference in the pattern of nutritional and veterinary care, especially since the animals in each period were distributed according to their weights and ages in a consistent manner. Since several studies were conducted to measure the dimensions of the body at the age of weaning, and with this the differences were limited to certain qualities compared to the results of the current study. The results of the current study are consistent with the study conducted by (Ahmed Ali et al., 2015) [14]. it was found the Height at withers, lump height, heart girth, lion girth, and length of the body in the weaning of the breeder, 59.55, 60.44, 73.55, 83.72 and 59.22, respectively.

The results based on gender revealed that there are non-statistically significant differences in the body's measurements represented by the length of the body, its height at withers, lump height, heart girth, and lion girth. This result appears to be not consistent with the existence of significant differences in the animal weight, the weight of the carcass, and the weight of the viscera. The measurements of the dimension of the body for the benefit of males despite the absence of significant difference and the weight of the animal is the result of all the contents of the body, but if we take dimensions for each individual adjective, it may not accurately express the mass of the body. The outcome of all body measurements, we will get the difference in weights between females and males. and the reason may be due to the superiority of males in the amount of muscle density (muscularity), which is considered one of the measurements of the body that is likely to be one of the reasons for the superiority of weight despite of non-significant differences in dimensions, because the testosterone hormone in males increases the muscle mass and bone density [15]. Studies also revealed that sheep treated with testosterone had a higher bone weight than sheep not treated with testosterone. [16]. the dimensions of the body are a measure of the amount of muscular development in the animal and a complement to the measure of weight as a standard for productivity [17].

This study observed notable seasonal variations in the growth hormone receptor (GHR) gene expression in local Iraqi sheep. GHR expression increased significantly during the second seasonal period, resulting in marked differences in body weights, dressing percentage, and carcass weight. Gender-based analysis revealed consistent male sheep's higher weights, influencing carcass weight, dressing percentage, and viscera weight. Specific body dimensions, such as lump height, showed significant differences between the two seasons. These findings underscore the crucial role of genetic factors in shaping sheep growth and development, offering practical insights for optimizing breeding and livestock management practices.

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