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Studying the Influence of gender and seasonal period variations on the Growth Hormone Receptor Gene and other biochemical blood parameters for local sheep

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Abstract

This study is aimed to investigate the influence of distinct variation of seasonal periods (October 2022 and January 2023), as well as gender disparities within a cohort of 50 local Iraqi sheep including 27 males and 23 females, aged (5 and 18 months). The primary focus was to establish the impact of these variables on the expression of the growth hormone receptors gene (GHR), growth hormone, thyroxin, and some biochemical components. The obtained results revealed a significant variation (P≤0.01) in the relative expression of growth hormone receptor gene GHR (1 for the first period and 6.896 for the second period). Notably, highly significant differences (P≤0.01) were observed in growth hormone (2.962 ± 0.0387 and 3.451 ± 0.016) and thyroxine (8.865±696.0 and 15.841±0.31) for the first and second periods respectively. Further analysis for seasonal periods disclosed significant difference (P≤0.01) in glucose, total protein levels, triglycerides and packed cell volume. Additionally, gender based analysis did not exert any significant impact in the direct expression (cycle threshold CT) of both the GHR gene and the housekeeping gene (ribosomal protein L19) as well as in both growth hormone and thyroxine levels. Gender investigation also exhibited non-significant effect on any of the studied biochemical blood parameters. These findings collectively illuminate that gene expression increased in the second seasonal period with GHR expression, growth hormones and thyroxine. While the levels of total protein, triglycerides, and glucose decreased in the second period than they were in the first period as well as gender analysis showed non-significant difference between all studied parameters.

Keywords: *Growth hormone receptor, gene expression, seasonal period, biochemical components*

Highlights:

1. Seasonal periods effect on gene expression of growth hormone receptors
2. Seasonal periods and growth hormone and thyroxine levels
3. Seasonal periods effect on some metabolic biochemical parameters
4. Real time PCR assay was used as a more modern and accurate technique.

Introduction

Sheep are considered one of the important pillars in the field of animal production due to their contribution to the production of meat and milk (1). the productive capability of Iraqi sheep encounter several challenges stemming from the reign's high temperatures causing stress, and the lack of natural pastures, as well as due to the limited adoption of modern technologies in genetic enhancement initiatives (2). The capability of an animal to show growing at different age stages, should be indicated by knowing the levels of growth hormone in the animal's body. Also, investigating how the growth hormone gene and the growth hormone receptors are expressed which is vital to their growth and development. In addition to above, investigation of some biochemical parameters and their associations might be another insights affected the growth of that animals. It is well known that growth hormone (GH) regulates postnatal growth, metabolic processes, and cell division. Moreover, stimulates bone and cartilage growth, and the growth of muscle and visceral tissue (3). Growth hormone, along with thyroxine, plays a key role in accelerating the metabolism (4). Biochemical blood components are also an important to assess the health status of the animal are also affected depending on the physiological state of the animal (5). This study was aimed to determine the effect of gender and seasonal periods on the level of gene expression for the growth hormone receptor gene, growth and thyroxin hormones levels, along with some other biochemical parameters.

Materials and methods

The study was conducted at the Physiology Laboratory of the Department of Animal Production Techniques/Al-Mussaib technical college. It was carried out In collaboration with the Wahaj Al-Dana Company for Biological Studies / Baghdad. The study involved the collection of 50 samples for local Iraqi sheep that obtained from the Mahmoudiya/Baghdad slaughterhouse. Samples were divided into two seasonal periods with 25 samples for the first period (1/10/2022 until 10/10/2022), and other 25 samples for the second period (1/1/2023 until 10/1/2023). For each sample, 5 ml of blood was collected from the jugular vein using a 5 ml sterile medical syringe, and 2 ml of blood was placed in a test tube containing an anticoagulant (EDTA) to prevent blood clotting and to measure the packet cell volume (PCV) and hemoglobin (Hb) and the rest of 3 ml blood was placed in a test tube (gel tube) and to extract serum in order to measuring hormones and biochemical compounds. for gene expression study, 1 mm biopsy of liver tissue was taken from animals and preserved in a test tube containing (DNA/RNA Shield™, etc.). Next, samples were transferred to the laboratory to handling the extraction process and

determine the level of gene expression using the real time PCR (RT-PCR). For the genetic study and RNA extraction, the process of extracting RNA from liver tissue samples was done using an RNA Mini Prip™ RNA extraction kit (Direct-zol) from ZYMO / USA, following the instructions in the leaflet accompanying the kit. The concentration of RNA was measured using the Nanodrop device (2 ul of the sample in the nanodrop device) the concentration of RNA is determined in ng/µl, while the purity is measured using a ratio of 280/260 O.D. (optical density) to determine the concentration of RNA, as the acceptable ratio for a reading of 280/260 is 1.7-1.9 (6). The ribonucleic acid (RNA) was converted into complementary nucleic acid (cDNA) using the special preparation kit (Prime Script TM mix), then RT-PCR technology was applied to measure the direct expression of the growth hormone receptor (GHR) gene using the following primers.

F: TTCTGGGAATCCTAAATTCACCAA

R: CTGTAAACTGTGATTAGCCCATC

In addition, biochemical analyses were performed using kits (Human Kit, Germany) to measure the level of thyroxine, glucose, cholesterol, total protein, and triglycerides. While for growth hormone measurement, A kit from VEDA-LAB, France was used to determine the level of growth hormone concentrations by follow the steps in the leaflet that attached to the issue.

Statistical analysis

Statistical analysis were performed using Spss ver 26 and SAS to examine and interpret the data. Data were expressed as mean ± SE, unpaired t-test was used to compare the mean values among the different studied groups. A p-value of ≤ 0.05 was considered significant.

Results

The results of the study indicated that there was a highly significant difference ($P \leq 0.01$) in the direct expression (CT) of the growth hormone receptor gene between the two studied seasonal periods, the first period was lower, represented by a higher number of threshold cycles than in the second period (29.790 ± 1.40, 23.856 ± 0.20) respectively. Also, the obtained results revealed a highly significant increase ($P \leq 0.01$) in the direct expression of the housekeeping gene (RPL19), as the expression level was higher in the second period (21.320±3340.) than in the first period (24.524±0.960) as shown in Table 1. For the relative expression, the results showed a significant increase in the second period compared to the first period (6.896 , 1).

Table (1): The effect of gender and seasonal period on growth hormone receptors gene expression, growth and thyroxin hormones, and some biochemical blood parameters.

Parameters	Seasonal Period		p-value	Gender		p-value
	First period	Second period		male	female	
Target gene (GHR)	29.790 ±1.40	23.856 ±0.20	**	26.378 ±1.0206	26.528 ±1.2507	NS

Reference gene (RPL19)	24.524 ±0.960	21.320 ±0.334	**	22.274 ±0.5296	23.461 ±1.0803	NS
$2^{\Delta ct}$	0.025	0.172		0.0581	0.119	
Relative expression	1	6.896		1	2.053	
Growth hormone	2.962 ±0.0387	3.451 ±0.016	**	3.165 ±0.0557	3.320 ±0.0597	NS
Thyroxin hormone	8.865 ±0.696	15.841 ±0.31	**	12.623 ±0.8177	12.7024 ±1.066	NS
cholesterol	34.269 ±5.119	37.608 ±1.587	NS	33.997 ±2.448	36.868 ±4.617	NS
triglycerides	21.433 ±1.516	17.426 ±1.488	*	19.512 ±1.447	18.570 ±1.779	NS
glucose	68.706 ±4.728	120.67 ±6.101	**	90.389 ±6.205	105.443 ±10.302	NS
Total protein	6.876 ±0.348	5.649 ±0.15	**	6.078 ±0.239	6.228 ±0.318	NS
PCV	42.57 ±1.811	46.36 ±0.959	*	42.85 ±1.336	43.9 ±1.512	NS
HB	14.162 ±0.602	14.008 ±0.287	NS	13.456 ±0.353	14.06 ±0.343	NS

($p \leq 0.01$)** ($p \leq 0.05$)* NS (Non-significant)

In terms of gender, the obtained results indicated that there was non significant difference in the cycle threshold level (CT) of direct expression of both the growth hormone receptor gene and the comparison gene, while the relative expression of the growth hormone receptor gene was higher in females than in males (Table 1). For growth hormone and thyroxine, the results of the study demonstrate a highly significant difference (with a p-value of ≤ 0.01) in the levels of both growth hormone and thyroxine between two distinct time periods. Specifically, during the first period, the average concentration of growth hormone was measured at 2.962 ± 0.0387, while in the second period, it rose to 3.451 ± 0.016. In terms of thyroxine levels, they increased from an average of 8.865 ± 696.0 in the first period to 15.841 ± 0.31 in the second period (table 1). The results also indicated that there is non-significant difference ($P \leq 0.05$) in the level of growth hormone and thyroxine between male and female of studied groups. For Biochemical characteristics of blood, the results of the study show a notable difference ($P \leq 0.05$) in various biochemical parameters between the first and second seasonal periods. Specifically, the triglyceride levels were 21.433±1.516 during the first period and decreased to 17.426±1.488 in the second period. Likewise, total protein levels were 6.876±0.348 during the first season and decreased to 5.649±0.15 during the second season. while glucose showed a decrease in his level

to reach 68.706±4.728 in the first season and increase to 120.67±6.101 in the second period. The same is true for packet cell volume as the level of the first period reached 42.57±1.811 and the level of the second period reached 46.36±9590. Moreover, results indicated that there was non-significant difference in terms of cholesterol and hemoglobin levels between the first and second seasonal periods

Discussion

The decrease in gene expression for the first seasonal period. might be due to heat stress in that timeframe since the first period was accompanied by a rise in temperatures. This heat stress might have inhibited gene expression, ultimately leading to weights being affected. The weight gain of animals is directly influenced by the fattening season, as the elevated temperatures during excessively hot months diminish the animals' appetite and their willingness to consume feed (7). Also, the decrease in gene expression in the first period may be related to the lack of available feed in the first period because it is known to be a period of drought.

In terms of gender, the obtained results indicated that there was non significant difference in the cycle threshold level (CT) of direct expression of both the growth hormone receptor gene and the comparison gene, while the relative expression of the growth hormone receptor gene was higher in females than in males (Table 1). This increase in gene expression of growth hormone receptors may be the result of direct stimulation of an increase in growth hormone in females, which is affected by the hormones progesterone and estrogen to cause follicular growth (8). Therefore, it is expected that growth hormone receptors will increase in the follicles for the purpose of completing the growth process in these follicles. The process of transcription and translation of growth hormone receptors is not only affected by steroid hormones but also by the level of nutrition and stages of physical development, which is reflected in the regulation of the sensitivity of cells to growth hormones (9).

for the hormones The significant increase in the level of growth hormone in the second period may be attributed to a decrease in heat stress since the second period was in the season of low temperatures (growth hormone may be affected by multiple factors such as changes in the length of periods of solar radiation, the role of solar radiation in regulating hormones, and changes in sleep patterns and physical activities between the summer and winter seasons (10), The lower temperature in the second period also led to an increase in the secretion of the hormone thyroxine, as studies indicate that its secretion increases to its maximum levels in the winter due to the cold weather and decreases to its lowest level in the summer due to the high temperature (11). In fact, there are many factors that affect the level of thyroxine, including age and weight. It has been found that the level of thyroxine is significantly related to the individual’s birth weight (12). Also, separating a young newborn from its mother after birth reduces thyroxine in newborns compared to newborns that remain with mothers (13). Studies revealed that there are non-significant differences between the sexes in young goats, but they are higher in adult females compared to males (T4 was significant) (9). In cashmere goats, T3 levels were lower in

females compared to males after 8 months of age. As for the T4 level, there were non-statistically significant differences between the sexes (14).

For Biochemical characteristics of blood The decreasing in the level of triglycerides and cholesterol (non-significant) it seems logical, as it is widely known that elevated level of growth hormone can contribute to decrease triglycerides and cholesterol in the body. This could affect it is attributed to the heightened metabolic rates and increased consumption in energy associated with elevated level of growth hormone which ultimately leads to reduction in levels of that substances. Also growth hormone enhance fat breakdown and decomposing from fat cells (15,16). As for total protein, studies have indicated that increasing growth hormone can increase the conversion (turnover) of some existing proteins and enhance their breakdown and formation of new proteins, thus affecting and reducing the levels of some proteins, such as total proteins and provide the body with energy, amino acids and maintain the process of growth (17). Moreover, it was observed that PCV level increased in the second season this may be due to the increase in growth hormone, which contributes to stimulating and growing red blood cells in the bone marrow which that need for tissues and the body for oxygen and thus leads to an increase in their size (18,19). The results also indicate that there is non-significant difference in other studied biochemical parameters between males and females which might be attributed to the influence of nutrition, environment, seasonal periods, and the health status of the animal.

Conclusions

This study clearly indicates an increase in the gene expression of the growth hormone receptor gene in the second seasonal period (January) growth hormones and thyroxine than in the first seasonal period (October). Therefore, the possibility of the animal growing faster in the cold months (the second seasonal period) and must be utilized with attention to graze the animal and providing good quality feed to help the animal grow faster. While the levels of total protein, triglycerides, and glucose decreased in the second period compared with the first seasonal period. The results also clearly showed the gender-based analysis showed a non-significant difference between all studied parameters.

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Conflict of interest

There is no conflict of interest.

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