



Relationship of some metabolic Hormones with Increased Live Body Weight of Male and Female of Karakul and Awassi Lambs

Thamer R. S. Aljubouri¹, Firas M. Al-Khafaji¹, & Mohammed Baqur S. Al-Shuhaib^{1*}

¹Department of Animal Production, College of Agriculture, Al-Qasim Green University, Al-Qasim, Babil 51001, Iraq.

*Corresponding author: mohammed79@agre.uoqasim.edu.iq

Received 17 March 2021; Accepted 26 May 2021; Available online 3 November 2021

Abstract: This study was conducted in the animal field of the Al-Kafeel Company from November 2019 till May 2020 to investigate the possible association between growth hormone (GH) and thyroxine (T4) with the growth traits in Awassi and Karakul sheep. The total number of animals which used in the study was 60 lambs, 28 (13 males and 15 females) from Karakul and 32 (18 males and 14 females) from Awassi. Blood samples were collected at birth, weaning, and six months of age, and both GH and T4 concentrations were measured. Results showed higher values of T4 for Karakul as compared with Awassi at birth and weaning. Karakul breed was also exhibited significantly higher values of GH over Awassi breed only at weaning, while, no significant differences were observed at birth and six months of age. Karakul lambs showed higher weights as compared with Awassi lambs at all studied periods. A highly significant ($p < 0.01$) positive correlation was observed between T4 concentration and the weight of lambs at most studied periods. Whereas, GH did not exhibit any correlation with growth traits measured in both breeds. The elevated T4 might be one of the reasons for superiority of Karakul over Awassi breed in live body weights. This high correlation between T4 and growth traits could be used in the early selection of lambs to improve the weights of sheep at marketing.

Keywords: Growth hormone, Growth traits, Sheep, Thyroxine Hormone, weight improvement

Introduction

The functions of the thyroid gland and its secreted hormones in small ruminants are essential to maintain the productive performance in sheep, such as growth, milk, and wool production. The circulating thyroxine (T4) and growth hormone (GH) can be considered as key indicators of metabolic activity, nutritional status, and livestock production (Todini *et al.*, 2007; Oberbauer, 2015). It is well known that both T4 and GH

have taken variable roles in regulating the balance of energy and protein metabolism, thermoregulation, and growth parameters in various tissues (Lopez-Rodriguez *et al.*, 2020). Sheep breeding has great economic importance in the Middle East in such a way sheep industry has become the main source of income for breeders in these regions. However, the growth of lambs is affected by many factors, including genetic factors, the birth season, the age of

dams, the sequence of birth, the sex of lambs and the weaning period (Jawasreh *et al.*, 2019). Improving sheep's growth traits is the main concern of many researchers, and growth trait is the most important economic factor in animals, which is controlled by several genes (Aljubouri *et al.*, 2020b; Imran *et al.*, 2021). One of the most prevalent sheep breeds in Iraq is Awassi, with 58.2% of prevalence over other breeds, such as Arabi (21.8%) and Karadi (20%) (Aljubouri & Al-Shuhaib, 2021). In addition, the Awassi sheep have the ability to survive and breed in conditions of drought and extreme climatic fluctuations (Al-Thuwaini *et al.*, 2020). Although Awassi sheep have a fat tail and fleece characteristics suitable for carpet, they have slower growth, lower fertility, and lower milk production than the other surrounding breeds in the region, such as Karakul and Assaf (Halil & Özbeyaz, 2020; Almahdawi & Altalib, 2020). However, Awassi sheep is a genetic wealth of great importance in the sheep industry in more than 30 countries in the Middle East as well as around the world (Al-Shuhaib *et al.*, 2019). Improving the productive performance of Awassi sheep under traditional breeding conditions faces difficulties resulting from nutrition deficiency due to the lower natural pastures, the spread of diseases, and exposure to heat stress conditions resulting from high or low temperatures (Ajam *et al.*, 2019).

The Karakul sheep is a middle-sized and chunky tail sheep that has been dominated in the majority of Iranian regions. This breed is being widely distributed in many other Asian countries. This breed is highly resistant to harsh environmental conditions and can be raised to produce milk, meat, and fiber (Ferro *et al.*, 2017). Noteworthy, Karakul sheep has a long

breeding season, with the possibility of giving three births in two years (Aljubouri *et al.*, 2020a). Recently, the Iranian Karakul sheep have been imported to Iraq and raised alongside Awassi in the same breeding stations. Hence, it is necessary to compare the metabolic performance of both breeds using two of the most important metabolic hormones as indicators for comparison. Considering the importance of metabolic T4 and GH hormones in improving the live body weight performance of sheep, the study aims to investigate the association between both hormones with the main growth traits of Awassi and Karakul sheep.

Materials & Methods

Animal resources

This study was conducted in the animal field of the Al-Kafeel Company (the Abbasid shrine, Barakat Aba Al-Fadl station, Karbala province) during the period extended from 1/11/2019 to 30/5/2020. This station was situated at a longitude of 32.6027° N, the latitude of 44.0197° E, and 32 m above mean sea level. The experiments were included a total of 60 lambs, 28 (13 males and 15 females) lambs of Awassi sheep, and 32 (18 males and 14 females) lambs from the Iranian Karakul. Each included lamb was numbered during the birth months (November and December, 2019). Ewes were isolated with their newborns after birth in places designated for the experiments. All lambs were placed during the first three days under observation to ensure that they suckled and consumed colostrum. After being numbered, the body measurements were conducted following the protocol described by (Everett-Hincks & Dodds 2008). Then, the weights of lambs were measured at three

periods of age (birth, weaning, and six months of age) for both analyzed breeds according to the protocol of (Zhao *et al.*, 2017).

Feeding lambs

The newborn lambs were left with their dams during the first two weeks of age. In the third week, the dams went out to graze and the newborn lambs remained in the sheds and merged with the mothers after their return from grazing until the next day during the first month. At the beginning of the second month, the lambs remained inside the sheds and were given 150 g of a concentrated diet with dams' milk consumed before and after their return from grazing. In the period extended from the third month of age until weaning (Three months years old), lambs are given a 200 g.Day-1 concentrate diet. This amount of diet had gradually increased with the increase of weight of lambs. Add to that, lambs were fed dams' milk until weaning. In the period between the fourth month to the sixth month of age, lambs were given a fattening concentrate (barley 50%, bran 30%, crushed corn 10% soybean cake 8%, salt 1%, premix vitamins 1%) at 2.5% of body weight and given as 500 g per lamb per day.

Blood collection

The blood samples were collected from the jugular vein of the lambs by a sterile medical syringe and placed in gel-containing test tubes (4 ml capacity). The serum was separated by using a clinical centrifuge at 3461 rpm for 6 minutes (EBA 20, Hettich, Germany). Blood samples were collected at the birth, weaning, and six months of age for both investigated breeds. Serum samples were kept frozen at a temperature of -20°C in sealed microcentrifuge

tubes (of 1.5 ml capacity) until the thyroxine (T4) and growth hormone (GH) being assessed.

Hormonal assays

Both hormones were measured using the enzyme-linked immunosorbent assay (ELISA) kit. All reagents and samples were brought to room temperature, 30 min before starting the assay procedure. The absorbance of hormones was measured at 450 nm using a microplate reader. Concentrations were presented as nmol.L⁻¹ for T4 and ng/ml for GH. A standard curve was generated for each hormone measured, and samples were interloped according to the manufacturer's instructions.

Statistical analysis

Statistical Analysis System -SAS (2012) was used in data analysis to study the effect of different factors on the studied traits according to a complete randomized design (CRD). The significant differences between the averages were compared with the Duncan (1955) polynomial test. The following mathematical model was used in the study:

$$Y_{ijk} = \mu + B_i + S_j + E_{ijk}$$

Where; Y_{ijk} is the phenotypic value of the traits, μ is the overall mean, B_i is the fixed effect of the breed ($i = Awassi, Karakul$), S_j is the fixed effect of the sex ($Male, Female$) and E_{ijk} is the random residual effect of each observation. p -values of less than 0.05 were considered statistically significant for all comparisons.

Results & Discussions

T4 and GH variables

Table (1) showed a highly significant effect ($p < 0.01$) for the level of T4 at birth (6.847, 5.241 nmol.L⁻¹) and weaning (4.500, 3.857 nmol. L⁻¹) of the Karakul breed as compared with the

Awassi breed respectively. The results showed that T4 concentrations of Karakul sheep (3.608 nmol.L⁻¹) did not exhibit a higher level of statistical significance over the Awassi sheep (3.230 nmol.L⁻¹) at the age of six months. GH

showed a significant ($p < 0.001$) superiority at the weaning age of Karakul (0.647, ng.ml⁻¹) as compared with Awassi breed, it reached (0.579 ng. ml⁻¹), while, its effect was not significant at birth and the age of six months.

Table (1): The levels of T4 and GH in Karakul and Awassi sheep in three periods of age (Mean ± SE).

Variable	Breed	N	Mean ± Std. Error	p-value
T4 at birth (nmol.L ⁻¹)	Karakul	28	6.847 ^a ± 0.343	0.0001
	Awassi	32	5.241 ^b ±0.184	
T4 at weaning (nmol.L ⁻¹)	Karakul	28	4.500 ^a ± 0.163	0.0161
	Awassi	32	3.857 ^b ± 0.195	
T4 at six months (nmol.L ⁻¹)	Karakul	28	3.608 ^a ± 0.201	0.1143
	Awassi	32	3.230 ^a ± 0.132	
GH at birth (nmol.L ⁻¹)	Karakul	28	0.642 ^a ± 0.037	0.1189
	Awassi	32	0.719 ^a ± 0.033	
GH at weaning (nmol.L ⁻¹)	Karakul	28	0.642 ^a ± 0.020	0.0550
	Awassi	32	0.591 ^b ± 0.017	
GH at six month (nmol.L ⁻¹)	Karakul	28	0.903 ^a ± 0.108	0.7290
	Awassi	32	0.853 ^a ± 0.097	
Birth weight	Karakul	28	4.328 ^a ± 0.135	0.0096
	Awassi	32	3.907 ^b ± 0.086	
Weaning weight	Karakul	28	24.321 ^a ± 1.006	0.0024
	Awassi	32	20.687 ^b ± 0.608	
six months' weight	Karakul	28	34.357 ^a ± 1.066	0.0001
	Awassi	32	28.843 ^b ± 0.794	

Values with different superscript letters within the same column differ significantly

On the other hand, lambs of the Karakul breed significantly ($p < 0.01$) exceeded the Awassi breed in live weights at birth (4.328 and 3.907 kg), weaning (24.321 and 20.687 kg), and six months of age (34.357 and 28.843 kg), respectively. The present study is in agreement with other reports that indicated the presence of a proportional association of T4 with live body weight in sheep (Blum *et al.*, 1980; Caldeira *et al.*, 2007; Koluman & Daskiran, 2011). Furthermore, T4 has represented a good indicator for the nutritional level due to its possible association with the status of feed intake in sheep (Abdel-Fattah *et al.*, 2013). This kind of association has been confirmed, since T4 showed a high correlation with live body weights in Karakul sheep at the first six months of age (Mirhoseini *et al.*, 2015). Furthermore, our study has also complied with other investigations performed on the Awassi sheep, in which significant differences in live body weights were identified in the same analyzed time intervals (Singh *et al.*, 2018). Our results are also consistent with Carlos *et al.* (2015), who referred to the elevation for T4 in Morada Nova sheep in Brazil. However, our results did not consistent with other studies that did not show any significant differences in T4 concentrations among various Asian sheep breeds (Sharma *et al.*, 2016). In contrast to our study, it was shown that there were no significant differences between the weights of the Karakul and Awassi breeds at the age of 3, 6, and 9 months, which may be due to several genetic and non-genetic factors (Aljubouri *et al.*, 2020c). Nevertheless, the presence of a higher level of T4 concentrations in the Karakul (having higher body weights) in comparison with Awassi sheep (having lower

body weights) may indicate a positive association between T4 and body weight in these breeds. For this reason, it is rational to say that the elevated T4 has been correlated with the increased live body weight indices in the investigated populations.

Association of T4 and GH levels with sex

It was found that the concentrations of T4 were not significant between both investigated breeds in both sexes at birth (6.011 nmol.L⁻¹ for Karakul, 5.973 nmol.L⁻¹ for Awassi), and at six months (3.548 nmol.L⁻¹ for Karakul, 3.282 nmol/L for Awassi). Whereas, the concentration of T4 showed significant differences ($p < 0.01$) at weaning age, in which the male lambs (4.500 nmol.L⁻¹) exceeded the female lambs (3.856 nmol.L⁻¹) for both breeds. In contrast to T4, there were no significant differences in the lambs' sex in the concentrations of GH in all three periods of age (Table 2).

Results showed that the male lambs significant ($p < 0.01$) exceeded female lambs in weights at weaning (23.964 and 21,000 kg) and the age of six months ($p < 0.05$) (32.992 and 29.125 kg) respectively. However, the sexual differences in live body weights did not reach the level of significance at birth for both breeds. Several reports have indicated the superiority of males over females in live body weights in the Iraqi Karadi sheep (Hama Khan *et al.*, 2019), and Turkish Karakul sheep (Halil & Özbeyaz, 2020). Furthermore, it has also been shown that the male lambs of the Iranian Baluchi breed have outperformed the female lambs in weights from birth to the age of six months (Binabaj *et al.*, 2021).

Table (2): The interaction of sex of lambs, level of T4 and GH, and live body weights of both Awassi and Karakul breeds (Mean \pm SE).

Variable	sex	N	Mean \pm Standard Error	p-value
T4 at birth (nmol.L ⁻¹)	M	29	6.011 ^a \pm 0.288	0.9296
	F	31	5.973 ^a \pm 0.314	
T4 at weaning (nmol.L ⁻¹)	M	29	4.500 ^a \pm 0.220	0.0160
	F	31	3.856 ^b \pm 0.147	
T4 at 6 months (nmol.L ⁻¹)	M	29	3.548 ^a \pm 0.174	0.2687
	F	31	3.282 ^a \pm 0.162	
GH at birth (nmol.L ⁻¹)	M	29	0.707 ^a \pm 0.037	0.2394
	F	31	0.669 ^a \pm 0.034	
GH at weaning (nmol.L ⁻¹)	M	29	0.607 ^a \pm 0.018	0.7346
	F	31	0.615 ^a \pm 0.018	
GH at 6 month (nmol.L ⁻¹)	M	29	0.851 ^a \pm 0.097	0.4674
	F	31	0.903 ^a \pm 0.108	
Birth weight	M	29	4.230 ^a \pm 0.129	0.1535
	F	31	3.993 ^a \pm 0.102	
Weaning weight	M	29	23.964 ^a \pm 0.97	0.0145
	F	31	21.00 ^b \pm 0.705	
six months' weight	M	29	32.99 ^a \pm 1.32	0.0051
	F	31	29.125 ^b \pm 0.72	

Values with different superscript letters within the same column differ significantly.

However, no significant effect of the sex of the lambs was reported on the live body weights and weight gain in the Syrian Awassi sheep (Aljubouri *et al.*, 2020b). The difference in the live body weight measurements between males and females with the progress of age may be

due to the increasing differences in the endocrine system and the role of the male hormones, which are essential for building bones and muscle growth (Karsenty & Olson, 2016). These key metabolic roles can be

attributed as estrogen works to inhibit the longitudinal growth of body bones while androgen acts as a structural hormone that promotes growth and speeds up the growth of bones (Ekambaram *et al.*, 2013; Karmakar *et al.*, 2018). This was probably owing to a greater activity of the pituitary gland in males promoted by sexual hormones (Kumar *et al.*, 2014). However, our results are not in agreement with Eshratkhah *et al.* (2010) who showed the absence of any noticeable difference of T4 values between both sexes of Moghani sheep.

Correlation between levels of metabolic hormones and growth traits

The results of the study indicate a highly significant positive correlation ($p < 0.01$) between the T4 concentration at birth with the weight of lambs at weaning ($r = 0.27280$, $P = 0.0350$), and at six months of age ($r = 0.33862$, $P = 0.0081$). A highly significant positive correlation ($p < 0.01$) was also observed between the level of T4 at weaning and the weight of the lambs at weaning ($r = 0.44245$, $P = 0.0004$) and at six months of age ($r = 0.36534$, $P = 0.0041$). However, no noticeable correlation was reported between GH concentration and growth characteristics in both breeds (Table 3).

A highly significant ($p < 0.01$) positive correlation between the weight of the lambs at birth and weaning ($r = 0.32369$, $P = 0.0116$) was observed. Another positive correlation between the weight of the lambs at birth and six months of age ($r = 0.21653$, $P = 0.0966$) was also observed. However, this correlation was not reached a significant level. Additionally, a highly positive correlation ($p < 0.01$) was recorded between the weight of the lambs at

weaning and the age of six months ($r = 0.81686$, $P = 0.0001$). The results indicated a positive and significant correlation ($p < 0.01$) between the concentration of T4 and live body weight at birth, weaning, and six months of age. The positive correlation was highly significant ($p < 0.01$) with live body weight for lambs at birth, weaning, and six months of age. This study is consistent with a study conducted on sheep that indicated a proportional increase in the level of T4 and live body weight with age (Carlos *et al.*, 2015).

This observation can be explained by the rapid requirements of the growing lambs to elevate T4 values in the blood and then binds to its receptors on the surface of the muscle cells to achieve better effectiveness at work and better biological functions (Haddad *et al.*, 2001). Thyroid hormones show pleiotropic effects on osteoblast function and differentiation, early pregnancy, and in a wide range of activities in metabolism, growth, and development (Candelotti *et al.*, 2015). This is also consistent with the studies that reported a positive correlation between birth weights with subsequent weaning and six-month weights in Madras red sheep, Carole \times Malpura sheep, Harnali sheep, and Egyptian Barki sheep (Gowane *et al.*, 2011; Ganesan *et al.*, 2013; Lalit *et al.*, 2016; Abd El Tawab *et al.*, 2020). Furthermore, it was shown that Akkaraman sheep recorded a tight correlation with T4 level, growth, and wool shearing capacity (Pehlivan *et al.*, 2020). In contrast with T4 results, our study refuted any correlation between the concentration of GH and the age of investigated sheep at birth, weaning, and six months' age. These results are also supported in Norduz Sheep (Karakuş *et al.*, 2017).

Table (3): Correlation between the level of metabolic hormones and the live body weights of Awassi and Karakul breeds.

Variable	r / p-value	birth weight	weaning weight	Six months weight
T4 at birth (nmol.L ⁻¹)	r	0.125	0.272	0.338
	p-value	0.338	0.035	0.008
T4 at weaning (nmol.L ⁻¹)	r	-0.044	0.442	0.365
	p-value	0.736	0.0004	0.004
T4 at six months (nmol.L ⁻¹)	r	0.051	0.452	0.419
	p-value	0.696	0.0003	0.001
GH at birth (nmol.L ⁻¹)	r	-0.064	-0.203	-0.140
	p-value	0.625	0.119	0.283
GH at weaning (nmol.L ⁻¹)	r	0.131	-0.015	0.077
	p-value	0.317	0.909	0.557
GH at 6 months (nmol.L ⁻¹)	r	-0.006	-0.088	-0.047
	p-value	0.961	0.501	0.717
Birth weight	r	-	0.323	0.216
	p-value		0.012	0.097
Weaning weight	r	-	-	0.816
	p-value	-	-	<.0001

p < 0.05: Significant, p >0.05: Not significant.

Conclusions

The high correlations between the elevated T4 values with the body weight gain in three different age periods indicate the possibility to employ this tight correlation in the breeding programs for early selection of lambs to improve lambs' weights at marketing age.

Conflict of interest

Authors declare that they have no conflict of interest.

Ethical approval

The experimental procedures of this study were approved by the Animal Committee of Al-Qasim Green University, Babil, Iraq.

Acknowledgements

This paper was partially supported by Al-Qasim Green University, Iraq.

ORCID

T. R. S. Aljubouri: 0000-0002-6853-3335

F. M. Al-Khafaji: 0000-0002-1223-6366

M. B. S. Al-Shuhaib: 0000-0002-6458-2068

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علاقة بعض هرمونات الأيض مع زيادة وزن الجسم الحي في ذكور وإناث حملان الكاراكول والعواسي

ثامر راضي صاحب الجبوري، فراس مهدي عبد الرضا الخفاجي ومحمد باقر صاحب الشهيب

قسم الإنتاج الحيواني، كلية الزراعة، جامعة القاسم الخضراء، العراق

المستخلص: تم إجراء هذه الدراسة في الحقل الحيواني لشركة الكفيل من نوفمبر - 2019 إلى أيار - 2020 للتحري عن العلاقة المحتملة ما بين هرمون النمو والثايروكسين مع صفات النمو في اغنام العواسي والكاراكول. استخدم في الدراسة 60 حملاً، 28 (13 ذكر و15 أنثى) من سلالة الكاراكول و 32 (18 ذكر و14 أنثى) من سلالة العواسي. تم جمع عينات الدم عند الولادة والفظام وعند عمر ستة اشهر، حُسب تركيزي كل من هرموني النمو والثايروكسين. بيّنت النتائج وجود مستويات أعلى للثايروكسين لحملان الكاراكول مقارنة بحملان العواسي عند الولادة وعند الفظام. أبدت الحملان العائدة لسلالة الكاراكول ارتفاعاً معنوياً في هرمون النمو مقارنة بحملان سلالة العواسي عند الفظام، بينما لم يتم ملاحظة أي فروقات معنوية بينهما عند الولادة وعند ستة أشهر من العمر. وأشارت النتائج الى ارتفاع معدلات اوزان حملان الكاراكول مقارنة بالحملان العواسية في كل الفترات الزمنية المدروسة. تم ملاحظة علاقة معنوية موجبة عالية ($p < 0.01$) ما بين تركيز الثايروكسين ووزن الحملان في معظم الأوقات الزمنية المدروسة. بينما ، لم تُظهر النتائج وجود علاقة بين هرمون النمو وصفات النمو التي تم قياسها في كلا الصنفين. ربما يكون ارتفاع مستوى الثايروكسين هو أحد الأسباب المسؤولة عن تفوق الكاراكول على العواسي في الاوزان الجسمية الحية. ويمكن أن تستخدم هذه العلاقة الكبيرة ما بين الثايروكسين وصفات النمو في الانتخاب المبكر للحملان لتحسين أوزان الأغنام عند التسويق.

الكلمات المفتاحية: هرمون النمو، صفات النمو، الأغنام، هرمون الثايروكسين، تحسين الوزن.