المجلة العلمية لجامعة الملك فيصل
The Scientific Journal of King Faisal University
العلوم الأساسية والتطبيقية
Basic and Applied Sciences

The Effect of the Seasons and Sex on the Structure and Activity of the Thyroid Gland of Dromedary Camels

Saeed Yassin Al-Ramadan
Department of Anatomy, College of Veterinary Medicine, King Faisal University, Al Ahsa, Saudi Arabia

تأثير المواسم والجنس على بنية ونشاط الغدة الدرقية للإبل العربية

سعيد ياسين الرمضان
قسم التشريح، كلية الطب البيطري، جامعة الملك فيصل، الأحساء، المملكة العربية السعودية

KEYWORDS
الكلمات المفتاحية
Camel, thyroid gland, thyroid stimulating hormone triiodothyronine and thyroxine
الإبل، الغدة الدرقية ، هرمون تحفيز الغدة الدرقية، ثلاثي أيودوثايرونين، الثيروكسين

RECEIVED الاستقبال	ACCEPTED القبول	PUBLISHED النشر
17/09/2020	18/10/2020	01/12/2020

https://doi.org/10.37575/b/vet/0032

Abstract

The dromedary camels are exposed to extreme weather conditions that affect their metabolic performance. The current research aims to study the effects of seasons and sex on the histological features of camel thyroids and T3, T4, and TSH levels. Twenty-eight adult camels were used in this study. The height of the follicular epithelium, the number of the follicles/field, and the diameter of the follicles showed seasonal variations (winter 10.5±0.46 μm, 4.79±2.12, 122±7.0 vs. summer 7.47±0.32 μm, 4.65±2.23, 130±10.0 μm). These parameters were measured in different sexes and found to be (male 9.2±0.66 μm, 5.47±2.0 follicle/field, 119±6.0 μm vs. female 8.4±0.69 μm, 3.16±1.2 follicle/field, 146±17.0 μm). Seasonal differences at the hormonal level were (winter T3: 1.14±0.12 ng/ml, T4: 28.87±1.36 μg/dl, TSH: 3.68±28 MIU/ml vs. summer T3: 0.95±0.10ng/ml, T4: 31.91±2.46μg/dl, TSH: 3.47±0.34MIU/ml). Sexual variations were also recorded in these hormones (males: T3: 1.37±0.34 ng/ml, T4: 30.14±2.45 μg/dl, TSH: 2.28±0.35 MIU/ml vs. females: T3: 1.06±0.06 ng/ml, T4: 25.8±2.20μg/dl, TSH: 3.93±0.20 MIU/ml). In conclusion, this study sheds some light on aspects of the changes in camel thyroids. There is still much to be done to discover more characteristics of this animal and how it adapts to the desert environment.

الملخص

تتعرض الإبل العربية لظروف جوية قاسية من شأنها أن تؤثر على أداء عمليات الأيض في أجسامها. يهدف هذا البحث إلى دراسة تأثير فصول السنة ونوع الجنس على الخصائص النسيجية للغدة الدرقية لدى الإبل ومستويات الهرمونات: ثلاثي أيودوثايرونين (T3) والثيروكسين (T4) والهرمون المنشط للغدة الدرقية (TSH). أُجريت عينة الدراسة على 28 من الإبل البالغة، وأظهرت النتائج اختلاف في قياسات ارتفاع الخلايا الظهارية وعدد الجريبات/المجال المجهري وقطرها بالتغيرات الموسمية كالتالي (10.5±0.46 مايكرومتر، 4.79±2.12, 122±7.0 مايكرومتر في الشتاء، مقابل 7.47±0.32 مايكرومتر، 4.65±2.23, 130±10.0 مايكرومتر في الصيف)، وتم أيضا تسجيل اختلافات في نفس هذه القياسات بين الذكور والاناث (لدى الذكر 9.2±0.66 مايكرومتر، 5.47±2.0 حويصلة، 119±6.0 مايكرومتر، مقابل 8.4±0.69 مايكرومتر، 3.16±1.2 حويصلة، 146±17.0 مايكرومتر للأنثى). في حين كان تأثير الاختلافات الموسمية على مستوى الهرمونات كالتالي (في فصل الشتاء، (T3): 1.14 ± 0.12 نانوجرام/ مل، (T4): 28.87 ± 1.36 ميكروغرام/ ديسيلتر ، (TSH): 3.68 ± 28 وحدة/ مل، مقابل الصيف: (T3): 0.95±0.10 نانوجرام/مل، (T4): 31.91±2.46 ميكروغرام/ ديسيلتر، (TSH): 3.47±0.34 وحدة/ مل)، سُجلت أيضا تأثير الفروقات الجنسية على الهرمونات كالتالي (بالنسبة للذكور: (T3): 1.37±0.34 نانوجرام/مل، (T4): 30.14±2.45 ميكروغرام / ديسيلتر، (TSH): 2.28±0.35 وحدة/ مل، مقابل (T3): 1.06±0.06 نانوجرام/ مل، (T4): 25.8±2.20 ميكروغرام/ ديسيلتر، (TSH): 3.93±0.20 وحدة/ مل للإناث). انتهت هذه الدراسة بتسليط الضوء على جوانب التغير التي تطرأ على الغدة الدرقية لدى الإبل وحيدة السنام، ولا يزال هناك الحاجة إلى مزيد من الدراسات التي يجب القيام بها عمله لكشف المزيد من الجوانب لهذا الحيوان وكيفية تأقلمه مع بيئته الصحراوية.

1. Introduction

The ecosystem of the desert has unique features such as extreme daily weather and seasonal climate. This system is also characterized by living creatures that have adapted to such ecological stressors. One of the largest and most tolerant animals living in the desert is the camel. This species is equipped with remarkable physiological and anatomical features, helping them survive in harsh environments.

It has been reported that the camel's metabolic rate is influenced by different factors such as ambient temperature, hydration, and light—dark cycle (Bouaouda et al., 2014; El Allali et al., 2013; Schmidt-Nielsen et al., 1967). Living in the desert puts more pressure on the inhabitants to regulate their body temperature and rationalize water metabolism. From this standpoint, one of the most important camel strategies to live in the desert is “adaptive heterothermy” (Bouaouda et al., 2014; Schmidt-Nielsen et al., 1957). Under normal conditions, the camel's body temperature fluctuates by 2 C°, while the range of fluctuation may reach up to 6 C° in case of high ambient temperature if accompanied by dehydration (Schmidt-Nielsen et al., 1957; Schmidt-Nielsen et al., 1967).

The metabolism is dependent on many factors, of which triiodothyronine (T3), thyroxine (T4), and thyroid-stimulating hormone (TSH) are the most important (McAninch and Bianco, 2014; Williams et al., 2019). In this respect, Nazifi and his team (1999) found that the camels' blood concentrations of T3 and T4 were significantly higher during the summer season than in the winter. The thyroid gland is distinguished from the rest of the endocrine glands by being the only one capable of storing its product outside the cells in the thyroid follicles. Those follicles are lined by simple low cuboidal to high cuboidal epithelial cells (Sellitti and Suzuki, 2014). The size of the follicles, as well as the height of the epithelium, are considered an indicator of the glandular activity. Banks (1993) mentioned that a follicle's activity is approximately inversely proportional to the diameter of the follicles. It has also been reported that the thyroid gland is considered hypoactive when the average composition of the thyroid gland is lined by squamous epithelium (Junqueira and Mescher, 2013).

Paradoxically, there are several reports about camel’s thyroid physiology and structure (Kausar and Shahid, 2006; Abdel-Magied et al., 2000 Atoji et al., 1999), but as far as the author knows, none of this research mentioned anything about the effect of the season, sex and pregnancy on the thyroid structure. However, in the current study, we hypothesized that the season and sex have a direct influence on the histological features of the thyroid gland and thyroid hormone levels.

2. Materials and Methods

Twenty-eight adult camels (5—18 years) were used in this study. Four of these animals were males and the rest were females. Among the female camels, three were pregnant. The samples were collected from the animals and brought to the Al-Omran Slaughter House. The procedures for collecting these samples were done according to the Ethical Guide for Handling Animals in the Slaughter Houses, Ministry Municipal and Rural Affairs, Saudi Arabia. Blood samples were collected from the jugular vein in 10 ml venipuncture tubes before slaughtering. The tissue samples were collected immediately after slaughtering.

Corresponding Author: Saeed Yassin Al-Ramadan salramadan@kfu.edu.sa , 00966594471666 المؤلف المراسل: سعيد ياسين الرمضان

2.1. Histological Study:

- <u>Tissue staining</u>: The thyroid gland was removed, and tissue samples were taken from the center of either thyroid lobe and were immediately fixed in 10% neutral formalin. After that, fixation samples were processed and embedded in paraffin wax. Sections were cut at 5 µm and stained with hematoxylin and eosin stain, according to Bancroft and Cook (1994). Staining was evaluated using a light microscope (Leica DM6000-B microscope), and histological images were obtained with a Leica DEC-420 digital camera (Leica Microsystems, Germany).
- <u>Histometry</u>: The microscopic measurements of the thyroid gland (height of the epithelium, follicles/field, and follicular diameter) were taken by using ImageJ software (http:/Sb-info.nih.gov/ij/).

2.2. Hormonal Assay:

Blood samples were centrifuged at 1200 g for 20 minutes, and then the serum was separated and stored at -20 C° until analysis.

The serum T3, T4, and TSH concentration were measured using an Enzyme-linked immunosorbent assay (ELIZA) kit (Medix Biotech INC, and the microplate reader MR 700 Dynatech Lab, USA). The procedure was conducted as recommended by the manufacturer.

2.3. Statistical Analysis:

All recorded data were analyzed by the SPSS-23 program (IBM Corp.©, 2015. IBM and SPSS, Armonk, NY: IBM Corp). The data were presented as range and mean ± standard error of mean. All data were subjected to an independent sample t-test. Differences were considered significant at $p < 0.05$.

3. Results

3.1. Histological Study:

<u>3.1.1. Seasonal Variation</u>

During winter, the follicular epithelium of the thyroid gland is cuboidal to columnar with spherical to oval nuclei (Fig.1 a, b). The height of the epithelium ranges between 8.0 to 13.0 µm with 10.5 ±0.46 µm on average. The number of the follicles/ field of the thyroid gland range between 1.8 to 8.4/field with an average of 4.79± 2.12 follicles/ field. The diameter of the thyroid follicles range from 94.5 to 174 µm, with an average of 122±7.0 µm (Table 1). However, the diameter tends to be larger in the subcapsular follicles (Fig. 1a).

The thyroid gland showed signs of proliferation, especially at the peripheral portion of the gland. These signs are manifested either as intrafollicular or as extrafollicular proliferation (Fig. 1c, d). The intrafollicular proliferation is manifested as enfolding of the follicular epithelium within the lumen. Progressive proliferation of the follicular epithelium results in the formation of new thyroid follicles (Fig. 1c). The extrafollicular proliferation of the glandular epithelium manifested as side budding of the follicles (Fig. 1d).

The thyroid follicular cells are low cuboidal to cuboidal in shape, and their nuclei are flat or spherical (Fig. 2a, b). The height of the epithelium ranges between 6.0 to 10.0 µm with an average of 7.47±0.32 µm. In the summer, however, the follicle/field range was 1.7 to 8.1, and the average was 4.65±2.23 follicle/field. The follicles' diameter ranged from 89.0 to 225 µm, averaging 130±10.7 µm (Table 1).

The signs for follicular formation were detected less during the summer than in winter. Extrafollicular budding could be detected (Fig. 2c). Another summer characterization of the camel's thyroid gland was detecting large-sized follicles more often (Fig. 2d). No significant differences were recorded between winter and summer in these parameters.

Fig.1: Sections from thyroid gland of adult camel during winter season, stained with H&E showing: (a) General view of the section note the capsule (arrow) and the size of the subcapsular follicles are larger in diameter than deeper follicles (X10) ; (b) Higher magnification showing the epithelium of the thyroid follicle (X100); (c) The proliferation of thyroid follicles by enfolding of follicular epithelium (arrow) (X63); (d) The proliferation of the thyroid follicles by budding (arrow) (X63).

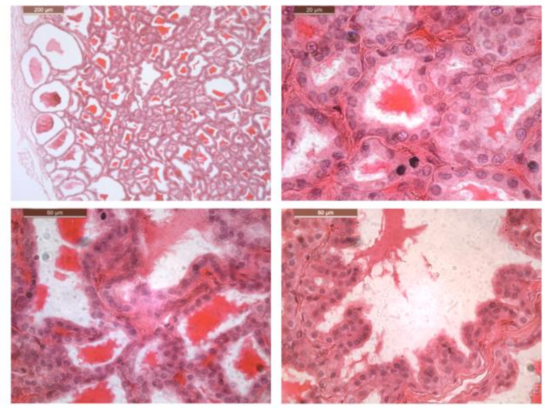

Fig.2: Sections from thyroid gland of adult camel during summer season, stained with H&E showing: (A) General view of the section note the capsule (arrow) and the size of the subcapsular follicles are larger in diameter than deeper follicles (X10); (B) Higher magnification showing the epithelium of the thyroid follicle (X100); (C) The proliferation of the thyroid follicles by budding (arrow) (X63); (D) Large size thyroid follicle seen more in summer season (X5).

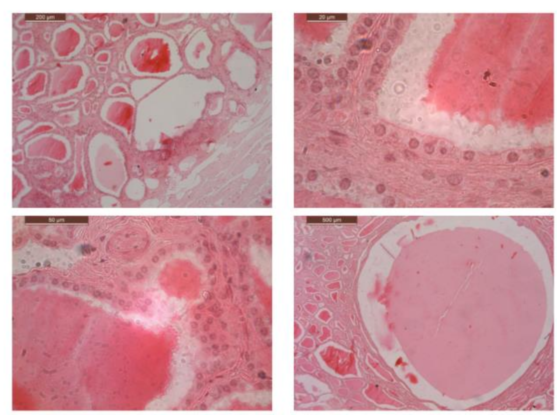

Table 1: Microscopic measurements of the camel's thyroid gland during winter and summer

Season	Winter		Summer	
Measurements	Range	Mean ± SEM	Range	Mean ± SEM
Height of epithelium µm	8.0-13.0	10.5±0.46	6.0-10.0	7.47±0.32
Follicle/Field	1.8-8.4	4.79±2.12	1.7-8.1	4.65±2.23
Follicular diameter µm	94.5-174	122±7.0	89.0-225	130±10.0

SEM: standard error of the mean: P<0.05.

<u>3.1.2. Sexual Variations</u>

There were some variations in the histological measurements recorded between males and females, although these differences were not statistically significant. The height of the male's follicular epithelium ranged between 7.0 to 13.0 µm with an average of 9.2±066 µm. The number of follicles/field ranged between 2.4 to 7.5/field, with an average of 5.47± 2.0 follicles/ field. The follicles' diameter ranged between 89.0 to 151.5 µm, with an average of 119±6.0 µm (Table 2). In the female, the height of the follicular epithelium ranged between 6.0 to 13.0 µm with an average of

8.4±069 μm. The number of the follicles/ field ranging between 1.8 to 5.2, with an average of 3.16± 1.2. The follicles' diameter ranged from 90.0 to 225 μm, with an average of 146±17.0 μm (Table 2).

Table 2: Microscopic measurements of the thyroid gland of male and female camels

Sex	Male		Female	
Measurements	Range	Mean ± SEM	Range	Mean ± SEM
Height of epithelium μm	7.0-13.0	9.2±0.66	6.0-13.0	8.4±0.69
Follicle/Field	2.4-7.5	5.47±2.0	1.8-5.2	3.16±1.2
Follicular diameter μm	89.0-151.5	119±6.0	90.0-225	146±17.0

SEM: standard error of the mean: P<0.05.

3.2. Hormonal Variation:

The analytical hormonal studies of the serum T3, T4, and TSH concentration of the camel are shown in tables 3 and 4. In general, the levels of T3 and T4 are 1.2±0.10 ng/ml and 29.18±1.22 μg/ml, respectively, whereas the level of the TSH was 3.63±0.20 MIU/ml. However, more analytical variations have been detected in terms of season and sex.

3.2.1. Seasonal Variations

During winter, the recorded level of T3 was more than that of the summer. In contrast, the level of T4 is significantly (p<0.05) less in winter vs. summer (21.00 ± 3.0 μg/dl vs. 30.66 ± 2.93 μg/dl). No significant variation in the TSH has been recorded, although it is higher in the winter (Table 3).

3.2.2. Sexual Variations

There were no significant differences in the levels of T3 and T4 between male and female camels. However, the thyroid-stimulating hormone showed a significant (P<0.05) increase in females (3.93 ± 0.20 MIU/ml) than in males (2.99 ± 0.16 MIU/ml) (Table 3).

Table 3: Seasonal and sexual variations of T3, T4, and TSH levels in adult camels

Hormone	Seasons			
	Winter	Summer	Male	Female
	Mean ± SEM	Mean ± SEM	Mean ± SEM	Mean ± SEM
T3 ng/ml	1.04 ± 0.06	0.95 ± 0.09	0.82 ± 0.04	1.06 ± 0.07
T4 μg/dl	21.00 ± 3.0*	30.66 ± 2.93*	30.47 ± 4.51	28.22 ± 1.89
TSH MIU/ml	3.89 ± 0.2	3.47 ± 0.34	2.99 ± 0.16*	3.93 ± 0.20*

SEM: standard error of the mean: P<0.05.

4. Discussion

Camels are often compared to other farm animals, but the reality is that camels live in a completely different environment than that of farm mammals. The natural habitat of a camel is arid and semi-arid deserts. This environment puts many challenges on camels' body systems to adapt to this harsh, fast-changing environment. One of these challenges is the pressure that is placed on the metabolism.

Since the thyroid gland is the leading player in the metabolic process, its tissue structures (T3 and T4) and product level vary. This relationship between thyroid activity and its histological structures has been well-established in many studies, some as early as 1926 (Cramer and Ludford).

The current study showed that some histometrical variations in the thyroid follicles of dromedary camels are related to seasonal changes. The diameter of the thyroid follicles is smaller, the number of follicles per field is larger, and the follicular epithelium is higher during the winter season than in summer. The size of the follicles and the amount of colloidal substance of the thyroid gland depends on the number of cells, which in turn varies according to the biological activity of the gland (Hartoft-Nielsen et al., 2005). This variation in the shape and size of the thyroid follicle and the height of the thyrocyte in relation to its activity have been recorded in other animal species. In the thyroid of European bison (*Bison bonasus*), the follicular epithelium is higher in the warm months than in cold months (Sawicki et al., 1992). In general, not only the height of the follicular epithelium or the size of the follicles are affected by the activity of the gland, but the number and texture of colloid material, the presence of blood and desquamated epithelial cell, as well as the staining affinity of the colloid, all are affected thyroid activity (Machado-Santos et al., 2013). In a species known for being poor thermoregulators, like the Iraqi buffalo (*Bubalus bubalis*), no significant variations were detected in the thyroid gland's histological features between summer and winter (Hussain and Al-Taay, 2009). Later authors found a slight increase in the follicular cell diameter in winter compared to summer. In the goat, the height of the epithelium of active follicles was higher in summer compared with the winter (Ali et al., 2020). However, this data is different from our result, which might be related to the sex of the animal sample, where the previous authors used adult males only. In the male goats, the height of the follicular epithelium was lower in prepubertal animals compared to pubertal animals and much lower in castrated male goats (Adhikary et al., 2003).

The variations in thyroid activity, recorded at the histology level, are confirmed by analytical measurements of the thyroid hormones (T3 and T4) in both seasons. The level of T3 (1.14±0.12 ng/ml) is higher during winter than in summer (0.95±0.10 ng/ml). On the other hand, the level of T4 is lower (28.87±1.36 μg/dl) in winter than in summer (31.91±2.46). Concerning the T3 level, our data is in accordance with previous results that showed the level of T3 as significantly higher in winter than summer (Tajik et al., 2013). Contrarily, the T4 level is different from the later authors, who found that T4 increased during the winter season as well. In another study, both T3 and T4 were higher in summer than in winter (Nazifi et al., 1999).

These variations between our findings and other published findings might be a result of different intrinsic and extrinsic factors of the subject samples. The levels of both T3 and T4 fluctuate with time. This is related to the ambient temperature, availability of food, reproductive status, and other factors like level of insulin and body weight, which all play a role in thyroid activity (Nakayama and Yoshimura, 2018; Muller and Botha, 1994). However, our data on camels is similar to earlier-recorded data (Yegil et al., 1978). The increased summer concentration of T4 was not accompanied by an increase in T3, suggesting a need for long-acting T4 rather than the quick-acting T3. Thus, the changes in metabolism from winter to spring would depend on the quick-acting T3 and later after heat acclimatization will depend on long-acting T4 (Yagil et al., 1978). Another explanation for less T4 during the winter season is less need for water, resulting in lesser metabolic demand (Yagil et al., 1978). Interestingly, in a species like the African striped mice (*Rhabdomys pumilio*), which live in a semi-arid environment, the level of T3 is negatively related to the wet season of winter when there is greater availability of food, as opposed to the dry season of summer (Rimbach et al ., 2017).

The level of T3 in male camels showed a slightly higher level when compared to females. This difference is similar to the previous recorded data from camels (Tajik et al., 2013). In contrast to the later authors, our findings showed that T4 levels were lower in females than in males. This variation might be due to the physiologic status of the female animals; none of the female animals in the current study were pregnant. In sheep, it has been reported that non-mated animals showed less T4 than mated animals (Yokus et al., 2006). The level of TSH was significantly higher in female camels than in males. The effect of gonadal steroids on the function of the thyroid hormone has been reported earlier (Tahboub and Arafah, 2009). In rats, a shift in TSH patterns was observed during sexual maturation (Banu and Aruldhas, 2002). This variation suggests a relation between the sex steroid and thyroid hormones, which in turn affects the pituitary output of TSH.

In conclusion, the current data from the thyroid gland of the dromedary camel showed unique histological features that could be related to seasonal variation. However, the magnitude of these variations was more affected by sex. In females, there was a tendency that reproductive status could also play a role in the thyroid gland activity. Further in-depth investigations need to be done, specifically in the male thyroid gland, in relation to the reproductive (rutting) and non-reproductive seasons.

Bios

Saeed Yassin Al-Ramadan

Department of Anatomy, College of Veterinary Medicine, King Faisal University, Al Ahsa, Saudi Arabia, salramadan@kfu.edu.sa, 00966594471666

Dr. Al-Ramadan is an associate professor of histology and cell biology. He is a graduate of Texas A&M University, USA. He has several publications in the field of anatomy, histology, and cell biology. The author is a member of several professional societies, and a supervisor and examiner for several graduate students. The author's areas of research interest are endocrinology, reproduction, biology of muscle development and immunity of camels. He is a member of the editorial board of Ruminant Science and a reviewer for several articles. Web of Science Researcher ID: AAW-4180-2020, ORCID: 0000-0002-3171-1855.

References

Abdel-Magied, E.M., Taha, A.A.M. and Abdalla, A.B. (2000). Light and electron microscopic study of the thyroid gland of the camel (Camelus dromedarius). *Anatomia, Histologia, Embryologia*, **29**(6), 331–6.

Adhikary, G. N., Quasem, M.A. and Das, S.K. (2003). Histological observation of thyroid gland at Prepubertal, Pubertal and Castracted Black Bengal Goat. *Pakistan Journal of biological Sciences*, **6**(11), 998–1004.

Ali, S. A., El-Sayed, S. A., Goda, N.I.A. and Beheiry, R.R. (2020). Morphological characteristics of the goat thyroid glands among summer and winter seasons. *Adv. Anim. Vet. Sci*, **8**(3), 252–9.

Allali, K. E., Achaâban, M.R., Bothorel, B., Piro, M., Bouâouda, H., Allouchi, M. E., ... & Pévet, P. (2013). Entrainment of the circadian clock by daily ambient temperature cycles in the camel (*Camelus dromedarius*). *American Journal of Physiology-Regulatory, Integrative and Comparative Physiology*, **304**(11), 1044–52.

Atoji, Y., Yamamoto, Y., Suzuki, Y. and Sayed, R. (1999). Ultrastructure of the Thyroid Gland of the One-humped Camel (Camelus dromedarius). *Anatomia, Histologia, Embryologia*, **28**(1), 23–6.

Bancroft, J.D. and Cook, H.C. (1994). *Manual of histological techniques and their diagnostic application*. London, UK: Churchill Livingstone.

Banks, W. J. (1993). *Applied Veterinary Histology*. Missouri, USA: Mosby-Year Book, Inc. St. Louis.

Banu, K.S. and Aruldhas, M.M. (2002). Sex steroids regulate TSH-induced thyroid growth during sexual maturation in Wistar rats. *Experimental and Clinical Endocrinology & Diabetes*, **110**(01), 37–42.

Bouâouda, H., Achâaban, M.R., Ouassat, M., Oukassou, M., Piro, M., Challet, E., Alali, K. & Pévet, P. (2014). Daily regulation of body temperature rhythm in the camel (*Camelus dromedarius*) exposed to experimental desert conditions. *Physiological Reports*, **2**(9), e12151.

Cramer, W. and Ludford, R.J. (1926). On cellular activity and cellular structure as studied in the thyroid gland. *The Journal of Physiology*, **61**(3), 398–408.

Hartoft-Nielsen, M.L., Rasmussen, Å.K., Feldt-Rasmussen, U., Buschard, K. and Bock, T. (2005). Estimation of number of follicles, volume of colloid and inner follicular surface area in the thyroid gland of rats. *Journal of Anatomy*, **207**(2), 117–24.

Hussin, A.M. and Al-Taay, M.M. (2009). Histological study of the thyroid and parathyroid glands in Iraqi buffalo (*Bubalus bubalis*) with referring to the seasonal changes. *Basrah Journal of Veterinary Research*, **8**(1), 26–38.

Junqueira, L.C. and Mescher, A.L. (2013). *Junqueira's Basic Histology: Text & Atlas*. New York, NY: McGraw-Hill Medical

Kausar, R. and Shahid, R.U. (2006). Gross and microscopic anatomy of thyroid gland of one-humped camel (*Camelus dromedarius*). *Pakistan Vet. J*, **26**(2), 88–90.

Machado-Santos, C., Teixeira, M.J., Sales, A. and Abidu-Figueiredo, M. (2013). Histological and immunohistochemical study of the thyroid gland of the broad-snouted caiman (*Caiman latirostris*). *Acta Scientiarum. Biological Sciences*, **35**(4), 585–9.

McAninch, E.A. and Bianco, A.C. (2014). Thyroid hormone signaling in energy homeostasis and energy metabolism. *Annals of the New York Academy of Sciences*, **1311**(n/a) 77–87.

Muller, C.J.C., Botha, J. A., Coetzer, W.A. and Smith, W.A. (1994). Effect of shade on various parameters of Friesian cows in a Mediterranean climate in South Africa. 2. Physiological responses. *South African Journal of Animal Science*, **24**(2), 56–60.

Nakayama, T. and Yoshimura, T. (2018). Seasonal rhythms: The role of thyrotropin and thyroid hormones. *Thyroid*, **28**(1), 4–10.

Nazifi, S., Gheisari, H.R. and Poorabbas, H. (1999). The influences of thermal stress on serum biochemical parameters of dromedary camels and their correlation with thyroid activity. *Comparative Haematology International*, **9**(1), 49–54.

Rimbach, R., Pillay, N. and Schradin, C. (2017). Both thyroid hormone levels and resting metabolic rate decrease in African striped mice when food availability decreases. *Journal of Experimental Biology*, **220**(5), 837–43.

Sawicki, B., Siuda, S. and Kasacka, I. (1992). Bisoniana 107. Microscopic structure of the thyroid gland in the European bison. *Acta Theriologica*, **37**(1-2), 171–9.

Schmidt-Nielsen, B., Schmidt-Nielsen, K., Houpt, T.R. and Jarnum, S.A. (1957). Urea excretion in the camel. *American Journal of Physiology-Legacy Content*, **188**(3), 477–84.

Schmidt-Nielsen, K., Crawford Jr, E. C., Newsome, A. E., Rawson, K. S. and Hammel, H. T. (1967). Metabolic rate of camels: effect of body temperature and dehydration. *American Journal of Physiology-Legacy Content*, **212**(2), 341–6.

Sellitti, D.F. and Suzuki, K. (2014). Intrinsic regulation of thyroid function by thyroglobulin. *Thyroid*, **24**(4), 625–38.

Tahboub, R. and Arafah, B.M. (2009). Sex steroids and the thyroid. *Best Practice & Research Clinical Endocrinology & Metabolism*, **23**(6), 769–80.

Tajik, J. and Sazmand, A. (2013). Serum concentrations of thyroid hormones, cholesterol and triglyceride, and their correlations together in clinically healthy camels (*Camelus dromedarius*): Effects of season, sex and age. *Veterinary Research Forum*, **4**(4), 239–43.

Williams, C.T., Chmura, H.E., Zhang, V., Dillon, D., Wilsterman, K., Barnes, B. M. and Buck, C.L. (2019). Environmental heterogeneity affects seasonal variation in thyroid hormone physiology of free-living arctic ground squirrels (*Urocitellus parryii*). *Canadian Journal of Zoology*, **97**(9), 783–90.

Yagil, R., Etzion, Z. and Ganani, J. (1978). Camel thyroid metabolism: Effect of season and dehydration. *Journal of Applied Physiology*, **45**(4), 540–4.

Yokus, B., Cakir, D.U., Kanay, Z., Gulten, T. and Uysal, E. (2006). Effects of seasonal and physiological variations on the serum chemistry, vitamins and thyroid hormone concentrations in sheep. *Journal of Veterinary Medicine Series A*, **53**(6), 271–6.