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, 7.47±2.32, 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±7.0 µm). Seasonal differences at the hormonal level were (winter: T3: 0.95±0.10 ng/ml, T4: 25.8±2.20 µg/dl, TSH: 3.68±28 MIU/ml vs. summer: T3: 1.37±0.34 ng/ml, T4: 31.9±2.46 µg/dl, TSH: 3.47±0.34 MIU/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: 2.86±0.35 MIU/ml vs. females: T3: 1.06±0.06 ng/ml, T4: 25.8±2.20 µg/dl, TSH: 3.93±0.20 MIU/ml). In conclusion, this study sheds some light on aspects of changes in camel thyroids. There is much still to be done to discover more characteristics of this animal and how it adapts to the desert environment.

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 (Bouaoua et al., 2014; El Allali et al., 2013; Schmidt-Nielsen et al., 1957). 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” (Bouaoua 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, Nazi 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. These 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 hypotrophic 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-Maged 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-Omrann 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.
2.1. Histological Study:
- Tissue staining: 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).
- Histometry: 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 (ELISA) 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:

3.1.1. Seasonal Variation
During winter, the follicular epithelium of the thyroid gland is cuboidal to columnar with spherical to oval nuclei (Fig. 1a, 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.

3.1.2. Sexual Variations
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 ± 0.66 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±0.69 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

<table>
<thead>
<tr>
<th>Measurements</th>
<th>Sex</th>
<th>Range</th>
<th>Mean ± SEM</th>
</tr>
</thead>
<tbody>
<tr>
<td>Height of follicles (µm)</td>
<td>Male</td>
<td>100-225</td>
<td>146±17.0</td>
</tr>
<tr>
<td></td>
<td>Female</td>
<td>100-300</td>
<td>225±20</td>
</tr>
<tr>
<td>Follicular diameter (µm)</td>
<td>Male</td>
<td>30.0-400</td>
<td>225±20</td>
</tr>
<tr>
<td></td>
<td>Female</td>
<td>22.5-300</td>
<td>225±20</td>
</tr>
</tbody>
</table>

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

<table>
<thead>
<tr>
<th>Hormone</th>
<th>Winter</th>
<th>Summer</th>
<th>Male</th>
<th>Female</th>
</tr>
</thead>
<tbody>
<tr>
<td>T3 ng/ml</td>
<td>1.4±0.1</td>
<td>1.14±0.12</td>
<td>1.2±0.10</td>
<td>1.2±0.10</td>
</tr>
<tr>
<td>T4 µg/ml</td>
<td>21.00 ± 3.0</td>
<td>30.66 ± 2.93</td>
<td>29.18±1.22</td>
<td>29.18±1.22</td>
</tr>
<tr>
<td>TSH MIU/ml</td>
<td>3.63±0.2</td>
<td>3.89 ± 0.2</td>
<td>30.66 ± 2.93</td>
<td>30.66 ± 2.93</td>
</tr>
</tbody>
</table>

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 colloid 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 (Rabulus 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 (Rhodabomys 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
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