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STUDIES ON PITUITARY (TSH)-THYROID (T3,T4,FT4) -LIVER AXIS DURING SEASONAL VARIATION AT TWO DIFFERENT AGES OF MALE CAMEL (CAMELUS DROMEDARIUS)

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SUMMARY

This study was undertaken to investigate the intricate relationship between pituitary, thyroid and liver of male camel. Thyroids and corresponding livers and blood at two ages during breeding and non- breeding seasons were collected from slaughter houses. Tissues were used for histological examination and evaluation of T3 and T4 content. Serum was used for determination of TSH, T3,T4 and FT4. Some fat and protein profiles were estimated. Serum activity of certain enzymes as ALT, AST, LDH, and ALP were also measured. The data showed that most of the thyroid follicles were large in old non-breeding and were small in young and old breeding animals. Mild to moderate fatty infiltration and small to medium sized lipid inclusions were common feature in hepatocytes of all tested animals especially in old age. TSH and T3 in old non-breeding were significantly decreased than old breeding. No significant variation between the different seasons and ages concerning FT4 while a significant decrease in serum T4 was observed in old non-breeding than young breeding camel. No significant variation in the amount of T3 and T4 in the liver extracts while thyroid extract obtained from camel in the non- breeding season showed a significant increased in T4 at both ages than breeding season at any age. Old camel had a significant increased in serum triglyceride, total lipids and cholesterol than young. No significant variation between the two different seasons and ages concerning total protein, albumin, globulin, albumin globulin ratio, ALP and LDH. In the breeding season, level of serum AST was significantly higher in old than young camel, while, old camel during non-breeding season showed a significant increase in serum ALT than young at any season. It is clearly that the old camel during the non-breeding season (summer), their physiological activities concerning pituitary (TSH), thyroid hormones(T3 and T4) and liver function parameters (fat metabolism, ALT and AST) were negatively affected.

INTRODUCTION

Thyroid hormone (TH) elicite an extraordinary multiplicity of biochemical, cellular, and physiological responses in the simplest to the most complex organisms. Thyroid cell growth and all the steps in the synthesis and secretion of TH were stimulated by the pituitary thyrotropin (TSH) (Khandwala and Lee, 2006). The diverse actions of the biologically active TH can be dividedinto two groups: growth and development, and regulation of metabolism (Tata, 2007). TH regulates the basal metabolic rate of all cells, including hepatocytes, and thereby modulate hepatic function. Liver in turn metabolizes the thyroid hormones and regulates their systemic endocrine effects. A complex relationship was existed between the thyroid gland and the liver in both health and disease (Meek et al., 2003). Thyroid dysfunction may perturb liver function, liver disease modulates thyroid hormone metabolism, and a variety of systemic diseases affect both organs (Meek et al., 2003). Tri-iodothyronine (T3) has a ten times greater affinity and ten times greater efficacy than thyroxine (T4) for the nuclear receptor, thus even though T4 was quantitatively secreted at much higher levels, it should be regarded as a pro-hormone that requires deiodination and conversion to T3 to become biologically active (Chadio et al., 2006).There are three groups of deiodinase enzymes that regulate thyroid hormone metabolism,(Bianco and Kim, 2006). Deiodination can occur in most if not all tissues, but the liver and the kidney show the highest deiodinating activity (Chadio et al., 2006). In addition to the central role in deiodination to activate and deactivate thyroid hormones, the liver performs specific functions relating to thyroid hormone transport and metabolism (Malik and Hodgson, 2002). Deiodination and conjugation were the principal pathways of thyroid hormone metabolism (Debaveye et al., 2008 and Kato et al., 2008). Liver synthesizes a number of plasma proteins that bind TH and thereby provide a large, rapidly exchangeable pool of circulating hormone(Yang et al.,2008). The free hormone component within plasma is in equilibrium with the protein-bound hormone, and it is this free fraction which accounts for the hormone's biological activities (Dayan 2001). However,the free hormone concentrations in different tissues vary according to the transport and deiodinase activity within specific tissues (Malik and Hodgson 2002). Although overt thyroid dysfunction is associated with some liver abnormalities, there is a dearth of information on liver function tests across thyroid function tests (Targher et al., 2008). Camel is known to be a seasonal breeder and, in the male camel, the breeding activity maximizes during the rutting period (winter and spring seasons). However, during the non-rutting period (summer and autumn), the breedin gactivity functioning thyroid and liver axis. Although there are a few reports on thyroid hormone concentrations in the almost ceases completely (Zia-Ur et al., 2007). Normal thyroid function is dependent ona normally

camel (Nazifi et al 2009), the studies on the relationship between the activity of thyroid hormones and the hepatic activity of camels are very scanty. The intricate relations between the pituitary TSH, thyroid gland and the liver during breeding and non-breeding seasons of two ages of camel in the present study were discussed.

MATERIALS AND METHODS

Male camel thyroids and corresponding livers during breeding (January and February, 2008) and non-breeding (May and June, G2008) were collected from many slaughter houses located in Qassim area and the average temperatures were 17-21 and 36-39C respectivily Twenty camels at two ages for each season were used; ten young age (3-5 years) and ten old age (13-15 years). Immediately after decapitation, two pieces from each pairs of the thyroid gland and two pieces from fixed liver lobe were obtained from the camel at slaughter. One piece of thyroid and one piece of liver were immediately fixed in 10% formalin, embedded in paraffin wax, sectioned at 5 µm and subsequently stained with Eosin and Hematoxylin. The another piece from thyroid and liver piece was perfused with the cold saline solution at 4°C and kept cold (154 mmol/L NaCl) and extracted separetely. Homogenization was performed with a homogenizer (Staufen, Germany) at 0-4 °C in 0.25 mol/L sucrose, 1 mmol/L EDTA and 0.05 mol/L TRIS-HCL solution, pH 7.4 (De-Waziers and Albercht, 1987 and Rossi et al., 1987). The homogenate were sonicated for 30 seconds at 10 kHz on ice (Takada et al., 1982). The sonicates were centrifuged in Beckman ultracentrifuge (90min, 8500Xg,4°C) and the supernatant was used for determination of T3 and T4 and expressed as ng /mg tissue.Blood was collected in separated clean centrifuge tubes, allowed to coagulate and serum was separated by centrifugation at 3000 r.p.m for 20 min. Serum specimens were quickly kept frozen at -20C until needed for analysis. Serum concentrations of triiodothyronine (T3) and thyroxine (T4), and thyroid stimulating hormone (TSH) were assayed by ELISA procedures using kits purchased from Immunotech Corporation, 90 Windom St. Boston, MA 02134. Free thyroxine (FT4)was assayed using Diagnostic Automation Kits USA ,Cat. No. 3122.Total cholesterol, Total lipids and Triglyceride were determined by Calorimetric, Enzymatic determination according to Zak (1957), Frings and Dum (1970) and Wahlfeld (1974) respectively. ALT(GPT) and AST(GOT) were determined by using Kits from BioMerieux according to the method of Reitman and Frankel (1957) and Lactic dehydrogenase(LDH) and Alkaline phosphatase (ALP) using Sigma Laboratory Kits according to Kachmar and Moss (1976) and Wright et al., (1972) respectively. Total protein was determined according to Cannon et al., (1974) using kits from

BioMerieux. Serum albumin was determined by Biuret and bromocresol green (BCG) dye binding method (Doumas et al., 1971) using kits from Roche Diagnostics GmbH, Mannheim and Randox Laboratories GmbH, Krefeldand. Serum globulin was calculated by subtracting the obtained albumin value from total protein. Albumin globulin ratio (A/G ratio) was also calculated. Data are expressed as the mean ±SEM. Mean differences between treatment groups were determined using Two way ANOVA to test for the effects of group and time and their interaction followed by T- test according to Snedecor (1971).

RESULTS

Gross studies of thyroid gland of camel revealed that the gland was located near the first three rings of trachea and consisted of two lobes (Fig. 1). An isthmus connected these lobes to each other. The colour of the gland was reddish brown. Histological studies indicated that, two types of follicles were identified, large and small. The large follicles were lined by low cuboidal epithelium having flattened nuclei and were assumed to be inactive cells. The small follicles were lined by high cuboidal epithelium with rounded nuclei, these were active cells. However, most of the follicles were large in old non-breeding and were small in young and old breeding (Fig.2, 3). Each follicle was filled with a gel-like material called colloid. The colloid is a storage form of follicular epithelial secretion. A well-developed connective tissue characterized the camel liver. Thick trabeculae were divided the liver parenchyma into lobules. Portal tracts and central veins are surrounded by a variable amount of fibrous tissue. A mild to moderate fatty infiltration, small to medium sized lipid inclusions were a common feature was present in hepatocytes of all animals especially in old age (Fig.4,5). Table (1) showed that, during non breeding season, a significant decrease in serum TSH levels of old camel than young ($P < 0.01$), and a significant decrease in old non-breeding animals than old breeding once ($P<0.05$). Serum T3 of old non-breeding camel showed a significant decrease than old breeding ($P< 0.001$) and young breeding animals ($P<0.05$). A significant decrease in serum T4 was observed in old non-breeding camel than young breeding ($P<0.05$). The obtained results showed that no significant variation between the different seasons and animal ages concerning free T4.The obtained results in table (2) showed that no significant variation among different seasons and camel ages concerning total protein, albumin, globulin , albumin globulin ratio, ALP and LDH. Concerning fat metabolism, the present data (table 2) showed that old camel in the non-breeding season had a significant increase in serum triglyceride than other groups ($P<0.001$), and there was a significant increase in old than young in the breeding season ($P<0.05$).Total lipids was significantly increased in old non breeding camel than young in the same season($P <0.01$) and

young in the breeding season (P <0.001). Cholesterol levels also showed a significant increased in older camel than younger in the corresponding season and older non- breeding than younger breeding. In the breeding season, levels of serum AST were significantly higher in old age than young camel (P <0.05). Meanwhile during non-breeding season, old camel showed a significant increased in serum ALT than young animals in both breeding and non breeding seasons (P<0.01) and (P<0.05) respectively.Data obtained in table (3) showed that, no significant variation in the amount of T3 and T4 in the liver extracts. Thyroid extract obtained from camel in the non- breeding season showed a significant increased in T4 at both ages than breeding season at any age.

Table(1): Serum Thyroid stimulating hormone (TSH), Triiodothyronine(T3), Thyroxine(T4)and free Thyroxine (FT4) levels of male camel at two different ages during breeding and non- breeding season (Thyroid function test).

Season&Age / Parameters	Breeding season		Non breeding season	
	Young (3-5 years)	Old (13-15 years)	Young (3-5 years)	Old (13-15 years)
Thyroid Stimulating H. (TSH) μU/ml	0. 37 ± 0.031	0.44 ±0.033*	0.49 ± 0.052[d]	0.32 ±0.037[d]*
Triiodothyronine (T3) ng/ml	1.40 ±0.19*	1.93 ±0.25[a]	1.34 ±0.21	0.84 ±0.08 [a]*
Thyroxine (T4) ng/ml	45.67 ±3.15*	38.22 ±4.16	43.74 ±2.15	35.17 ±2.55*
Free thyroxine (FT4) ng/ml	10.11 ±1.32	11.56 ±1.33	9.67 ±1.28	10.20 ±2.04

In the same row, values having the same mark (a) ,(d) and (*) are significantly different at P <0.001,P <0.01and P < 0.05 respectively.

Tab-e(2): Blood and liver function parameters of male camel at two different ages during breeding and non breeding season.

Season&Age / Parameters	Breeding season		Non- breeding season	
	Young (3-5 years)	Old (13-15 years)		Young (3-5 years)
Total protein gm/dl	7.130±1.04	6.94±0.92	Total protein gm/dl	7.130±1.04
Albumen gm/dl	3.140±0.21	3.33±0.09	Albumen gm/dl	3.140±0.21
Globulin gm/dl	4.140±0.51	4.16±0.22	Globulin gm/dl	4.140±0.51
Albumen/Globulin ratio	0.970±0.11	0.91±0.32	Albumen/Globulin ratio	0.970±0.11
Triglycerides mg/dl	49.24±4.99c*	68.13±5.13b*	Triglycerides mg/dl	49.24±4.99c*
Total lipids mg/dl	460.3±32.65a	531.2±60.43	Total lipids mg/dl	460.3±32.65a
Cholesterol mg/dl	28.43±1.69b*	41.14±3.97*	Cholesterol mg/dl	28.43±1.69b*
GOT (AST) U/L	109.22±6.81*	143.34±5.55*	GOT (AST) U/L	109.22±6.81*
GPT(ALT) U/L	29.54±3.02 a	31.29±6.13	GPT(ALT) U/L	29.54±3.02 a
ALP (U/L)	100.21±8.11	89.11±6.11	ALP (U/L)	100.21±8.11
LDH (U/L)	692.7±38.15	552.3±42.17	LDH (U/L)	692.7±38.15

In the same row, values having the same mark (a,b,c) ,(d) and (*) are significantly different at P <0.001,P <0.01and P < 0.05 respectively.

Table(3): Triiodothyronine(T3)and Thyroxine(T4) in thyroid and liver extracts of male camel at two different ages during breeding and non- breeding season.

Season&Age / Extracts		Breeding season		Non- breeding season	
		Young (3-5 years)	Old (13-15 years)	Young (3-5 years)	Old (13-15 years)
Thyroid extract	T3 ng/mg	52.58±8.13	43.47±5.65	41.12±7.11	55.73±4.12
	T4 ng/mg	589.12±42.8b d	465.62±37.4ac	943.22±62.1cd	893.13±57.5ab
Liver extract	T3 ng/mg	6.23±1.02	5.26±0.86	6.28±1.10	4.92±0.76
	T4 ng/mg	4.13±0.66	5.17±0.74	5.14±1.02	5.80±1.21

In the same row, values having the same mark are significantly different at P < 0.001.

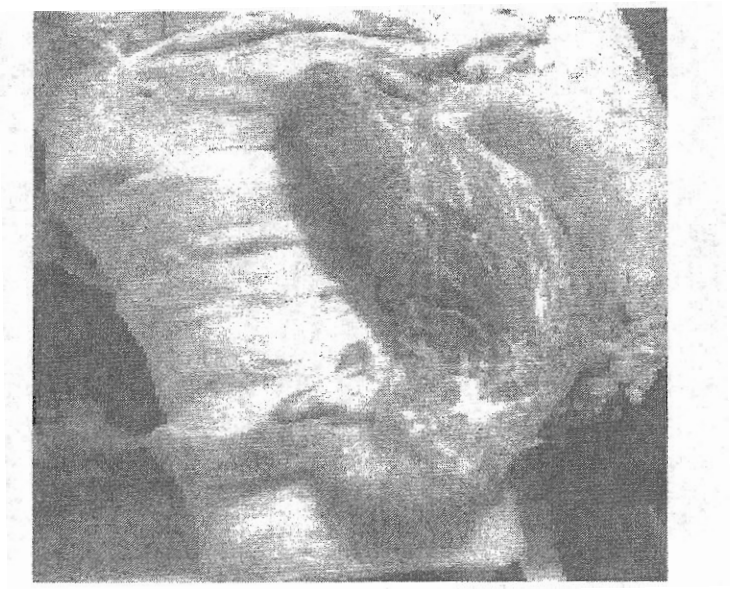

Fig (1) : Showing thyroid lobe located near the first three rings of trachea.

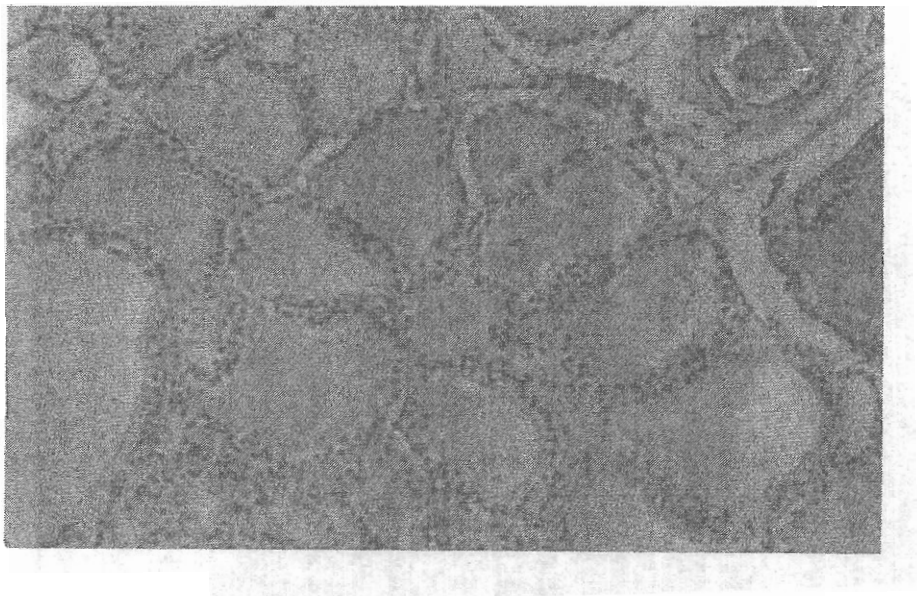

Fig (2) : Thyroid follicles of the young breeding male camel. Most follicles were small size.H and E stain (X 20).

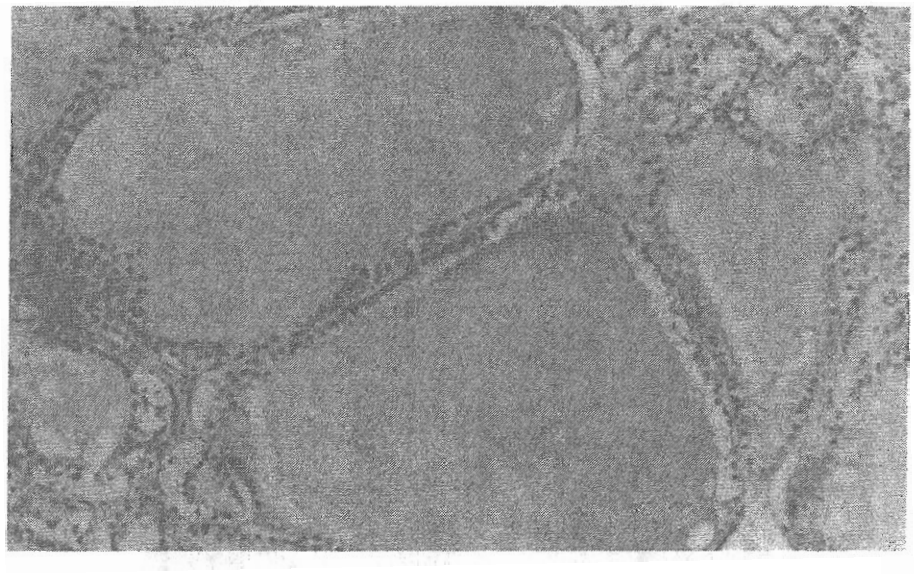

Fig (3) : Thyroid follicles of old non-breeding male camel . Most follicles were large size. H and E stain (X 20).

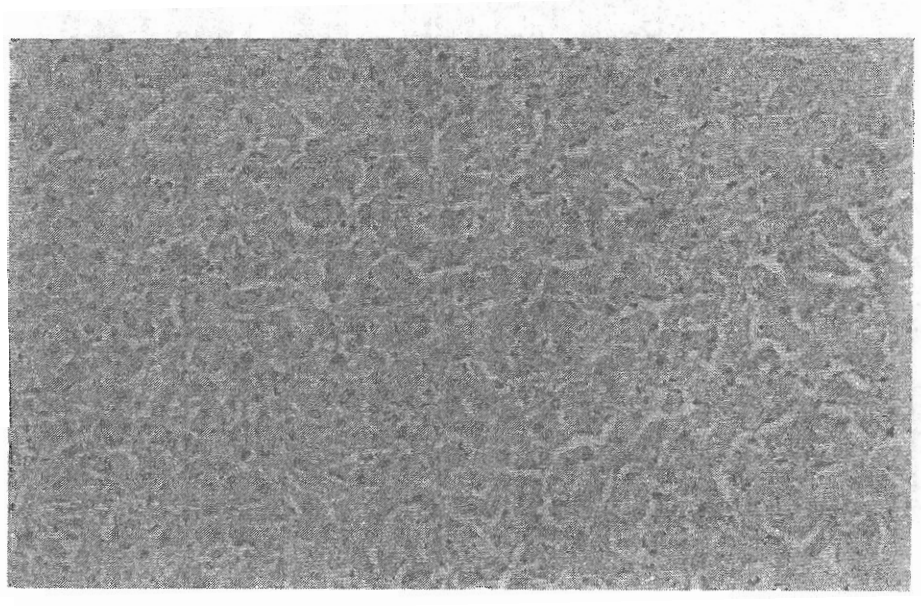

Fig . (4) : Hepatocyte of young breeding male camel. A mild fatty infiltration, and small sized lipid inclusions were present. H and E stain (X 20).

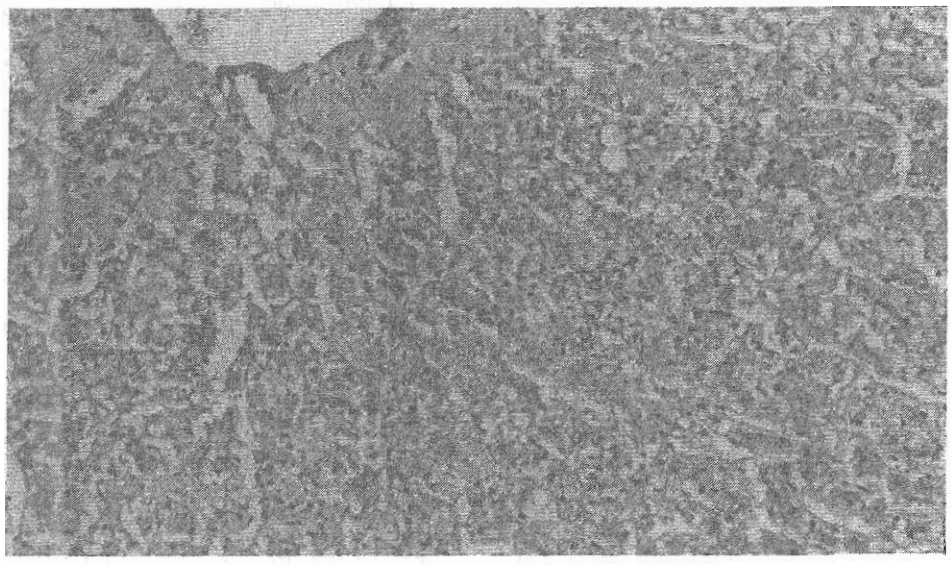

Fig. (5): Hepatocyte of old non-breeding camel. A moderate fatty infiltration and medium sized lipid inclusions were present .

DISCUSSION

Thyroid hormones play a pivotal role in the mechanisms permitting the animals to live and breed in the surrounding environment. Appropriate thyroid gland function and their hormonal activity are considered crucial to sustain the reproductive and productive performance in domestic animals (Todini, 2007).Many endogenous and environmental factors are able to affect thyroid activity and hormone concentrations in blood, acting at the level of hypothalamus, pituitary and/or thyroid gland, as well as on peripheral monodeiodination(Todini, 2007).The simultaneously effect of age as endogenous and breeding season as environmental climatic factors, on modulating camel thyroid gland activity and/or liver and thyroid extracts were discussed. Gross study of thyroid gland of camel was in concordance with the findings of Kausar and Shahid (2006). Histological studies revealed similar results as previously reported in camel by (Abdel-Magied et al., 2000 and Kausar and Shahid, 2006). Most of the follicles were large in old non-breeding and small in young and old breeding. Gross and histological study of liver were agreed with the findings of Lalla and Drommer (1997) and Endo et al., (2000). A mild to moderate fatty infiltration and small to medium sized lipid inclusions were present in hepatocytes of all animals especially in old age. Siddig (2002) reported that fat content of the camel liver was changed with age and body weight.Data obtained showed that,

within the same season, the old non breeding camel showed a significant decrease in serum TSH than young without any change in serum T3, T4 and FT4. Age and climatic conditions were affected pituitary TSH but each alone not affected the level. Within the same age, TSH and T3 in old non-breeding were significantly decreased than old breeding and this may indicated that the climatic conditions affect the T3 beside TSH. Same results were obtained in dromedary by Abdel–Samee and Marai (1997), Nazif et al., (2007) and Zia-Ur-Rahman et al., (2007) and in Llama by Gauly et al., (1997). They mentioned that the seasonal pattern of blood TH levels often showed maximal values during winter (cold months) and minimal during summer (hot months) and such decline may help the camel to reduce its endogenous heat production during summer. During summer thyroid hormones decrease in the blood because TH is the primary endocrine stimulator of non-shivering thermogenesis in animals (Collin et al., 2005 and Silva, 2005). On the other hand, cold stress in ram lambs (Doubek et al., 2003) and shearing (Merchant and Riach, 2002) induced increases in blood TH levels. However, contrasting results have been reported (Ashutosh et al., 2001 and Yokus et al., 2006). Recently, it has been found that photoperiod regulates the expression of type II deiodinase gene in the mediobasal hypothalamus of the Saanen goat, hence seasonally affecting the bioavailability of TH for the reproductive neuroendocrine axis (Yasuo et al., 2006). The obtained results showed that no significant variation between the different seasons and ages concerning FT4 while a significant decrease in serum T4 was observed in old non-breeding camel than young breeding. TH level in rat was gradually decreased with age as reported by Gromakova and Konovalenko (2004). Concerning

the liver and thyroid extracts, the obtained data showed that no significant variation in the amount of T3 and T4 in the liver extracts. Thyroid extract obtained from camel in the non- breeding season showed a significant increased in T4 at both ages than breeding season at any age. Mendel et al., (1988) reported that the liver extracted 5–10% of plasma T4 during a single passage, this value was much higher than can be accounted for by the amount of free T4 delivered to the liver, indicating that a substantial amount of protein-bound T4 was available for uptake. Peeters, et al., (2005) reported that , the relationship between serum and tissue TH levels was weakest for liver T4 compared with the other TH in liver and muscle. This might suggest that other factors than serum concentrations, such as deiodination and regulation of transport, were more important in the regulation of liver T4 concentration than in the regulation of liver T3concentration.Liver is the major site for cholesterol and triglyceride metabolism, and the thyroid hormones play an integral part in hepatic lipid homeostasis. Concerning parameters of fat metabolism, data showed that age was the main factor affecting measurements. Old camel showed a significant increase in serum triglyceride than young one in the two seasons .Total lipids was significantly increased in old non breeding camel than young in the

same season and young in the breeding season. Cholesterol levels also showed a significant increase in older camel than younger in the corresponding season and older non- breeding than younger breeding. There was a little information about the serum lipids in dromedary camels, however, There were contradictory findings regarding the relation between serum thyroid hormones and cholesterol and triglycerides. The serum cholesterol level generally varies inversely with thyroid activity (Gueorguieva and Gueorguiev, 1997). In contrast, the concentrations of thyroid hormones were not correlated with cholesterol levels in camels and goat (Wasfi et al., 1987 and Nazifi et al., 2002 and 2007). There was no information about the serum lipids in dromedary camels in relation with age, however, Kleinveld (1996) reported that , in humans there was a statistically significant increase in the concentrations of serum cholesterol and triglyceride in advanced age. Hugi and Blum (1997) reported that, in calves, the concentration of cholesterol increased transiently with age, but triglycerides did not show a consistent change. The changes obtained in serum levels of fat metabolic profile during the dry season may indicated that the nutritional status could induce significant changes in the dromedary camel while the available forage during the green season improved the body condition and the blood metabolic profile as previously reported by Amin et al.,(2007).The obtained results showed no significant variation between the two different seasons and ages concerning total protein, albumin, globulin , albumin globulin ratio. This data agree with the work of Abdel –Samee and Marai (1997). Lin et al., (2003)) reported that the major site of plasma protein production is the hepatocyte, and several plasma proteins including haptoglobin and lipoproteins are up-regulated by THS. In the breeding season, level of serum AST was significantly higher in old than young camel, while, old camel during non-breeding season showed a significant increase in serum ALT than young at any season. This suggests that liver function may be partially affected by heat stress as mentioned previously by Abdel –Samee and Marai (1997) or by hypothyroidism in older age (Targher et al., 2008) as the increases in activities of these enzymes have been reported to be associated with hepatic necrosis and other histopathological changes (Khang and Wiktorsson, 2004). However, no significant variation among different seasons and ages concerning ALP and LDH.From the obtained results, it can be concluded that old camel (which is the most tolerant farm animal to hot climate conditions) during the non-breeding season which occurs during summer, the physiological activities concerning pituitary(TSH), thyroid hormones(T3 and T4) and liver function parameters (fat metabolism, ALT and AST) were negatively affected.

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دراسات على محور الغدة النخاميه (الهرمون المحفز للدرق) – الدرقية (الثيروكسين الثلاثى والرباعى والرباعى الحر) – الكبد أثناء الاختلافات الموسميه لعمرين مختلفين لذكور الجمال وحيدة السنام .

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الملخص العربى

أجريت هذه الدراسة لتحديد العلاقة بين الغدتين النخاميه والدرقية والكبد فى ذكور الجمال وحيدة السنام . تم جمع الغدة الدرقية والكبد والدم لعمرين مختلفين أثناء مواسم التزاوج ومواسم الخمول الجنسى من المسالخ بمنطة القصيم .أستخدمت أنسجة الدرقية والكبد للكشف النسيجى ولتقدير محتواها من هرمونات الدرقيه . أستخدم المصل لتقدير الهرمون المحفز للدرق (TSH) وهرمونات الدرقية الثيروكسين الثلاثى والرباعى والرباعى الحر (T3, T4 , FT4) . تم ايضا قياس وظائف الكبد على بعض صور أيض الدهون والبروتين تم قياس نشاط بعض انزيمات فى المصل مثل ALT, AST , LDH, ALP . أظهرت النتائج أن معظم حويصلات الدرقية كانت كبيره فى الاعمار للجمال أثناء فترة الخمول الجنسى وصغيرة أثناء الاعمارالصغيرة والكبيرة أثناء فترة النشاط الجنسى . كانت ظاهرة التسرب الدهنى والاجسام الدهنية الخفية الى المتوسطةعامة فى خلايا الكبد لكل الحيوانات التى تمت دراستها وخاصة فى الاعمار الكبيره . وكان هناك نقص معنوى فى الهرمون المحفز للدرق والثيروكسين الثلاثى فى مصل الابل الكبيره أثناء موسم الخمول الجنسى عن الكبيره أثناء موسم التزاوج . لم يكن هناك فرق معنوى فى مستويات الثيروكسين الحر بينما كان هناك خفض معنوى فى الثيروكسين الرباعى فى الاعمار الكبيره عن الصغيرة أثناء مواسم التزاوج . لم يكن هناك فرق معنوى فى محتويات الكبد من الثيروكسين الثلاثى والرباعى بينما محتويات الدرق للجمال الكبيرة أثناء الخمول الجنسى أظهرت زيادة الثيروكسين الرباعى للاعمار المختلفة عن الفترة النشاط الجنسى أظهرت النتائج زيادة معنوية فى الجلسريد الثلاثى والدهن الكلى والكوليسترول فى الاعمار الكبيرة عن الصغيرة . لم يكن هناك فروق معنوية فى الحيوانات التى تمت دراستها على البروتين الكلى والالبيومين والجلوبيولين والنسبة بينهما والانزيمين LDH, ALP . كانت مستويات الانزيم AST مرتفعة فى مصل الإبل الكبيرة عن الصغيرة بينما مستويات الانزيم ALT مرتفعة فى الكبيرة أثناء موسم الخمول الجنسى عن الصغيرة فى اى موسم . خلصت الدراسة الى انه من الواضح ان الابل الكبيرة (وهى التى تعتبر من اكثر الحيوانات مقاومة للمواسم الحارة) أثناء موسم الخمول الجنسى (الصيف) يتغير النشاط الفسيولوجى للغدة النخامية (الهرمون المحفز للدرق) والدرقية (الثيروكسين الثلاثى والرباعى) وبعض مفردات وظائف الكبد (أيض الدهون وأنزيم AST, ALT) .