Influence of Pregnancy Stages on Thyroid Status and Blood Metabolites in Desert Ewes Raised under Range Conditions in West Kordufan State, Sudan

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DISCLAIMER: The authors declare that they have no conflict of interest.

Authors' contributions

IAA and AOA designed the experiment. IAA performed the experiment and statistical analysis of data and wrote the first version of the manuscript. AOA, AMA WEE, OHA and AOA confirmed scientific authentication of data and participated in preparation of the manuscript. All authors revised and approved the final version of the manuscript.

ABESTRACT:

This study aimed to evaluate the influence of the stages of pregnancy in two ecotypes of sheep, located in different grazing areas on thyroid status and certain blood metabolites. Eighty clinically healthy ewes (40 Hamari and 40 Kabashi ecotype) aged 2-5 years were used in the study. For each ecotype, the ewes were divided into four groups according to the reproductive status. Group (A): 10 ewes non-pregnant non-lactating (dry) served as the control. Group (B): 10 ewes in the early stage of pregnancy (first trimester). Group (C): 10 ewes in mid-stage of pregnancy (second trimester). Group (D): 10 ewes in the late stage of pregnancy (third trimester). Blood samples were used to measure serum levels of thyroid stimulating hormone (TSH), thyroxine (T_4), triiodothyronine (T_3), plasma glucose concentration and serum triglyceride levels. The study detected that Hamari ecotype was supplied with salt and minerals block during watering by its owners. The results showed that in both ecotypes, the serum TSH levels were significantly ($P<0.05$) higher during the early stage of pregnancy then, it decreased significantly ($P<0.01$) du as the pregnancy advanced. While serum T_4 levels were higher during the early stage of pregnancy, then it decreased significantly ($P<0.01$) during the late pregnancy in both ecotypes. Serum T_3 level was significantly ($P<0.001$) higher in both ecotypes during early and mid-pregnancy, then declined to the lowest value during the late stage. The plasma glucose level was significantly ($P<0.001$) lower during mid and late pregnancy in both ecotypes. There was a significant ($P<0.05$) decrease in serum triglyceride level when pregnancy advanced in both ecotypes. Kabashi ecotype showed significantly higher serum triglyceride levels in the control group ($P<0.001$) during early pregnancy stage ($P<0.01$) and mid pregnancy stage ($P<0.05$) compared to the values of serum triglyceride levels in Hamari ecotypes. This study indicated that pregnancy altered thyroid status in both ecotypes, however, the influence of pregnancy on the thyroid status was less in the Hamary ecotype which supplemented by mineral and salt blocks. Therefore, supplementation with minerals during pregnancy might enhance the metabolic profile on the natural pasture grazing.

Keywords: Thyroid. Glucose.Trigyceride. Desert Ewes.

INTRODUCTION

Sudan is one of the largest countries in Africa, characterized by a great number of livestock, vast areas of range and cultivated land. Sheep are multiple purpose animals, providing meat, milk and skin. They are raised under nomadic condition with traditional methods of management and natural grazing [1]. sheep population is estimated at 41 million, which represent over 36% of the livestock in the country. Most are desert sheep distributed across the low rainfall Savannah, semi-desert and desert zones [2]. The desert sheep production in the marginal area in Kordufan constitutes an important source of income for the nomadic tribes. There are many ecotypes of desert sheep in Kordufan State; however the major ecotypes are Hamari and Kabashi sheep [3].

About 90% of sheep are owned by the traditional producers who mainly depend on the natural grazing to raise their animals [4]. The nutritional limitation, low nutritive value of the forages, high ambient temperature, scarcity of feed and water have a great effect on sheep production [5], which could be associated with the activity of the thyroid gland.

Thyroid hormones (TH) influence most cells of the body and regulate growth rate chemical reactions [6]. The fundamental role of thyroid hormone in the body is attributed to the stimulation of metabolic activity by increasing the circulating hormones, particularly T_3 and T_4 plasma concentrations to sustain and improve animal nutrition and production. However, thyroid dysfunctions are considered important endocrinopathies in both human and animals [7, 8]. Pregnancy is a dynamic process characterized by dramatic physiological changes [9]. During pregnancy, thyroid activity is reported to be changed in most of the mammalian species investigated [10]. Serum biochemical metabolites might be altered by pregnancy; changes in the concentrations of serum biochemical profiles in different physiological conditions have been investigated in animal [10]. During pregnancy, several metabolic changes and adaptations take place [11]. Despite the importance of thyroid dysfunction, no data is available on sheep in Sudan, probably; there were no obvious clinical signs (goiter). However, studies have showed subclinical changes in thyroid activity, which could affect productivity in animals [12]. On the other hand, studies in West Kordufan state showed clinical and subclinical colloid goiter in camels [13]. The prevalence of thyroid disorder was also, high in humans in North Kordufan [14]. The aim of the present study was to evaluate the influence of pregnancy stage in two different ecotypes of desert sheep (Hamari and Khabashi), located in different grazing areas, on thyroid status and certain blood metabolites.

MATERIALS AND METHODS

Study area

The present study was conducted in West Kordufan State, that is located in Southwestern part of the region of Kordufan, Sudan, between latitude 11^{o} 20’ North and 32 ^{o} 22-30^{o} 27 East, and lies between the South Kordufan, North Kordufan and East Darfur state [15]. The Southern part of the state is characterized by heavy rainfall, vegetation, trees and heavy clay soils. The Northern part is a medium-range rain and sandy soil [15]. In the present study, samples were collected from Southern and Northern locations: Qubaiesh and Alnuhod areas.

Animals and Experimental plan

Eighty clinically healthy ewes (40 Hamari and 40 Kabashi ecotype) aged 2-5 years were used in the study. For each ecotype, the ewes were divided into four groups according to the reproductive statues. Group (**A**): 10 ewes non-pregnant non-lactating (dry) ewes served as a control. Group (**B**): 10 ewes in early stage of pregnancy (first trimester). Group (**C**): 10 ewes in mid stage of pregnancy (second trimester). Group (**D**): 10 ewes in late stage of pregnancy (third trimester). Pregnancy was diagnosed by palpation and the information obtained from the owners.

Grazing and Management of animals

Animals were maintained on natural pasture grazing. However, Hamari ecotype was supplied salt and minerals block during watering by its owners. While in the area of Kabashi ecotype there was a shortage of water, dry matter and poor management. During the summer season, the flocks spent more time near the watering point and the animals were watered 2-3 times per week.

Blood samples collection

Blood samples were collected by jugular venipuncture once at 8:00 a.m. Ten (10) ml of blood was collected and immediately 2 ml of blood was transferred to a test tube containing sodium fluoride as anticoagulant for determination plasma glucose concentration. The rest of blood samples were allowed to stay at room temperature for 3 hours and then centrifuged at 3000 r.p.m. for 15 min. Hemolysis-free serum samples were separated and transferred to clean plastic vials, and immediately frozen at -20C$^{\circ}$ for TSH, T4, T3, total protein and triglycerides measurement. Thyroid function tests were carried out by measuring serum levels of TSH, thyroxin (T4) and triiodothyronine (T3) using microplate immune-enzymetic assay Kit-Antrim OIS available from (Fortress Company). Plasma glucose concentration was determined by the enzymatic method using a kit (Biosystem-Spain), as described by Rognoni and Ronchini [16]. The serum total proteins concentration was determined by the Biuret methods as described by

King and Wootton [17]. Serum creatinine concentration was measured by spectrophotometer described by Fabian and Ertingshausen [18].

Statistical Analysis

For data obtained in the present study, the mean values ± standard deviation were computed and analyzed using statistical package for social science (SPSS) version 21. One-way (ANOVA) test and t-test were used to assess the significant difference among the groups. Significant differences were considered at ($P \leq 0.05$).

RESULTS

The results showed that in both ecotypes, serum TSH level increased significantly ($P<0.05$) during early stage of pregnancy, afterwards it decreased numerically with advance of pregnancy. However, there was no significant difference in serum TSH values between Hamari and Kabashi ecotypes (Fig. 1, Table 1). Serum T_4 levels increased numerically during early stage of pregnancy in both ecotypes, then the serum levels of T_4 decreased significantly ($P<0.01$) during the late stage of pregnancy in both ecotypes (Fig. 2, Table 2). Serum T_3 level increased significantly ($P<0.001$) in Hamari ecotype and numerically in Kabashi ecotype during the early and mid-stages of pregnancy, then the serum T_3 levels declined to lowest value during late stage in both ecotypes. Interestingly, the highest values of serum T_3 were observed only in Hamari ecotype in the control group ($P<0.01$) as well as during the early stage of pregnancy ($P<0.001$) ($P<0.001$) (Fig. 3, Table 3). In both ecotypes, the Plasma glucose level decreased significantly ($P<0.001$) in mid and late stage of pregnancy. However, there was no significant difference in plasma glucose level between Hamari and Kabashi ecotypes (Fig. 4, Table 3). The effect of stages of pregnancy and ecotype on serum triglyceride concentration in Hamari and Kabashi ewes were showed in Fig 5 and Table 3. In both ecotypes, there was a significant ($P<0.05$) decrease in serum triglycerides concentration with the progress of pregnancy, particularly during the late stage compared to the early and mid-stages of pregnancy. The effect of ecotype shows that there was a significant (P<0.001), (P<0.01) increase serum triglyceride concentration in Kabashi ecotype in empty and the during the early stage of pregnancy, respectively.

DISCUSSION

During pregnancy, thyroid activity was reported to be changed in sheep [19]. In the present study, the results indicated that thyroid status influenced by pregnancy and ecotype in desert ewes (Hamari and Kabashi) raised under range conditions. The data indicated that there was a significant increase in serum TSH concentration with advancing pregnancy, then the value of serum TSH declined during the late pregnancy in both ecotypes. This pattern of response could be attributed to the elevated estrogen concentration, which could influence the liver activity to increase the serum thyroxin binding globulin (TBG) concentration, elevated TBG concentration leads to a decrease in free T_4 concentration, which results in increased TSH secretion by the pituitary gland [20]. An increase in concentration of TSH leads to increase in serum T_4 and T_3 values ultimately decrease TSH concentration by the negative feedback [21]. Similar pattern was observed in human [22, 23, 24] and in goats [25]. Noteworthy, a study in camels do not show a significant difference in TSH concentration between pregnant and non-pregnant dromedary camels [26].

Serum T_4 concentration increased numerically in early and mid-stages of pregnancy, then the value of T_4 decreased significantly during the late pregnancy in both ecotypes. This decrease is probably due to the growth fetus which plays a competitive role higher thyroid activity, iodine affinity and uptake than maternal ones [27]. Furthermore, the decrease in serum T_4 may be attributed to an increase in T_4 deionization by deiodinase type 2 in the placenta, which plays a critical role in the delivery of T_4 to the fetus [28]. The current observations are in agreements with previous studies in ewes [29, 30, 31]. Similar studies indicated a decrease in the maternal serum T_4 concentration during late pregnancy in goats [27,32] and crossbreed dairy cows [33]. However, earlier studies did not show significant change in thyroid activity in ewes during different stages of pregnancy [34, 35]. Regarding the ecotype, the present study revealed that during late pregnancy, Hamari ecotype showed higher value of T_4 compared to respective value of Kabashi ecotype (. The higher serum T_4 in Hamari ecotype could be to mineral supplementation offered to Hamari ecotype. Serum T_3 showed the same pattern of serum T_4

during different stages of pregnancy in both ecotypes, but the change in hormone value was numerical and not significant in Kabashi ecotype. The decrease in serum T_3 values during late pregnancy is probably attributed to the negative energy balance [19]. The current results are in line with previous studies in ewes [19, 30]. Studies on goat and in crossbreed dairy cow also indicated a decrease in the maternal serum T_3 concentration with advancing pregnancy [27, 32, 33]. Other investigations did not show any significant effect in the T_3 value in ewes among different stages of pregnancy [34, 35]. In the current study, the mean plasma glucose concentration decreased significantly ($P<0.001$) with advance of pregnancy in both ecotypes (Fig. 4, Table 4). The decrease could be attributed to mobilization of maternal glucose into fetal circulation through an active transport process across the placenta. A decrease in glucose level with advance of pregnancy was also reported by Jacob and Vadodaria [36], who attributed it to pregnancy glucose requirements for both the dam and the fetus. The reduction in glucose concentration during late pregnancy was observed in several studies in ewes [37, 38, 39] and in goats [25]. In contrast, other studies reported an increase in glucose concentration with the advance of pregnancy [40].

The results obtained indicated a significant decrease in serum triglycerides concentration in late pregnancy in both ecotypes (Fig. 5, Table 5). Previous studies indicated decrease in insulin concentration during late pregnancy combined with lower sensitivity of adipose tissue to insulin and increase in somatotropin may reduce the synthesis of triglycerides in the adipose tissues and favor their mobilization during this period [41, 42]. This result may indicate that the plasma lipids content is sensitive and it is affected by many factors. A study by [43] confirmed that the amount and composition of the plasma circulating lipids of dairy cows were dependent upon number of physiological variables, includes the nature of the diet, time since feeding, age, ecotype, pregnancy and stage of lactation. In the present study, many factors could influence the triglycerides concentration in the body especially, feed intakes and feeding regimes. Previous studies confirmed decline in triglycerides concentration with advance of pregnancy [39, 44]. On the other hand, some researchers showed a gradual increase in serum triglycerides concentrations with the advance of pregnancy [37, 41]. In the current study, the lower mean values of serum triglycerides concentration in Kabashi compared to Hammari is probably due to poor handling and management for Kabashi ecotype.

CONCLUSION

It was concluded that pregnancy induces variable alterations in thyroid status associated with change in certain relevant blood metabolites. In both sheep ecotype, there was a significant increase in serum TSH, T_4 and T_3 values in early and mid stages of pregnancy then, declined significantly during the late stage of pregnancy, and there was a significant increase in serum T_3 concentrations in Hamari in dry ewes and during the early stage of pregnancy due to iodine and minerals supplementations. Moreover, serum metabolic profile test can be applied as an excellent trend for the assessment of the nutritional status during pregnancy to avoid the nutritional disorders associated with the pregnancy.

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Table 1. Effects of stages of pregnancy and ecotype on TSH concentration (mIU/L) in Hamari and Kabashi ewes (n = 80)

Stages of pregnancy	Ecotype		
	Hamari	Kabashi	LS
Dry	$^{A}0.28\pm0.07^{a}$	$^{A}0.22\pm0.05^{a}$	NS
Early Pregnancy	$^{B}0.36\pm0.10^{a}$	$^{B}0.28\pm0.09^{a}$	NS
Mid Pregnancy	$^{AB}0.29\pm0.04^{a}$	$^{B}0.35\pm0.10^{a}$	NS
Late Pregnancy	$^{A}0.27\pm0.09^{a}$	$^{AB}0.25\pm0.08^{a}$	NS
LS	*	*	

$^{A, B:}$ Mean values within the same column with different superscripts (capital) are significantly different.

$^{a, b}$: Mean values within the same row with different superscripts (small) are significantly different.

LS: Level of significance * $P<0.05$

NS: Not significant

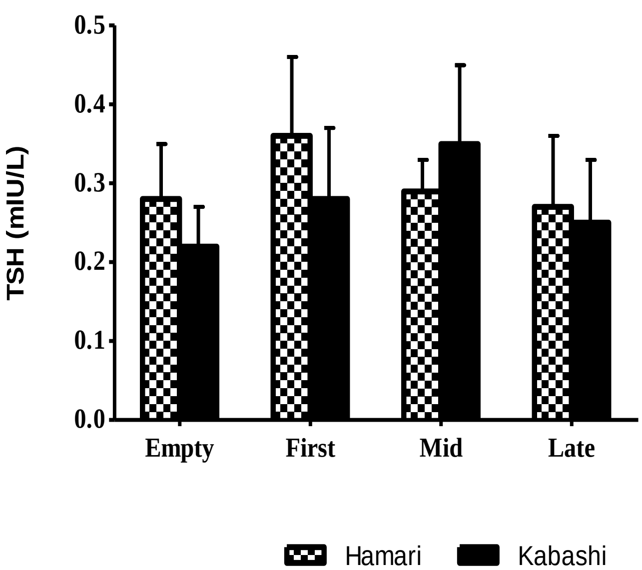

Figure 1: Effects of stages of pregnancy and breed on TSH in ewe

Table 2. Effects of stages of pregnancy and ecotype on serum T_4 concentration (μg/dL) in Hamari and Kabashi ewes (n = 80)

Stages of pregnancy	Ecotype		
	Hamari	Kabashi	LS
Dry	$^{A}5.37±1.18^{a}$	$^{A}5.05±0.93^{a}$	NS
	$^{A}6.40±1.74^{a}$	$^{A}5.48±1.30^{a}$	NS
Early Pregnancy	$^{A}5.49±1.29^{a}$	$^{A}4.89±0.60^{a}$	NS
Mid Pregnancy	$^{B}4.15± 0.65^{a}$	$^{B}3.90±0.66^{b}$	*
Late Pregnancy			
LS	**	**	

$^{A, B:}$ Mean values within the same column with different superscripts (capital) are significantly different.

$^{a, b}$: Mean values within the same row with different superscripts (small) are significantly different.

LS: Level of significance ***** P< 0.05 ****** P< 0.01

NS: Not significant

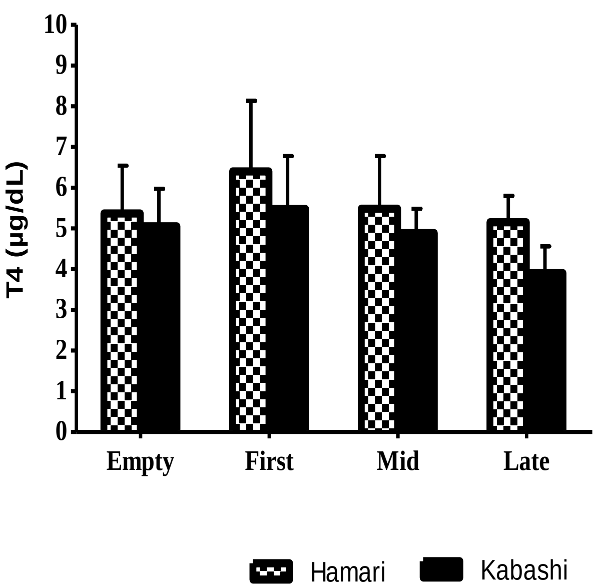

Figure 2: Effects of stages of pregnancy and breed on serum thyroxine hormone T_4 in ewe

Table 3. Effects of stages of pregnancy and ecotype on serum T_3 (ng/mL) in Hamari and Kabashi ewes (n = 80)

Stages of pregnancy	Ecotype		
	Hamari	Kabashi	LS
Dry	^{A}1. 02±0.30^{a}	^{A}0.30 ±0.08^{b}	**
Early Pregnancy	^{B}2.35±0.57 ^{a}	^{A} 0.48 ±0.22^{b}	***
Mid Pregnancy	^{C}1.50±0.87^{a}	^{A} 0.50 ±0.45^{a}	NS
Late Pregnancy	^{D}0.35±0.12^{a}	^{A} 0.34 ±0.12^{a}	NS
LS	***	**	

$^{A, B, C, D:}$ Mean values within the same column with different superscripts (capital) are significantly different.

$^{a, b}$: Mean values within the same row with different superscripts (small) are significantly different

LS: Level of significance ** P<0.01 *** P<0.001

NS: Not significant

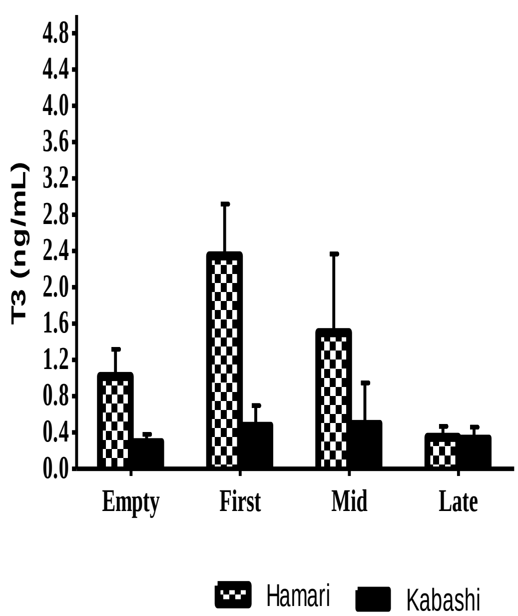

Figure 3: Effects of stages of pregnancy and breed on serum triiodothyronine T_3 in ewe

Table 4. Effects of stages of pregnancy and ecotype on plasma glucose (mg/dL) in Hamari and Kabashi ewes (n = 80)

Stages of pregnancy	Ecotype		
	Hamari	Kabashi	LS
Dry	^{A}52.9±4.13^{a}	^{A}51.4±5.89^{a}	NS
Early Pregnancy	^{AB}44.3±6.48^{a}	^{A}49.8±5.43^{a}	NS
Mid Pregnancy	^{B}42±6.67^{a}	^{B}43±7.08^{a}	NS
Late Pregnancy	^{CB}39±6.69^{a}	^{B}40.3±5.55^{a}	NS
LS	***	***	

$^{A, B, C:}$ Mean values within the same column with different superscripts (capital) are significantly different.

$^{a, b}$: Mean values within the same row with different superscripts (small) are significantly different.

LS: Level of significance ******* P<0.001

NS: Not significant

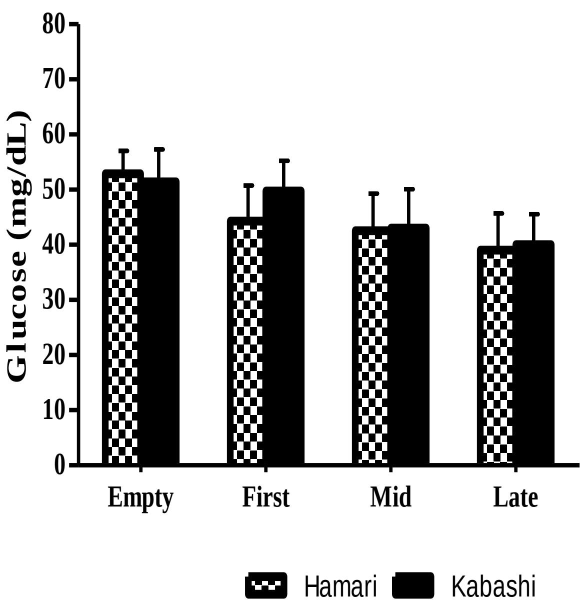

Figure 4: Effects of stages of pregnancy and breed on plasma glucose in ewe

Table 5. Effects of stages of pregnancy and ecotype on serum triglycerides (noml/dL) in Hamari and Kabashi ewes (n = 80)

Stages of pregnancy	Ecotype		
	Hamari	Kabashi	LS
Dry	$^{A}0.65\pm0.12^{a}$	$^{A}1.06\pm0.21^{b}$	***
Early Pregnancy	$^{A}0.64\pm0.11^{a}$	$^{A}0.99\pm0.23^{b}$	***
Mid Pregnancy	$^{A}0.61\pm0.12^{a}$	$^{A}0.87\pm0.29^{b}$	*
Late Pregnancy	$^{B}0.45\pm0.09^{a}$	$^{B}0.35\pm0.17^{a}$	NS
LS	*	*	

$^{A, B}$: Mean values within the same column with different superscripts (capital) are significantly different.

$^{a, b}$: Mean values within the same row with different superscripts (small) are significantly different.

LS: Level of significance ***** P<0.05 ****** P<0.01 ******* P<0.001

NS: Not significant

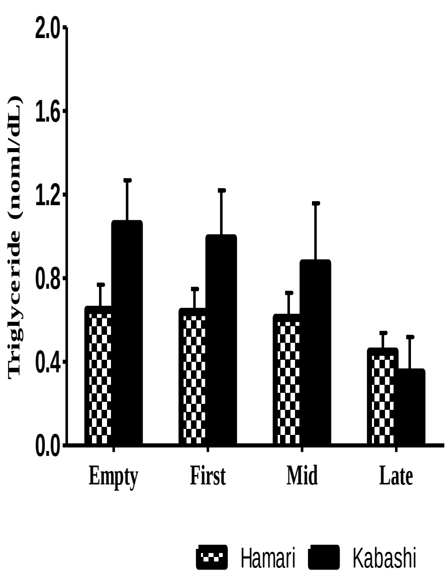

Figure 5: Effects of stage of pregnancy and breed on serum triglyceride in ewe