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Endogenous and seasonal factors influencing circulating thyrotropin concentration in Red Sokoto and Sahel goats

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Abstract

Through regulating thyroid physiology, thyrotropin influences the activities of thyroid hormones, including thermogenesis and adaptation to ambient thermal challenges. This study aimed at evaluating the influences of some endogenous factors (breed, sex, age and body mass index; BMI) and season on circulating thyrotropin concentration in Red Sokoto and Sahel goats. A total of 120 goats were sampled, comprising Red Sokoto (n = 20) and Sahel (n = 20) goats in each season during the peak of the cold-dry (CDS), hotdry (HDS) and rainy (RAS) seasons in a tropical Savannah climate. Results revealed that among Red Sokoto goats, bucks had significantly higher (P<0.05) circulating thyrotropin concentration than does during the HDS, while adults had significantly higher values than kids during the RAS. Significant (P<0.05) breed difference was only observed during the HDS, with the Red Sokoto goats having higher values among adult bucks and doelings but lower values than Sahel goats among buck-kids and adult does. Significant (P<0.05) seasonal variation was observed only in male Sahel goats with buck-kids having lower values during RAS as compared with CDS and HDS, while adult bucks had higher values during the RAS as compared with the HDS. A significant positive correlation (r = 0.29; P<0.001) was observed between thyrotropin concentration and BMI in the overall population. It was concluded that circulating thyrotropin concentration was higher in male and adult goats, and the effects of season and breed were sex and age-dependent. Moreover, the direct relationship between thyrotropin concentration and BMI may reflect the role of thyrotropin in the accumulation of body energy reserve in the savannah goats.

Keywords: Age, Breed, Body mass index, Sex, Thyroid-stimulating hormone

Introduction

One livestock product from Northern Nigerian with great economic value that attracted global attention

during the colonial era was the skin of the Red Sokoto goat (RSG), exported as the famous Morocco leather.

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It acquired this name because it was transported across the Sahara by caravans controlled by Moroccan merchants (Adebayo, 1992; Blench, 1999). For this reason, the SRSG is described as 'the most famous African goat that no one has heard of'. On the other hand, the Sahel goat, which is also a Savannah goat type like the Red Sokoto goat, has been described as the sheep-like breed of goat, found in the Sahel belt of West Africa, as it is distributed from Senegal to Sudan (Blench, 1999). All year-round, goats and other livestock inhabiting some regions of West African, including the Northern Guinea Savannah, experience a combination of environmental conditions which have been classified into three major seasons, namely: the cold-dry (harmattan; CDS), hot-dry (HDS) and rainy (RAS) seasons (Igono & Aliu, 1982; Habibu et al., 2017). The challenge of the CDS is the cold-dry wind, heavy dust-laden particles, and wide fluctuations in day and night ambient temperatures, while that of the HDS is the high ambient temperatures that predispose livestock to heat stress (Minka & Ayo, 2014; Habibu et al., 2017; Sejian et al., 2019).

Thyrotropin or thyroid-stimulating hormone (TSH) is a pituitary hormone that influences most activities of thyroid hormones by binding to the TSH receptors in the thyroid gland to induce the release of thyroxine (T₄) and a limited amount of tri-iodothyronine (T₃; Klein, 2019; Ertek, 2021). Besides, this basic function, TSH has been linked to obesity in humans and immune system in humans and mice (Smith et al., 1983; Kruger et al., 1989; Bell et al., 2000; Valyasevi et al., 2002; Al-Musa, 2017; Klein, 2019). Seasonality in circulating T₃ and T₄ in goats is physiologically manipulated to maintain body temperature within normal range, irrespective of season (Habibu et al., 2016a). Circulating T₃ and T₄ has been reported to be higher in Red Sokoto than Sahel goat kids (Habibu et al., 2016a; Sejian et al., 2019). Similarly, circulating T₃ and T₄ have been shown to be higher in growing goats than adults, while does have higher values than bucks (Colavita et al., 1983; Todini et al., 1992; Todini, 2007). Probably, due to the anabolic effect of T₃ and T₄ on protein synthesis, especially in growing subjects (Capen & Martin, 2003), the correlation between body mass index (BMI) and both T₃ and T₄ was positive in goats (Habibu et al., 2016a). While the influences of breed, sex, age, BMI and season on circulating T₃ and T₄ have been well reported in goats (Todini et al., 1992; Todini, 2007; Habibu et al., 2016a; 2017), there is a paucity of information on the influence of these factors on TSH in goats. This is despite the fact that circulating T₃ and T₄ are under the influence of TSH (Todini, 2007). Understanding how breed, sex, age, BMI and season collectively influence both thyroid hormones and TSH may significantly complement the existing knowledge on the mechanism through which different breeds, sexes and age groups of livestock cope with seasonal changes. The aim of this study was to evaluate the influences of breed, sex, age, BMI and season on circulating TSH concentrations in Red Sokoto and Sahel goats.

Materials and Methods

Experimental site and animal management

The study was carried out in the Small Ruminant Research Section of the National Animal Production Research Institute (NAPRI), Ahmadu Bello University, Shika, Zaria, Nigeria, and located on latitude 11⁰ 12' N, longitude 7^o 33' E and an altitude of 610m above sea level. The goats were housed in a half-walled pen, roofed with galvanized metal sheet, and the floor was covered with concrete. The pens were well-ventilated and had dimensions of 6.1, 6.1 and 2.2m for length, width and height, respectively. The pens had a stocking density of 35 kids. The health status of the goats was evaluated based on behaviour, appetite and faecal consistency. Routinely, the goats were screened by a trained staff member for haemoparasites and helminths (using floatation and sedimentation tests). Only clinically healthy animals were used for the study. Both breeds of goat were housed within the same environment. Oestrous synchronisation of the does for breeding was carried out using controlled internal drug release (CIDR) and the bucks were allowed to mate the does naturally. The kids were provided with milk by directly suckling their dams and weaned at 3 months of age. The adult goats were fed on Digitaria smutsi hay as a basal diet. All the goats were supplemented with a concentrate ration of ground maize (30%), cottonseed cake (36%), maize offal (20%), wheat offal (10%), bone meal (2.5%) and salt (1.5%) at 3% body weight per day. Good-quality drinking water was provided ad libitum.

Experimental design

The adult Red Sokoto and Sahel goats used for the study were homogeneous for age (4 to 5 years), live weight (25 \pm 1.6 kg) and body condition score (2.9 \pm 0.1). The age of kids for both breeds ranged between 2 to 4 months. Body condition was scored by the same person adopting a six-point scale method (Santucci *et al.*, 1991). The goat kids were sampled at the peak of the CDS, HDS and RAS during the months of December, April and July, respectively. A total of 120

goats were used in the study. In each season, 40 different sets of goats (20 goats from each of the two breeds) of both sexes were sampled. For each of the breeds, five (n = 5) goats of different sexes (buck and does) and age groups (adults and kids) were used in every season. Five millilitres (5 ml) of blood sample was obtained from each goat in the morning hours, between 09:00 and 11:00 h (GMT + 1), through the jugular vein into vacutainer tubes containing potassium ethylenediaminetetraacetic acid. The adapted study protocol and animal experimentation followed the international guidelines for animal welfare.

Morphometric dimensions of the goats for the determination of BMI were obtained using a measuring tape. Body length was measured as the length from the external occipital protuberance to the base of the tail, while withers height was measured as the length from the surface of a platform to the withers as described by Hassan & Ciroma (1992). Body mass index was then determined using the mathematical formula described by Tanaka *et al.* (2003):

BMI = Body Weight (kg) / {Withers Height (m) / Body Length (m) × 10}

Values of ambient temperature and relative humidity in the morning and afternoon hours of the study period were obtained from the Meteorological Unit, Institute of Agricultural Research, Ahmadu Bello University, Zaria, Nigeria; located about 6 km away from the experimental site. The temperature-humidity index (THI) was then calculated (Ravagnolo $et\ al.$, 2000): THI = $(1.8 \times T + 32) - \{(0.55 - 0.0055 \cdot RH) \times (1.8 \times T - 26)\}$; where: T = ambient temperature (°C) and RH = relative humidity (%).

Concentrations of circulating TSH were determined from the serum using a commercial enzyme-linked immunoassay kit (AccuBind ELISA Microwells; Monobind Inc® California, USA). Assay sensitivity was 0.078 µIU/mL and the intra-assay coefficients of variation for normal levels of TSH were less than 10%. All assays were performed following the manufacturer's instructions.

Data analysis

The statistical package used was GraphPad Prism version 5.01 for windows (2007) from GraphPad Software, San Diego California, USA. Values obtained were expressed as mean (±SEM). The 3rd and 97th percentiles were calculated as the of circulating TSH because of the skewed data distribution. Comparisons between breeds and sexes were done using Student's t-test, while one-way analysis of variance, followed by Tukey's post hoc test were used to compare values among the seasons. Values of P < 0.05 were considered significant.

Results

The mean values of meteorological parameters in the study area are presented in Table 1. The highest (P < 0.05) mean ambient temperature and THI were recorded during the afternoon hours of the HDS. While the highest (P < 0.05) relative humidity was recorded during the RAS in the morning hours, the lowest (P < 0.05) THI was recorded in the morning hours of the CDS.

The normal range of TSH concentrations in Red Sokoto and Sahel goats are presented in Table 2. The overall mean values of TSH concentrations in Red Sokoto tended to be lower (P = 0.13) than that of Sahel goats (0.76 vs $0.82 \,\mu IU/mI$). Similarly, the

Table 1: Mean values of meteorological parameters during the cold-dry, hot-dry and rainy seasons

Parameters	Cold-dry		Hot-dry		Rainy	
	Morning	Afternoon	Morning	Afternoon	Morning	Afternoon
Ambient Temperature (°C)	17.45	29.12 ^x	25.00	39.11 ^y	23.30	30.79 ^x
Relative Humidity (%)	20.06	16.23	49.99	25.51	76.61	71.42
THI	56.96 ^x	73.13 ^x	70.97 ^y	85.21 ^y	71.21 ^y	80.31 ^y

Values with different superscript (x,y) indicate significant (P < 0.05) difference between seasons. THI = temperature-humidity index

Table 2: Normal range of TSH (μ IU/ml) in the goats studied

	Mean ± SEM	Median	Min-Max	Р3	P97	Skewness	Kurtosis	Range
RSG	0.76 ± 0.02	0.72	0.17 -1.61	0.50	1.50	1.69	5.23	0.50 - 1.50
SG	0.82 ± 0.03	0.72	0.51 - 1.9	0.51	1.56	1.74	3.04	0.51 - 1.56
Total	0.79 ± 0.02	0.72	0.17 -1.90	0.51	1.50	1.79	4.12	0.51 - 1.50

RSG – Red Sokoto Goat; SG – Sahel Goat

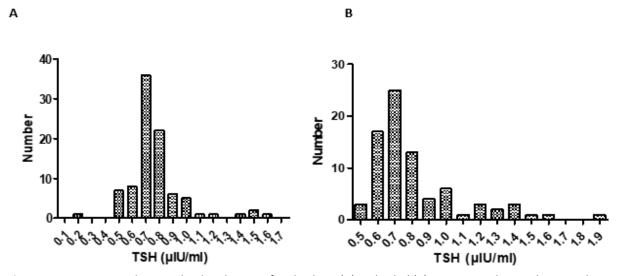


Figure 1: Histograms indicating the distribution of Red Sokoto (A) and Sahel (B) goats according to their circulating concentrations of TSH

normal range of TSH concentrations in Red Sokoto was lower than that of Sahel goats (0.50 - 1.50 vs 0.51– 1.56 $\mu\text{IU/ml}$). The distribution of Red Sokoto and Sahel goats with respect to their TSH concentrations (Figures 1 and 2) showed more skewed distribution, but lower kurtosis in Sahel than Red Sokoto goats.

Effects of age and sex on circulating concentrations of TSH in Red Sokoto and Sahel goats during the CDS, HDS and RAS are presented in Tables 3 and 4, respectively. In Red Sokoto goats, significantly lower (P < 0.05) value of circulating TSH was observed in kids compared with adults during the RAS and in the overall data. Similarly, in Red Sokoto goats, serum TSH concentration was significantly higher (P < 0.05) in males than in females during the HDS.

There was no significant difference between age groups and sexes in the values of circulating TSH in Sahel goats. The effects of breed and season on circulating concentration of TSH in buck-kids and doelings are shown in Figures 3 and 4, respectively. The circulating concentration of TSH was significantly lower (P < 0.05) in Red Sokoto than Sahel goats during

The circulating concentration of TSH was significantly lower (P < 0.05) in Red Sokoto than Sahel goats during the HDS in buck-kids, while in doelings, the value was higher (P < 0.05) in Red Sokoto than Sahel goats. In buck-kids of Sahel goats, serum concentration of TSH was significantly lower (P < 0.05) in the RAS compared with the CDS and HDS. While in doelings of Sahel goats, serum concentration of TSH was significantly lower (P < 0.05) during the HDS compared with the RAS.

Figures 5 and 6 show the effect of breed and season on circulating TSH concentration in adult bucks and

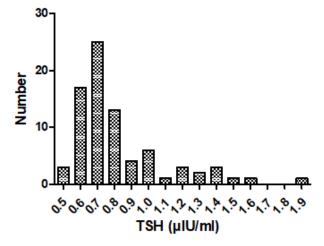


Figure 2: Histogram indicating the distribution of both Red Sokoto and Sahel goats according to their circulating concentrations of TSH

does, respectively. Like in the kids, significant effect of breed on circulating TSH concentrations in adult goats was only observed during the HDS. Circulating concentration of TSH was significantly higher (P < 0.05) in Red Sokoto than Sahel goats during the HDS in bucks, while in does, the value was lower (P < 0.05) in Red Sokoto than Sahel goats. Similarly, significant seasonal variation was observed only in male Sahel goats. The buck-kids of Sahel goats had significantly lower (P < 0.05) serum concentration of TSH during the RAS compared with the CDS and HDS, while the adult bucks had higher (P < 0.05) values during the RAS as compared with the HDS.

Table 3: Effect of age on serum concentration of TSH (μIU/ml) in Red Sokoto and Sahel goats in different seasons

Season	Red Sokoto		Sahel		
	Young	Adult	Young	Adult	
Cold-dry	0.73 ± 0.02	0.77 ± 0.02	0.98 ± 0.01	0.75 ± 0.02	
Hot-dry	0.74 ± 0.02	0.73 ± 0.03	0.76 ± 0.04	0.72 ± 0.04	
Rainy	0.69 ± 0.05^{a}	0.93 ± 0.10^{b}	0.81 ± 0.09	0.92 ± 0.09	
Total	0.72 ± 0.02^{a}	0.81 ± 0.04^{b}	0.80 ± 0.04	0.81 ± 0.04	

Values of the same parameters with different superscripts (a,b) are statistically significant (P < 0.05).

Table 4: Effect of sex on values of serum TSH (μIU/ml) in Red Sokoto and Sahel goats in different seasons

Season	Red Sokoto		Sahel		
	Male	Female	Male	Female	
Cold-dry	0.76 ± 0.02	0.75 ± 0.02	0.89 ± 0.06	0.85 ± 0.08	
Hot-dry	0.77 ± 0.03^{a}	0.70 ± 0.02^{b}	0.77 ± 0.05	0.71 ± 0.04	
Rainy	0.80 ± 0.06	0.81 ± 0.09	0.78 ± 0.09	0.93 ± 0.08	
Total	0.78 ± 0.03	0.76 ± 0.04	0.81 ± 0.04	0.81 ± 0.04	

Values of the same parameters with different superscripts (a,b) are statistically significant (P < 0.05).

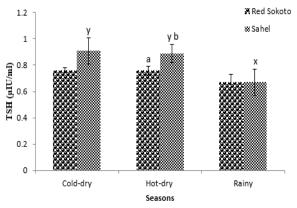


Figure 3: Effects of breed and season on circulating concentrations of TSH in buck-kids. Bars with different alphabets are statistically significant (P < 0.05) for breeds (a,b) and seasons (x,y)

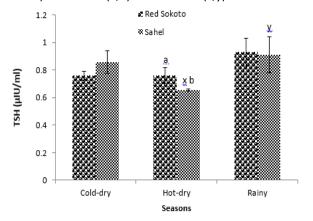


Figure 5: Effects of breed and season on circulating concentrations of TSH in adult bucks. Bars with different alphabets are statistically significant (P < 0.05) for breeds (a,b) and seasons (x,y)

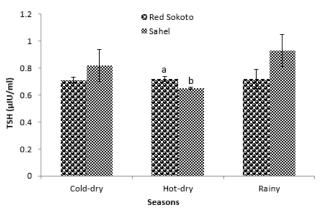


Figure 4: Effects of breed and season on circulating concentrations of TSH in doelings. Bars with different alphabets are statistically significant (P < 0.05) for breeds (a,b)

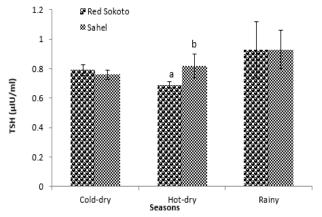


Figure 6: Effects of breed and season on circulating concentrations of TSH in adult does. Bars with different alphabets are statistically significant (P < 0.05) for breeds (a,b)

The association between BMI and circulating concentrations of TSH in young and adult goats are presented in Table 5, while the overall association between BMI and circulating concentrations of TSH is presented in Figure 7. A significant positive correlation ($r=0.29;\ P<0.001$) was observed between TSH concentration and BMI in the overall population. Significant (P<0.05) correlation was only observed in kids with the correlation being positive (r=0.62) during the HDS in Sahel goats, but negative (r=0.58) during the RAS in Red Sokoto goats.

Discussion

The skewness for a normal distribution is zero, and any symmetric data should have a skewness near zero. The distribution of Sahel goats had a higher skewness and skewed to the right tail, towards the higher values. Thus, the Sahel goats had a higher mean value. Kurtosis is a measure of whether the data are heavy-tailed or light-tailed relative to a normal distribution. The high Kurtosis in Red Sokoto than Sahel goats reflects the heavy-tail in the histogram which indicates the existence of more outliers in Red Sokoto than Sahel goats. It is well established that the

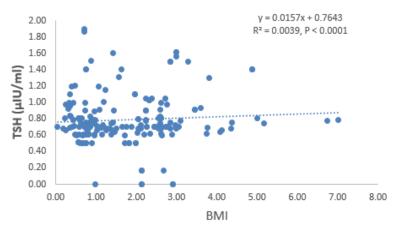


Figure 7: Overall Pearson's correlation coefficient between BMI and circulating concentrations of TSH in goats

normal range of circulating concentration of a given biological molecule is dependent on the assay method used and the population of the subject concern (Schnabl *et al.*, 2008). To our knowledge, values of circulating TSH concentration have not been reported in any breed of Nigerian goats.

The values reported in the current study are lower $(0.78\pm0.03\,\text{vs}\,3.36\pm0.10\,\mu\text{IU/ml})$ than those reported in Angora goats (Pehlivan & Dellal, 2017). Assay technique, breed difference and variations in geographical adaptation may be responsible for the low mean values of circulating TSH concentration in Nigerian Savannah goats as compared to Angora goats.

In goats, meteorological parameters outside the thermoneutral zone for THI (65 – 75) and ambient temperature (24 – 30°C) may induce thermal stress (Lu, 1989; Hamzaoui *et al.* 2013). In the present study, the ambient temperature and THI were higher than the thermoneutral zone in the afternoon hours of the HDS, suggesting heat stress; while in the morning hours of the CDS, the THI was lower, suggesting cold stress. Unlike in sheep, a paucity of data exist on the influence of endogenous and exogenous factors on

circulating levels of TSH in goats (Nouri et al., 2006; Al-Damegh, 2012). The influence of breed on concentration of circulating TSH has been previously reported in sheep (Al-Damegh, 2012). In the current study, breed difference was only observed during the HDS and the effect did not follow a definite pattern according to sex or age. Concentration of circulating TSH was higher in Sahel than Red Sokoto goats in buck-kids and adult does; while lower values were recorded in Sahel than Red Sokoto goat in doelings and adult bucks. Even though the cause of these findings is

Table 5: Pearson's correlation coefficient between BMI and circu	ulating concentrations of TSH in young
and adult goats	

Season		Body mas	s index	
	Youn	g	Adul	t
TSH	Red Sokoto	Sahel	Red Sokoto	Sahel
Cold-dry	-0.27	0.241	-0.08	0.35
Hot-dry	-0.41	0.62*	-0.17	-0.36
Rainy	-0.58*	0.193	0.31	0.36

Values with asterisk (*) are statistically significant (P < 0.05)

not understood, this endocrine pattern suggests that breed difference in circulating TSH levels is mainly expressed when ambient heat load increases and the pattern is influenced by sex and age. Cumulatively, the endocrine pattern expressed during the HDS may reflect the challenges posed by heat stress on the hypothalamus-pituitary-thyroidal axis (Nouri *et al.*, 2006; Habibu *et al.*, 2017). Lesions in the thyroid gland have been reported to be more during summer than winter in apparently healthy sheep (Nouri *et al.*, 2006).

The current study demonstrated a lower circulating TSH concentration in female compared to male Red Sokoto goats during the HDS. This suggests sexual dimorphism in the response of goats to the change in circulating TSH concentration due to effect of heat stress. Also, since the higher circulating concentration of T₄ has been reported in females than males (Todini et al., 1992; Todini, 2007), it is possible that the negative feedback inhibition of TSH secretion from the pituitary may be responsible for the low TSH in females (Capen & Martin, 2003; Todini, 2007). The findings of the current study in Red Sokoto goats disagrees with the report of Pehlivan & Dellal (2017) in which concentration of circulating TSH did not significantly differ between male and female goats, irrespective of season. But agrees with findings of the current study in Sahel goats where sexual dimorphism was not observed in all seasons. This suggests that the expression of sexual dimorphism in TSH activity in goats may be breed and seasonal dependent.

In the current study, the lower concentration of circulating TSH in kids than adults of Red Sokoto goats is probably due to a negative feed-back effect resulting from higher concentration of circulating thyroid hormones in growing than adult goats (Colavita *et al.*, 1983; Ashraf *et al.*, 2021). However, the result of the current study contradicts that of Khan *et al.* (2001) in which circulating TSH was higher in children compared to adults; whereas, the concentration of circulating thyroid hormones was lower in children compared to adults.

On the other hand, the effect of season on concentration of circulating TSH was only expressed in the male Sahel goats, and was not observed in female Sahel and Red Sokoto goats. While the Sahel buck-kids had higher values during the CDS and HDS than the RAS, the adult male Sahel goats had low values during the HDS than the RAS. This seasonal fluctuations suggest the existence of an on-going endocrinological adaptation in the buck-kids and adult male Sahel goats due to exposure to the Guinea

Savannah climate as compared with the female Sahel and the Red Sokoto goats. The Sahel goats are indigenous to the Sahel belt of West Africa, and are until recently, adapted to the Guinea Savannah climate (Gall, 1996; Habibu et al., 2017). Similarly, previous studies have shown low adaptability of Sahel kids to the seasons of the Guinea Savannah as compared to the Red Sokoto kids (Habibu et al., 2016a, b). Lower concentrations of circulating T₃ and T₄ during the HDS as compared with the CDS and RAS has been reported in adult bucks of Sahel goats (Habibu et al., 2017); whereas, the values showed no significant seasonal fluctuation in kids of Sahel goats (Habibu et al., 2016a). Thus, it could be inferred that during the HDS, Sahel bucks simultaneously reduce the production of TSH and thyroid hormones to reduce thermogenic heat production and minimise the impact of heat stress (Indu et al., 2014; Gupta & Mondal, 2019). In the Sahel buck-kids, however, the higher values of circulating TSH may reflect increased production due to reduced responsiveness of the thyroid gland to TSH resulting from heat stressinduced glandular lesions (Nouri et al., 2006) or reduction in glandular secretory activities (Ali et al., 2020). The high values of circulating TSH during the CDS may be a physiological response to cold stress; aimed at increasing production of thyroid hormones to maintain normal body temperature (Habibu et al., 2016a; Kuzmenko et al., 2021). This difference in the seasonal response of young and adult goats may be due to the weak response of buck-kids to the challenge of heat stress, given their physiological immaturity. Similarly, in agreement with the findings of the current study in Red Sokoto goats, no significant seasonal fluctuation in concentration of circulating TSH was observed in Angora goats (Pehlivan & Dellal, 2017).

Growth is accompanied by an increase in BMI, and BMI is a good indicator of nutritional status or body energy reserve in animals and also shows strong direct association with body condition score (Tanaka et al., 2003: Estrada-Cortes et al., 2009; Salazar-Cuytun et al., 2020). In the present study, a negative association was observed between BMI and TSH concentration in young Red Sokoto goats during the RAS, while a positive correlation was observed in young Sahel goats during the HDS and the overall data. In a similar trend, Solanki et al. (2013) reported a positive correlation, while Milionis & Milionis (2013) reported a negative correlation between BMI and TSH concentration in humans. The positive correlation between BMI and TSH is probably due to the role of TSH in stimulating pre-adipocyte differentiation

Valyasevi et al., 2002; Schäffler et al., 2005). The findings of the current study suggest that circulating TSH concentrations may affect BMI and the relationship may be influenced by breed and age. In conclusion, lower concentration of circulating TSH was observed in female compared to male Red Sokoto goats during the HDS. Similarly, breed difference was only observed during the HDS and seems to be age and sex dependent. This suggests that breed difference and sexual dimorphism in circulating TSH in these breeds is mainly expressed when the goats are exposed to high ambient heat load. Concentration of circulating TSH was lower in kids than adult Red Sokoto goats. The effect of season was age dependent, such that values were higher in young goats during the HDS and CDS, while lower values were recorded in adult goats during the HDS. The positive correlation between BMI and TSH is probably a reflection of its role in the accumulation of body energy reserve.

which results in adipogenesis (Bell et al., 2000;

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Conflict of interest

The authors declare that there is no conflict of interest.

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