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Influence of Season and Oestrous Cycle Phase on Serum Progesterone and Thyroxine Profiles in Savanna Brown Goats

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Abstract: *The study was carried out to determine the effect of seasonal changes and oestrous cycle phase on serum progesterone (P4) and thyroxine (T4) profiles in Savanna Brown (SB) goats. Ten (n = 10) cycling SB goats with mean body weight and body mass index of 20.17 ± 0.2 kg and 6.2 ± 0.4, respectively were allotted to 2 groups of 5 animals each per season of study (Hot-rainy, HRS and cold-dry, CDR). Mean oestral serum P4 (ng/mL) concentrations were as follows: HRS: 0.05 ± 0.04 vs CDS: 0.05 ± 0.07; dioestral: HRS: 0.52 ± 0.02 vs CDS 0.62 ± 0.08. Combined mean of oestral and dioestral serum P4 concentrations were, HRS: 0.05± 0.02 vs CDS: 0.57 ± 0.06. Mean oestral serum T4 (ng/mL) concentrations were,HRS: 75.83 ± 4.13 vs CDS: 85.10 ± 3.90; dioestral: HRS: 78.70 ± 2.72 vs CDS: 92.35 ± 4.01 ($P < 0.001$). Combined mean of oestral and dioestral serum T4 concentrations were, HRS: 77.42 ± 2.35 vs CDS: 89.93 ± 2.90 ($P < 0.001$). In conclusion, mean serum T4 concentrations were significantly higher during CDS than HRS season cycles. While, peak serum T4 level coincided with peak P4 activity at mid- and late dioestrus/proestrus phases of both HRS and CDS cycles.*
Keywords: *Goats, Oestrous cycle, Progesterone, Season, Thyroxine,*

I. Introduction

The Nigerian goat population is the largest in Africa and the fourth largest in the world after India, China and Pakistan [1]. The Savanna Brown (SB), also known as Red Sokoto goat constitutes about 60 per cent of the Nigerian goat population and is predominantly found in the arid and semi–arid regions of the Northern Guinea Savanna zone of Nigeria [2,3]. They are year–round breeders with age and weight at first oestrus being 4-6 months and 10-18 kg, respectively [3]. Oestrous cycle length of about 19 - 21 days [4], and oestrus duration of 21-26 hours had been reported in SB goats [4,5,6,7]. The ovaries are the main source of progesterone P4 in goats and serum P4 concentration is an important index of ovarian activity [8,9] and pregnancy diagnosis in goats ([10,11,12]. Ovarian activity and P_4 secretion in goats is affected by many factors including season [13,14]. The influence of season on corpus lutuem function and follicular population had been reported in SB goats [15,16]. Thyroid hormones (THs), play a pivotal role in the process of adaptation that enable animals to live and breed despite the periodic changes in season. This is particularly important in the free-ranging and grazing animals such as traditionally reared small ruminants, whose main physiological functions (feed intake, reproduction, hair growth) are markedly seasonal [17,18]. Thyroid hormones act on many different target tissues, stimulating oxygen utilisation and heat production in every cell of the body. The overall effects are to increase the basal metabolic rate, cellular uptake of glucose, protein synthesis, lipid metabolism and stimulation of cardiac and neural functions [19,20]. In the temperate regions of the world, studies have implicated THs in the manifestation of endogenous seasonal rhythms of neuroendocrine reproductive activity in sheep and goats, and many species of birds [21,22,23]. Thyroid hormones act during a limited period, late in the breeding season, to permit transition to seasonal anoestrus [24,25,22]. They also operate as part of a complex intercellular network, sharing signalling pathways with other hormones, and affecting other hormone systems [26]. There is increasing evidence that THs can affect the sex steroid hormone axis and vice versa [27,28]. However, there is paucity of information on the relationship between seasonal changes, ovarian activity and thyroid function in SB goats. Therefore, this study was carried out to evaluate the effect of oestrous cycle phase and seasonal changes on serum concentrations of P4 and T4 in SB goats during the HRS and CDS season cycles.

II. Materials And Methods

2.1 Experimental location

The study was carried out at the Small Ruminant Research Programme, National Animal Production Research Institute (NAPRI), Shika, Ahmadu Bello University, Zaria, Nigeria. Shika is located between latitudes 11 and 12° N and between longitudes 7 and 8° E at an altitude of 640 m in the Northern Guinea Savanna zone.

The average annual rainfall in Shika is approximately 1100 mm, and mainly during the months of April to October. The maximum ambient temperature range in Shika is 27 – 35 °C depending on season [29].

2.2 Experimental animals

Ten (n = 10) multiparous apparently healthy and cycling Savanna Brown (SB) does, aged between 2–5 years (2-4 parity) with a mean body weight of 20.17 ± 0.2 kg and body mass index of and 6.2 ± 0.4 [30], respectively were used for experiment. The does had been weaned of their kids for at least eight weeks prior to the commencement of the experiment. Each doe had shown at least one oestrus before it was included in the study. The animals were randomly alloted into two groups of five animals per season of study (n = 5). They were kept in semi-open concrete floor pens. Digitaria smutsi hay was provided ad libitum as basal diet, while supplementary concentrate ration of approximately 15% crude protein made up of ground maize (12%), cotton seed cake (24%), wheat offal (62%), bone meal (1.5%) and salt (0.5%) was provided at 300g/head/day between the morning hours of 9.00-10.00 am and evening hours of 4.00-5.00 pm. Water and mineral salt lick were also provided ad libitum.

2.3 Seasons of experiment

The classification of seasons adopted for this study had earlier been described [31]. The HRS season experiment was carried out between April and May, while the CDS experiment was conducted between December and January. The meteorological data during the seasons of the study were collected from the Institute for Agricultural Research (IAR), Ahmadu Bello University, Zaria, located about 3 km from the site of the experiment.

2.4 Oestrus synchronization and heat detection

The animals were synchronised with a single injection of 7.5 mg $PGF_{2\alpha}$ (Lutalyse® PHARMACIA). The injection was administered by deep intramuscular route in the thigh muscle. Animals that did not respond to the first injection were given a second injection of 7.5 mg $PGF_{2\alpha}$ 11 days later. Immediately, following the first treatment with $PGF_{2\alpha}$, a mature apronised buck was placed in the flock for heat detection. The heat detection was carried out daily during the morning hours of 8.00 am - 9.00 am and evening hours of 4.00 pm -5.00 pm. Standing to be mounted was the single criterion used to confirm that a doe was in estrus.

2.5 Blood sampling

All the animals (n = 5/season) were bled by jugular venipuncture during the first oestrous cycle following synchronization with $PGF_{2\alpha}$. The animals were bled at day 0 and 1 of the first oestrus, twice weekly subsequently, and finally on day 0 and 1 of the second oestrus following treatment with $PGF_{2\alpha}$. The day 0 and 1 samples were considered oestral samples for the first and second post-synchronization oestrus. Blood sampling was carried out between the morning hours of 8.00 am - 9.00 am and prior to the first feeding of the day. At each sampling, 10 mL of whole blood was collected and centrifuged at 2000 x g for 10 min. The serum was then decanted and divided into two portions. One serum portion each, was used for analyses of P4 and T4, respectively.

2.6 Progesterone assay

A commercially prepared ELISA kit (CLINOTECH® DIAGNOSTICS, Canada) was used for the quantitative determination of serum P_4. The assay was carried out as described by the manufacturers of the kit. The sensitivity of the test kit is 0.05 ng/mL, while its specificity is 100% for progesterone. Progesterone concentrations equal to or greater than 0.1 ng/mL was considered as evidence of luteal activity [7].

2.7 Thyroxine assay

A commercially prepared ELISA kit (DIAGNOSTIC® AUTOMATION INC. California) was used for the quantitive determination of serum T4. The sensitivity of this assay kit is estimated to be 0.4 µg/dL.

2.8 Data analysis

Oestrus cycle length and oestrus duration were expressed as mean ($\pm$ SEM) days and hours, respectively. The data were compared between sampling days, oestrous cycle phases and seasons. Data on mean serum P_4 and T_4 were analysed using Student's t Test, ANOVA and Tukey's post hoc test. Pearson's coefficient of correlation analysis was used to determine the relationship between serum concentrations of P4 and T4 during the oestrous cycle. The statistical package used was Graphpad Prism version 4.0 (2003) for Windows from GraphPad Software, San Diego, California, USA (www.graphpad.com) [32]. Values of $P < 0.05$ were considered significant.

III. Results

3.1 Meteorological conditions

Mean (±SEM) maximum and minimum ambient temperatures (T° C) were 33.10 ± 0.30 vs 21.32 ± 0.20 and 30.90 ± 0.33 vs 13.11 ± 0.22 during the HRS and CDS, respectively (TABLE 1). Maximum and minimum relative humidity (%) were 71.10 ±1.23 vs 55.30 ± 1.50 and 17.5 ± 0.60 vs 14.0 ± 0.50 during the HRS and CDS, respectively. Rainfall (mm) was 13.33 0.27 vs 0.00 in the HRS and CDS, respectively.

3.2 Oestous cycle length and oestrus duration

Mean oestrous cycle length (days) and oestrus duration (hours) were 22.20 ± 0.96 vs 22.80 ± 1.32 and 27.20 ± 4.08 vs 30.40 ± 6.40 during the HRS and CDS, respectively (TABLE 2).

3.3 Serum progesterone profile

Mean serum P_4 (ng/mL) concentrations in oestral phase were 0.50 ± 0.04 and 0.50 ± 0.07 in the HRS and CDS, respectively (TABLE 3). Mean dioestral P_4 (ng/mL) concentrations in the HRS and CDS were 0.52 ± 0.02 and 0.620 ± 08, respectively. Mean P_4 profile during HRS cycles declined at day 1, rose to a moderate plateau between days 4 and 7 and then declined to lower levels on day 13. (Fig. 1). Thereafter, it rose again to a moderate peak on day 16 and then declined gradually towards the end of the cycle between days 22 and 24.

Mean progesterone profile in CDS cycles also declined between days 1 and 4; rose to a peak on day 13 and declined again towards lower levels at day 16. Thereafter, P4 level rose again to another peak at day 22 and then declined sharply towards baseline level between days 23 and 24. In general, P_4 levels were higher in CDS than HRS cycles, especially at mid-dioestral (day 13) and late dioestral (day 22) phases of the oestrous cycle.

Mean oestral T_4 (ng/mL) concentrations were 75.83_± 4.13 ng/ml and 85.10 ± 3.90 in HRS and CDS cycles, respectively (TABLE 4). Mean dioestral T_4 (ng/mL) concentration was higher in the CDS than HRS cycles (92.35 ± 4.01 vs 78.70 ± 2.73; P<0.001). Similarly, the combined mean of oestral and dioestral T_4 (ng/mL) concentrations was higher during CDS than HRS cycles (89.93 ± 2.90 vs 77.42 ± 2.35; P<0.001). In

HRS cycles, mean T_4 level gradually rose from day 1 to peak on day 10, declined to lower a level on day 13, rose again consistently to peak at day 22 and then declined towards basal levels between days 23 and 24 (Fig. 2). Serum T_4 profiles during CDS cycles showed peaks at days 4 and 10, a decrease by day 16 and another peak on days 22 and 24. In general, T4 levels were higher in CDS than HRS cycles, especially at metoestral (day 4), mid- (day 10) and late dioestral (day 22) phases of the oestrous cycle. There was no significant correlation between mean serum concentrations of P4 and T4 during the oestrous cycle in both seasons (TABLE 5).

IV. Discussion

The relatively higher P_4 levels observed in this study in the CDS as compared to the HRS season cycles suggests greater level of corpus luteum (CL) activity during the CDS than the HRS season. This may imply that the CDS season CL attains bigger size and secretory activity than the CL of the HRS season. Similarly, higher P_4 levels had been reported in Anglo-Nubian does during breeding (autumn) than non-breeding season (spring) [33]. In a related study, higher follicular and CL activity had been reported in the CDS than HRS season in SB does[15,16]. High peripheral P_4 concentrations are correlated with CL size and morphology [34,35]. Therefore, it can be inferred from the present study that CL function was greater in the CDS than HRS season cycles. The relatively higher P_4 level observed in the CDS season of this study may suggest higher probabality of occurrence of multiple ovulations/CL in the CDS than in HRS season cycles. This is because higher follicular turnover [36], and P_4 levels are associated with multiple ovulations and twining in goats [37,738], and ewes [39].

The predominance of peak P_4 levels in the early, mid and late dioestral phase confirms the tendency for higher P_4 concentrations in the dioestral as compared to oestral phase of the oestrous cycle in goats [6,12,40,9]. Mid-to-late cycle increase in CL function is a result of increased luteal cell activity/turnover, rather than increased luteal tissue formation [41,35]. The general decline in P_4 level between day 0 and day 7 of the CDS season cycles as opposed to the short-term decline between day 0 and day 1 of HRS season cycles suggests that the process of $PGF_{2\alpha}$–induced luteolysis was longer in the CDS than HRS season. This phenomenon may be a function of bigger CL size or numbers in the CDS than HRS season. Corpus luteum size correlates with increased P_4 production and peripheral concentrations of P_4 [42,43,44,35]. The relatively low mean P_4 concentrations (<1.0 ng/mL) observed in this study, irrespective of seasonal difference, agree with an earlier report of low serum P4 concentrations in postpartum SB does [9]. A similar observation of low level CL function had been reported in Zebu cattle treated with $PGF_{2\alpha}$ at proestrus [45]. This may suggest that the secretory potential of a post-$PGF_{2\alpha}$ CL is lower than that of a spontaneous CL. The P_4 values observed in this study are much lower than earlier reported for non-pregnant cyclic SB does [6]. This may be due to differences in assay methods used for the determination of P_4 between the two studies.

The significantly higher T4 levels observed during the CDS as compared to the HRS cycles is indicative of higher thyroid gland activity during the CDS than HRS cycles. This is suggestive of higher metabolic potential in SB goats during CDS than HRS cycles. This finding agrees with earlier reports of increased TH secretion in colder seasons in cattle, sheep and pigs [46,47,48,49]. In contrast however, higher T4 levels in summer than in winter had been reported in Cashmere goats [50], while no significant difference was observed between cold and warm season plasma T4 activity in Sahel ewes [51]. The seasonal disparity between and within species in T4 activity may be due to factors that affect thyroid function such as seasonal cyclicity in feed intake, body weight and reproductive status [52,53,54]. In general, seasonal variation in thyroid gland activity with maximal T4 concentrations in colder than hotter months had been reported in most domestic species [55,56,57,58]. Similarly, higher T4 levels have been reported during increasing daylength (spring) and lower levels during decreasing daylength (autumn) in temperate sheep and goats [59,60,61,62]. This means that T4 activity is higher in the non-breeding season than in the breeding season in temperate sheep and goats [33].

Since circulating THs level represent a relevant metabolic index of the nutritional, growth and reproductive status of an animal [63,53,54,64], the significantly higher T4 levels observed in the CDS as compared to the HRS season in the present study may suggest better nutritional, metabolic and reproductive status of SB goats during CDS than HRS season. This is supported by the relatively higher P4 level and longer oestrous cycle length and oestrus duration recorded in the CDS than HRS season cycles of this study. In relation to oestrous cycle phase, peak T4 levels were observed mainly at the metoestral (day 4; HRS), mid- (day 10) and late dioestral (day 22) phases of the HRS and CDS cycles, respectively. This pattern of occurrence of T4 activity suggests that thyroid gland function may be affected by oestrous cycle phase in the SB goats, with higher T4 activity occurring predominantly during the mid- and late dioestral/proestrus phases of both CDS and HRS season cycles. It also suggests that irrespective of seasonal difference, a cyclical rhythm in peak T4 activity occurred during the oestrous cycle in SB goats. A rhythmical pattern of peak T4 activity with higher levels in early and lower levels at late pregnancy have also been reported in goats [51,57,65,18,66]. The mid- and late dioestral/proestrus phases of oestrus cycle are characterized by heightened ovarian activity in goats such as peak corpus luteum fuction [6,12,40] and the second to third follicular wave growth and selection [36,67].

There was no significant difference between mean oestral and dioestral T4 concentrations in both HRS and CDS season cycles of this study. However, mean dioestral serum T4 concentration was significantly higher in CDS than HRS season cycles. This observation is in contrast with the findings of earlier workers, who observed higher T4 levels in oestral than dioestral phase in goats [33], and sheep [68]. However, higher levels of the biologically more active triiodothyronine (T3) have been reported in the luteal phase in ewes [68]. These variations may be due to differences in voluntary feed intake [69,17,18], age [70,71,72], season and circadian rhythm [73,55,74,18].

Even though, there was no significant correlation between serum concentrations of P4 and T4 in both seasons, a tendency for peak levels of P4 and T4 to coincide at mid- (day 10 – day 13) and late dioestral phases (day 22) of both CDS and HRS season cycles was observed. Thyroid hormones are known to increase the functional capacity of all cells of the body through enhancement of basal metabolic rate [20,19]. Similarly, enhanced secretion of P4 and oestradiol-17β production had been reported in granulosa cells cultured in T3 augmented media [75,76]. Therefore, the tendency for peak T4 level to coincide with peak P4 activity observed in this study is suggestive of a T4 mediated increase in metabolic activity of the luteal cells resulting in higher rate of P4 secretion from the CL at the mid- and late dioestral/proestrus phases of both CDS and HRS season cycles.

V. Conclusion

It is concluded that mean serum T4 concentrations were significantly higher during the CDS than HRS season cycles; and peak serum level of T4 coincided with peak P4 activity during mid-(day 10–day 13) and late dioestral/proestrus phases of both CDS and HRS cycles.

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Table 1. Meteorological conditions during the early hot-rainy and cold-dry season periods of the experiment.

Meteorological conditions	Seasons	
	Early hot-rainy	Cold-dry
RH max (%)	17.5 ± 0.60	71.10 ± 1.23
RH min (%)	14.00 ± 0.50	55.30 ± 1.50
AT max (TC)	30.90 ± 0.33	33.10 ± 0.30
AT min (TC)	13.11 ± 0.22	21.32 ± 0.20
Windspeed (Km/d)	160.10 ± 6.41	200.50 ± 5.31
Sunshine duration (h)	7.00 ± 0.35	7.78 ± 0.30
Rainfall (mm)	0.00	13.30 ± 2.70

± - SEM
RH – Relative humidity
AT – Ambient temperature
Max – Maximum
Min - Minimum

Table 2. Mean (± SEM) oestrous cycle length and oestrus duration in Savannah Brown goats during early hot-rainy and cold-dry seasons (n=5 per season).

Season	Cycle length (Days)	Oestrus duration (Hours)
Early hot-rainy	22.20 ± 0.96	27.20 ± 4.08
Cold-dry	22.80 ± 1.32	30.40 ± 6.40

Table 3. Mean (± SEM) serum progesterone concentrations during the oestrous cycle in Savannah Brown goats in the early hot-rainy and cold-dry seasons. (n=5 per season).

Seasons	Progesterone (ng/L)		Overall mean (± SEM)
	Oestral phase	Dioestral phase	
Early hot-rainy	0.50 ± 0.04	0.52 ± 0.02	0.50 ± 0.02
Cold-dry	0.50 ± 0.07	0.62 ± 0.08	0.57 ± 0.06

Table 4. Mean (±SEM) serum thyroxine concentrations during the oestrous cycle in Savannah Brown goats in the early hot-rainy and cold-dry seasons. (n=5 per season).

Seasons	Thyroxine (ng/L)		Overall mean (± SEM)
	Oestral phase	Dioestral phase	
Early hot-rainy	75.83 ± 4.13	78.70 ± 2.73[a]	77.42 ± 2.35 [a]
Cold-dry	85.10 ± 3.90	92.35 ± 4.01[b]	89.93 ± 2.90 [b]

a,b: Values with different superscripts within column are statistically significant (P< 0.001).

Table 5. Correlation of coefficient (r) between serum concentrations of progesterone and thyroxine during the oestrous cycle in Savannah Brown goats in the early hot-rainy and cold-dry seasons. (n=5 per season).

Season	R	P value	Significance
Early hot-rainy	0.0872	0.564	NS
Cold-dry	0.0767	0.596	NS

NS – Not significant (P > 0.05)

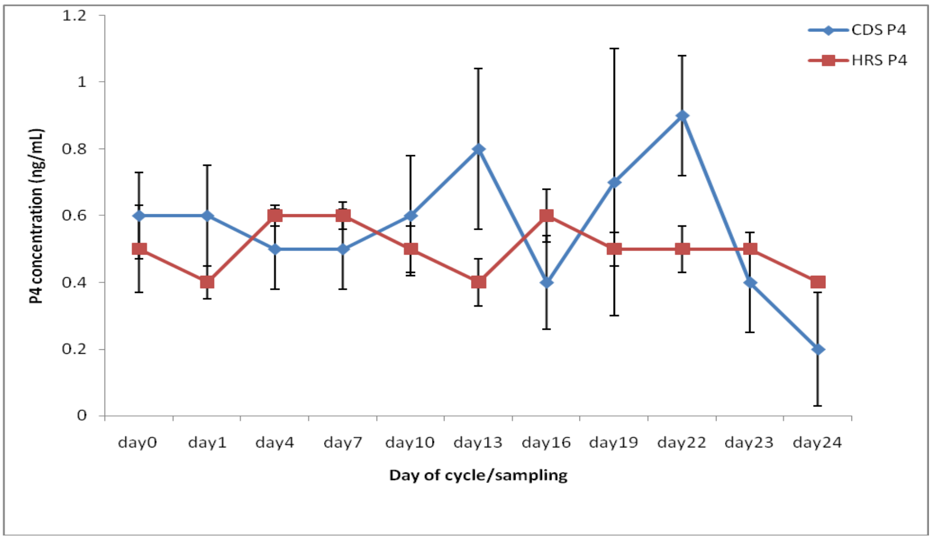

Figure 1. Pattern of serum progesterone profiles during the oestrous cycle in Savannah Brown goats in the early hot- rainy and cold-dry seasons.

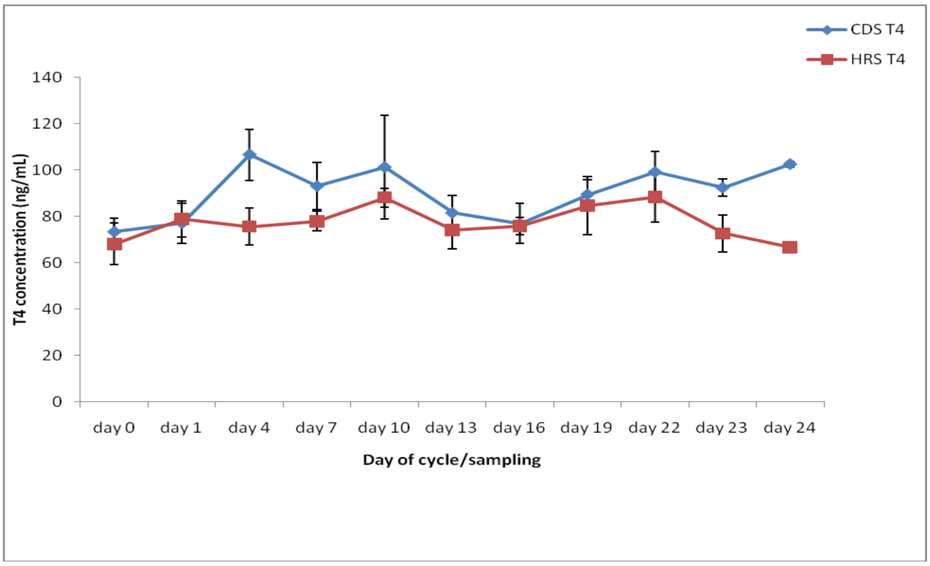

Figure 2. Pattern of serum thyroxine profiles during the oestrous cycle in Savannah Brown goats in the early hot- rainy and cold-dry seasons.