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Research Article

Annual and Seasonal Variations of Testicular and Pituitary-Thyroid Axis Activities in Bucks Native to Sahara Desert

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Abstract

This study was conducted to assess comparative annual and seasonal patterns described in morphometric parameters, sexual and thyroid activities in indigenous bucks native to Sahara Desert. Seven adult bucks were used to establish seasonal patterns of body weight, testicular volume, plasma testosterone concentration measured by radioimmunoassay and pituitary-thyroid axis activity estimated by plasma concentrations of TSH, FT3 and FT4 measured by enzyme-linked fluorescent assay. The results showed monthly and seasonal variations characterized by a minimum in January for body weight, from December to February for testicular volume, followed by plasma testosterone concentration in March; they progressively increased to their respective maxima reached in July, August and September when ambient temperature and photoperiod were the highest. In October, there was a rapid fall in testosterone levels, preceding that of body weight and testicular volume (P<0.001). Plasma TSH showed no significant variations, while the monthly rhythms of FT4 and particularly FT3 (P<0.001) were inverted to the sexual cycle, since the highest values occurred in winter (January) and the lowest in summer (July). These results suggest morphometric and hormonal interrelations in this indigenous seasonal breeder goat in order to survive and reproduce in its natural arid environment.

Keywords: *Buck, Sahara Desert, Seasonal variations, Testicular activity, Thyroid activity*

Sahra Çölü'ne Özgü Antilopların Testis ve Hipofiz-Tiroid Aksı Aktivitelerinin Yıllık ve Mevsimsel Değişimleri

Öz

Bu çalışma, Sahra Çölü'ne özgü yerli antilopların morfometrik parametreler, seksüel ve tiroid aktivitelerinin tanımlanan karşılaştırmalı yıllık ve mevsimsel modellerinin araştırılması için yapılmıştır. Vücut ağırlığı, testis hacmi, radyoimmünoassay ile ölçülen plazma testosteron konsantrasyonu ve TSH, FT3 ve FT4 plazma konsantrasyonlarının enzim işaretli floresan yöntemiyle ölçülmesiyle belirlenen hipofiz-tiroid eksen aktivitesinin mevsimsel modellerini belirlemek için yedi adet yetişkin antilop kullanıldı. Sonuçlar, vücut ağırlığının Ocak ayında, testis hacminin Aralık ile Şubat ayları arasında ve plazma testosteron konsantrasyonunun Mart ayında minimum seviyelerde yer alarak aylık ve mevsimsel varyasyonlar sergilediğini; çevre sıcaklığının ve fotoperiyodun en yüksek olduğu Temmuz, Ağustos ve Eylül aylarında bu değerlerin maksimum seviyelere aşamalı olarak yükseldiğini gösterdi. Ekim ayında, vücut ağırlığı ve testis hacminden önce testosteron seviyelerinde hızlı bir düşüş saptandı (P<0.001). Plazma TSH miktarı önemli bir değişiklik göstermezken, FT4 ve özellikle de FT3'ün (P<0.001) aylık ritimleri, en yüksek konsantrasyonlarının kışın (Ocak) ve en düşük konsantrasyonlarının yazın (Temmuz) saptanması nedeniyle seksüel siklusun tersine döndü. Bu sonuçlar, bu yerli mevsimlik damızlık antilopların doğal kurak ortamlarında hayatta kalma ve üremeleri için morfometrik ve hormonal ilişkiler sergilediğini göstermektedir.

Anahtar sözcükler: *Antilop, Sahra Çölü, Mevsimsel değişim, Testiküler aktivite, Tiroid aktivitesi*

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Introduction

The goat is one of the most adaptable livestock species distributed worldwide across a large variety of climatic and geographical areas [1]. In Algeria, most of them are raised mainly in the arid and semi-arid regions. Their adaptation to periodic environmental changes results in the appearance of biological rhythms, particularly reflected by breed differences in the time of maximum body weight (BW), testis volume, testicular and thyroid activities [2].

The photoperiod has been suggested as the main factor influencing the seasonal reproduction. The length of the day is transduced by the pineal gland into a chemical message via its rhythmic secretion of melatonin, which regulates the hypothalamic-pituitary-gonadal function [3]. Furthermore, the reproduction is a costly process in terms of energetics for the male which may reflect competition for access to females, aggressive behavioral, territorial defense, and paternal behavior [4].

The central mechanisms regulating reproduction and energy balance by environmental factors are tightly linked. Indeed, several investigations have targeted the interrelationships between reproduction and hypothalamic-pituitary-thyroid axis, in relation with the annual cycle [5,6]. These studies showed that the thyroid hormone (TH) are required for seasonal transition from breeding to non-breeding state [5], and for metabolic adjustments in response to heat stress [6]. Understanding the physiological mechanisms involved in this regulation is an important issue to breeding optimization. Males with better sexual behavior under high temperature conditions can be selected for the development of breeds adapted to arid climate [2].

By using scientific methods, the production of this indigenous ruminant will be increased in the future in order to improve the nutrition of Saharan populations and the preservation of this local well adapted species. So, the purpose of this work is to characterize annual and seasonal patterns in (1) BW, (2) sexual activity as estimated by testicular biometry and plasma concentration of testosterone and (3) pituitary-thyroid axis activity, measured by thyroid stimulating hormone (TSH) and TH plasma concentrations in bucks reared in their natural biotope, under arid climate conditions.

Material and Methods

Ethical Approval

The methodology protocols of the present study followed the ethical concepts in animal experimentation, recommended by FELASA and approved by the Algerian Ministry of Higher Education and Scientific Research (Executive Decree 10-90 supplementing the Algerian government decree 04-82) and the AASEA (45/DGLPAG/DVA.SDA.14).

Site of Study and Meteorological Data

The study was carried out at Béni Abbès research station located in the Algerian Sahara Desert (30°07′ N, 2°10′ W, elevation 495 m, South West of Algeria), characterized by cold and humid winter, and hot and dry summer *(Table 1; Fig. 1)*. The temperature-humidity index (THI) was calculated using the formula of Mader et al.[7]:

$$THI = 0.8 \times AT + [(RH \div 100) \times (AT - 14.4)] + 46.4$$

AT: Ambient temperature (°C), RH: Relative humidity (%).

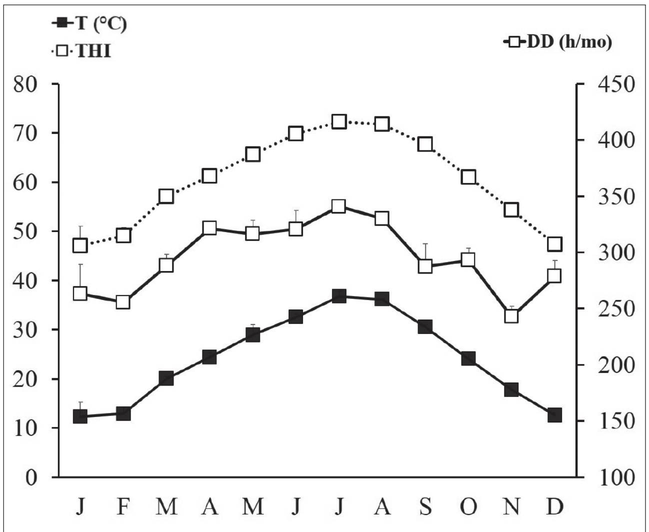

Fig 1. Annual variations of temperature, temperature-humidity index and daylight duration from the Béni-Abbès region (30°07′ N, 2°10′ W, elevation 495 m, South West of Algeria) during the experimental period (mean±sem). **T:** Temperature; **THI:** Temperature-humidity index; **DD:** Daylight duration; **mo:** month. Data were obtained from the Algerian meteorological center

Table 1. *Meteorological seasonal data (minimum - maximum values) from the Béni-Abbès region (30°07′ N, 2°10′ W, elevation 495 m, South West of Algeria) during the experimental period*

Seasons	Ambient Temperature (°C)	Relative Humidity (%)	Temperature Humidity Index	Daylight Duration (h/month)	Precipitation (mm/month)
Winter	4-19	26-63	47-64	186-314	0-42
Spring	20-33	15-37	63-80	305-378	0-27
Summer	20-41	12-31	67-87	304-365	0-6
Autumn	12-31	22-47	55-79	230-322	0-5

Animals

Seven bucks (3-4 years old; BW: 30-48 kg) were used in this study, which was conducted over 2 consecutive years. The animals were clinically healthy and have typical libido characteristics, they were reared under open shelter, undergoing the influence of all natural variations of climatic factors. Bucks were fed forage and a mixture of oats and barley (about 1 kg per buck) once daily (4:30 p.m.) with ad libitum access to water and mineral salt licks.

Morphometric Parameters

The BW and testicular morphometric parameters were measured twice a month during two consecutive years. The large and small diameters for the left and right testes were measured with a sensitive caliper in order to calculate the testicular volume according to a mathematical model (TVM) [8]:

TVM (cm^3) = $4/3 \times \pi \times ab^2$

where a and b represent respectively half of the large and small diameters.

The testicular volume was also estimated with a volumetric technique by water immersion [9]; the animal being in a standing position, the two testes were immersed in a calibrated 2-liters beaker filled with lukewarm water; the testicular volume is given by the water displacement (TVW in mL).

Blood Sampling

Blood samples (5 mL) were collected at 9:00 a.m. every 15 days during two consecutive years, by jugular vein puncture in heparinized vacutainer tubes (lithium heparinate, Venoject Terumo; Leuven, Belgium) and kept on ice before centrifugation at 4000 x g for 10 min at 4°C. Plasma samples were separated rapidly and stored at -20°C for further analyses.

Testosterone Assay

Testosterone assay was performed by radioimmunoassay (RIA), after extraction of steroids with a mixture of ethyl acetate - cyclohexane (v/v), using a specific antibody raised in rabbit by immunization with carboxymethyl-oxime of testosterone coupled to bovine serum albumin [10]. The specificity of this method was estimated by measuring the cross-reactivity of the antibody with dihydroxytestosterone (47%), androstenedione (16%) and less than 0.5% with other steroids; the sensitivity was 0.02 ng/mL and the intra- and inter-assay coefficients of variation were 5.1 and 9.3%, respectively.

TSH and Thyroid Hormones Assays

Plasma TSH, FT3 and FT4 concentrations were determined by Enzyme Linked Fluorescent Assay (ELFA) (VIDAS Vitek ImmunoDiagnostic Assay System, Biomérieux, Lyon, France). The principle of the assay associates the immuno-enzymatic method with a final fluorescence detection step, using mouse monoclonal immunoglobulins anti-TSH (Ref: 30 441, TSH3) and sheep antibody for anti-FT3 (Ref: 30 402, FT3) and anti-FT4 (Ref: 30 459, FT4N), labelled with alkaline phosphatase. The assay sensitivity was 0.005 µIU/mL for TSH, ≤0.7 pmol/L for FT3 and = 1.11 pmol/L for FT4. No interference was seen for TSH antibody with other hormones (<0.01% with FSH, LH and hCG). For FT4 antibody no interference observed up to the concentration of 12.51 µg/L for L-triiodothyronine and 2.28 µg/L for D-thyroxine. The cross reactivity of FT3 antibody was of 0.2% with L-thyroxin. The intra- and inter-assay coefficients of variation were respectively 2.44 and 4.30% for TSH, 4.83 and 5.28% for FT3 and 5.40 and 8.49% for FT4 (kit values).

Statistical Analysis

Data are expressed as the means±sem. The bimonthly values were grouped by month and then by seasons (the two years combined) determined conventionally as follows: winter from December 21st to March 20th, spring from March 21st to June 20th, summer from June 21st to September 20th and autumn from September 21st to 20th December. Statistical evaluation of differences was done by analysis of variance (ANOVA), using IBM SPSS Statistics 25 (Copyright IBM and its licensors 1989, 2017), including months or seasons as fixed factors and animals as random factors. Normality and homogeneity of variance were respectively investigated by Shapiro-Wilk and Levene's test. The difference between the variables was calculated post hoc using the Tukey test. Correlations were calculated between the months and seasons by means of Pearson's correlation coefficient (r). A probability level of $P<0.05$ was used for significance.

Results

Evolution of Monthly Patterns

Body Weight

The monthly BW values *(Fig. 2)* were the lowest in January, increased progressively till April (+16.4% vs January, $P<0.01$) and the maximum body mass was reached in July (+22.6% vs January, $P<0.001$). It was followed by a progressive decrease until December (-16.3% vs July, $P<0.01$), leading to highly significant annual variations.

Testicular Volume

The monthly variations of testicular volume paralleled that of BW *(Table 2; Fig. 2)*, whether evaluated by measuring testicular diameters mathematically TVM ($r=0.823$, $P<0.01$) or by water immersion TVW ($r=0.908$, $P<0.01$). The TVM showed a sensitive increase in May (+22.5% vs January, $P<0.01$), and the maximum was reached in August (+39.3% vs January, $P<0.001$). In September, TVM fell significantly

(-31.4% vs August, P<0.001) and progressively until the minimum occurring in December (-36.2% vs August, P<0.001). The TVW showed the same pattern as that of the TVM *(Fig. 2)*. However, the values always remained at a significantly higher level than TVM, characterized by a minimum in February and a maximum in July (+38.0% vs February, P<0.001). The correlation coefficient *(Table 2)* shows a highly significant positive relationship between the two methods of measure (r=0.805, P<0.01).

Plasma Testosterone Concentration

The pattern of monthly values of plasma testosterone concentration *(Fig. 3)* showed annual variations (P<0.01) with a minimum level in March, a gradually increase until June (+447% vs March, P<0.01) and a peak reached in September (+601% vs March, P<0.001); then a gradual decrease from October (-61.3% vs September, P<0.01) until December (-76.3% vs September, P<0.001). The correlation coefficient *(Table 3)* shows a significant positive relationship (P<0.05) with BW (r=0.616) and TVW (r=0.637).

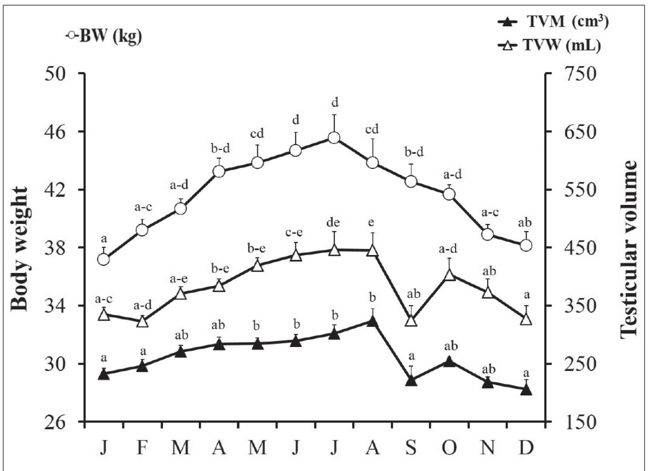

Fig 2. Annual variations in body weight and testicular volume in the buck reared under arid environment (mean±sem, n = 7). Measurements taken twice a month during two consecutive years were grouped by month for each buck. **BW:** Body weight; **TVM:** Testicular volume by measure according to a mathematical model; **TVW:** Testicular volume by immersion in water. Different superscripts indicate significant differences between months (P<0.05)

Plasma TSH and Thyroid Hormones Concentrations

Plasma concentration of TSH showed no significant variations *(Fig. 3)*. FT3 and FT4 showed similar patterns of evolution through the year (P<0.001), with maximum values in January, followed by a progressive decrease from February (-12.6% for FT3 and -10,7.0% for FT4) until April (-48.4% for FT3 and -23.7% For FT4 vs January, P<0.001); the decrease becomes significant in May vs January (-45.2% for FT3 (P<0.001) and -18.8% for FT4 (P<0.01)). A gradual increase appears from November (+82.3% for FT3 and +38.9% for FT4 vs October) until December (+149% for FT3 vs October and +40.9% for FT4 vs October). A significant increase was observed only in December for FT3 (+107% vs July, P<0.001).

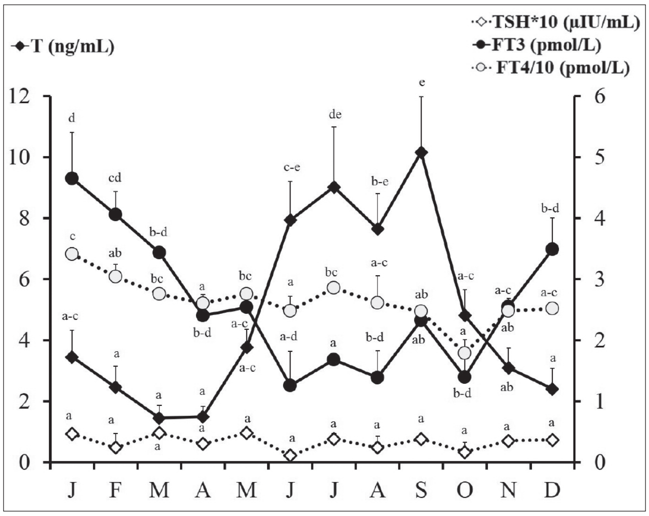

Fig 3. Annual variations of plasma testosterone, TSH and thyroid hormones (FT3 and FT4) concentrations in the buck reared under arid environment (mean±sem, n = 7). Levels measured twice a month during two consecutive years and grouped by month. **T:** Testosterone; **TSH:** Thyroid stimulating hormone (mean values multiplied by 10); **FT3:** Free triiodothyronine; **FT4:** Free thyroxine (mean values divided by 10). Different superscripts indicate significant differences between months (P<0.05)

Table 2. *Coefficients of correlation between mean monthly body weight, testicular and thyroid activities in the buck reared under arid environment (n=7)*

Parameter	BW	TVM	TVW	T	TSH	FT3	FT4
BW	1						
TVM	0.823**	1					
TVW	0.908**	0.805**	1				
T	0.616*	0.336	0.637*	1			
TSH	-0.327	-0.328	-0.119	-0.030	1		
FT3	-0.600*	-0.184	-0.624*	-0.578*	-0.216	1	
FT4	-0.357	-0.175	-0.140	-0.024	0.868**	0.022	1

Data were obtained from the Algerian meteorological center
***BW:** Body weight; **TVM or TVW:** Testicular volume; **T:** Testosterone; **TSH:** Thyroid stimulating hormone; **FT3:** Free triiodothyronine; **FT4:** Free thyroxine. Correlations were calculated between the months by means of Pearson's correlation coefficient; * indicates statistical significance at P<0.05; ** indicates statistical significance at P<0.01*

***Table 3.** Coefficients of correlation between mean seasonal body weight, testicular and thyroid activities in the buck reared under arid environment (n=7)*

Parameter	BW	TVM	TVW	T	TSH	FT3	FT4
BW	1						
TVM	0.925	1					
TVW	0.992**	0.879	1				
T	0.764	0.665	0.813	1			
TSH	-0.580	-0.376	-0.571	-0.046	1		
FT3	-0.769	-0.516	-0.842	-0.886	0.395	1	
FT4	-0.368	-0.098	-0.384	0.079	0.954*	0.351	1

***BW:** Body weight; **TVM or TVW:** Testicular volume; **T:** Testosterone; **TSH:** Thyroid stimulating hormone; **FT3:** Free triiodothyronine; **FT4:** Free thyroxine. Correlations were calculated between the seasons by means of Pearson's correlation coefficient; * indicates statistical significance at P<0.05; ** indicates statistical significance at P<0.01*

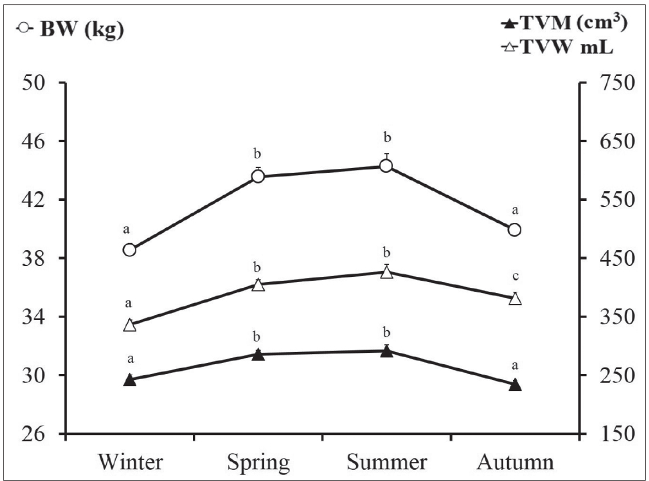

Fig 4. Comparative seasonal profiles of body weight and testicular volume (TVM and TVW) in the buck reared under arid environment (mean±sem, n = 7). **BW:** Body weight; **TVM:** Testicular volume by measure according to a mathematical model; **TVW:** Testicular volume by immersion in water. Data grouped by season including all experimental years. Different superscripts indicate significant differences between seasons (P<0.05)

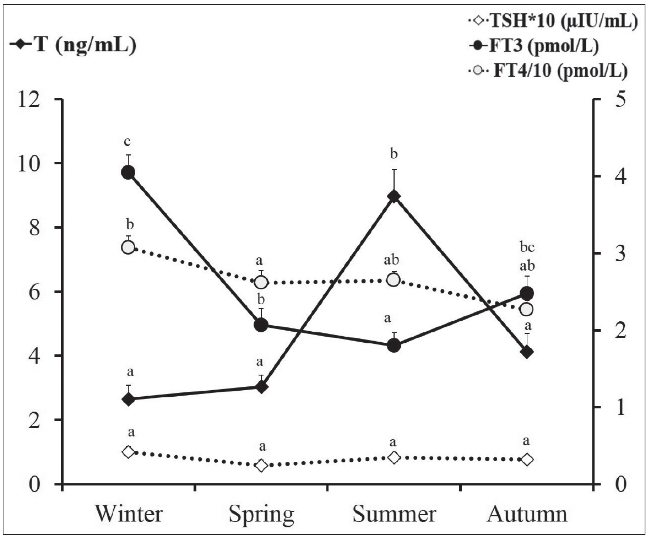

Fig 5. Comparative seasonal profiles of plasma concentration of testosterone, TSH and thyroid hormones (FT3 and FT4) in the buck reared under arid environment (mean±sem, n = 7). **T:** Testosterone; **TSH:** Thyroid stimulating hormone (as mean values multiplied by 10); **FT3:** Free triiodothyronine; **FT4:** Free thyroxine (as mean values divided by 10). Data grouped by season including all experimental years. Different superscripts indicate significant differences between seasons (P<0.05)

Evolution of Seasonal Patterns

When grouping values by season, the patterns exhibited important seasonal variations but the differences were indeed attenuated. The lowest values of the BW *(Fig. 4)* being observed in autumn-winter and the highest in spring-summer (+14.9% winter vs summer, P<0.001). The seasonal difference (summer vs winter) for testicular volume were +19.9% (P<0.001) for TVM and 26.7 for TVW (P<0.001) *(Fig. 4)*. A significant positive correlation *(Table 3)* was observed with the TVW and BW (r=0.992, P<0.01).

The seasonal pattern of plasma testosterone *(Fig. 5)* showed a gradual increase from a minimum in winter to a maximum in summer (+239%, P<0.001). These variations were positively correlated *(Table 3)* with those of morphometric parameters as BW (r=0.764), TVM (r=0.665) and TVW (r=0.813). The plasma thyroid hormones *(Fig. 5)* showed also significant seasonal variations, particularly for FT3 (P<0.001), characterized by highest values in winter and the minimum was occurred in summer (-55.5% vs winter, P<0.001). The pattern of FT4, showed a significant diminution in spring (-13.8% vs winter, P<0.05) which paralleled that of TSH (r=0.954, P<0.05) *(Table 3)*.

Compared Patterns Between Testicular and Thyroid Activities

The monthly *(Table 2; Fig. 3)* and the seasonal *(Table 3; Fig. 5)* variations of testicular and thyroid activities revealed a reversed evolution between the concentrations of testosterone and TH, particularly that of FT3. The two annual cycles were significantly opposite, the Pearson correlation coefficient was r=-0.578 (P<0.05). The FT3 showed also negative correlation with that of BW (r=-0.600, P<0.05) and testicular volume (r=-0.624, P<0.05) *(Table 2)*.

Discussion

In this study, we found that Desert bucks, living in their natural arid environment, show important annual and seasonal variations in their BW, which paralleled those of the reproductive activity, as measured by the testicular volume and plasma testosterone concentration. These variations were reported in several ungulate species living in the same conditions of arid and semi-arid areas of Algeria, especially in rams [11] and camel [12]. The appearance of the seasonal rhythm of BW, although the bucks received the same diet, is probably related to the physiological state and the reproductive performance, regardless of feeding level, as reported by Zarazaga et al.[13]. Indeed, the BW gain was observed when the males are sexually inactive, corresponding to the seasonal anestrous of the females [14,15], which appears mainly in winter. In other hand, the minimum and the maximum of testicular volume and plasma testosterone concentrations were also observed respectively in summer and winter, indicating a seasonal rhythm in the reproductive cycle parallel to that of BW. Theses variations were probably related to environmental changes, particularly the photoperiod. Indeed, during the elongation of the daylength (late winter), the concentration of testosterone was lowest, while the highest value was observed during the day shortening (late summer), showing that this male goat is a "short day breeder". It is known that the pineal melatonin, an endocrine index of daylength, is used to relay photoperiodic information to the reproductive axis and serves as a master regulator of breeding. The day shortening increase melatonin, causing decreased TSH-β subunit expression in pituitary pars-tuberalis, which affects sex hormone levels [16]. In our study, no clear change in plasma TSH between seasons. The lack of rhythm probably reflects unimportant role of peripheral TSH in the regulation of seasonal reproduction, confirming the findings of several authors who report that peripheral TSH secreted by pituitary pars-distalis, from the classical hypothalamic-pituitary-thyroid axis, does not depend on the photoperiod [17]. However, the TH follows a seasonal pattern, the minimum being observed in summer and the maximum in winter, which was inverse to that of testicular activity. The increase observed in winter, probably promotes changes in the neuroendocrine axis, which result in transition from the breeding to the non-breeding season, as reported by Yasuo et al.[18]. Moreover, the reduction in summer may reflect a metabolic adaptation to harsh environment, characterizing arid regions. In effect, in a previous work done on the same breed, we have reported high levels of cortisol in summer [19], thus decreasing the release of TH in order to reduce basal metabolism [2]. It is difficult to discriminate between the respective role of temperature and photoperiod on the seasonality of thyroid activity, in different environmental conditions [20]. However, these variations affect the energy metabolism that results in seasonal changes in BW, which is positively correlated with reproductive success. Knowledge on such physiological adaptive strategies, allows the monitoring and manipulation of testicular and thyroid activities, in order to improve animal health, welfare and production of this indigenous buck.

In conclusion, the buck native to Algerian Sahara Desert is a "short day breeder", it follows annual and seasonal rhythms of its reproductive activity correlated to BW, and opposed to that of thyroid activity. This species limits its reproductive activity to specific period of time in a year in order to survive and to maximize the success of reproduction and survival of their offspring.

Availabity of Data and Materials

The authors declare that data supporting the study findings are also available to the corresponding author.

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Conflict of Interest

The authors declared that there is no conflict of interest.

Author Contributions

N. Chergui: Conceptualization, Methodology, Validation, Formal analysis, Investigation, Writing - Original Draft, Writing - Review & Editing, Visualization. N. Boukenaoui-Ferrouk: Visualization, Review & Editing. S. Charallah-Cherif: Methodology & Visualization. F. Khammar: Resources, Methodology & Visualization. Z. Amirat: Conceptualization, Methodology, Investigation, Resources, Writing - Original Draft, Visualization, Review & Editing, Supervision. P. Mormede: Conceptualization, Writing - Original Draft, Review & Editing, Visualization.

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