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SEASONAL LEVELS OF CORTISOL, TRIIODOTHYRONINE AND THYROXINE IN MALE AXIS DEER

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Abstract—1. Seasonal plasma levels of thyroxine (T_4), triiodothyronine (T_3) and cortisol were investigated between November and June in seven penned male Axis deer.

2. No distinct seasonal variation of cortisol has been detected. The levels oscillated between 1 and 5 μg/dl.

3. The stress of immobilization and sampling had little effect on cortisol levels. Concentrations remained mostly stable in three consecutive samples taken 10 min apart.

4. T_3 concentrations were stable between November and March (average values 110–120 ng/dl). After a sharp decline in April (average 70 ng/dl), a strong rebound in May and June was observed.

5. A distinct seasonal peak of T_4 (highest individual value, 12.1 μg/dl) was detected in March. After a sharp decline in April (lowest individual value, 4.5 μg/dl) a strong rebound followed in May.

INTRODUCTION

In the last twenty years circannual variations of hormonal levels were reported in numerous boreal species of the deer family, the Cervidae (Lincoln, 1985; Bubenik, 1986; Bubenik and Schams, 1986). On the other hand, very little endocrine research has been performed on the deer of the temperate or tropical regions which, in a number of species, are more numerous than the representatives of the boreal group (Lincoln, 1985). So far, endocrine studies have been performed only in one tropical species, the Rusa deer (*Cervus rusa timorensis*) (van Mourik and Stelmasiak, 1984 a, b, 1986).

One of the tropical deer species kept often in zoological gardens and parks all around the world is Axis deer (*Axis axis*) known also as an Indian chital (Mohr, 1932; Ables, 1977). Despite the detailed observations on the time course of seasonal events, such as reproduction, antler cycle, pelage exchange, behavior, nutrition etc. (Ables, 1977) no attempts have ever been made in this species to investigate blood levels of hormones.

The adaptation of individual deer species to specific environmental conditions is often reflected in the pattern of endocrine secretion (Bubenik, 1986). In boreal regions the photoperiodic regulation of seasonality in cervids is relatively well understood (Bubenik and Smith, 1987). On the other hand, data on even basic circannual events (such as the reproduction) are very scanty for deer living in the tropical areas (Stuwe, 1985).

In order to fill the gap in our knowledge and to provide the basic data on the levels of hormones in tropical deer species we have decided to investigate seasonal levels of hormones in plasma of Axis deer introduced into a temperate region of southern Texas. This article will report data on T_3, T_4 and cortisol levels; another article (Bubenik *et al.*, in preparation) will deal with seasonal levels of LH, FSH, PRL and testosterone.

MATERIALS AND METHODS

Animals and sampling procedure

Seven male Axis deer (*Axis axis*) ranging in age from 1 to 4 years, were sampled between November and June. The animals, (identified by letters "M" to "T") were obtained from an exotic animal farm located 40 km north of San Antonio, Texas and transferred to Kingsville, TX (latitude 27:3° North). The bucks were fed pelleted, standard deer food ration supplemented by fresh sorghum plants and branches with leaves of deciduous trees. The deer were immobilized once a month between 8:00–9:00 hr by Rompun (xylazine hydrochloride) (2–3 mg/kg) and then three consecutive blood samples (10 min apart) were collected from the jugular vein into pre-heparinized tubes. Three samples were taken to compensate for sometimes large variation of blood levels occurring in deer within a short period of time (Bubenik *et al.* 1985). Blood was kept on ice until it was centrifuged (usually within 1 hr); plasma was then frozen for further assays. After blood sampling the animals were injected intravenously with xylazine antidote yohimbine hydrochloride (0.3 mg/kg) to speed up the recovery from anaesthesia.

One buck (M) died in November during the transport and another expired in April. The experiment had to be terminated in June, when we lost two more bucks due to post-immobilization hyperthermia.

Radioimmunoassays. Plasma total T_3, T_4 and cortisol levels were measured in duplicate samples using highly specific radioimmunoassays (RIA) (RIA Inc. Scarborough, Ontario, Canada). Intra-assay and inter-assay variability were less than 10% and good correlations were found between sample volume and measured hormone levels for all assays. The lowest detectable levels were as follows: T_3, 12 ng/dl; T_4, 0.02 μg/dl and cortisol, 0.5 μg/dl. The percentage binding of T_3 was 43.2 $\pm$ 0.2 (mean $\pm$SE) and for

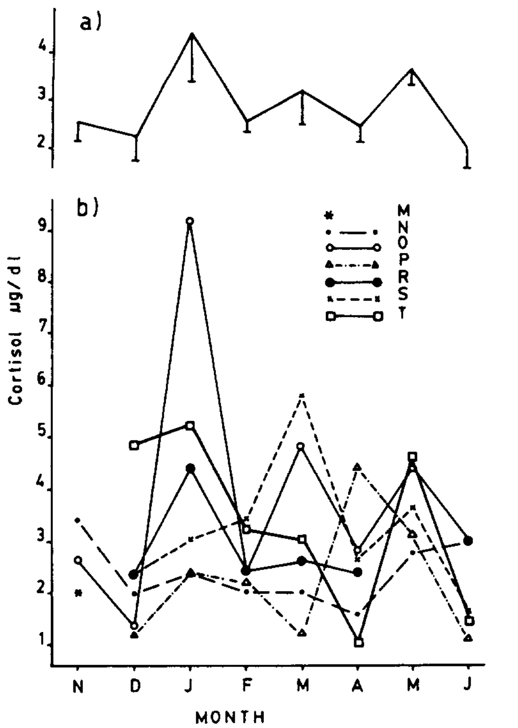

Fig. 1. (a) Monthly average of seasonal levels of cortisol in male Axis deer. Significant differences are given in Table 1. (b) Individual seasonal levels of C in seven Axis bucks ("M" to "T"). (Each value represents an average of three consecutive samples.)

T_4 59.8 ± 1.7. All hormone assays were carried out within three months of the sample collection.

Statistics. Data were analysed by the Randomized Complete Block Design (blocked by deer, months as treatment) followed by the Fisher's least significant test (Ray, 1982). Due to the small number of deer, November values were not compared statistically. Because of the complicated nature of the design (different number of animals in various months) no statistical analyses were made of the difference between 1st, 2nd and 3rd samples taken each month.

RESULTS

Cortisol

Seasonal levels. (Fig. 1a) Average monthly levels do not indicate any clear seasonal rhythm. Despite the large standard error, the January peak was statistically significant ($P < 0.05$) compared to December, February, April and June. The May value was also significantly higher than concentrations in December, April and June (Table 1). However, values of individual animals (Fig. 2b) show that January peak is significant mostly because of extremely high values of animal "O". May concentrations are significant only against three other months, mainly because of rather coincidental high values of animals "O", "P" and "T". Average seasonal concentrations of cortisol (C) in the Axis deer varied between 2 and 4.5 μg/dl (Fig. 1a) and individual values (Fig. 1b) exhibited a range with minimum at 0.7 and maximum at 10.4 μg/dl.

Stress effect. Despite the heavy dose of immobilizing drugs, the deer tried to struggle, sometimes violently, in an attempt to escape. However, compared to stressed white-tailed deer (Smith and Bubenik, in preparation) or rusa deer (Van Mourik and Stelmasiak, 1984a), the levels of cortisol in most animals were surprisingly low (Fig. 2) and in a clear majority of cases did not change from first to third sample. Out of 43 samplings, in only six cases is there a clear rapid increase of cortisol levels from first to third sample (4 times in animal "T" and 2 times in buck "O"). On the other hand, a rapid decline in cortisol concentrations was observed once in the animal "S" and once in "R". (Bucks "O" and "T" were both yearlings; "S" and "T" were adult bucks, 3–4 years old). In some months, such as February and April, the cortisol values were maintained in an extremely narrow range.

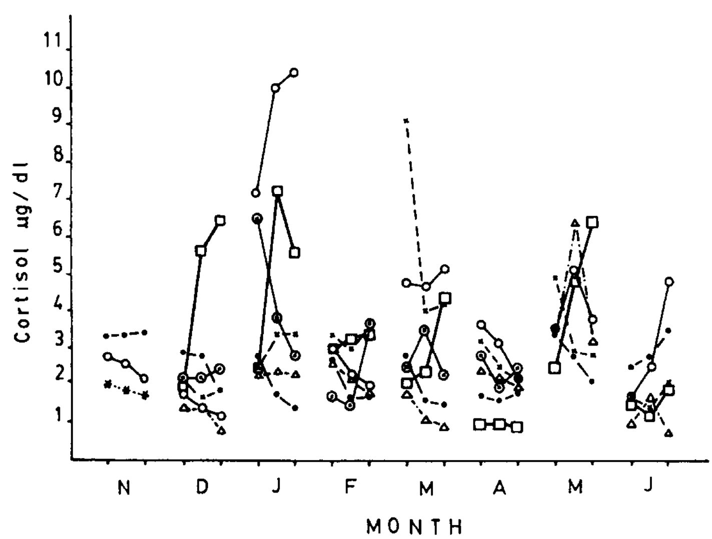

Fig. 2. Monthly concentrations of cortisol in three samples taken 10 min apart. The symbols used are the same as in Fig. 1.

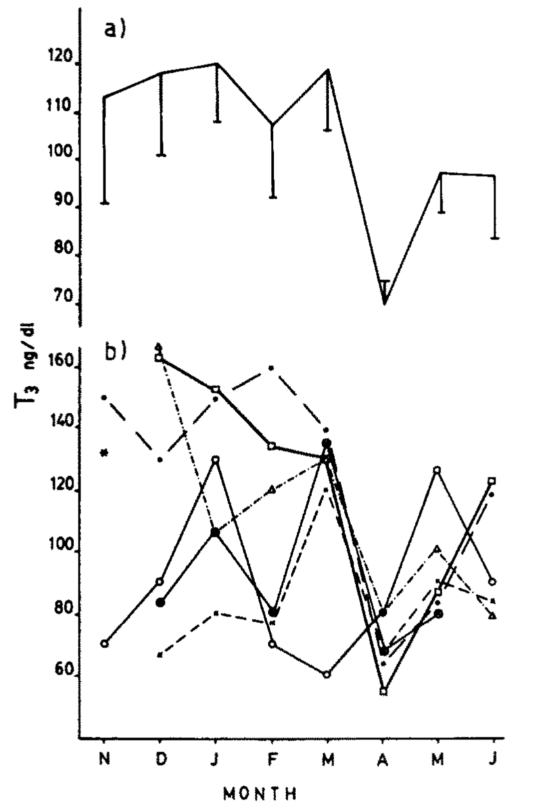

Fig. 3. (a) Monthly averages of seasonal levels of T_3. Significant differences are provided in Table 1. (b) Individual seasonal levels of T_3. The symbols are the same as in Fig. 1. (Each value represents an average of three consecutive samples.)

In addition to usual presentation of monthly means and standard errors we have also decided to include individual measurements. As each particular buck quite often exhibits a unique, individual type of response to sampling circumstances, presentation of averages only might create a false impression (see Figs 1, 3 and 4). Wild animals are not inbred and therefore respond less uniformly to identical situations. Presentation of individual data, combined with observations on somatic and physiological responses of the species are therefore often more revealing than publication of averages and standard errors.

Triiodothyronine (T_3)

Average values of T_3 (Fig. 3a) indicate relatively stable T_3 concentrations between November and March. After a sharp decline in April which was significantly different ($P < 0.05$) from December, January, February and March (Table 1), a rebound of T_3 concentrations was detected in May and June. Individual graphs (Fig. 3b) present rather variable levels between November and February. Except for buck "O", all other deer exhibited high T_3 levels in March, followed by a sharp decline in April in all animals.

Thyroxine (T_4)

Average levels of T_4 (Fig. 4a) were slowly rising between November and February and then increased rapidly between February and March. A rapid drop of T_4 values in April was followed by a rebound in May and June. The March peak was significantly different ($P < 0.05$) from all other months investigated. The minimum concentrations in April were significantly different ($P < 0.05$) from all other months but December (Table 1). All individual graphs (Fig. 4b), except the one of buck "R", followed the same trend as the average values.

DISCUSSION

The original aim of this study was to investigate for the first time the entire circannual cycle of various hormones in blood of Axis deer. Unfortunately, the very flighty nature of this species (Mohr, 1932) and the high ambient temperature in southern Texas (reaching over 40°C at midday in July and August) forced us to abandon the investigation prematurely. However, despite the fact that we have obtained data for only an 8-month period, several observations made in this study appear to be important enough to justify the publication of our results.

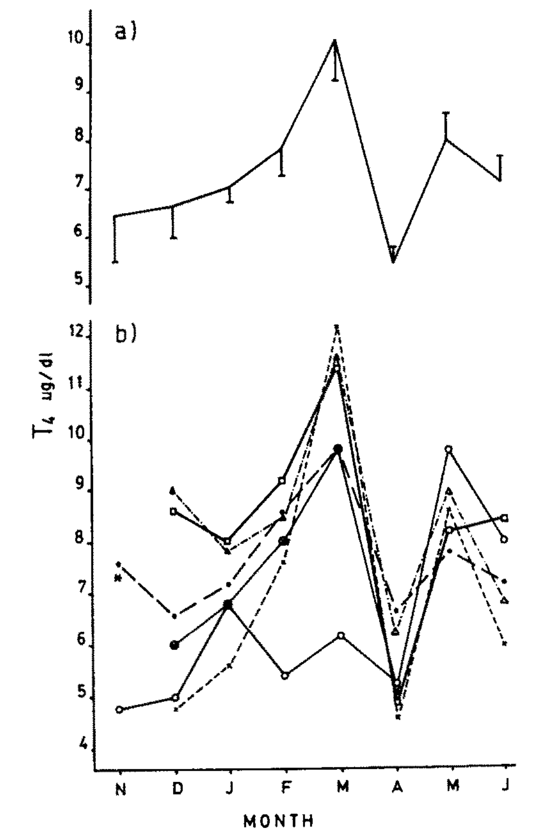

Fig. 4. (a) Monthly averages of seasonal levels of T_4. Significant differences are provided in Table 1. (b) Individual seasonal levels of T_4. The symbols given are the same as in Fig. 1. (Each value represents an average of three consecutive samples.)

Table 1. Plasma levels of cortisol, triiodothyronine (T_3) and thyroxine (T_4) (mean ±SE) in Axis deer (*Axis axis*)

		Cortisol		T_3		T_4	
	N*	X	SE	X	SE	X	SE
Nov.	3	2.54	0.40	113.6	21.6	6.46	0.87
Dec.	6	2.27[a]	0.50	117.1[a]	17.1	6.67[a]	0.71
Jan.	6	4.46[b]	1.03	120.6[a]	11.9	7.05[a]	0.33
Feb.	6	2.59[a]	0.22	106.9[a]	15.1	7.86[a]	0.56
Mar.	6	3.24[a]	0.70	118.7[a]	12.1	10.16[b]	0.87
Apr.	6	2.53[a]	0.30	70.2[b]	4.3	5.50[c]	0.30
May	5	3.74[b]	0.34	97.2[a]	8.1	8.32[a]	0.58
Jun.	5	2.03[a]	0.40	96.4[a]	11.39	7.23[a]	0.43

N = number of deer used.
*Three plasma samples were taken from each deer, 10 min apart.
Means in columns with different letters are significantly different ($P < 0.05$).

Cortisol

Plasma levels. Average seasonal concentrations (2–4.5 ng/dl), as well as the range of C values in individual samples (0.7–10.4 ng/dl) were very close to C concentrations reported by our group for white-tailed deer, where average seasonal level varied between 1 and 6 μg/dl (Bubenik *et al.*, 1975; Bubenik and Leatherland, 1984) and individual concentrations ranged from 0.5 to 18 μg/dl (Bubenik *et al.*, 1984). Similar ranges were also reported for white-tailed deer by other investigators (Wesson *et al.*, 1979; Seal *et al.*, 1983). These values were also in the range reported for humans (20–40 μg/dl) (Vierhapper *et al.*, 1981).

On the other hand, the C concentrations in another tropical deer species, the Rusa deer, exhibited a much higher range of values. The average baseline value reported was 22 μg/dl and individual values ranged from 9.6 to 92 μg/dl (van Mourik and Stelmasiak, 1984a). These higher levels of C were closer to levels found in sheep, where individual values ranged from 10 and 900 μg/dl (Fulkerson and Tang, 1979; Lincoln, Almeida, Klandorf and Cunningham, 1982). These data indicate that C concentrations in various deer species are very individual and differences between them might be considerable. In addition, as each deer species might respond differently to similar stressors, conclusions based on comparisons of incomplete data between various cervids might be erroneous.

Stress response. Variations between C levels in three samples taken 10 min apart (Fig. 2) revealed surprisingly stable concentrations. The few exceptions (where C values have risen rapidly) indicate that the adrenal gland of Axis deer is capable of secreting more glucocorticoids upon the stimulation but for unknown reasons it is not doing it readily.

The Rusa deer is a relatively calm cervid; the Axis is the most excitable and the white-tailed deer is slightly calmer than the Axis (Bubenik, personal observation). Maximal levels of C in Rusa deer achieved upon stimulation with 6 I.U. of ACTH reached 1320 μg/dl (van Mourik and Stelmasiak, 1984b). A similar maximal stimulation in white-tailed deer, achieved with 20 I.U. of ACTH reached only 35 μg/dl (Bubenik, 1986; Smith and Bubenik, in preparation). In white-tailed deer individuals which most obviously exhibited the signs of distress (such as hyperventilation, panic behavior, resistence to immobilization, etc.) very often did not respond at all to ACTH administration. On the other hand, in well-adjusted individuals, 20 I.U. of ACTH induced 8–10 times increase in levels of C (Bubenik, 1986; Smith and Bubenik, in preparation). Finally, ACTH-induced C secretion in wild white-tailed deer was not different between individuals from an extremely high density population (expected to be socially stressed) and control, low density population (Seal *et al.*, 1983).

The fact that the calmest individuals and the least excitable species (such as the sheep and the Rusa deer) exhibit the highest variations of C levels and the volatile individuals and excitable species (such as the Axis and white-tailed deer) have the lowest rate of C increase to stress, appears contradictory to the accepted attitude that the level of adrenal secretion indicates the degree of stress (Christian *et al.*, 1960; Seal *et al.*, 1983).

All these results indicate that perhaps we need a critical reevaluation of the role of the deer adrenal gland in the stress response. As no diurnal variation of C was demonstrated in cervids (Bubenik *et al.*, 1984; Van Mourik and Stelmasiak, 1984a), three other factors could influence the scope of the adrenal response: (1) the species, (2) the character of the individual and (3) the type of stress (acute vs chronic, psychological vs somatic). If the range of secretion of C is determined by the species, the individual character of the deer and the type of stress may substantially modulate the adrenal response. Based on one set of data, we may speculate that the higher weight of adrenal gland found in stressed wild populations (Christian *et al.*, 1960; Bubenik and Bubenik, 1967) might not be the result of hyperproduction of corticoids but rather an opposite case—the compensatory attempt to increase production in the gland which is unable to perform sufficiently.

Triiodothyronine. Average seasonal levels of T_3 in axis deer (Fig. 3a) (70 and 120 ng/dl) were almost identical to seasonal T_3 levels found in Soay rams (88–113 ng/dl) by Lincoln and co-workers (1982) and in wild white-tailed does maintained on a semi-starvation diet (77 ng/dl) (Watkins and Ullrey, 1983). In captive deer fed *ad libitum*, such low levels were found only in castrates (Bubenik and Leatherland, 1984). On the other hand, in intact bucks, seasonal variation was very substantial ranging from 150 to 280 ng/dl in one study (Bubenik and Leatherland, 1984) and 95 to 223 ng/dl in another (Bubenik *et al.*, 1986). Similar levels (228 μg/dl) were also detected in penned, well-fed white-tailed does (Watkins and Ullrey, 1983).

Thyroxine. Average seasonal concentrations of T_4 in Axis deer (Fig. 4a) (5.5 and 10 μg/dl) were in a range reported in Soay rams (6–7 μg/dl) (Lincoln *et al.*, 1982) and poorly fed white-tailed does (5.4 μg/dl). In well-fed white-tailed does, the T_4 levels were found at 14.4 μg/dl (Watkins and Ullrey, 1983) and seasonal variation in our group of penned bucks exhibited levels which ranged from 11 to 14 μg/dl (Bubenik and Bubenik, 1978), 14 to 17 μg/dl (Bubenik and Leatherland, 1984) and 11 to 20 μg/dl (Bubenik *et al.*, 1986). The blood level of thyroid hormones in boreal deer are linked to nutritional status and thermogenesis (Bahnak *et al.*, 1981; Watkins and Ullrey, 1983). The seasonal levels of T_3 and T_4 are usually higher during the spring and summer and low during the autumn and winter (Watkins and Ullrey, 1983; Bubenik and Leatherland, 1984). However, as no shift of distinctly seasonal variations of T_3 was observed in white-tailed deer in which other seasonal parameters such as reproduction, antler cycle and pelage exchange were altered by melatonin administration (Bubenik *et al.*, 1986), it appears that seasonal variation of thyroid activity is either temperature-dependant or it is guided by an endogenous rhythm. As all our deer were fed *ad libitum*, the relatively low levels of thyroid hormones in Axis deer might indicate the low priority of thermogenesis in tropical species. The acute problem for animals in hot and humid climates is hyperthermia. Therefore, the 50% decline in levels of thyroid hormones in April which coincide with the beginning of the hot period in southern Texas, might have been caused by the reduction of thyroid activity, responding to warming of ambient temperature. Unfortunately, the data on the thyroid activity during the hottest months of the year (July and August) have not been obtained.

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