BREEDING BEHAVIOUR AND TESTOSTERONE LEVEL OF MALE SPOTTED DEER

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requirement for the degree of

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DECLARATION

I hereby declare that the thesis, entitled **"BREEDING BEHAVIOUR AND TESTOSTERONE LEVEL OF MALE SPOTTED DEER"** is a bonafide record of research work done by me during the course of research and that the thesis has not previously formed the basis for the award to me of any degree, diploma, associateship, fellowship or other similar title, of any other University or Society.

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CONTENTS

Chapter No.	Title	Page No.
1	INTRODUCTION	1
2	REVIEW OF LITERATURE	4
3	MATERIALS AND METHODS	17
4	RESULTS	25
5	DISCUSSION	51
6	SUMMARY	60
	REFERENCES	64
	ABSTRACT	

LIST OF TABLES

Table No.	Title	Page No.
1	Season wise birth	26
2	*Season wise breeding*	26
3	Alpha I	30
4	Alpha II	30
5	Velvet I	31
6	Velvet II	31
7	Diurnal frequency of behaviours in different phases of antler cycle	33
8	Behavioural score for animals in different stages of antler cycle	34
9	Diurnal frequency of behaviours in breeding stage	35
10	Behavioural score for animals in the breeding stage	35
11	Harem composition	38
12	Average daily breeding activities	38
13	Score chart for behavioural data collection	39
14	Weekly breeding score for each animal	40
15	Testosterone levels of animals	42
16	Cortisol level of animals	45

LIST OF FIGURES

Figure No.	Title	Page No.
1	Season wise birth	27
2	Season wise breeding	27
3	Alpha I and Alpha II	28
4	Velvet I and Velvet II	28
5	Velvet shedding stage	29
6	Velvet shedding stage with blood vessels exposed	29
7	Behaviour frequencies in different stages of antler cycle	36
8	Testosterone and behaviour score for Alpha I	36
9	Harem Size	43
10	Testosterone levels of four animals during the study period	43
11	Cortisol level of Alpha I	46
12	Cortisol level of Alpha II	46
13	Cortisol level of Velvet I	47
14	Cortisol level of Velvet II	47
15	Cortisol levels of four animals during the study period	48

16	Testosterone and breeding score of Alpha I	49
17	Testosterone and breeding score of Alpha II	49
18	Testosterone and breeding score of Velvet I	50
19	Testosterone and breeding score of Velvet II	50

Introduction

1. INTRODUCTION

The spotted deer or axis deer (*Cervus axis axis)*, which is considered as the most beautiful deer, comes under the order artiodactyla and family cervidae. The axis deer originally comes from the dense forests of India, and is the most commonly found member of the deer family in India, where it is called chital. It is distributed across the entire country except in the extreme northern regions.

The coat colour is bright rufuos fawn profusely spotted with white at all ages and all seasons. Usually they are seen in herds of 10 to 30, at least with two to three stags. The adults grow to a height of approximately 90 cm at the shoulders and can weigh up to 85 kg. The males of the group are having antlers with two or three tines or branches on each antler. Females are smaller and usually without antlers.

Life expectancy ranges from 20 to 30 years. Despite being one of the favourite prey species of predators such as tigers and leopards and only giving birth to a single fawn at a time, their population is quite big.

Their diet consists of all kinds of vegetation but grass is favourite one. They also eat the antlers that they shed for their rich nutrients. These deer can be seen, unlike many of their cousins, in dense jungle as well as open grasslands. The criterion behind their distribution is the presence of water.

Generally they are prolific breeders (Cheeran *et al.*, 2005). The axis deer by and large do not have a fixed breeding season. They breed at any season and usually a single offspring is produced. Usually the sexual maturity is reached at the age of 12 to14 months.

In males the antler cycle is initiated by velvet antler formation. In this stage thickly furred skin (velvet) will be developing over the seal of the antler. This stage is found to be steaming up stage of males, in which they gain more body weight and do not show any breeding activities. This stage lasts for a few

months and after the shedding of velvet it culminates in the breeding stage. Such males will be having hardened antlers and will be emitting loud bellows while going after the receptive female of the herd. Bucks with hardened antlers and in the rutting condition may be found throughout the year. Each buck seems to have it's own breeding cycle which may not be synchronized with the other bucks in the herd.

Behavioural studies are of great importance in increasing our understanding and appreciation of animals. In addition to providing knowledge about the complexity and density of behaviour, such studies also provide information crucial to improvements in the welfare of animals maintained in captivity. In this respect, development of ethogram and behavioural score which is associated with the androgen level can help a lot in interpreting complex behaviour of the males of this species.

Relatively seasonality is pronounced in chital (*Axis axis*) of all age classes in southern India. Raman (1998) reported that usually only the adult male of the species attained peak hard antler during the months, where most conceptions occurred. Native to India, they have been exported to a few regions like Australia, Texas, and Chile and only very little published information is available on the breeding performance, behaviour, frequencies, seasonality and hierarchical status of males of this group.

Methods to identify and measure faecal testosterone and glucocorticoid metabolites have been successfully used in a number of domestic livestock and some wild species. In case of cervids, reproductive behaviours and the faecal steroid concentrations show overt seasonal fluctuation (Li *et al.*, 2005). The antler cycle, as a male secondary sexual characteristic, is closely linked to the sexual cycle and its timing is controlled by reproductive hormones (Fennessy *et al.*, 1988). But in the case of axis deer stags, the correlation of antler cycle with testosterone level and the reproductive behaviour is not well established. The

better understanding of the breeding behaviour of this particular group of cervids may help in doing favouable manipulations in their breeding patterns.

The population of deer in general and spotted deer in particular poses a problem of unmanageable numbers in captive and other confined facilities. Effective ways are yet to be worked out to reduce the breeding frequency of such problem population. In a closed population if limited numbers of males are performing breeding according to their hierarchical status, there is a possibility of reducing the breeding intensity of the herd. One practical method to prevent the breeding is to vasectomise the males of higher social order. This will curtail its fertility while keeping the sexual desire intact, which in turn will contain their population size for a period of time.

The proposed project was meant to find out whether there is a hierarchical order existing in the male population in the captive herd and whether the males of higher hierarchy prevent the breeding of other males. This knowledge can be used to develop strategies, for prevention of breeding over a desired period. A better understanding of the breeding behaviour in relation with the testosterone profiles will be helpful in so many other ways like evolutionary aspects, comparative aspects and many more.

So, the proposed project was undertaken with the following objectives.

1. Identification of social hierarchy and harem formation, if any.
2. Estimation of faecal testosterone levels before and during the breeding stage.
3. Breeding performance, preferences and frequencies of alpha to lower order males.
4. Correlation between testosterone level and behavioral pattern.
5. Estimation of faecal cortisol levels during breeding and non-breeding seasons.

Review of Literature

2. REVIEW OF LITERATURE

2.1. SELECTION OF STUDY SEASON

2.1.1. Photoperiodicity and Season

Within the herd, there was little evidence of a clear seasonal synchrony in the antler cycle. Detailed information obtained from 4 stags indicated that there was a fixed relationship between stage of the antler cycle and testis diameter; minimum testis diameter occurred 1-2 months after antler casting whereas maximum testis diameter occurred when stags were in hard antler. Changes in body weight, circumference of the neck and plasma testosterone concentrations largely paralleled those of testis diameter. (Loudon and Curlewis. 1988). They concluded from the study that there is little or no seasonal photoperiodic entrainment of the antler and testicular cycles of males in population of axis deer.

In red deer (*Cervus elphus*) antler casting occurred at the end of long days and cleaning at the end of short days as observed by Suttie *et al.* (1989). It is considered that antler cycles were due to the ability of the stags to vary the release of LH and testosterone in response to changes in the artificial photoperiod.

In male roe deer (*Capreolus capreolus*) the concentrations of plasma testosterone increased under natural photoperiod between March and August and the sexual cycle of the male appears to be initiated by androgens rhythm in winter and is then maintained by hormonal charger resulting from increasing photoperiod in spring as reported by Sempere *et al.* (1992).

Monfort *et al.* (1993) observed that the gonadal activity peaks during the winter and the spring as day length increases.

Raman (1998) observed that the seasonality was pronounced in chital *(Axis axis)* of all age classes in southern India. He also observed that only the

adult males of the species attained peak hard antler during the months where most conceptions occurred.

Tiwari *et al.* (2002) observed that the mating time was observed from mid December to January last. They also observed that delivery usually took place during rainy season, June to early August when there is maximum humidity in the environment.

Willard and Randel (2002) noted that on the separation of axis stags into their respective seasons versus antler status categories revealed that 96.2% of the stags harvested during the summer and autumn were in hard antler, in contrast to only 23.3% during winter and spring indicating the seasonality of antler cycle.

Singh (2005) noted that the time at which the axis deer stags shed their antler varies in different localities and breeding takes place in all seasons.

2.1.2. Seasonal Synchronisation of Antler Cycle

Bubenick *et al.* (1991) observed that the antler cycles of six adult axis deer were relatively synchronized within the herd under artificial photoperiod.

2.2. IDENTIFICATION OF DOMINANT MALE POPULATION

In red deer *(Cervus elaphus)* antler growth is related to rank position. The dominant males of a socially stabilized group will produce larger antlers than sub ordinate males as reported by Bartos *et al.*(1998)

Roed *et al.* (2002) found that in polygynous species like reindeer, the male reproductive success is often correlated with the dominance status of the individual males and sex ratio in the population.

Saseendran *et al.* (2003) observed that in a population of sambar deer, males were found to control the group lead by alpha male. Alpha male was identified by it's good physical appearance, sharp and long antlers, positioning at vantage points to take the major share of feed and it's mates.

2.3. BEHAVIOURAL OBSERVATION

Gizejewski (2003) noticed that in the premating stage in red deer, all the behavioural elements typical of stags during the rutting time like aggression, roaring, characteristic smell, anxiety all appeared in succession.

Cheeran (2004) recorded that the spotted deer are prolific breeders.

The reproductive behavior in male Pampas deer had two peaks, the first in December-January, characterized by predominately anogenital sniffing, flehmen, urine sniffing, chasing and mounting behavior, and the second peak in July-September (behavior primarily related to gland marking) (Pereira *et al.*, 2005).

2.3.1. Ethogram

An ethogram is a complete catalogue of all behaviour and vocalizations occurring in a species (Banks, 1982).

In selecting measure of a particular behavioral study, it is useful to know the array of behaviour which the animal is capable of showing, a largely complete description of such a array is called an ethogram and is necessarily based on an extensive study of that species and they can be very useful if the behaviour description is precise enough (Fraser and Broom, 1990).

2.4. PHYSICAL AND BEHAVIOURAL CHANGES

2.4.1 Antler Characteristics

Emotional and endocrine status may be more crucial for antler development than nutritional conditions as reported by Bubenick and Bubenick (1987).

The antlers are temporary, which are shed after the rutting period is over, then regrow and remain in velvet (covered by soft skin) for sometime. The antlers branch and give rise to tines or twines. As the males get ready for breeding the

soft skin or velvet is rubbed against tree trunks to get the hard and sharp antlers exposed. During this period the stags (males) are in rut and starts searching for does or hinds (Mathur, 2005).

2.4.2 Weight Gain

Entire bucks of fallow deer exhibited pronounced live weight gains over spring and summer months (October to February.) and rapid live weight losses over the rutting period (Asher *et al.* 1987).

The antler length, body weight and chest girth were maximal during pre rut (December to January) in eld's deer stags as observed by Monfort *et al.* (1993).

The relative weight loss in males of red deer during rutting season was maximum for the animals in the prime age and the intensity was lower in younger and senescent males as noted by Yaccoz *et al.* (2002).

2.4.3. Other Changes

Gosch and Fischer (1989) found that the testicular volume started to increase in July/August, peaked just before the rut, declined until December to 50% of maximum, persisted at this level up to February/March and reached minimal volume after antler casting in late April. There was no apparent age effect on the seasonality of testis size fluctuations. Velvet shedding and antler casting occurred at about 80% and 25%, respectively, of maximal testis volume.

2.5. BREEDING PREFERENCES AND FREQUENCIES

As the breeding time approaches the fight among adult males establishes a winner male dominant, scent marks the territory and defends it (Mathur, 2005).

In species in which dominance hierarchies are formed either by males or by females, higher-ranking animals usually engage in more sexual behavior than do lower ranking animals. Among the males, there is considerable evidence that

dominant members of the group are usually sexually more active than the subordinates (Estep and Dewsbury, 2005).

2.6. HAREM FORMATION

. Males reproductive success depends on his harem size and the length of time for which he can defend the harem and this in turn depends on his body size and fighting ability as recorded by Krebs and Davies (1984).

2.7. ESTIMATION OF HORMONE LEVELS

Current literature discusses mostly the role of androgens and glucocorticoids, two groups of hormones that were previously implicated in rank based behavior as reported by Bartos *et al.* (1998).

Certain androgens are essential for proper function of centers involved in antler growth has been documented in castrates as reported by Bubenick and Bubenick (1987).

2.7.1. Non-invasive Technique

Wasser *et al.* (1991) has stated that faecal steroid analysis offers the potential of addressing many timely integrative problems in reproduction and conservation biology. There have been few efforts to interrelate behavioural ecology with endocrine activity in free-living mammals, primarily because of the unavailability of necessary tools. Feacal steroid analysis may be important for understanding the complex inter relationship between animals physiology and its environment.

Wasser *et al.* (1995) reported that the faecal steroid measures provide a useful means of characterizing fertility, ovulation and pregnancy in female maned wolves.

Faecal steroid measurement is a valuable non –invasive tool for assessing reproduction, environmental stress and aggression in populations of captive and free living animals as reported by Khan *et al.* (2002)

2.7.2. Testosterone Level

2.7.2.1. Season and Testosterone

Van Mourik *et al.* (1986) observed that the plasma testosterone concentrations in mature, male rusa deer (*Cervus rusa timoresis*) showed a minor elevation in autumn (May) and reached maximal levels in late winter – early spring (August) coinciding with the rut.

In entire bucks of fallow deer, serum testosterone concentrations increased during late summer (January to March) peaked to and 12 ng/ml respectively immediately before the onset of rut. (April) as observed by Asher *et al.* (1987).

Bubenik *et al.* (1987) found that in intact white tailed deer bucks, testosterone remained low (below 3.5 m mol/l) from February to August and then rose significantly ($P < 0.01$) till November with a peak of 36.78mmol/l) and then declined from November to January.

Asher and Peterson (1991) found the testosterone pulse frequency were low (0-2pulses /24h) in January and increased (5-7pulses /24h) in February in adult male fallow deer (*Dama dama*). By March and April (pre rut and rut periods respectively) there were episodic surges in plasma testosterone concentrations.

Bubenick *et al.* (1991) observed that the testosterone concentration exhibited a distinct seasonal pattern, minimum in December (0.1 ng /ml) maximum in may (1.75 ng/ml)

Bubenick. (1992) reported that the antler cycle of cervids is more or less tied to the seasonal variation of androgens. The timing, intensity of growth, and the length of the antler cycle vary between various cervids.

In red deer stags, the mean leutinising hormone (LH) and testosterone pulsatically and plasma concentration varied seasonally. Testosterone pulse frequency was more from March to November and testosterone pulse amplitude fell down from March to November (5.3ng/ml –1 to undetectable) although there was a conspicuous peak in July. (Mid winter) of almost 5 ngml/l as observed by Suttie *et al.* (1992)

Monfort *et al.* (1993) found that in Eld`s deer stags from autumn, there is small but detectable pulses in testosterone and during the winter transition into the breeding season, testosterone surges that lasted for 2-3 hours. High amplitude, low frequency testosterone surges were also observed during spring and basal testosterone concentrations decreased during summer.

High frequency blood sampling revealed markedly seasonal patterns of secretion of testosterone and LH, with hybrid Mesopotamian and European fallow deer exhibiting an apparent earlier onset of high amplitude testosterone 'surges' in February (late summer) compared to those occurring in April (Autum) for European males; as observed by Asher *et al.* (1996).

It is fairly well established that the androgens are the most important hormones involved in the development and mineralisation of the normal antlers. Photoperiodically controlled antlerogenisis begins during the period characterized by minimal levels of testosterone in blood. When the amount of androgens begins to rise, the antlers are usually fully developed and ready to be completely mineralized. The completion of hardening of antler bone precedes the rutting season by several weeks during which testosterone levels increases further as reported by Bubenick and Bubenick (1990).

The influence of seasonal changes on reproductive endocrine function has been most intensively examined in the cervidae. Seasonal elevations in testosterone have been associated with increases in sexual activity, aggressiveness, and testicular size. These factors being maximally coincident with the female cyclicity (Wildt, 2005).

2.7.2.2. Antler Cycle and Testosterone

Lincoln and Kay (1979) found that in the intact red deer (*Cervus elaphus*) stags plasma LH and testosterone concentrations changed during the year, the LH levels were maximal from September to November coinciding with the time of peak testicular activity and the mating season.

In red deer stags the pedicle initiation was associated with increasing plasma testosterone levels in response to changes in LH secretion and the antler development occurred when testosterone levels were low or decreasing. Cleaning of the velvet was associated with high levels of plasma testosterone and antler casting occurred when plasma testosterone concentrations were low or undetectable and prolactin levels were high or increasing as found out by Suttie *et al.* (1984).

The levels of serum testosterone reflected the inter-relationship between the antler and it`s sexual cycles with very low testosterone levels occurring at casting and during velvet antler growth. The levels were higher at antler cleaning and then increased to a maximum at the rut before declining to reach the nadir at casting. The results are consistent with a hypothesis that the antler cycle, as a male secondary sexual characteristic, is closely linked to the sexual cycle and its timing is controlled by reproductive hormones. Low plasma concentrations of testosterone, even after LH stimulation, are consistent with the hypothesis that testosterone is unnecessary as an antler growth stimulant during growth (Fennesssy *et al.*, 1988).

Asher *et al.* (1989) observed that in adult male fallow deer during the rutting period (March and April) episodic secretion of testosterone manifest as surges in plasma concentration of 4-6 h duration, and during the rut, the surges of plasma testosterone occurred at similar times of the day. Plasma profiles in May indicated very low concentrations of testosterone secretion in the immediate post rut period.

Suttie *et al.* (1991) found that the LH and testosterone secretion generally followed the same pattern. When the pedicles were growing, the LH and testosterone pulsatile secretion increased but the pulse frequency of both the hormones fell during the velvet antler growth in red deer stags (*Cervus elaphus*).

It is well established that the major landmarks of the antler cycle such as the mineralization and casting, are closely connected to the variation in androgens, particularly testosterone (Bubenick, 1992).

Suttie *et al.* (1995) reported that velvet antler growth can occur without testosterone stimulation during the period of velvet growth, but the timing of antler growth is linked to the annual cycle.

Bubenick *et al.* (1997) noted that the male reproductive axis is sequentially activated; the peak of testosterone values observed in December and March was not significant. They have also observed that the variation of testosterone level correlated relatively well with the antler cycle on male rein deer.

Higher testosterone levels were higher and lower follicle stimulating hormone (FSH) levels was reported in dominant males during ruti male Pudu.(*Pudu pua*) as reported by Bartos *et al.* (1998).

The low level of energy provided in food to mothers during their pregnancy period significantly reduced peak levels of testosterone in their male offspring (Bubenick *et al.*, 1999).

Roelants *et al.* (2002) observed that testosterone showed a maximum concentration during rut. (July or August) and the testosterone peak coincides with maximal meiotic intensity in August.

A temporary increase in testosterone values was recorded at the time of antler regrowth in fallow deer, the peak being significantly high. ($P<0.01$) A plasma androgen concentration at least above a minimal threshold level is a necessary pre requisite for normal antler growth in fallow deer as observed by Bartos *et al.* (2000).

The growth and mineralization of the antlers correlate with the seasonal variation of serum androgens (Bubenick *et al.*, 2005). Plasma testosterone concentrations of testosterone are minimal during antler growth, then rapidly increase during antler mineralization and reach peak levels shortly before the breeding season.

2.7.3. Cortisol Levels

The major rutting period of *Axis axis* in February to March was characterized by the changes in differential leukocyte count, elevations in serum muscle enzymes and lower serum cortisol levels (Chapple *et al.*, 1991).

Monfort *et al.* (1993) found that in eld`s deer stags (*Cervus eldithamin*) cortisol was secreted episodically at a rate of approximately 6 peaks per hour. They have also found out that eld`s deer stags lack a distinct seasonal rhythm of cortisol secretion.

Suttie *et al.* (1995) reported that cortisol responses were higher in red deer stags from late velvet antler growth to peak rut, compared with the times of antler casting and early velvet growth.

Cortiol and LH did not show any significant difference between dominant and subordinate males of pudu as reported by Bartos *et al.* (1998).

Bubenik *et al.* (2002) noted that the cortisol levels exhibited wide seasonal variation (9-45 ng/ml) without any peak or difference in levels among the groups.

The cortisol concentrations and the antler cycle link were not apparent. (Bartos *et al.*, 1998).

Measuring the glucocorticoids in faeces is a new, non-invasive approach. Faecal samplings offer the possibility of gathering information on cortisol concentrations in wild animals and prove to be an extremely useful manage-mental tool for identifying circumstances that causes stress (Merl.*et al.*, 2000, Von der Ohe and Servheen, 2002).

2.8. TESTOSTERONE AND BEHAVIOUR

Testosterone levels are correlated with aggression (Creel *et al.*, 1992) male social dominance (Koren *et al.*, 2002), mating also stimulates testosterone secretion. (Sapolsky, 1993) therefore testosterone is also expected to peak during the mating season.

Hormones influence behaviour and are also influenced by behaviour (Creel *et al.*, 1996). Monitoring their levels can therefore provide insights into mechanistic aspects of behaviour.

Finishing antler growth coincides with territory establishment,which may reflect peak agonostic activity. Agonostic activity in mammalian males is usually androgen dependant as repoted by Bartos *et al.* (1998).

Testosterone levels were clearly higher in dominant over subordinate males during the rut(March)and somewhat also higher in spring(October). This is in agreement with other authors who linked social aggression to high levels of plasma testosterone for red deer stags ,rein deer bulls, for white tailed deer bucks as reported by Bartos *et al.* (1998).

Reproductive behaviours and the Faecal steroid concentrations showed overt seasonal fluctuations in pere david's deer. There were statistically significant correlations between some male reproductive behaviours, such as anogenetal sniffing, urine spraying, bellowing, antler adorning, chasing, handling hinds, mounting and copulating with faecal testosterone concentration as observed by Li *et al.* (2001).

Androgens are generally responsible for species-specific arrays of reproductive behaviour, ranging from aggression related to mate or territory defense to scent marking, courtship and copulation. Not only do androgens stimulate reproductive behaviour, but social factors can also cause an increase in hormone levels. Mating activity stimulates acute increase in testosterone. (Asa, 2005).

There were significant correlations between fecal testosterone and reproductive behavior (r=0.490), and between fecal testosterone and antler phases (r=0.239) (Pereira *et al.*, 2005).

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Measuring the glucocorticoids in faeces is a new ,non invasive approach.Faecal samplings offers the possibility of gathering information on cortisol concentrations in wild animals and prove to be an extremely useful managemental tool for identifying circumstances that causes stress (Merl.et al.,2000, Von der Ohe and Servheen, 2002).

Among the males, those that achieve high ranks in their groups generally have a higher level of testosterone than those at the bottom of the hierarchy. Mates are an important resource and are cause of aggression. Sexual aggression is highly seasonal. The correlation of breeding and aggressiveness is seen among ungulates (Mathur, R, 2005).

It is fairly well established that the androgens are the most important hormones involved in the development and mineralisation of the normal antlers.Photoperiodically controlled antlerogenisis begins during the period characterised by minimal levels of testosterone in blood. At this time, when the amount of androgens begins to rise, the antlers are usually fully developed and ready to be completely mineralized. The completion of hardening of antler bone preceeds the rutting season by several weeks during which testosterone levels increased further as repoted by Bartos *et al.* (1998).

Materials and Methods

3. MATERIALS AND METHODS

The present study was carried out in Zoological garden Thrissur, (longitude $76^0$15`E latitude$10^0$32`N) that is having an area of about 4.5 acres, during the period from December 2004 to May 2005. 192 spotted deer maintained in the zoo were used for the study.

3.1 SELECTION OF THE SEASON OF STUDY

Season war birth occurred among the spotted deer of the zoo over a period of past 11 years were collected from birth register maintained in the zoo. Data were analyzed and winter season followed by monsoon season were identified as the season having maximum number of births. Seasonal breeding activity was deduced by reducing the gestation period from the date of birth. The data revealed that monsoon season (35%) followed by summer (31%)were found to be the season having maximum breeding activity during the last 11-year period. Based on the result of preliminary study and easiness to observe and collect faecal samples, winter and summer season were selected as the study period for the present work.

3.2. IDENTIFICATION OF MALE POPULATON

Out of the 45 males, four males in higher order of hierarchy were identified, based on their body size, antler-developing capacity to take vantage positions and dominance. And the animals were named as alpha I, alphaII, velvet I and velvet II in chronological order of dominance. The breeding behaviour and testosterone levels of the group were studied based on the behavioral responses of these animals with the female herd.

3.3. BEHAVIOURAL OBSERVATION

A preliminary study was conducted to familiarize with subjects and their behaviour for developing the method of measurement and compiling a detailed ethogram. The behavioural observations were made from 06:00 to 18:00 hours during the period of observation to coincide with the daily routine of animals. Behavioural observations were called 'events' when the behavioural pattern is of short duration and 'states' when behavioural pattern is of relatively long duration (Martin and Bateson, 1993). A total of 144 hours of observation in each group with a total of 72 hours of observation on each animal were recorded. A total of 436 hours of behavioural observation was made for the present study.

Observing the focal animals for one hour and continuously recording all the frequencies and intensities of selected behaviour from five meter distance, collected behavioural data. An ethogram of behaviour to be recorded was prepared as given in the table. The observation period was sub divided into 12 short periods of one-hour duration. Each focal animal was observed for five days by selecting one periods of each day. During the six days of observation in prior to breeding, breeding and post breeding periods was observed in all timings of the day. A total of 24 hours of observation in the breeding season, pre breeding and post breeding stages on each animal were recorded.

3.3.1. Recording Method

3.3.1.1 Focal Animal Sampling /Focal Sampling

Focal animal sampling is a sampling method in which, all occurrences of specified actions of an individual, or a specified group of individuals, are recorded during each sampling period and a record is made of the length of each sampling period and for each focal individual, the amount of time during the sample that is actually in view (Altmann, 1974).

Focal sampling means, observing one individual for a specified period of time and recording all the instances of it's behaviour usually for several different categories of behaviour (Martin and Bateson ,1993).

The above definitions for focal animal sampling were used for the behavioural observations made during the study.

Ethogram

Ethogram which is a catalogue of descriptions of the descrete, species typical behavioural patterns that form the basic behavioural repertoire of the species (Lehnar, 1987)for the present study was developed from the diverse array of behavioural descriptions for provided by various authors. The ethogram for the present study is given in Table.

Score chart for evaluating the ethogram is given in Table 13.

Behavioural data collection chart.

Ethogram

Sl.no.	**Behaviour**	**Description.**
1.	Feeding and drinking	Ingestion of food and consumption of water. Involves the individual gathering of food/water with mouth.
2.	Rumination.	Individuals engage in rumination of food.
3.	Rubbing/grooming.	Individuals lick the body/rubs against substrate such as tree/wall.
4.	Head high.	Head with antlers are held high.
5.	Vocalization.	Bellowing, mate calls.

Table continued .....

6.	Territory marking.	Chasing away the other males from certain area by the superior males of the herd.
7.	Fighting.	Fighting among the superior males, superior and inferior males etc. by clashing the antlers.
8.	Urine spraying	Continuous dribbling of urine when penis is erected.
9.	Masturbation or penis jerking.	Erected penis repeatedly hitting ventrum.
10.	Resting.	Individuals will not show any specific activity and will be lying down.
11.	Sniffing.	Sniffing the vulva of females.
12.	Chasing the female.	Males going after the receptive female of the herd.
13.	Mounting.	Mounting over the female in attempt to mate.
14.	Service.	Thrust and ejaculation.
15.	Flehmen.	The flattened surface of the tip of the nostril is brought to the mouth and the nostril tip is placed in contact with the roof of the muzzle.
16.	Other solitary behaviours.	Individual is engaging in a behaviour not covered in the ethogram descriptions above.

3.4. PHYSICAL AND BEHAVIOURAL CHANGES

The animals were observed from velvet forming stage to the antler shedding stage. The breeding behaviour, activity pattern etc of the animals were noted over the study period.

3.5. HORMONE LEVEL ESTIMATION

3.5.1 Feacal Samples

3.5.1.1. Collection and Storage

Faecal samples were collected once weekly from two superior animals of each season and thus including a total from four animals for a period of six

months. Faecal pellets of all the animals were taken within 1 hour after voidance. Faecal pellets were placed in a polyethylene bag and stored at -20^0 C till the preparation for extraction and RIA analysis.

3.5.2. Extraction For RIA

Frozen faecal samples were dried in freeze drier (ishlin)-50° C until dry. Each sample was powdered and mixed thoroughly. A 0.2 g of the sub sample was mixed with 5 ml of 90-percentage ethanol in a test tube and vortexed briefly. The tubes were boiled in a water bath (90 °C) for 20 minutes, adding ethyl alcohol to avoid it from boiling dry. The extract was brought up to pre boil levels with 90-percentage ethanol and centrifuged at 1500 rpm for 20 minutes. The extracts were poured off in to another storage vial. To the remaining faecal powder 90 percentage ethanol was added again and vortexed for 30 seconds and centrifuged at 1500 rpm for 15 minutes. The first and second extracts were combined and dried down and reconstituted in one ml of methanol and vortexed for a brief period. The methanol extracts were stored at -20° C until RIA analysis. (Brown, 2000)

3.5.3. RIA for Faecal Testosterone and Cortisol

Faecal testosterone concentrations were determined using clinical assay TM gamma coat TM (^{125}I) testosterone RIA kit (Radium vial de Mare, Pomezia, Italia).

Faecal cortisol estimation was done by Clinical assays ^{TM} Gamma coat ^{TM} Cortisol ^{125} I RIA kit.

3.5.4. Principle

The unknown concentration of a hormone is determined by measuring how much of a known amount of the radioactively labeled hormone H, binds to a fixed quantity of anti H antibody in their presence of H. This competition

reaction is easily calibrated by constructing a semi-logarithmic curve indicating how much H binds to the antibody as a function of H.

3.5.5 Procedure for estimation of Testosterone

1. To the bottom of the appropriate gamma coat tube the following were added.
2. 100 µl of testosterone control serum blank and testosterone serum standards. (0,0.25,0.5,1.5,6.0,15.0 ng/ml)
3. 100 µl of samples (extracted faecal samples) were added to each tube.
4. 500 µl of radioactive conjugate reagent was added to each tube and mixed on vortex mixer set at low speed.
5. The tubes were incubated for 1 hour in a 37° C water bath.
6. The contents of the tubes were decanted after incubation.
7. The tubes were counted in a Gamma counter. (1470 RIA Calc WTZ program 3.6 Automatic gamma counter) for one minute suited for I^{125}.
8. A standard curve was plotted with counts per minute (CPM) values and testosterone standards. (0, 0.25, 0.5, 1.5, 6.0, 15.0 ng/ml) on semi logarithmic graph paper.
9. The counts per minute (CPM) bound for each tube corresponding to each samples were used to interpolate the unknown values from the standard curve.

3.5.6 Procedure for Estimation of Cortisol

1. To the bottom of the appropriate duplicate gamma coat tubes the following were added.

a) 10µL of cortisol serum blank (0µg/dL) and cortisol serum standards. (1, 3, 10, 25 &60 µg/dL)

b) 10 µL of extracted faecal sample was added to each tube.

2. 1.0 ml of tracer buffer reagent was added to, each tube and mixed in a vortexed mixer set at a low speed.

3. The tubes were incubated for 45 minutes in a 37± 2°C water bath.

4. The contents of the tube were decanted after incubation.

5. The tubes were counted in gamma counter. (1480 WIZZARD ™ automatic gamma counter) for one minute with windows suitably suited for iodine -125.

6. A standard curve was plotted with contents per minute (CPM) values and cortisol concentration standards 1, 3, 10, 25 and 60(µg/dL) on semi logarithmic graph paper.

7. The counts per minute (CPM) bound for each tube corresponding to each sample were used to interpolate the unknown values from the standard curve.

3.6. BREEDING PREFERENCES AND FREQUENCIES

Each male identified were observed for a total of six days. The frequencies of sniffing, flehmen, chasing, mounting, service etc were noted.

3.7. HAREM FORMATION

The harems formed by all the dominant animals were observed for a period of six days. Number of females, number of velvet forming males, number of males in full developed velvet, number of males in hardened antler etc were noted.

3.8. CORRELATION BETWEEN ANDROGEN LEVEL AND BEHAVIOUR

The behavioural scores were compared with the weekly hormonal levels of each animal.

3.9. STATISTICAL ANALYSIS

The statistical analysis was done as described by Snedecor and Cochran (1994).

Results

4. RESULTS

4.1. BREEDING SEASON

The seasonal breeding activity was obtained by analyzing the birth record of spotted deer in the zoo for the last 11 years. Maximum number of breedings occurred in the chronological order. 35% in monsoon followed by 31% in summer, 19% in winter and 15% in post monsoon. The data are presented in Tables 1 and 2 and Figures 1 and 2.

4.2. IDENTIFICATION OF MALE POPULATON

The four males identified according to their hierarchical status among the 45 males were named as alpha I, alpha II, velvet I and velvet II and is presented in Figure 3 and 4. In Figure 5 the velvet shedding stage is depicted and the blood vessels exposed during this stage is shown in Figure 6.

4.3. PHYSICAL CHANGES.

4.3.1. Antler characteristics

Breeding activity is preceded by the development of antler, initiated by pedicle formation. The different phases of antler formation among the experimental deer were observed and recorded. Average time of existence of the full-developed antler was 11 weeks and the average time of antler cast state was eight weeks. The average time of existence of full-developed velvet antler was eight weeks and the time from the starting of velvet shedding to final hardened antler was four weeks. Different phases of antler cycle among the experimental deer are presented in Tables 3 to 6.

Table 1. Season wise birth

Season	1993	1994	1995	1996	1997	1998	1999	2000	2001	2002	2003	Total
Winter	9	16	6	8	5	7	9	9	6	6	6	87
Summer	6	0	0	0	7	2	0	5	10	10	18	58
Monsoon	0	2	2	2	6	6	20	8	10	10	13	79
Post monsoon	0	2	6	2	4	3	6	12	4	4	2	45
Total	15	20	14	12	22	18	35	34	30	30	39	

Table2. Season wise breeding

Season	1993	1994	1995	1996	1997	1998	1999	2000	2001	2002	2003	Total
Winter	0	0	0	2	4	4	16	7	6	2	7	48
Summer	0	9	10	5	9	3	11	13	6	6	5	77
Monsoon	13	10	2	5	7	8	4	8	16	2	13	88
Post-monsoon	0	1	2	0	2	3	4	4	4	5	14	39
Total	13	20	14	12	22	18	35	32	32	15	39	

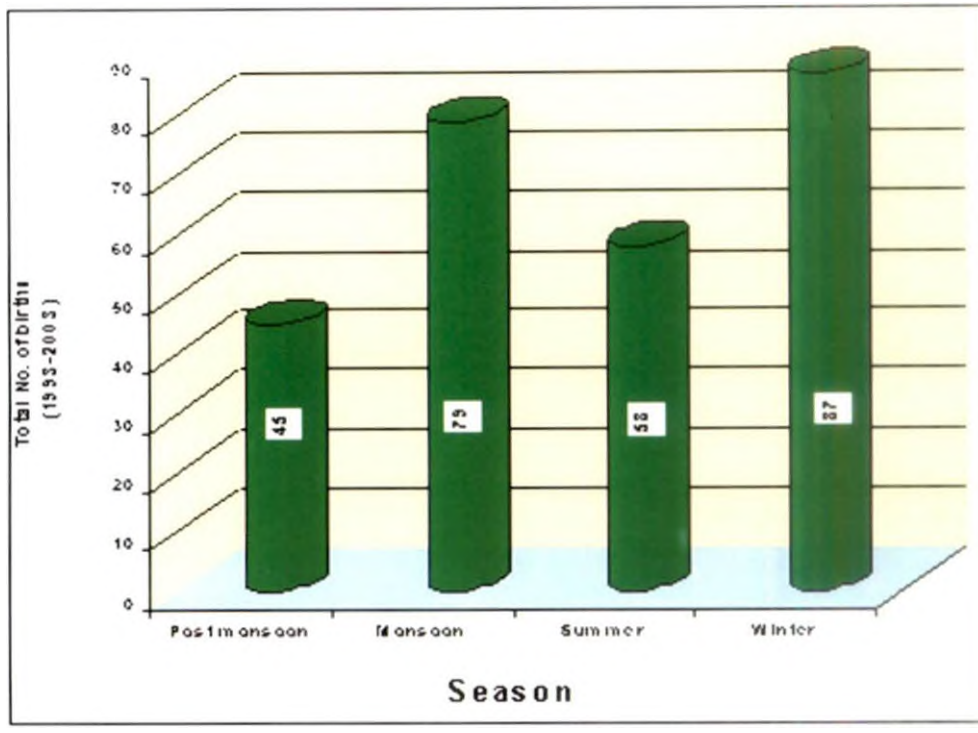

Fig.1. Season wise birth

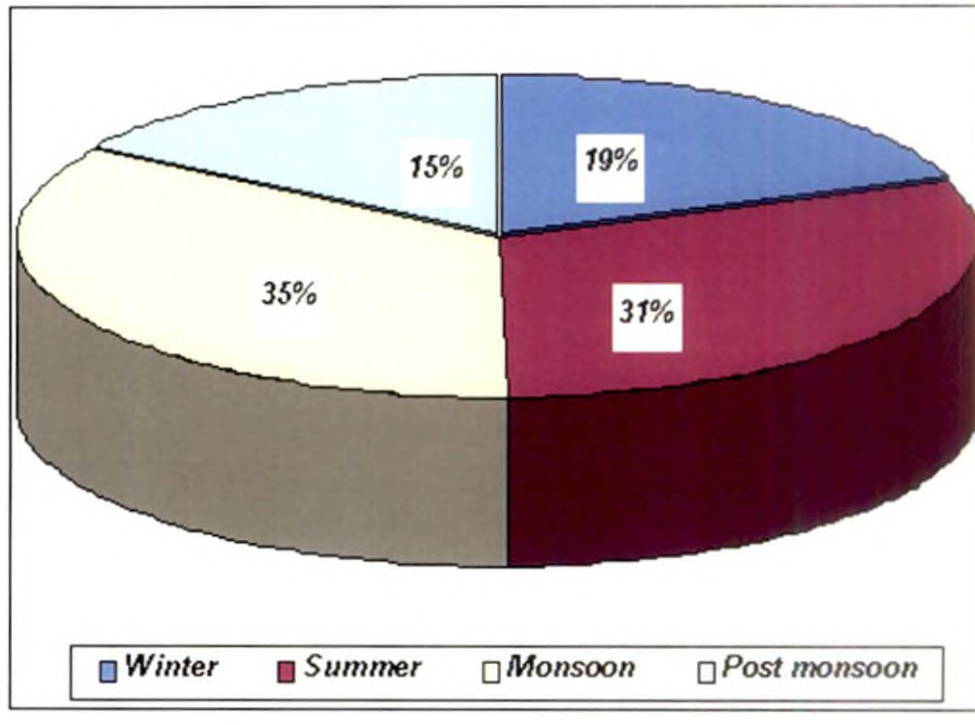

Fig. 2. Season wise breeding

Fig. 3. Alpha I and Alpha II

Fig. 4. Velvet I and Velvet II

Fig.5. Velvet shedding stage

Fig.6. Velvet shedding stage with blood vessels exposed

Phases of antler cycle of dominant males

Table 3. Alpha I

December				January				February				March				April				May			
I	II	III	IV	I	II	III	IV	I	II	III	IV	I	II	III	IV	I	II	III	IV	I	II	III	IV
HA*	HA	HA	HA	HA	HA	HA	HA	HA	HA	HA	HA	CA*	CA	CA	CA	CA	CA	CA	CA	PF*	PF	PF	PF

Table 4. Alpha II

December				January				February				March				April				May			
I	II	III	IV	I	II	III	IV	I	II	III	IV	I	II	III	IV	I	II	III	IV	I	II	III	IV
HA	HA	HA	HA	HA	HA	HA	HA	HA	HA	CA	CA	CA	CA	CA	CA	CA	CA	CA	CA	PF	PF	PF	PF

*

PF - pedicle formation
VA - velvet antler (full developed)
HA - hardened antler (full developed)
CA - casting of antler
VS - velvet shedding

Table 5.Velvet I

December				January				February				March				April				May			
I	II	III	IV	I	II	III	IV	I	II	III	IV	I	II	III	IV	I	II	III	IV	I	II	III	IV
VA*	VA	VA	VA	VA	VA	VA	VA	VS*	VS	VS	VS	HA	HA	HA	HA	HA	HA	HA	HA	HA	HA	HA	HA

Table 6.Velvet II

December				January				February				March				April				May			
I	II	III	IV	I	II	III	IV	I	II	III	IV	I	II	III	IV	I	II	III	IV	I	II	III	IV
VA	VA	VA	VA	VA	VA	VA	VA	VS	VS	VS	VS	HA	HA	HA	HA	HA	HA	HA	HA	HA	HA	HA	HA

It was also observed from the study that there was synchronization of antler cycles of the alpha I and alpha II and velvet I and velvet II.

4.3.2. Body size

The males in velvet forming stage were found to have more body size corresponding to the superior males in the rutting stage. The males with well developed antler in velvet stage were found to spend more time in feeding and were found to be the most masculine animals among the group.

4.3.3. Face colour

The black colour of the face was the darkest in alpha I male followed by alpha II in the breeding season. In the case of velvet-I and velvet-II males, the face colour, especially the colour of the muzzle became darker after the shedding of the velvet and development of hardened antler.

4.4. BEHAVIOURAL OBSERVATION

Frequency of behaviour listed in the ethogram for the study was observed at different stages of the antler development in males. The diurnal frequency of behaviours during pedicel formation stage, velvet stage, velvet shedding stage, hardened antler stage and casting stages are presented in Table 7. Frequencies of behaviour in different stages are presented in Figure 7. Respective scores of dominance in different stages were worked out and is presented in Table 8. Maximum score obtained was in the hardened antler stage or rutting stage.

The rutting stage was found to be the most dominant and aggressive stage. To identify the most dominant male at the most aggressive rutting stage, the diurnal behaviour of each dominant male was recorded and is presented in Table 9.

Table.7. Diurnal frequency of behaviours in different phases of antler cycle

Stage	Flehmen	Mounting	Fighting	Service	Chasing	Rumination	Sniffing	Grooming	Rest	Feed	Territory	Bellow
Pedicel formation	0	0	1	0	0	6	0	3	4	7	0	0
Velvet antler(full)	2	0	4	0	0	5	9	3	5	8	0	0
Velvet shedding.	5	0	4	0	2	2	11	2	2	4	1	0
Hardened antler (rut)	8	7	12	4	6	2	63	11	7	3	1	6
Antler casting.	2	1	4	0	0	2	9	3	4	4	0	2

Table.8. Behavioural score for animals in different stages of antler cycle

Stage	Flehmen	Mounting	Fighting	Service	Chasing	Rumination	Sniffing	Grooming	Rest	Feed	Territory	Bellow
Pedicel formation	0	0	1	0	0	3	0	1	3	3	0	0
Velvet antler(full)	1	0	1	0	0	4	1	1	2	4	0	0
Velvet shedding.	2	0	1	0	1	5	6	1	5	1	2	0
Hardened antler (rut)	4	1	3	2	2	2	3	2	3	2	3	3
Antler casting.	1	1	1	0	0	5	1	1	3	1	0	1

Table 9. Diurnal frequency of behaviours in breeding stage

Animal	Flehmen	Mounting	Fighting	Service	Chasing	Rumination	Sniffing	Grooming	Rest	Feed	Territory	Bellow
ALPHA 1	10	9	15	1	5	2	87	10	6	5	3.	5
ALPHA II	10	8	19	2	14	0	68	10	3	4	0	2
VELVET1	6	3	3	6	6	6	47	17	2	3	0	11
VELVET II	5	17	11	8	8	0	50	5	3	1	2	15

Table 10. Behavioural score for animals in the breeding stage

Animal	Flehmen	Mounting	Fighting	Service	Chasing	Rumination	Sniffing	Grooming	Rest	Feed	Territory	Bellow
ALPHA 1	5	1	3	1	1	5	5	1`	1	3	5	1
ALPHA II	5	1	5	1	1	0	3	1	3	1	1	1
VELVET1	3	1	1	3	3	3	1	3	5	1	1	5
VELVET II	3	1	3	1	3	0	1	1	3	1	3	3

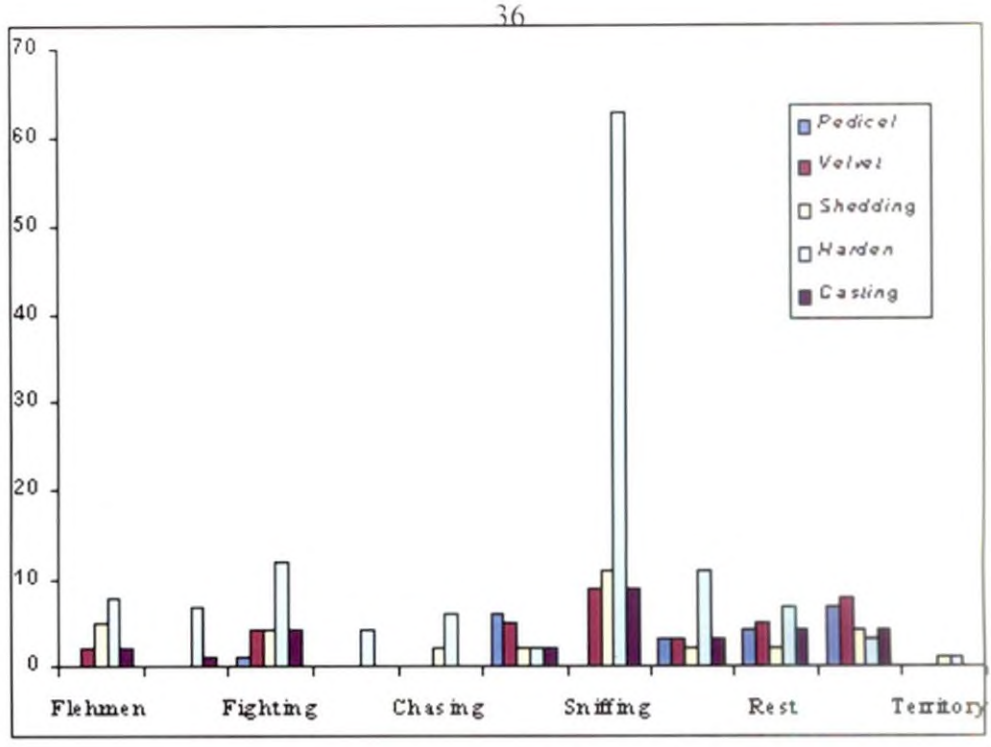

Fig. 7. Behaviour frequencies in different stagesof antler cycle.

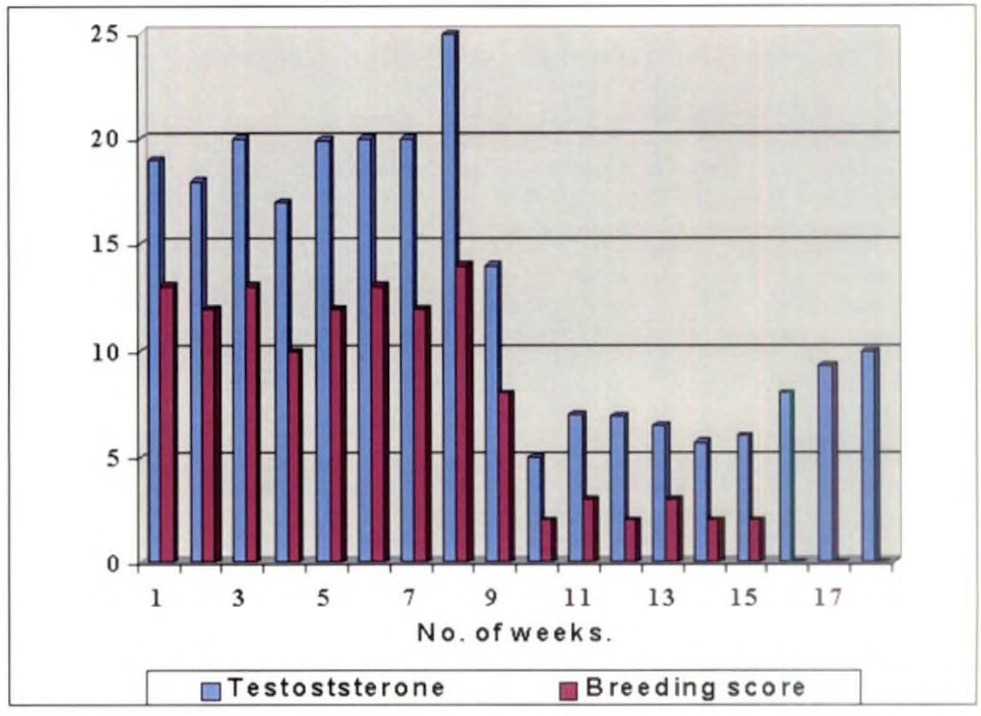

Fig. 8. Testosterone and behaviour score for Alpha I

The respective score arrived for each stag as per the frequency of diurnal behaviour in the ethogram is presented in the Table 10. The male alpha I was found to be dominant with a higher score of 32 and is given in the Figure 8

4.5. HAREM FORMATION

Harem formed by the dominant males was observed during the rutting stage. The composition of the harem of each stag is given in Table 11. Most dominant male alpha I formed the largest harem with maximum mean number of 19 females and with a mean number of only five male stags in velvet stage. The maximum harem size was seen in alpha I which was 26, followed by alpha II and velvet I, which were 16 as shown in Figure 9.

4.6. BREEDING BEHAVIOUR, PREFERENCES AND FREQUENCIES

4.6.1. Breeding behaviour

Frequency of behaviours exhibited exclusively during breeding season was observed among the experimental group in winter and summer season and is presented in Table 12. The weekly breeding scores for each animal are presented in Table 14. Breeding efficiency of the individual males were evaluated and ranked using the score chart as given in Table 13. Among the breeding behaviours observed maximum frequency was observed for sniffing which was 70.78% followed by Flehmen 8.98%, mounting 7.86 %, chasing 6.74% and service 5.61%. Maximum percentage of service was observed in velvet I followed by velvet II and alpha I. But the breeding related activities were more in case of alpha-I followed by alpha II and velvet I.

Table 11. Harem composition

Sl. No.	Animal	Velvet antler.	Hardened antler male.	Males with developed velvet	Females.	Harem size (No. of animals).
1	Alpha 1	5	0	0	19	26
2.	Alpha 2	4	0	0	12	16
3.	Velvet 1	7	0	0	9	16
4.	Velvet 2	1	1	0	13	15

Table 12. Average daily breeding activities

Season	Animal	Sniffing	Flehemen	Chasing	Mounting	Service
Winter	Alpha I	87	10	5	9	3
	Alpha II	68	10	4	8	2
Summer	Velvet I	47	6	6	3	6
	Velvet II	50	5	8	7	4

Table 13. Score chart for behavioural data collection

Sl. No.	BEHAVIOUR (Diurnal)	SCORING		
		5	3	1
1	Feeding and drinking.	1-4 times	5-8	9-12
2	Rumination.	1-3	4-6	7-9
3	Rubbing or grooming	21-30	11-20	1-10
4	Bellowing	11-15	6-10	1-5
5	Territory marking.	3-5	1-2	0
6	Fighting.	16-21	10-15	<10
7	Resting	1-2 hrs	3-5 hrs	5-7 hrs
8	Sniffing	71-90	51-70	<50
9	Flehemen.	9-12	5-8	1-4
10	Chasing females	9-12	5-8	1-4
11	Mounting	21-30	11-20	1-10
12	Service.	9-12	5-8	1-4
13	Other solitary	9-12	5-8	1-4

Table 14. Weekly breeding score for each animal

No.	Animal	December				January				February				March				April				May			
		1	2	3	4	1	2	3	4	1	2	3	4	1	2	3	4	1	2	3	4	1	2	3	4
1.	Alpha1	13	11	12	13	10	10	12	13	12	14	9	8	2	3	2	2	3	2	1	2	0	0	0	0
2.	Alpha2	7	7	8	8	9	9	6	5	4	5	1	2	1	1	2	1	1	1	2	1	0	0	0	0
3.	Velvet1	1	1	2	2	3	2	2	3	5	4	5	8	10	10	9	9	11	8	8	8	10	8	9	10
4.	Velvet2	1	1	1	2	2	2	3	3	2	3	3	5	8	6	5	8	6	9	5	6	5	6	6	7

4.6.2. Breeding preferences

More interest in females and other breeding related activities were shown by alpha I followed by alpha II and velvet I.

4.6.3. Breeding frequencies

The breeding frequency was more in velvet I with an average of six per day, followed by velvet II with four and alpha I three.

4.7. HORMONE LEVEL ESTIMATION

4.7.1. Faecal testosterone level

Faecal testosterone levels of four dominant males in the two seasons are given in Table 15. The mean testosterone concentration in the pedicel formation stage was 8.55±0.44ng/g of dry weight of faeces (ng/g) and concentration in the velvet stage was about 4.74±0.15ng/g. In the velvet shedding stage, the testosterone concentration was 17.77±0.71ng/g. In the rutting stage of velvet I and velvet II, the concentrations were about 19.64±0.86ng/g and 16.08±0.47ng/g respectively and for alpha I and alpha II the levels were 19.2±0.97ng/g and 12.8±1.58ng/g. The mean testosterone concentration in the casting stage was 6.37±0.30ng/g.

The mean testosterone concentration in the stags which were in rutting stage during summer was 17.95±0.65ng/g and in winter rutting males the concentration was 16.19±0.18ng/g.

The faecal testosterone levels were maximum in the rutting stage followed by the velvet shedding stage. The testosterone levels were lowest in the velvet stage followed by antler casting stage. During the time of pedicel formation, the concentration showed an increasing trend (Figure 10).

Table 15. Testosterone levels of animals

Season	Animal	December				January				February			
		1	2	3	4	1	2	3	4	1	2	3	4
Winter	Alpha 1	19	-	18	20	-	17	19.9	20	20	24.9	-	14
	Alpha 2	-	12	12.9	15	16.5	20	12	-	7	7	7	-
Summer	Velvet 1	3	-	3.2	4	4.9	4.2	3.2	10	19.1	18	20	-
	Velvet 2	3.1	-	3.8	4.1	-	4.7	6.5	7	15.5	18	16	-

Table continued....

March				April				May			
1	2	3	4	1	2	3	4	1	2	3	4
5	7	6.9	-	6.5	5.7	-	6	8	9.3	-	10
5.5	5.8	6	-	6.3	5.8	9.3	-	7	8.1	-	8.9
20	23	21	20	24.5	17	17	-	19.9	18	16	-
18.3	-	15.8	16	16.5	18	14.9	-	14	15	16.2	-

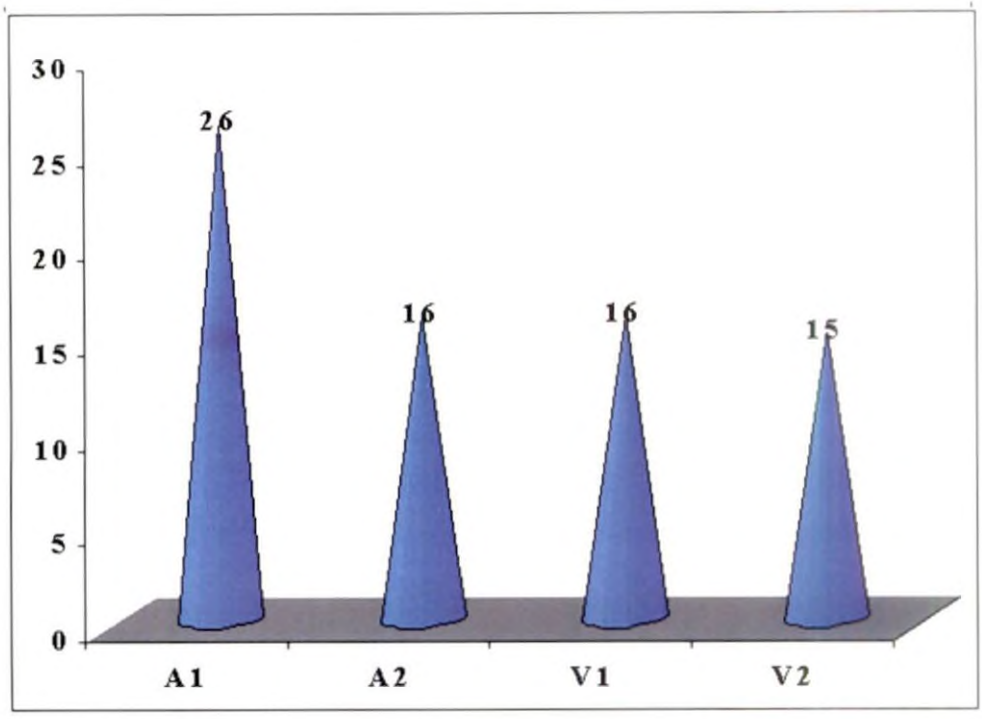

Fig. 9. Harem size

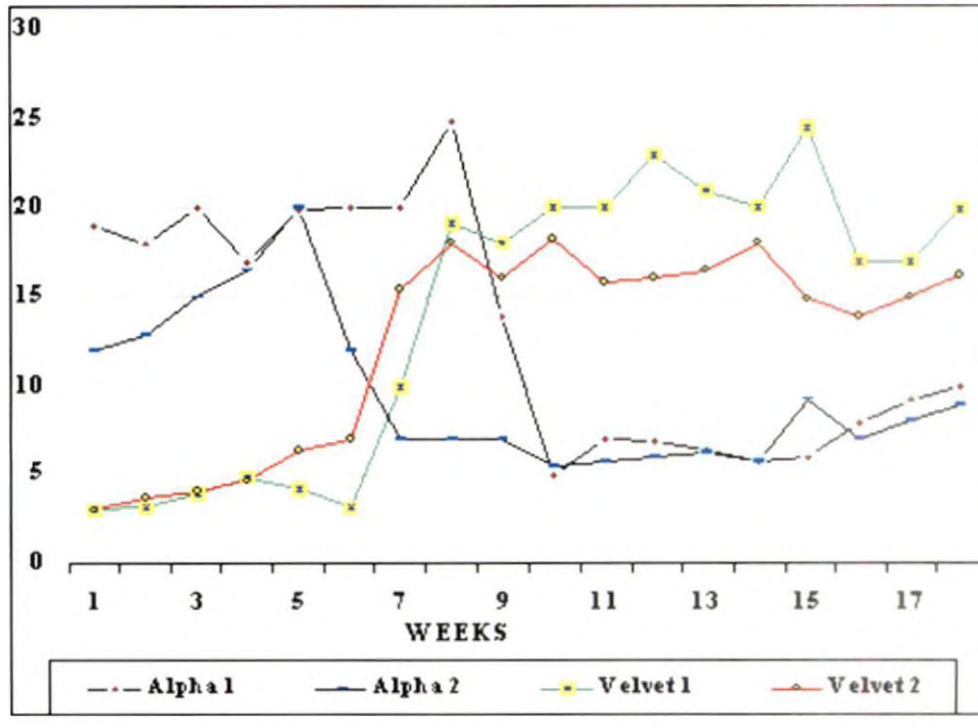

Fig. 10. Testosterone level of the four animals during the study period

4.7.2. Faecal cortisol level

Individual differences in the basal and peak values of faecal cortisol metabolites were observed. Mean cortisol concentration in the pedicel formation stage was 118.67±3.99ng/g and in velvet stage, it was 337.77±2.78ng/g. In the velvet shedding stage, the mean cortisol concentration decreased to 110.83±8.83ng/g. The mean cortisol concentration in the rutting stage was 121.07±6.88ng/g and in the casting stage the concentration was 114.54±5.57ng/g (Table 16).

In the summer rutting males, the cortisol concentration was 167.53±7.55ng/g and in winter rutting males the concentration was 140.35±13.59ng/g. The cortisol levels of the four dominant males namely alpha I, alpha II, velvet I and velvet II are shown in Figures 11, 12, 13 and 14. Comparisons of cortisol levels of the four animals are given in Figure 15.

There was no correlation between the breeding score and cortisol in any of the stages of sexual cycle. Similarly no correlation was found between the faecal cortisol level and faecal testosterone level. Feacal cortisol concentration was found to be maximum in the velvet stage followed by rutting stage, pedicel formation stage, casting stage and velvet shedding stage.

4.8. CORRELATION BETWEEN ANDROGEN LEVEL AND BEHAVIOUR

A positive correlation (ρ=0.878, $p<0.01$) between the breeding score and faecal testosterone was observed in animals of velvet stage. In the animals of velvet shedding stage also a positive correlation (ρ=0.94, $p<0.01$) between the testosterone level and breeding score was obtained. In the rutting stage a positive correlation (ρ= 0.817, $p<0.01$) between the testosterone level and breeding score was obtained. But in the antler casting stage and antler re-growth stage no significant correlation was seen. Comparison of faecal testosterone level and breeding score of individual animals are presented in the Figures 16, 17, 18 and 19.

Table 16. Cortisol level of animals

Season	Animal	December				January				February			
		1	2	3	4	1	2	3	4	1	2	3	4
Winter	Alpha 1	120	-	140	100	-	100	125	120	196	200	100	300
	Alpha 2	-	85	90	100	110	200	160		100	300	110	-
Summer	Velvet 1	320	-	380	335	400	375	300	305	90	105	100	380
	Velvet 2	-	-	305	360	-	300	320	311	120	150	100	-

Table continued....

				April				May			
1	2	3	4	1	2	3	4	1	2	3	4
110	90	110	-	96	106		120	125	110	-	105
100	94	120	-	160	133	140		125	117	-	130
100	160	200	170	185	200	170	-	190	187	150	-
100	-	196	200	130	140	140	196	170	195	200	-

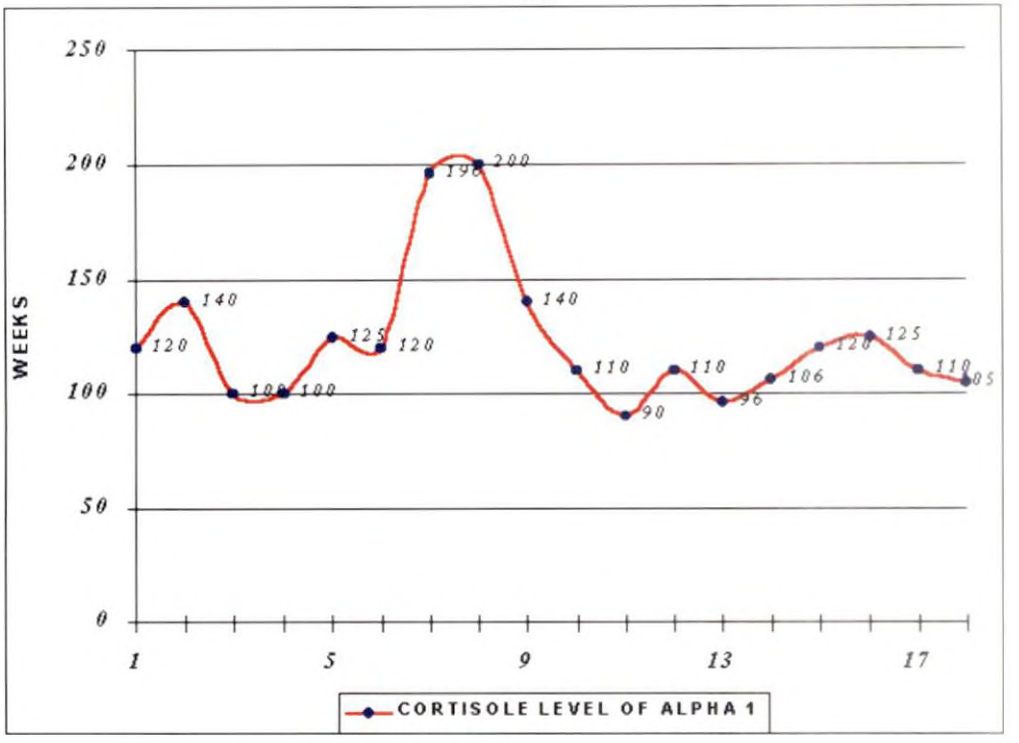

Fig. 11. Cortisol level of Alpha I

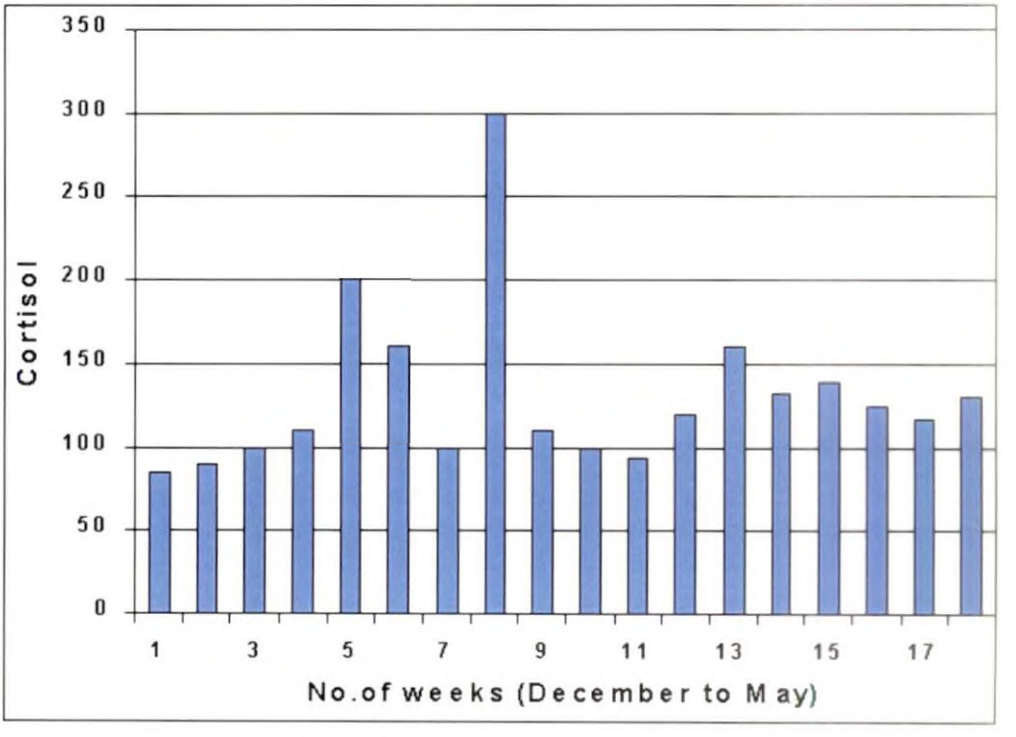

Fig. 12. Cortisol levels for Alpha II

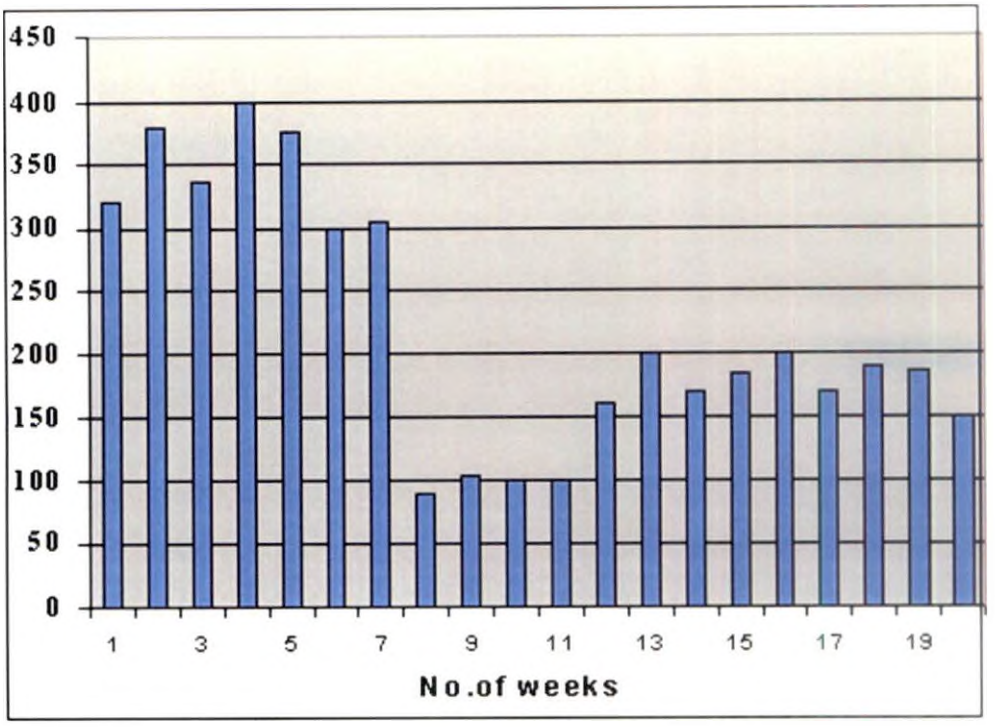

Fig. 13. Cortisol level of Velvet I

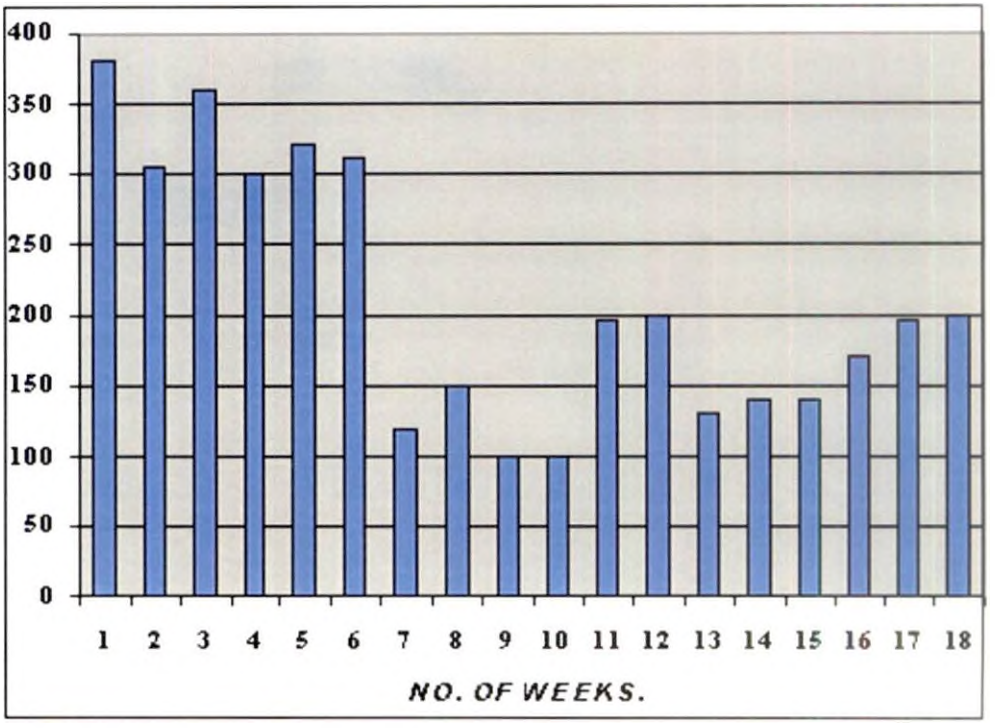

Fig. 14. Cortisol level of Velvet II

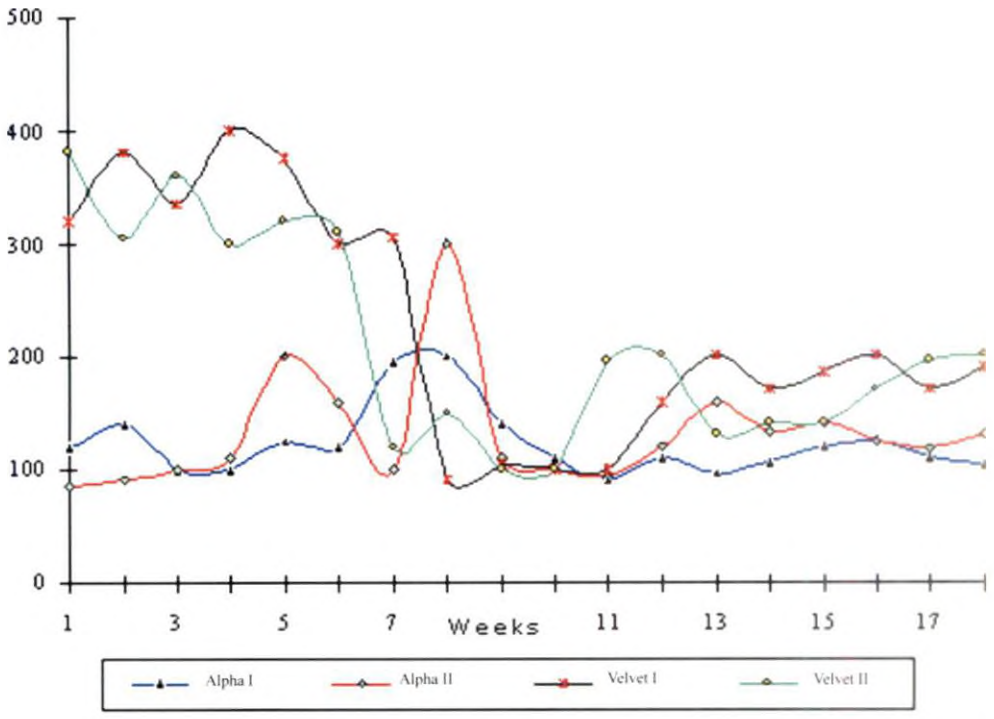

Fig. 15. Cortisol level of the four animals during the study period.

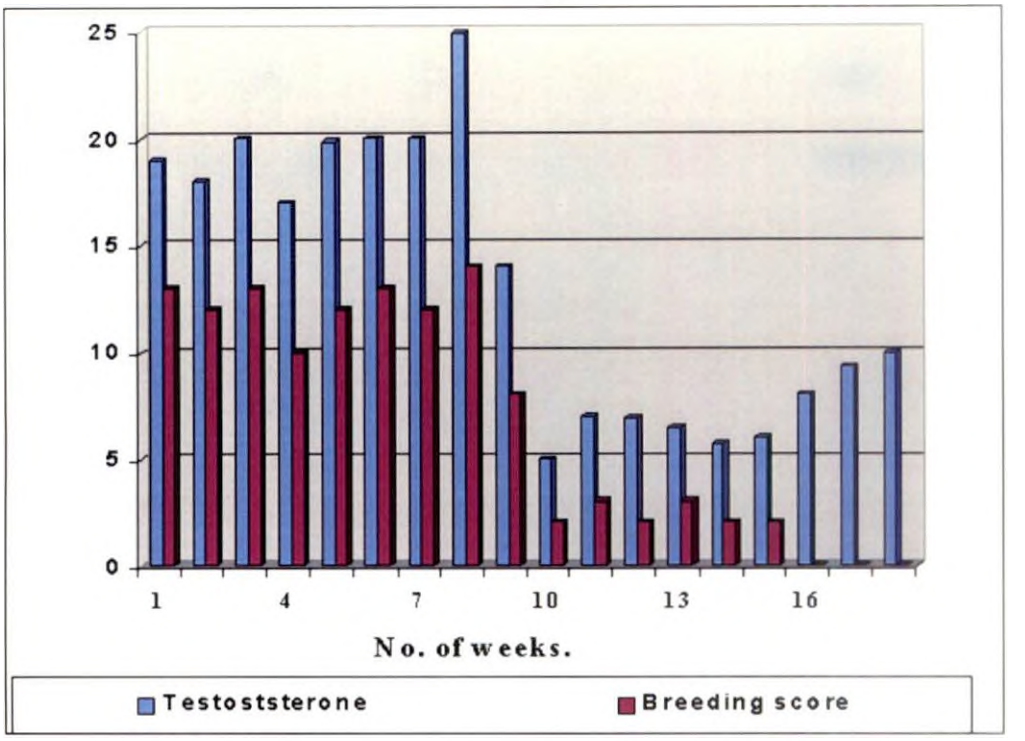

Fig. 16. Testosterone and breeding score of Alpha I

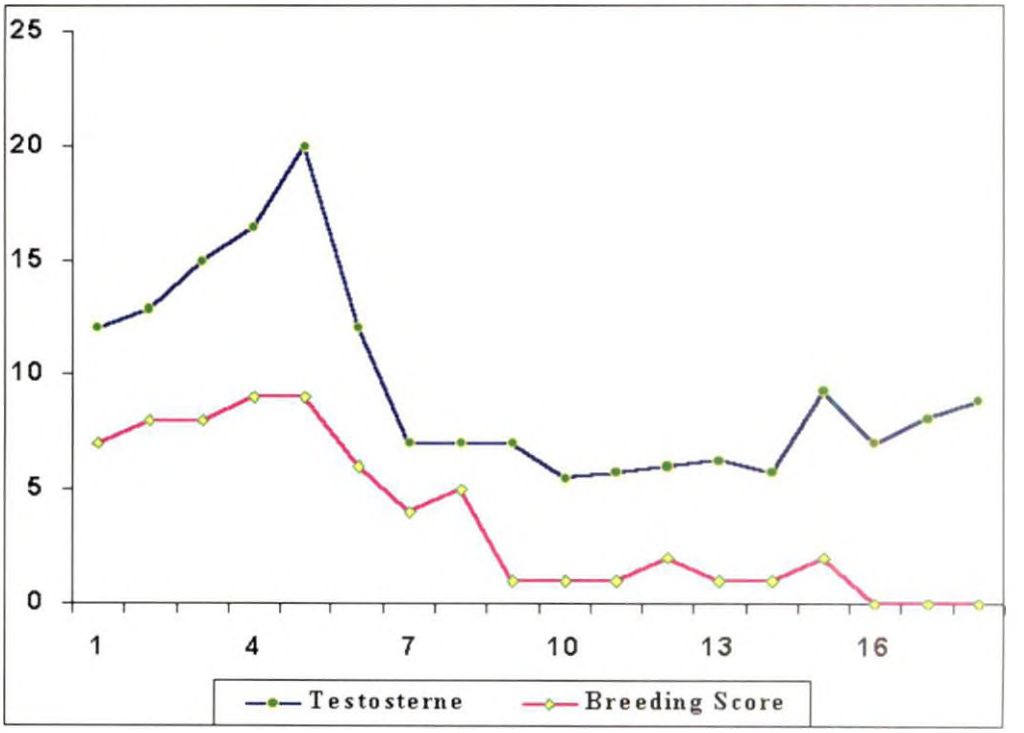

Fig. 17. Testosterone and breeding score of Alpha II

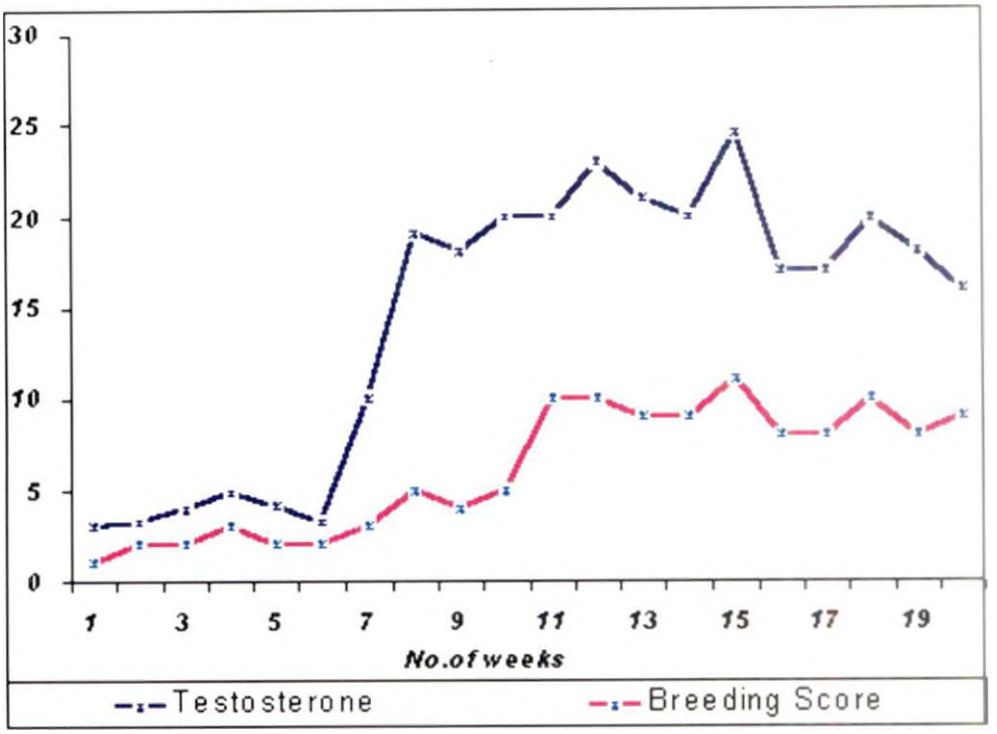

Fig. 18. Testosterone and breeding score for velvet I

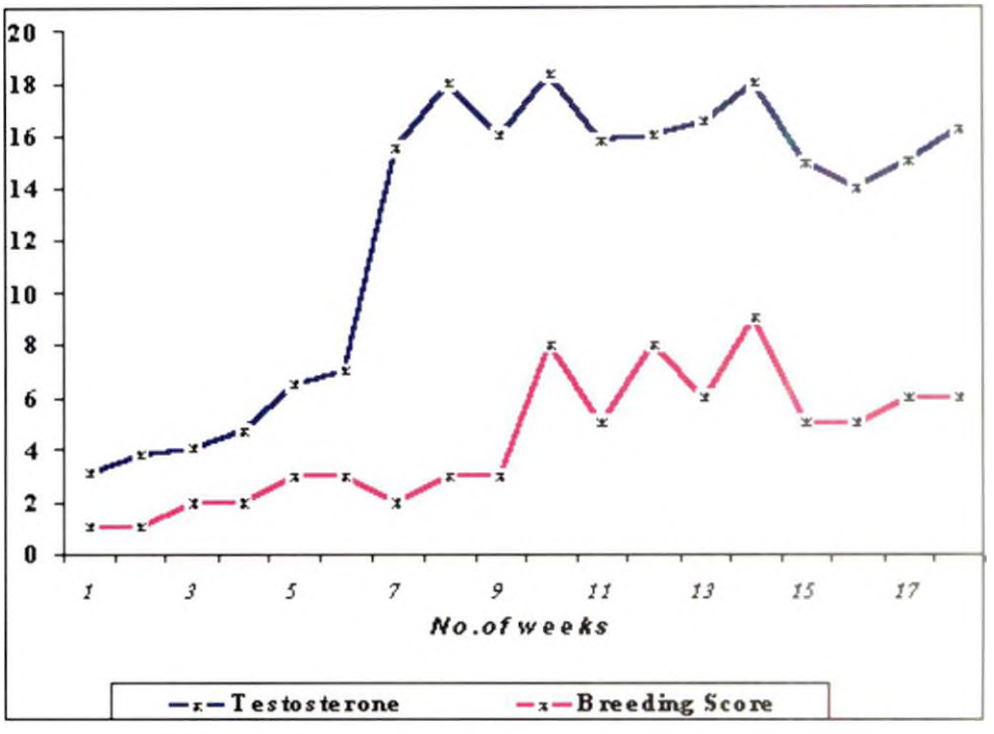

Fig. 19. Testosterone and breeding score for velvet II

Discussion

5. DISCUSSION

5.1. BREEDING SEASON

There is no photoperiodicity for breeding in spotted deer as both in monsoon and summer equally high breeding frequencies were observed. In the present study breeding occurred in all seasons, which indicates the absence of seasonality in breeding as reported by Raman (1998) from the spotted deer herd of South India. There seems to have a relation between the low atmospheric temperature and high humidity for the births among the spotted deer as maximum number of births occurred in winter (35%) and monsoon (31%) in the present study. This was supported by Tiwari *et al.* (2002) who reported that in axis deer delivery took place during rainy season, June to early August when there is maximum humidity in the enviourment.

5.2. IDENTIFICATION OF MALE POPULATON

Present study indicated that there might be more than one superior male in the breeding stage at any point of time. Saseendran *et al.* (2003) observed that in a population of Sambar deer, males were found to control the group lead by alpha male. The male population in the higher border of hierarchy was identified based on their body size, antler development capacity and capacity to take vantage positions and dominance. The superior male population was identified based on its good physical appearance, sharp and long antlers, positioning at vantage positions to take major share of feed and its mates.

The antler cycle of the superior males in each season was found to be relatively synchronized. This is supported by the findings of Bubenik (1992) who reported that the antler cycles of the axis deer were relatively synchronized with - in the herd. But Loudon and Curlewis (1988) reported that with in the herd, there was little evidence of a clear seasonal synchrony of the antler cycle.

5.3. PHYSICAL CHANGES

5.3.1. Antler Development

Phase of antler development was found to have a definite correlation with dominance and breeding stage. In the present study, dominant males in rutting season had the largest antlers among the group. This finding is supported by the study of Bubenick *et al.* (1987) that the largest antlers are found in deer, which were dominant in the previous rut. It was established that dominant males of a socially stabilized group will be having the lager antlers than the sub ordinates (Bartos *et al.*, 1998). Velvet shedding in the case of axis deer was observed to have peeling of velvet, exposing blood vessels and is prolonged for weeks. Among the experimental group, it took four weeks to shed velvet where as Van Mourick (1986) reported a shedding duration of several weeks in male rusa deer. The average time of existence of fully developed hardened antler was about 12 weeks for axis deer in the present study. Among the male rusa deer, antlers are mineralized in August and cast in December (Van Mourick, 1986).

5.3.2. Weight Gain

Weight gain was more among the animals in fully developed velvet stage compared to its contemporaries. A comparative weight loss was observed during rutting stage. These findings are in agreement with the findings of Asher *et al.* (1987) who observed pronounced live weight gain over spring and summer months in entire bucks of fallow deer and rapid live weight losses over the rutting period. Maximum body weight and chest girth were seen during pre rut (Monfort *et al.*, 1993) and a relative weight loss in rutting period were findings similar to present study (Yoccoz *et al* ., 2002).

5.3.3. Face Colour

In the present study, the colour changes of the face; especially the muzzle gets darkened in males of rutting stage.

5.4. BEHAVIOURAL OBSERVATION

Focal animal sampling technique developed by Martin and Bateson (1993) was used in the present study to record the behaviour of males.

The behaviour indicative of mating desire was more fully exhibited by the stags in the rutting stage. Using a common yardstick of behaviour scoring method could identify stags in the rutting stage.

The average behavioural score for alpha I and alpha II in the rutting stage was about 32 and 27 respectively. In the full-developed velvet antler stage the behavioural score was 14 and 13 for velvet I and velvet II. After the velvet shedding or in the rutting stage, the score was 30 for velvet I and 27 for velvet II.

In the velvet shedding stage the average behavioural score was about 21 for velvet I and 16 for velvet II. In the antler casting stage it was about 15 for alpha I and alpha II. In the pedicle formation stage, the score was 11 for alpha I and 10 for alpha II.

In the rutting season of alpha I and alpha II, velvet I and velvet II the behavioural scores were very high. But in the velvet stage (full developed) the behavioural scores were comparatively less in the case of velvet I and velvet II. In the casting stage the score is less and still lesser in pedicle initiation stage. In the velvet shedding stage it is in between.

Fraser and Broom (1990), opined that dominant animal at the most dominant stage can be identified by an ethogram, which is a capable of showing the complete array of behaviour.

These findings agree with the findings of Li *et al.* (2001) who observed that the reproductive behaviour showed overt seasonal fluctuations in Pere david's deer. These findings also agrees with the findings of Gizejewski (2003) who noticed that in the premating stage of red deer, all behavioural elements like aggression, roaring, characteristic smell, anxiety etc appeared in succession. This finding is supported by the study of Pereira *et al.* (2005) also.

Animals in the pedicle formation stage did not show any breeding activities and spent most of the time for feeding. This was in agreement with the findings of Monfort *et al.* (1993), which was evidenced by maximum body weight during pre rut (December to January) in eld's deer stags.

Animals in the velvet stage were not found to be very active and did not show any interest in females. They were very cautious and were found to be moving away from other males and no fighting was observed usually. The animals in the velvet forming stage were found to be very interested in feed. Asher *et al.,* 1987, record similar observations.

Corresponding with the velvet shedding stage, stags showed interest in females and exhibited reproductive behaviours like sniffing and flehemen, agnostic behaviour and more intensities and frequencies than in the velvet stage. These findings agrees with the findings of Gizejewski (2003) who noticed that in pre mating stages of red deer all behavioural elements appeared in succession.

Where as in the animals of in the antler casting stage, reproductive behaviours of the stags were drastically decreased in comparison with the well-pronounced and conspicuous breeding stage or rut. Finally, it lost all the interest in females. No records are available explaining the behaviour at this stage.

5.5. HAREM FORMATION

Breeding stage was preceded by harem formation by the rutting male. The average harem size in the breeding stage of alpha I was 26, in the case of alpha II

was 16 and velvet I and velvet II was 16 and 15 respectively. In the case of superior animals the harem size was more compared to next animal in the higher order of hierarchy. This coincides with the observations that axis deer formed harems of size 10 to 30 and that the males reproductive success depends on the harem size and the length of time for which he can defend the harem (Krebs and Davies, 1984).

5.6. BREEDING BEHAVIOR PREFERENCES AND FREQUENCIES

5.6.1. Breeding Behaviour

The superior animals in the breeding stage were found to be very active and engaged mainly in breeding activities. The superior animals of the herd had given the mate calls and threat calls. Fighting with other males for breeding and for marking its territory was observed. This was similar to the findings of Mathur (2005) that as the breeding time approaches, fight among the adult male establishes a winner male dominance, scent marks the territory and defends it.

5.6.2. Breeding Preferences

It was observed that the higher-ranking animals among the spotted deer stags engaged in more sexual behaviour than do lower ranking animals. The mean frequency of breeding activities and breeding of the animals are given in Table 12. The score for breeding and related activities was more in alpha I, that is 13 followed by velvet I and alpha II which is 11. More interest in females and mean frequency of breeding related activities were more alpha I but the success rate of breeding was comparatively less. This is supported by the findings of Estep and Dewsbury, (2005) and Mathur (2005) that in species in which dominant hierarchies are formed, higher-ranking animals showed more sexual activity than do lower ranking.

5.6.3. Breeding Frequencies

Hierarchical status and the niche of the particular stag determined its breeding success. Among the experimental group, velvet I had the breeding frequency of six per day followed by four per day in velvet II and three per day for velvet II. This may be correlated with the higher level of testosterone seen in males which came to the breeding stage in summer as mating activity stimulates an acute increase in testosterone as recorded by Asa, 2005.

Season was found to have some influence in breeding frequency. In the present study, during summer, there was a high rate of breeding among animals. The higher hormonal production especially testosterone during the hot sunny summer season may be the season for this high rate.

5.7. HORMONE LEVEL ESTIMATION

5.7.1. Faecal Testosterone

The faecal testosterone values varies with the stage of the antler cycle. Highest faecal testosterone values of 19.64±0.86 ng/g dry weights of faeces (ng/g) were found during the rutting stage and was maximum in velvet I. This is in agreement with the findings of Lincolon and Kay (1979) that the high testosterone levels coincide with the time of peak testicular activity and the mating season. This is also supported by Asher *et al.* (1989) that there was surge release of testosterone during rut. The higher level of testosterone in the rutting stage of males is in accordance with the findings of Bartos *et al.* (1998) that testosterone levels were high in dominant animals during rut. Roelants *et al.* (2002), record similar observations.

During the casting of the antler, the level decreased to about 6.53± 0.50 to 6.18± 0.31 ng/g. The decreasing faecal testosterone level in the antler casting

stage in the present study coincides with the findings of Suttie *et al.* (1984) that antler casting occurred when plasma testosterone concentrations were low. This finding also agrees with Asher *et al.* (1989) that plasma profiles indicated a very low concentration of testosterone in the immediate post rut period.

During the time of antler re-growth, the values shot up to 9.1± 0.59 and 8± 0.55(ng/g) in case of alpha I and alpha II respectively. The increasing plasma testosterone concentrations during regrowth stage in deer stags is similar to the observations of Suttie *et al.*, 1984 that in red deer stags the pedicle initiation was associated with increasing plasma testosterone levels in response to changes in LH secretion. Similar findings are recorded by Bubenik and Bubenik (1990) and Bartos *et al.* (2000).

In the velvet forming stage the average concentration was about 4.64±. 93(ng/g) incase of velvet I and 4.87± .64(ng/g) in the case of velvet II. This also agrees with findings of Suttie *et al.* (1984) that the antler development occurred when testosterone levels were low or decreasing. This is supported by findings of Bubenik *et al.* (2005) also.

In velvet shedding stage also, the concentration was comparatively higher than that of the other stages like casting, regrowth, antler development etc. The average concentration of faecal testosterone in the velvet shedding stage was 19.03±. 58 (ng/g) in velvet I and 16.5±. 76(ng/g) in case of velvet II. Suttie *et al.* (1984) that cleaning of the velvet was associated with high levels of plasma testosterone, do similar observations. This finding is supported by Suttie *et al.* (1991), Fennessy *et al.* (1988), Bubenik *et al.* (2005).

In the rut and pre rut periods there were episodic surges in plasma testosterone concentrations (Asher *et al.*, 1991) and it coincides with the observations of the present study. A slightly higher level of testosterone found in the males velvet I and velvet II, which came to breeding stage in summer than alpha I and alpha II which were in the breeding stage in winter. The above fact is

in accordance with Asher *et al.* (1987) that serum testosterone concentrations increased during summer in entire bucks of fallow deer. This is in agreement with the findings of Bubenik *et al.* (1991) that testosterone concentration exhibited a distinct seasonal pattern, minimum in December (0.1ng/ml) and maximum in May (1.75ng/ml). But it disagrees with the findings of Monfort *et al.* (1993) that the basal testosterone concentrations decreased during summer.

The variation in the testosterone levels in various stages of antler cycle is supported by the findings of Bubenik (1992, 1997, and 2005) that major landmarks of the antler cycle such as mineralisation and casting are closely connected to the variation in androgens, particularly testosterone.

5.7.2. Faecal Cortisol

The lesser concentration of faecal cortisol in rutting stage compared to breeding is similar to the findings of Chapple *et al.* (1991) that major rutting period in axis deer there was lower serum cortisol levels. There was no seasonal rhythm of cortisol secretion as supported by the findings of Monfort *et al.* (1993). The higher concentration of faecal cortisol seen in the deer stags is similar to the findings of Suttie *et al.* (1995) that cortisol responses were higher in red deer stags from late velvet antler growth to peak rut compared to the times of antler casting and early velvet growth.

There was no correlation between the reproductive behaviour and faecal cortisol level, similar to the findings of Bartos *et al.* (2000) that cortisol concentrations and antler cycle link were not apparent. No distinct difference in faecal cortisol concentrations between animals in the first position and second position of hierarchy. This is in accordance with the fact that cortisol concentrations did not show any significant difference between dominant and subordinate males as reported by Bartos *et al.* (2000).

5.8. CORRELATION BETWEEN ANDROGEN LEVEL AND BEHAVIOUR

The positive correlation (ρ=. 878, p<. 01) between the breeding score and faecal testosterone was observed in animals of velvet stage. In the animals of velvet shedding stage also a positive correlation (ρ=. 94, p<. 01) between the testosterone level and breeding score was obtained. In the rutting stage a positive correlation (ρ=. 817, p<. 01) between the testosterone level and breeding score was obtained. This is supported by the findings of Koren *et al.* (2002) that the testosterone levels are correlated with the male social dominance and observations by Sapolsky (1993) that mating also stimulates testosterone secretion. From this study it is evident that hormones influence sexual behaviour and is also influenced by behaviour (Creel *et al.*, 1996).

Androgens are generally responsible for species specific arrays of reproductive behaviours, ranging from aggression related to mate or territory defense to scent marking, courtship to copulation. Not only androgens stimulate behaviour, but social factors also can cause an increase in hormone levels. Mating activity stimulates an acute increase of testosterone. These findings are similar to the findings of Li *et al.* (2001) that there were statistically significant correlations between some reproductive behaviours such as anogenital sniffing, urine spraying, bellowing, antler adorning, chasing, handling hinds, mounting and copulating with faecal testosterone level. Pereira *et al.* (2005) has also made similar findings like there were significant correlations between fecal testosterone and reproductive behavior (r=0.490), and between fecal testosterone and antler phases (r=0.239).

But in the antler casting stage and antler regrowth stage no significant correlation was seen. This is similar to the findings of Li *et al.* (2001) that reproductive behaviours and the faecal steroid concentrations showed overt seasonal fluctuations in pere david`s deer.

Summary

6. Summary

The axis deer is one of the tropical deer species and is native to India. In most of the zoos and other captive facilities this species of animals often multiply to unmanageable numbers. Methods of containment of such situations have to be astute and ethically sound. So a thorough understanding of the breeding behaviour of this species is essential in this regard. Only meager studies are reported to reveal its blood parameters, hormone levels, breeding behaviour and the likes. Hence the present work was aimed to make a detailed understanding of the breeding behaviour of the males of captive axis deer in relation to the testosterone profile in order to evolve a successful method to curtail the exploring population in captivity.

The study was carried out in a captive population of spotted deer in Government Zoo, Thrissur, from December 2004 to May 2005. Selection of the study season was made by analysing the birth register of the spotted deer in the zoo. Seasonal breeding activity was deduced by reducing the gestation period from the date of birth. Accordingly, monsoon season followed by summer season showed the maximum breeding activity. However, for ease of observation and collection of faecal sample the study was done in winter and summer. Out of a total of 45 males, four males in the higher order of hierarchy were identified and selected for the study. Behavioural data collection was done by focal animal sampling for one hour during different periods of the day. A total of 436 hours of observation was carried out. The main sets of behaviour observed were feeding, rumination, sniffing, flehemen response, chasing of females, mounting, service, fighting, grooming, resting, territory marking and bellowing.

Four males in the higher order of hierarchy were identified as alpha I,alpha II, velvet I and velvet II. A detailed observation of these animals was done. The maximum behavioural score of 30 was obtained for alpha I and velvet I followed by 27 for alpha II and velvet II.

Physical and behavioural changes of the animals in different stages of the antler cycle were observed and a characteristic difference between the breeding and the non-breeding stage was observed. The weight gain was more in the velvet forming stage where as the blackness of the face was more in the breeding stage. (Hardened antler stage) .The average time for velvet shedding was observed as four weeks and the time for which the fully developed antler remains was eight weeks. From the antler casting to re-growth it took eight weeks.

The faecal sample of the dominant male animals were collected and stored at -20° C. It was processed and analyzed for faecal testosterone and cortisol by radio immunoassay.

The mean faecal testosterone level in the breeding season was about 19.2±0.97 ng/g of dry weight of faeces for alpha I and 12.8±1.58 ng/g dry weight faeces for alpha II. The mean testosterone concentration in the casting stage was about 6.18±0.31 ng/g dry weight of faeces for alpha I and 6.53±. 50ng/g dry weight of faeces for alpha II. Mean testosterone concentration in the antler regrowth stage was about 9.1±0.59ng/g dry weight of faeces in alpha I and in the case of alpha II it was ,8±0.55ng/g dry weight of faeces. In case of velvet I the mean testosterone concentration in the velvet stage was about 4.64±0.93 ng/g dry weight of faeces and in the case of velvet II was about 4.87±0.64 ng/g dry weight of faeces .In the velvet shedding stage of velvet I and velvet II, the testosterone concentration was 19.03±.58 ng/g dry weight of faeces and 16.5±.76ng/g dry weight of faeces. In the rutting stage of velvet I and velvet II the concentration was about 19.64±.86 ng/g dry weight of faeces and 16.08±.47ng/g dry weight of faeces. Faecal testosterone levels in each individual during the breeding season were significantly increased than before the breeding season and after the breeding season.

The faecal testosterone levels were maximum in the rutting stage followed by the velvet shedding stage. The testosterone levels were lowest in the

velvet stage followed by antler casting stage. During the time of velvet regrowth, the concentration was showing increasing trend.

Individual differences in the basal and peak values of faecal cortisol metabolites were observed. The mean cortisol concentration in the rutting stage of alpha I and alpha II were about 137.89±12.31ng/g dry weight of faeces and 143.13±.26.52ng/g dry weight of faeces respectively. The mean cortisol concentration in the casting stage was about 105.33±4.42ng/g dry weight of faeces for alpha I and 122.43±8.89in the case of alpha II. The mean cortisol concentration in the antler re-growth stage was about 113.33±6.02ng/g dry weight of faeces and in the case of alpha I and it was 124±3.79ng/g dry weight of faeces in case of alpha I.In case of velvet I, the mean cortisol concentration in the velvet stage was about 345±15.07ng/g dry weight of faeces and in the case of velvet II was about 329±13.45ng/g dry weight of faeces .In the velvet shedding stage of velvet I and velvet II, the mean cortisol concentration was 98.03±4.41 ng/g dry weight of faeces and 123± 14.55ng/g dry weight of faeces. In the rutting stage of velvet I and velvet II, the concentration was about 171.2± 9.49ng/g dry weights of faeces and 163 ± 12.37ng/g dry weight of faeces.

There was no correlation between the breeding score and cortisol in any of the stages of sexual cycle. Similarly no correlation was found between the faecal cortisol level and faecal testosterone level. Feacal cortisol concentration was found to be the maximum in the velvet stage followed by rutting stage, antler regrowth stage, casting stage and velvet shedding stage respectively.

The mean frequency of breeding activities were more in velvet I which is six, followed by four in velvet II and three in alpha I and two in alpha II. The score for breeding behaviour were more in alpha I (13) followed by 11 for alpha II and velvet I .Lowest score of nine was observed in velvet II.

The mean number of animals in the harem of alpha I was about 26 and in the case of alpha II was about 16. But in the case of velvet I and velvet II there was a significant difference in harem size.

A positive correlation (ρ=. 878, p<. 01) between the breeding score and faecal testosterone was observed in animals during velvet stage. A similar correlation was also observed during velvet shedding stage and rutting stage.But in the antler casting stage and antler re-growth stage no significant correlation was seen.

In summary, the correlation between behavioural observation and faecal testosterone values makes this technique a promising tool that can be used as a reliable means to interpret the breeding behaviour in relation to the testosterone level of higher order males. Such a non-invasive tool can be helpful in controlling the problem of population explosion of many wild species kept under captive facilities vis-à-vis desirable maneuver in their breeding activities. The methods like vasectomy or chemical sterilization of superior animals can be used effectively to control the population size, as it will maintain the hierarchical order of males in the herd during breeding season and will prevent the breeding by other males also.

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BREEDING BEHAVIOUR AND TESTOSTERONE LEVEL OF MALE SPOTTED DEER

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ABSTRACT

Captive population of spotted deer (*Cervus axis axis*) maintained in zoological gardens, Thrissur were utilized to study the breeding behaviour and testosterone level of males. Out of a total of 45 males, four males in the higher order of hierarchy were selected for the study. A total of 436 hours of observation were carried out by focal animal sampling technique using a behaviour score sheet. Faecal samples were collected from these animals and stored at -20 °C, until it was extracted for measurement for faecal testosterone and cortisol by radioimmunoassay.

Seasonal breeding activity was deduced by reducing the gestation period from the date of birth and monsoon season (35 %) followed by summer season (31%) were selected as the seasons of maximum breeding. Raining season was avoided to eliminate the errors in sample collection and observation of the herd was done in winter and summer.

Four males in the higher order of hierarchy were identified based on their body size, antler size, capacity to take the vantage positions and dominance. Animals were named as alpha I, alpha II, velvet I and velvet II. The dominant male of the group was found to develop the longest antlers among the group. Similarly, the body size was more in the velvet forming stage. The blackness of the face, especially the area around the muzzle increases from non-breeding to the breeding stage. The antler cycle of males includes different stages like pedicle formation, velvet growth, velvet shedding, hardened antler stage (rutting stage) and antler cast state.

Largest harem size was observed in alpha I(26)followed by alpha II(16).The most frequently observed breeding activity was sniffing 70.78% followed by flehmen 8.98%.Maximum score for breeding behavior was obtained for alpha I(13)followed by alpha II (11).But the actual breeding frequency was more in velvet I (6) followed by velvet II ,which is four per day.

The mean testosterone concentration in the pedicel formation stage was 8.55±0.44ng/g of dry weight of faeces (ng/g) and concentration in the velvet stage was about 4.74±0.15ng/g. In the velvet shedding stage, the testosterone concentration was 17.77±0.71ng/g. In the rutting stage of velvet I and velvet II, the concentration was about 19.64±0.86ng/g and 16.08±0.47ng/g respectively and for alpha I and alpha II the level was 19.2±0.97ng/g and 12.8±1.58ng/g. The mean testosterone concentration in the casting stage was 6.37±0.30ng/g.

The mean testosterone concentration in the stags which where in rutting stage during summer exhibited a testosterone level of 17.95±0.65ng/g and in winter rutting males the concentration was 16.19±0.18ng/g.

Individual differences in the basal and peak values of faecal cortisol metabolites were observed. Mean cortisol concentration in the pedicle formation stage was 118.67±3.99ng/g and in velvet stage, it was 337.77±2.78ng/g. In the velvet shedding stage, the mean cortisol concentration decreased to 110.83±8.83ng/g. The mean cortisol concentration in the rutting stage was 121.07±6.88ng/g and in the casting stage the concentration was 114.54±5.57ng/g. There was no correlation between the breeding score and cortisol in any of the stages of sexual cycle. Similarly no correlation was found between the faecal cortisol level and faecal testosterone level.

A positive correlation (ρ=. 878, p<. 01) between the breeding score and faecal testosterone was observed in animals during velvet stage. A similar correlation was also observed during velvet shedding stage and rutting stage. But in the antler casting stage and pedicel formation stage no significant correlation was seen.

The results of the present study suggests that in captive herds of spotted deer, non invasive method can be successfully used to find out the testosterone levels, which can be integrated with breeding behaviour to have a better understanding of the breeding patterns of the group. This knowledge of breeding behavior in relation to testosterone level can be effectively used to adopt some measures to contain the population size.