BEHAVIOURAL RESPONSES IN CAPTIVE MALE ASIAN ELEPHANTS (*Elephas maximus*) TO SPECIFIED STRESSORS

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Thesis submitted in partial fulfilment of the requirement for the degree of

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DECLARATION

I hereby declare that the thesis entitled **"BEHAVIOURAL RESPONSES IN CAPTIVE MALE ASIAN ELEPHANTS (*Elephas maximus*) TO SPECIFIED STRESSORS"** 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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CERTIFICATE

Certified that the thesis entitled **"BEHAVIOURAL RESPONSES IN CAPTIVE MALE ASIAN ELEPHANTS (*Elephas maximus*) TO SPECIFIED STRESSORS"** is a record of research work done independently by **Dr. G. Vivek**, under my guidance and supervision and that it has not previously formed the basis for the award of any degree, fellowship or associateship to him.

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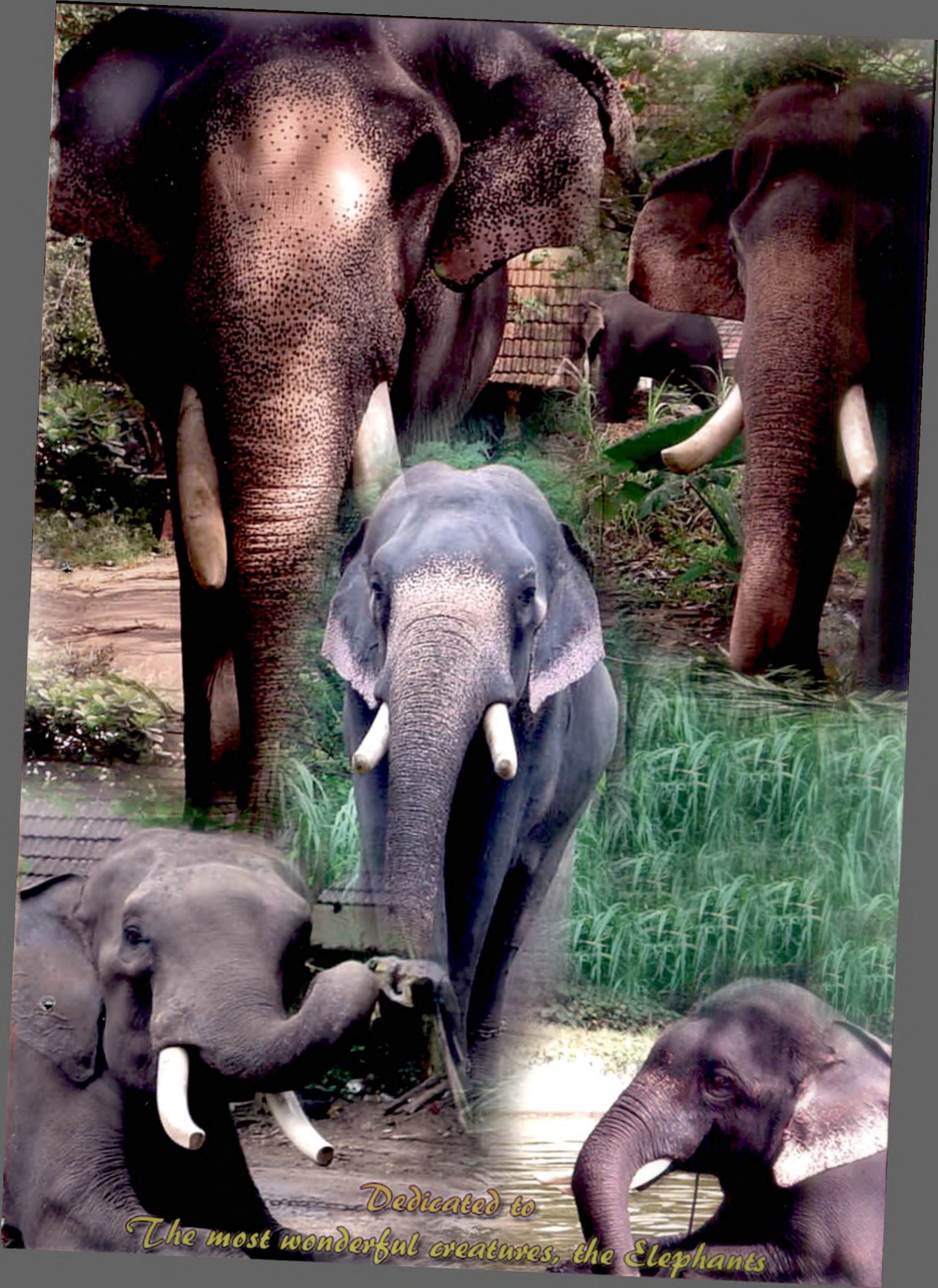
Dedicated to
The most wonderful creatures, the Elephants

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Introduction

1. INTRODUCTION

An object of worship, a beast of burden, gentle in captivity, dangerous in the wild, the companion of mahouts, an envoy of peace, loved, feared and hated, the elephant has a glorious and an infamous association with man in Asia. For its sheer contrast and splendor, this association is unequalled by any other interaction between animal and man in the world.

Mankind has harnessed the strength and intelligence of elephants for hundreds of years. One of the earliest references to the domestication of the Asian elephant dates back to 1200 BC in Egypt (Douglas-Hamilton and Douglas-Hamilton, 1975).

Elephant symbolizes the Indian ethos. It has been very closely associated with the religion, myths, history and cultural heritage of the country for centuries. The Asian elephant is unique, being the only species of wild animal that, after a few month of training by man, behaves towards him with patience and understanding. It participates in man's religious, cultural, and social activities, lending dignity and grace as each occasion demands, as though it had learnt all about it in the jungle (Santiapillai and Jackson, 1990).

Population of Asian elephants in India is estimated at 28,000. India has by far the largest remaining population of the Asian elephant in the world. According to recent estimates there are about 3400-3600 domesticated elephants in India, and Kerala state has about 612 domesticated elephants (Bist, 2002). Asian elephant is highly endangered species throughout its range including India. Presently, large-scale development programmes have destroyed the elephant's habitat, and there is no future for the elephant except in a few protected areas and one has to consider seriously the possibility that the Asian elephants will be known as mainly a domesticated animal in the twenty first century (Daniel, 1998).

Asian elephants (*Elephas maximus*) are threatened by habitat reduction, human interference, and population fragmentation. Assessing the welfare status of captive and free living elephants is a growing area of concern as ethical questions are raised about the psychological well-being of animals maintained for research,

economic, education, or entertainment purposes. These factors emphasize the need to assess the adequacy of environmental and husbandry conditions for optimal behaviour, welfare, and health in these species (Brown *et al., 1995*).

The conservation of the elephant in Asia is of international significance. The Asian elephant is thus a "flagship" species, whose conservation will ensure the maintenance of biological diversity and ecological integrity on a large scale. One-third of the endangered Asian elephant (*Elephas maximus*) population worldwide is living in captivity and captive management is becoming increasingly important to ensure the survival of this species (Schwarzenberger *et al.*, 2001).

The care and management of elephants in captivity has become an intricate combination of art and science and, the importance of furthering behavioural research for the benefits of elephants is obvious. Behavioural studies are of great importance in increasing our understanding and appreciation of animals. In addition to providing knowledge about the diversity and complexity of behaviour, such studies also provide information crucial to improvements in the welfare of animals maintained in captivity.

The assessment of an animal's general health and condition may provide a short-term, preliminary assessment of the levels of stress in an animal. However, it is insufficient to assess welfare as a whole. Physiological findings should therefore be correlated with behavioural observations while assessing the stress and welfare status of an animal. In advancing the care and management of elephants, behavioural studies cannot stand alone, the benefits of regular hormone assessment is also important to learn more, and to enhance the well being of the elephants (Schulte, 1999).

There have been meager studies to investigate the possibility of using non-invasive methods to assess adrenocortical activity in elephants (Dathe *et al.*, 1992; Brown *et al.*, 1995). Methods to identify and measure faecal and urinary glucocorticoid metabolites have been successfully used in a number of domestic livestock and some wild species. Knowledge about scientific management of captive Asian elephants is meager. Welfare of tamed/captive elephants is a priority requirement today for the protection of the species. Captive elephants can serve as a

platform for hands-on research directly benefiting the conservation of wild elephants (Lair, 1997). A synthesis of approach to measure health, physiological studies and behaviour is essential to quantify the welfare and ensure the well-being of these animals. In such a context, this study aimed to,

1. Ensure the long-term survival and to evolve management plans to optimize conditions for behaviour and health, thus ensuring the well being of elephants maintained in the captivity.
2. Identify behavioural patterns indicative of stress in captive elephants and relate their occurrence to environmental stressors.
3. Measure glucocorticoid metabolites in elephant faeces and urine, and investigate the methods biological relevance including its potential use as a tool in assessing welfare.
4. Assess the suitability of measuring faecal and urinary cortisol metabolites as a non-invasive method to monitor stress in elephants.
5. Relate the fecal and urinary glucocorticoid metabolite concentrations with the behavioural aspects and environmental/imposed stressors.

Review of Literature

2. REVIEW OF LITERATURE

The environment stimuli that lead to an imbalance of homeostasis are "stressors", and the corresponding defense reactions of organisms are "stress responses". Stressful stimuli can induce adrenocorticotrophic hormone (ACTH) release. This in turn, increases the synthesis and secretion of cortisol.

Hormones influence behaviour and are also influenced by behaviour (Creel *et al.*, 1996). Monitoring their levels can therefore provide insights into the mechanistic aspects of behaviour. Glucocorticoid release in response to an acute stressor is rapid and transient, leading to behavioural changes and the changes are an integral component of the stress response. Rapid behavioural transitions induced by glucocorticoids should be regarded as key components in the stress response.

Hormones, such as glucocorticoids, released into the blood stream during stressful events, can potentially provide a quantitative index of stress. Samples derived from handled animals are problematic because stress may alter blood hormonal levels (Koren *et al.*, 2002). Urine levels of cortisol or cortisol metabolites can remain elevated for some hours. Measuring glucocorticoids in faeces is a new, non-invasive approach to measure stress. Faecal samplings offers the possibility of gathering information on cortisol concentrations in wild animals and prove to be an extremely useful management tool for identifying circumstances that causes stress (Merl *et al.*, 2000; Von der Ohe and Servheen, 2002).

2.1 ANIMAL WELFARE, STRESS AND BEHAVIOUR

Behavioural characteristics are useful indices of welfare because they are often the first or the only apparent indications of stress. Also they can be assessed by direct observation. They also provide insight into probable sources of stressors and an animal's strategies for coping with them (Kilgour, 1978)

The term 'welfare' refers to the state of an individual in relation to its environment, as regards its attempts to cope with its environment (Broom, 1986a; Fraser and Broom, 1990). Both failure to cope with the environment and difficulty in coping are indicators of poor welfare (Gonyou, 1986).

It is well established that physiological and psychogenic stress can have a disruptive effect on the behaviour of mammals. Assessing an animal's stress physiology is therefore essential for the understanding and improvement of animal well-being (Clark *et al.*, 1997a,b) as well as to monitor the impacts of socially mediated and environmental stressors on behaviour, health and reproduction in primates (Breazile, 1987; Bahr *et al.*, 2000).

When individual vertebrates lose grip on their life conditions stress symptoms appear and their welfare becomes problematic. The highly dynamic patterns of the homeostatic mechanism activated during stress makes it difficult to deduce any simple relationship between stress and welfare. In captivity, the originally highly adaptive physiological and behavioural mechanisms may no longer be functional in regulatory capacity leading to a decrease in welfare (Wiepkema and Koolhaas, 1993). Impaired growth, body damage, disease, adrenal activity, and behaviour anomalies are some of the indicators of poor welfare (Broom, 1991).

The issue of animal welfare has been most closely associated with ethology. Applied ethology, the scientific study of behaviour, ethology and welfare, has been used to gather appropriate information involved in studying the behaviour of animals that are managed in captivity (Gonyou, 1994).

Swanson (1994) indicated that, farm animal well-being can been assessed by physiological measures, various health parameters and animal behaviour, and implies that a multi-disciplinary approach is needed to assess and define animal welfare.

Veasey *et al.* (1996) on comparing the behaviour of zoo housed animals with wild conspecifics as a welfare tool indicated that, to make an assessment of welfare, behavioural comparisons with wild animals should be used in conjunction with other techniques in the investigation of the welfare of captive animals.

Inappropriate social conditions are known to lead to social stress and adrenal activation in some captive species, which, in turn, may compromise the immune system, behaviour and reproduction (Mendoza *et al.*, 2000).

Determining whether environmental conditions facilitate or compromise coping is key to ensuring animal well-being and the major focuses of zoo animal

welfare science will be the measurement of well-being based on combination of assessment criteria related to biological functioning, natural behaviour, and subjective emotional states. An assay system capable of assessing adrenal activity (measurement of faecal corticosteroids) as a potential indicator of stress in the black and white rhinoceros is increasingly being used to investigate the effects of various stressors on animal physiology (Brown *et al.*, 2001).

2.2 STRESSORS

Stressors stimulate the secretion of adrenocorticotrophic hormone (ACTH) from the pituitary and that of corticosteroids from the adrenal cortex. During chronic stress conditions the adrenal cortex allows the hypersecretion of glucocrticoids even without the increment of ACTH (Aguilera *et al.*, 1996) and trigger behavioural changes (Korte *et al.*, 1993; Bahr *et al.*, 1998).

Wiepkema and Koolhaas (1993) indicated that different stressors evoke their own and often specific stress responses (behaviourally and physiologically). Chronic stress of the organism is characterized by the fact that some stressors have a long lasting after-effect resulting from the permanent presence of the stressor itself. In many cases of chronic stress the symptoms involved are often restricted to certain times of the day.

In response to stressors, the central nervous system of mammals evokes physiological responses that ultimately result in activation of the hypothalamo-pituitary-adrenocortical (HPA) axis and the sympatho-adrenal axis and subsequently the secretion of cortisol. Usually the degree of increase in cortisol or corticosterone in plasma, is regarded as indicative of stressful conditions i.e., greater concentration interpreted as more stressful (Minton, 1994).

Welfare in more approachable fashion can be defined as focusing upon objective measures of health or stress, or observable changes in behaviour, while tending to downplay the mental state of the animals (Rushen, 1996).

Sapolsky *et al.* (1997) indicated that, cortisol is critical for an individual's adaptation to acute physical stressors; thus, a number of studies have been focused on the factors that mediate glucocorticoid secretion.

Garaï (1997) defined that behavioural stressors could be social stress such as bullying, new environment, overcrowded conditions, insecurity, etc. which elicit a specific reaction by the animal and termed "stress related behaviours" for any behaviours which appear to be a reaction to a motivational state within the functional contexts of insecurity and fear, although physical causes of stress, such as drought, adverse weather conditions, nutritional deficiencies and their influence on the general behaviour which cannot be totally overlooked.

Orchinik (1998) used the term "stressor" to refer to any threat to homeostasis and the term "stress response" to refer to nonspecific physiological responses to a stressor and indicated that there are marked species and individual differences in what constitutes a stressor.

Collier *et al.* (2000) in a study on the physiological responses to stress, indicates that stress is an external event or condition which results in a strain on a physiological system and major stressors for animals are associated with animal handling, housing and feeding practices.

Morrow *et al.* (2000) in their study on urinary corticosteroids as an indicator of stress in dairy cattle, indicates that, metabolic excretion profile of cortisol to accurately reflect a physiological event, corresponding changes in circulating hormones and the respective excreted metabolites must be established and verified, and with respect to cortisol, this can be achieved by artificially elevating circulatory plasma cortisol concentrations by administering ACTH or by inducing physiological and/or psychological stress (e.g., transport, restraint, translocation, confinement).

If the stressor is consistent and at regular intervals, physiological adaptation of the glucocrticoid response to the threat may occur, leading to lower observed concentrations of glucocorticoids than would be expected and on the other hand, irregular, novel, or severe stressors, such as those often associated with human disturbances, might be an added source of variation of glucocorticoid levels (Von der Ohe and Servheen, 2002).

2.2.1 Disease

The link between altered behaviour and the diseased state is so close that there is a tendency for obviously abnormal behaviour to be used as the identification for particular disease (Fraser and Broom, 1990).

Increased urinary corticosteroids have been recorded in response to psychological stressors (Carlstead *et al.*, 1992; Rebdo, 1993) and disease such as pasteurellosis in rocky mountain big horn sheep (Miller *et al.*, 1991b).

Carlstead *et al.* (1999) stated that "stress has been implicated as an underlying causal factor for many of the disease syndromes identified in rhinoceros maintained in captivity" and that research to identify the stressors should be undertaken.

Stress has been shown to lead to increased susceptibility to infectious diseases and induce lymphocytopenia due in part to alterations of immune function (Von der Ohe and Servheen, 2002).

2.2.2 Musth and Aggression

Musth is an annual period of heightened aggressive and sexual behaviour accompanied by the broadcast of odoriferous, behaviourally influential messages from secretions of the temporal gland (Rasmussen *et al.*, 2002), urine dribbling, elevated androgen secretion for periods of a few weeks to several months, increased bouts of travel and greater interest in females (Poole and Moss, 1981; Hall-Martin, 1987). Most healthy male elephants experience musth once a year, but males of poor condition may not exhibit musth for up to 4 years. Males in good condition are more likely to enter musth (Chandrasekaran *et al.*, 1992) and it is affected by nutritional status, because a common method of suppressing musth is water and food deprivation (Jainudeen *et al.*, 1972; Cooper *et al.*, 1990; Schmidt, 1993; Scot, 2002).

Aggressive interactions produce prolonged physiological changes in individuals and ethological field studies describe the time course of the agonistic behaviour and showed that the stress response can be detected in the faeces by determining faecal glucocorticoid metabolites (Wallner *et al.*, 1999).

Schulte (2000) opined that adult males still experience musth in captivity and remain at the same location for long periods and rarely interact with other males. Thus, they can neither establish dominance out of musth nor supercede the hierarchy when in musth. Furthermore, males do not compete for selection by a female. Hence, the desirable qualities of musth in captive males differ from wild males and the apparent benefits of musth may be lost.

Schwarzenberger *et al.* (2000) in a study on Asian and African elephants showed that there is an elevation in the level of faecal cortisol metabolites during musth in males of both species and indicated the advantage of faecal steroids, which even in the most aggressive bulls can be used.

Scott (2002) indicates that several behaviours increased in frequency and duration in musth males. When the males were in musth, they tended to explore more of their yard area and spend more time investigating the area compared to when they were not in musth. There also was an increase in ear flapping during musth. She also observed that the mature male elephants generally performed fewer than three flehmen responses per hour to urine of any type, whereas the pubertal male generally performed more than four flehmen responses per hour.

2.2.3 Season

Cavigelli (1999) in a study on behavioural patterns associated with faecal cortisol levels in ring-tailed lemurs reviewed that, faecal cortisol measures can be used to assess seasonal and individual differences in adrenal activity, and that this measure could provide a means for quantifying physiological stress in free-ranging animals.

Das *et al.* (1999) indicated that, in stressful conditions, the number of physiological and behavioural responses varies in intensity and duration in relation to the environmental factors.

Kotrschal *et al.* (2000) by ACTH challenge and "social stimulation" studied the effects of physiological and social challenges in different seasons on faecal corticosterone in male domestic geese and found that faecal corticosterone metabolites increased approximately 40 fold to 150 fold after ACTH challenge.

Mader (2000) opined that, changes in facilities and management strategies do not need to eliminate environmental stress completely, but rather minimize the severity of the environmental challenge and aid the animal in adapting to it.

Foley *et al.* (2001) in their study on non-invasive stress and reproductive measures of social and ecological pressures in free-ranging African elephants showed that faecal cortisol metabolite concentrations were significantly higher in the dry season and suggested that measures of glucocorticoid metabolites in the faeces could provide indices of physiological stress and are ideally suited for monitoring long-term effects of social disruption poaching and a variety of other management concerns.

Millspaugh *et al.* (2001) quantified faecal glucocorticoid concentrations among free-ranging elk by subherd, sex, and season and determined their relationship to various environmental conditions. Faecal glucocorticoid measures were least in winter (Mean = 17.41 ng/g for bull subherds and Mean = 18.9 ng/g for cow subherds) and increased to peak concentrations in summer (Mean = 33.6 ng/g for bull subherds and Mean = 34.21 ng/g for cow subherds).

Pravosudov *et al.* (2002) indicated that baseline levels of corticosterone and the magnitude of adrenocortical response to acute stress are known to vary seasonally. Their results suggest that factors other than photoperiod are also responsible for the observed seasonal changes in baseline levels of corticosterone, whereas photoperiod is directly involved in regulation of adrenocortical stress response.

Huber *et al.* (2003) investigated the effects of season on glucocorticoid production on a non-invasive basis by measuring concentrations of cortisol metabolites in feces of undisturbed red deer and indicated that season exerts a significant effect on faecal glucocorticoid excretion, and faecal glucocorticoid excretion varied seasonally with a peak during December and January.

2.2.4 Restraint and Handling

Friend *et al.* (1985) in a study on the comparison of four methods of calf confinement found that basal cortisol and adrenal response to exogenous

adrenocorticotrophic hormone (ACTH challenge) were elevated due to confinement and isolation stress.

Pain is a sensation that is itself extremely aversive. Perceived pain is a part of the state of an individual; such a perception may have other effects on the state, but the greater the pain, the poorer the welfare. Careful measurement of behaviour can give a good indication of the extent of pain (Broom, 1991).

Lay *et al.* (1992) indicated that, the physiological indicators of stress: plasma cortisol concentrations and heart rate were elevated on handling and restraint of crossbred cattle for branding.

Rebdo (1993) in a study to assess the effect of tethering on environmentally induced stereotypies and urine cortisol concentrations in heifers, concluded that, tethering leads to high urine levels of cortisol and increased levels of stereotypies.

Tame animals that are accustomed to frequent handling and close contact with people are usually less stressed by restraint and handling than animals that seldom see people. Cattle that have been trained and habituated to a handling procedure may be completely calm and have baseline cortisol and heart rate measurements during handling and restraint (Grandin, 1997).

Rushen (1996) opined that the nature of the relationship between the animals and handlers can have a major impact on animal welfare. Many of the handling treatments that animals find aversive are performed by humans, and are likely that animals learn to associate aversive handling with people.

While measuring stress in wildlife to better understand the physiological impact of potential management implications on human-induced stress found that the glucocorticoid concentrations increase as a result of restraint in carnivores (Moe and Bakken, 1997; Ogburn *et al.*, 1998; Beerda *et al.*, 1999).

Animal pain, an aversive sensory and emotional experience, changes the animal's physiology and behaviour to reduce or avoid damage and to reduce the likelihood of recurrence and to promote recovery. Unnecessary pain occurs when the physiological and behavioural responses to it are unsuccessful in alleviating it (Molony and Kent, 1997).

Merl *et al.* (2000) demonstrated an elevated level of cortisol metabolites in faecal samples due to pain after castration or colic in horses.

Measuring urinary and faecal steroid excretion has the advantage of providing an integrated index of cortisol production over the period preceding sampling with the data being less influenced by the natural episodic nature of hormone secretions or animal handling (Morrow *et al.*, 2000).

Schwarzenberger *et al.* (2000) in a study on Asian and African elephants indicated that training as a management practice showed an elevation in the level of faecal cortisol metabolites.

Dehnhard *et al.* (2001) in an experiment on roe deer used capture and veterinary treatment of animals as experimental stresses which resulted in a 7.5-fold increase in faecal metabolites (1200 ± 880 ng/g) compared to baseline concentrations. They concluded that administration of long acting tranquilizer (LAT) reduced stress response significantly, resulting in only a four-fold increase of faecal metabolites (650 ± 280 ng/g).

While evaluating repeated handling and the use of a mask on the cortisol concentration of Brahman cattle during restraint in a squeeze chute Andrade (2001) found that handling was reflected by a decrease in cortisol concentration. Cortisol values tended to be higher when animals were restrained without a mask.

Lensink *et al.* (2001) investigated the influence of handling on calves' behavioural and physiological (cortisol, heart rate) reactions and concluded that both heart rate and blood cortisol level increased significantly after handling.

McAfee *et al.* (2002) in a study on stereotypic behaviour in horses indicated that social isolation has been associated with weaving behaviour in stabled horses.

Behavioural and physiological changes are used for assessment of pain in animals. Molony *et al.* (2002) indicated that acute pain following surgical procedures in lambs showed significant changes in the physiology and behaviour.

2.2.5 Travel

Parrot *et al.* (1997) in a study on stress hormone response of sheep to road transport following two different loading procedures, showed that they exhibited

increase in plasma cortisol concentrations, and concluded that, transport appeared to be more stressful than loading.

Palme *et al.* (2000) in their study on transport stress in cattle, found that, in control group, the concentration of faecal 11,17-DOA were within the range throughout the whole experiment, whereas in transport group, the maximal concentrations occurred 12 hours after the start of the experiment.

Palme and Möstl (2000) opined that, transportation and painful situations such as colics in horses were well reflected in faecal 11,17-DOA concentrations and thus, the established method of faecal glucocorticoid measurement should be a valuable non-invasive tool in a variety of research tools such as animal welfare and in ethological studies.

Schwarzenberger *et al.* (2000) in a study on Asian and African elephants to evaluate various stressors associated with management practices showed that training and transport are conditions, during which elevated cortisol levels are expected and indicated that 11,17-dioxoandrostane assay is suitable to measure faecal cortisol metabolite concentrations in both species.

Denhard *et al.* (2001) in an experiment on roe deer used transportation of animals as experimental stresses which resulted in a 7.5-fold increase in faecal metabolites (1200 ± 880 ng/g) compared to baseline concentrations.

Lensink *et al.* (2001) while investigating the influence of transport on calves' behavioural and physiological (cortisol, heart rate) reactions, concluded that both heart rate and blood cortisol level increased significantly after transport and returned to baseline within two hour after transport.

Toscano *et al.* (2001) in a study on the transportation of circus elephants found that, the exercise and stimulation the elephants receive during the walk is very beneficial for the overall health of the animals and, when proper care is taken, the transport of circus elephants does not compromise the well-being of the animals even during relatively extreme environmental conditions.

2.3 NON-INVASIVE STRESS MONITORING

Millspaugh *et al.* (2002) indicated that non-invasive methods based on urinary cortisol and faecal cortisol metabolites (Miller *et al.*, 1991a; Saltz and White, 1991; Creel *et al.*, 1997; Wasser *et al.*, 2000) to quantify stress that offer advantages over traditional invasive methods.

Glucocorticoid concentrations in blood are widely used as a parameter of stress in animals (Morton *et al.*, 1995). However, blood collection itself causes stress. Since glucocorticoids are metabolized in the liver and the metabolites are excreted via the urine and the faeces (Brownie, 1992), collection of urine and faecal samples is easy and feedback free as there is no need to handle the animal and concluded that measuring faecal 11,17-DOA should be a valuable tool for non-invasive monitoring of disturbances in hares (Palme and Möstl 1997; Cooper, 1998; Teskey-Gerstl *et al.*, 2000).

Wallner *et al.* (1999) reviewed the difficulties in collecting plasma samples and the effects of the collection procedure itself, especially under free or semi free conditions, have led investigators to seek non-invasive approaches. The procedures for analyzing various steroids in urine and in faeces are well established in primates (Stavisky *et al.*, 1995; Whitten and Russel, 1996).

Anticipatory reactions and responses to the collection of samples that are not specific for stress may bias real stress responses and lead to misinterpretation. Non-invasive sampling procedures may minimally disturb the subject under study and, thus, partly prevent anticipatory or stress nonspecific responses (Beerda *et al.*, 1996).

Faecal hormone assays are used in a variety of disciplines to examine stress in felids (Graham and Brown, 1996), cheetah (Jurke *et al.*, 1997), rodents (Harper and Austad, 2000), elk deer (Millspaugh *et al.*, 2001) and in a diverse array of non-domestic and avian species (Wasser *et al.*, 2000). Furthermore, faecal glucocorticoid assays reflect an integrated level of circulating glucocorticoid over time, rather than a point sample independent of short-term fluctuations, and therefore might provide a more accurate assessment of long-term stress (Goyman *et al.*, 1999; Millspaugh *et al.*, 2001).

Although considerable interspecies differences exist in the amounts of steroid metabolites excreted via faeces or urine (Palme *et al.,* 1996) and the metabolites formed (Palme and Möstl, 1997; Möstl *et al.,* 1999; Teskey-Gerstl *et al.,* 2000), alternative non -invasive methods for the determination of faecal cortisol metabolites utilizing a newly established enzyme immunoassay measuring 11,17-dioxoandrostanes, a group of cortisol metabolites have been developed as an integrated measure of adrenal activity (Palme *et al.,* 1999).

Wasser *et al.* (1996) developed and validated a non-invasive method to quantify faecal steroids as a tool for monitoring long-term ovarian activity in free-ranging African elephants and found that majority of steroid metabolites was excreted in faeces than in urine. Intra sample variation in faecal hormone concentrations was substantially reduced by extracting well mixed faecal powder from freeze-dried samples, taken from the central or premixed portion of the wet sample.

Bahr *et al.* (2000) indicated that there is difficulties in collecting blood and the negative effects of the procedure itself pose serious limitations to this approach, particularly for free-ranging and group-living animals in which repeated capture and restraint are not feasible (Sapolsky and Share, 1998). As a non-invasive alternative, analysis of the predominant glucocorticoid metabolites excreted in urine and/or faecal samples would therefore offer a considerable advantage for assessing adrenal function in both captive and free-ranging primates.

Faecal samples are non-invasive, easy to collect year-round, and provide an integrated measure of all cortisol secretion during the previous one to two days (Harper and Austad, 2000).

Stead *et al.* (2000) concluded that measuring 11,17-DOA is a valuable tool for non-invasive monitoring of adrenocortical activity in African elephants which could help optimize the capture, transport and husbandry (Graham *et al.,* 2001) and be useful in investigating stress in free-ranging situations.

Schatz and Palme (2001) indicated the advantage of feedback-free sampling methods such as corticoid determination in urine, saliva and faeces as a non-invasive approach for monitoring physiological responses to stress (housing, handling and

human-animal interaction), since blood sampling is impractical in zoo and wild animals and the sampling of blood can itself induce a stress response.

Faecal steroid analyses are becoming more popular among both field and laboratory scientists, because of the benefits associated with sampling procedures that do not require restraint, anesthesia, and blood collection include less risk to both subject and the investigator, as well as the potential to obtain endocrine profiles that do not reflect the influence of stress, and the collection of physiological data permits the evaluation of the relationship between endocrine status and behaviour (Stavisky *et al.*, 2001; Möstl and Palme, 2002).

2.4 BEHAVIOUR STUDIES

The method of direct observation plays a unique role in the behavioural sciences. It is at once the necessary link between laboratory research and "real-world" behaviour, and the bane of our aspirations for more accurate, more objective information about behaviour (Altmann, 1974).

Because of adaptation to a new environment, loss of comfort and well-being may be acute or chronic. To avoid injuries in the new environment, animals may modify or reduce the frequency of behaviour (Gonyou, 1986).

Animal behaviour has been defined as the interaction of an animal with its environment. An abnormal behaviour might help an individual to cope, but it is still an indicator that the animal's welfare is poorer than that of another animal that does not have as much difficulty in coping (Fraser and Broom, 1990; Broom, 1991). In the use of behavioural criteria in research assessing an animal's perception and response to the environment, careful consideration must be given to design, analysis, and interpretation (Gonyou, 1991).

Behavioural studies can be an important element to successful captive management of a species and can be even more critical for developing effective conservation strategies for many species (Stevens and Hutchins, 1993; Kleiman, 1994).

Brown (2000) in a study on reproductive endocrine monitoring of elephants for assisting captive management, indicated that, continued studies should focus on

developing behavioural indices of stress for elephants, used in conjunction with physiological measures (e.g., corticoid analyses) to develop mitigating actions to improve environment or management conditions. Similar views were indicated by Wallner *et al.* (1999) in a study to document stress in barbary macaques.

Stead *et al.* (2000) in their preliminary investigations into the application of the non-invasive techniques to assess welfare in captive African elephants showed good correlation between behavioural observations, environmental stressors and faecal glucocorticoid metabolite concentrations.

Foley *et al.* (2001) in their study on free-ranging African elephants, correlated cortisol metabolite concentrations with behavioural measure of dominance by conducting 20-min focal samples on each adult female in three groups, recording all feeding behaviour and aggressive interactions initiated and received on a monthly basis over a period of three years and showed that the cortisol metabolite concentrations were highest among lowest-ranking animals.

Behavioural observations are also a type of "assay" that is used to quantify animal biological responses. As with physiological measurements, methods of behavioural observation should be validated and selected based on the objectives of the particular study (Mitlöhner *et al.*, 2001).

Schmid *et al.* (2001) studied the behavioural reactions and change in cortisol levels on introduction of foreign female Asian elephants into an existing group and found that, all elephants showed behavioural changes to the process of introduction. Two of the elephants showed an increase in stereotypes and one a reduction in stereotypes. Cortisol measurement in urine showed that, one elephant having positive correlation between locomotion and cortisol levels, another showing positive correlation between stereotypies and cortisol levels.

Stavisky *et al.* (2001) in a study on female cynomolgus monkeys, conducted regular behaviour observations and revealed that, serum cortisol values were significantly correlated with behaviour across the sampling periods.

Stavisky *et al.* (2001) in a behavioural study on group-housed macaques, made behavioural observations in half hour samples composed of three 10-min adlibitum samples to determine rates of aggressive, submissive, and affliative

interactions, and ten instantaneous scan samples to determine the percentage of time animals spent engaged in a set of behaviours including eating/drinking, grooming, stereotypic behaviours, resting, alertness, locomotion, and play, following procedures previously described. Data were recorded by hand by a single observer over the entire collection period.

Lynch *et al.* (2002) in their study on individual and seasonal variation in faecal cortisol levels of wild male tufted capuchin monkeys correlated behavioural data with the change in faecal steroid metabolites.

Wielebnowski *et al.* (2002) in their study on impact of social management on adrenal and behavioural activity in the cheetah compared behavioural frequencies among all females before and after pair separation and revealed that grooming activity decreased, whereas pacing and flehmen increased when females were housed in pairs.

2.4.1 Behaviour in Captivity

Captivity frees elephants from the selective pressures imposed by predation and for the most part parasitism; and greatly limits the range of new habitats to explore in the course of development which may modify adaptive behaviour and impact the degree and type of social behaviour exhibited. Yet, captive and wild elephants demonstrate many of the same behaviours. Hence, behavioural studies on captive animals may be relevant to wild populations and the detail of data available to researchers on individual animals held in captivity is typically much greater than for wild individuals, although the relevance to the wild must be considered when making generalizations (Adams and Berg, 1980; Schulte, 2000).

Kleiman (1992) indicated the importance of behaviour research in zoos because; the results from classical descriptive behavioural research and basic behavioural research has enormous potential to contribute positively to the science of animal management, animal welfare, long-term breeding programs, conservation biology, and the advancement of scientific theory.

Behavior is an acknowledged indicator of well-being. When we strive to provide optimal care for captive animals by providing for their physical and

psychological well-being, reducing or eliminating abnormal behavior is an issue that cannot be overlooked or shortchanged (Laule, 1993).

Environmental enrichment involves increasing the stimulus value of the home environment by increasing its complexity and can generally be considered an improvement in the biological functioning of captive raised animals (Newberry, 1995).

Elephants in captivity have been restrained by chaining and as a result of chaining, species-typical behaviours, such as foraging for food, social interactions, play behaviour, and locomotion are greatly restricted. This inability to perform species-typical behaviours may contribute to the development of stereotypies in elephants (Wiedenmayer and Tanner, 1995).

Carlstead *et al.* (1999) in a study on behaviour of black rhinoceros in United States zoos concluded that behavioural assessments of individual animals are a valuable tool for investigating potential causes of well-being in zoo animals.

Friend (1999) in a study on circus elephants to characterize changes in their behaviour prior to receiving feed, water, and being removed from the picket line for performances found that the elephants spent an average of 33 ± 1.2 per cent of observations eating, and stereotypic behaviour increased in the 15-min period immediately prior to water, performances and hay, when compared to their frequency during the three preceeding15-min periods indicating 'anticipation' of water and performances, and a lack of substrate to manipulate or eat in regards to hay.

Schulte (2000) opined that, understanding of captive elephant behaviour is important not only for the maintenance of a viable captive populations, but also to enhance the survival and management of wild populations, and large scale studies on the captive population should lead to new discoveries and offer fresh solutions to the problems confronting the management of elephants.

Elephants in captivity are easier to observe than their wild counterparts because they are acclimated to the daily presence of human visitors and staff and the design of viewing areas typically allows for unobtrusive viewing of behaviours (Scott, 2002).

2.4.2 Ethogram Development

In general, animals that can perform normal behaviour are more likely to achieve better welfare than those that cannot. If normal behaviour should be accommodated, then we should know what normal behaviour is for each species. This is the basis of studies that develop ethograms of our livestock (Banks, 1982).

Development of complete ethograms for our agricultural species and increased knowledge of the effects of various degrees and methods of confinement on their behaviour are required to develop optimal environments for livestock production (Harstock, 1982).

Dellmeier *et al.* (1985) in their study compared the behaviour of calves confined by four different methods to determine if differences existed among treatment groups with respect to behaviours displayed or their frequency and duration, and to contribute towards development of a complete ethogram for cattle.

Ethogram, which is a catalogue of descriptions of the discrete, species typical behaviour patterns that form the basic behavioural repertoire of the species (Lehner, 1987).

In selecting measures for a particular behavioural study it is useful to know the array of behaviours which the animal is capable of showing. A largely complete description of such an 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).

Weller and Bennet (2001) in a study on the behaviour of captive ocelots, studied the behaviours derived from an ethogram and collected data by observing focal animals for 15 minutes continuously recording all occurrences. They recorded duration for nine of the behaviours (walk, run, climb, jump, pace, stand, sit, lie awake and lie asleep) and frequencies for concurrent behaviours (investigate, sniff, spray, scrape, sharpen claws and cheek rub).

Scott (2002) in a study on captive male elephants, closely observed male elephant behaviour in captivity, and designed a detailed ethogram to distinguish different behaviours, thus characterizing behaviours indicative of, and unique to,

musth and, expanded this behavioural context into anatomical and physiological aspects of musth.

2.5 BEHAVIOURAL MEASURES

Stereotypies are typically observed in situations of conflict or frustration and claimed to be an adaptive function. Once stereotypies are established, the situation is different and any environmental disturbance, in particular the presence of a human observer, can result in a higher incidence of the prevalent stereotypies (Dantzer, 1986).

Stereotypies can be defined as unvarying, repetitive behaviours that have no obvious goal or function. They are associated with sub-optimal housing or management systems (Mason, 1991) and are thought to develop due to the inability of the animals to control their environment (Fraser and Broom, 1990; Broom, 1991; Carlstead, 1996). They tend to increase in frequency in elephants before being fed, being watered, and performing and suggest that this may indicate an "anticipation" of performances (Friend, 1999).

Stereotypies such as weaving are a cause of concern about the welfare of the performer as they tend to occur in animals faced with environmental restrictions which pose insoluble problems relating to the control of their environment (Mason, 1991).

Typically, wild elephants will spend between 16 and 20 hours a day feeding, with peaks in the morning, afternoon, and around midnight (Vinod and Cheeran, 1997). Free ranging elephants will sleep four to five hours a day, either in a recumbent or standing position, usually between 0200 and 0700 hours and again during hottest part of the day (McKnight, 1995).

A study of four European circuses indicated that, although 19 of 29 elephants displayed stereotypies both when chained and penned, stereotypies occurred less frequently when the elephants were penned (Schmidt, 1995).

Brockett *et al.* (1999) in a study on the nocturnal behaviour of unrestrained African elephants observed the elephants for 172 h for the behaviours of eating, lying, standing, and walking by all occurrence scan sampling technique and found

out that all the behaviours were performed for equal no of observation time. They concluded that increased activity is performed in the evening hours and the number of unrestrained hours be increased for elephants in the evening hours.

Fischbacher and Schmid (1999) studied the stereotypic behaviour of spectacled bear by every minute scan sampling technique and reported the frequency, i.e., the number of scans an animal was performing a certain behaviour during 1 hour of observation, was reported as an independent datum. Other behavioural categories observed were resting, walking, eating, manipulating feeding devices and interacting socially.

Friend (1999) in a study on circus elephants used the 1995 and 1996 observation data sets to calculate relative time budgets for the periods of observation. The raw counts were converted to 'percentages of observation'. The activity of the focal elephants used in the time budgets was summarized to determine the incidence of the various behaviours during the period when the elephants were not subject to disturbance by the activity of people.

Friend and Parker (1999) in a study on behaviour of picketed circus elephants found that, stereotypic weaving and head bobbing were most prominent and were affected by older age and food availability. The increase in stereotypic behaviour observed prior to water and performances probably reflect elements of arousal and motivation.

Gruber *et al.* (2000) in their study to determine the effect of penning and chaining on circus elephant behaviour found that the elephants engaged in more comfort, ingestion and locomotion activities and fewer social interactions and stereotypies when penned than when chained. Variation in stereotypic activity was related to age, with younger elephants more likely to show stereotypic activity than older elephants.

Mitlöhner *et al.* (2001) opined that continuous observations are an accurate method for behavioural measurement, and made focal animal sampling (using continuous sampling of individuals) of feeding, lying, standing, drinking and walking behaviours in heifers and indicated that one heifer was representative of the entire pen.

Zimmermann *et al.* (2001) studied the behaviours of the offspring of intensely managed animals in extensively managed conditions. Locomotion, standing, lying, grazing and drinking behaviour of the animals was recorded. The behavioural data were converted to percentage of observations. The percentages of time spent grazing in spring, summer, autumn and winter were 48.85, 40.96, 48.10 and 31.71 per cent, respectively. In winter the percentage of time spent standing was highest (43.06 per cent) and the percentage of time spent lying was lowest (10.42 per cent) and concluded that cattle habituated to tethered conditions are very capable of adapting to life outdoors.

Mills and Davenport (2002) in a study on the control of stereotypic weaving behaviour in horses, found that all test subjects were seen to weave and engage in at least one other behaviour described as stereotypies.

Scott (2002) indicated that, there was an increase in the frequency of stereotypic behaviours in the captive male elephants during musth.

2.6 GLUCOCORTICOIDS AND STRESS

Glucocorticoid action has been implicated in impaired growth due to skeletal muscle and lymphoid atrophy, as well as depressed body weight due to both reduction of food intake and a decrease in food utilization efficiency (Klasing, 1985).

The assessment of levels of cortisol is an established way to investigate stress since these hormones reflect the activity of two important stress responsive axes: the hypothalamic-pituitary-adrenal (HPA) axis and the sympatho-adrenal-medullary (SAM) axis. Objective physiological measures of animal responses to environmental stressors often are used for evaluation of the presence and degree of stress, and of subsequent adaptations that may occur (Hahn *et al.,* 1990; Downing and Bryden, 2000).

Routine monitoring of gonadal and adrenal steroids in captive animal populations provides valuable information regarding their physiology, health status and the impact of environmental factors and managemental practices. Therefore, noninvasive faecal steroid assays have been developed to monitor concentrations of

excreted steroids in captive species (Lasley and Kirkpatrick, 1991; Schwarzenberger *et al.*, 1996; Terio *et al.*, 2002).

Sapolsky (1993) in relation to the adrenocortical axis found that in many primate species, cortisol may be secreted as a direct response to an environmental or social stress. It may also be considered an enhancer for rapid physical response, as it mobilizes circulating glucose to provide readily available energy for muscular activity.

Faecal steroid analysis has become a more widely appreciated field technique. Applications have expanded to consider steroid concentrations in relation to stress (Creel *et al.*, 1996; Boinski *et al.*, 1999) and aggression in males (Brockman *et al.*, 1998; Lynch *et al.*, 2002).

Adaptation to stressful events is associated with an increased production and secretion of glucocorticoids from the adrenal cortex into the blood (Clark *et al., 1997a,b*). In contrast to the concentration in blood, which is influenced by the stressful sampling itself, and which reflects a momentary situation, the collection of urine or faeces allows the monitoring of previous stressful conditions, without needing to handle the animals. The determination of metabolites in urine samples is hampered by the difficulty of obtaining the samples and faecal samples can easily be collected from the ground, and the contents have been mixed in the gut, thus providing an integrated measure of a few hours. As cortisol itself is absent in the faeces, an enzyme immunoassay measuring 11,17-dioxoandrostanes (11,17-DOA) has been established to measure faecal cortisol metabolites in farm animals for non-invasive monitoring of adrenocortical activity (Palme and Möstl, 1997; Hofer and East, 1998; Palme *et al.*, 1999; Palme *et al.*, 2000; Teskey-Gerstl *et al.*, 2000).

Wallner *et al.* (1999) showed that immunoreactive cortisol and 11-oxoetiocholanolone found in the faeces can indeed determine the endocrine response of the adrenal gland after a social stressor in Barbary macaques.

Glucocorticoids induce a variety of behavioral and physiological changes that presumably help the animal respond appropriately to the situation. Understanding seasonal modulation of glucocorticoid release has far-reaching

importance for both the physiology of the stress response and the short-term survival of individual animals (Romero, 2002).

Glucocorticoids have a number of effects on the body that can be vital for coping with a stressor. Glucocorticoid hormones released during stressful events initiate numerous physiological reactions that enable the animal to cope with the stressor. For this reason, measurement of these hormones has the potential to yield important information regarding animal well-being (Von der Ohe and Servheen, 2002).

Wielebnowski *et al.* (2002) in their study on impact of social management on reproductive, adrenal and behavioural activity in the cheetahs, tested the utility of faecal corticoid excretion as an index of stress in intensely managed, controlled environment and indicated that faecal corticoid monitoring has been useful for assessing adrenal activity of animals in captivity.

2.6.1 Glucocorticoid Measurement

Stress responses involve the release of glucocorticoids, such as cortisol, from the adrenal glands, and a change in cortisol concentrations commonly is used as a physiological indicator of stress (Moberg, 1985).

Cortisol can be measured in blood, but because secretion is dynamic, analysis of excreted corticoid metabolites often is a better indicator of overall adrenal activity. Assays for cortisol or its metabolites in elephants have been validated for urine (Brown and Lehnhardt, 1995; Brown *et al.*, 1995), faeces (Wasser *et al.*, 1996) and saliva (Dathe *et al.*, 1992).

The pulsatile secretion of cortisol, which peaks in the early morning hours and falls in the evening hours, requires blood collection to occur before 11.00 AM in order to most accurately reflect cortisol activity. Because faecal corticosteroids represent the sum of cortisol secretion and metabolism over many hours, it has been assumed that the diurnal pattern of cortisol secretion will be dampened in faecal extracts, making it possible to collect samples at any time of day (Stavisky *et al.*, 2001).

Glucocorticoid concentrations are typically measured by radioimmunoassay or enzyme immunoassay, and the specific procedure used for sample preparation, hormone extraction, and assay vary from study to study (Von der Ohe and Servheen, 2002).

2.7 EXTRACTION AND STORAGE FOR FAECAL AND URINE CORTISOL

Brown *et al.* (1995) developed a method for preserving urine to allow storing unfrozen samples for determination of cortisol and creatinine. Urine samples were stored in tubes containing absolute ethanol (10 per cent), sodium azide (0.1 per cent) or distilled water (control), and frozen at -25^{0}C. They found that there were no differences between ethanol and sodium azide in their ability to preserve cortisol or creatinine activity.

Bamberg *et al.* (2001) in a study on determination of corticosterone metabolites in faecal samples of male rats for possible non-invasive monitoring of stressful situations; extracted the faeces with 0.5 g of homogenized faeces suspended in 5ml methanol (80 per cent) and centrifuged (Palme *et al.,* 1996), and the supernatant diluted 1:10 with assay buffer for the estimation of radioactivity.

Wasser *et al.* (1996) in a study on excretory fate of estradiol and progesterone in African elephants, extracted faeces by boiling 0.18-0.2 g lyophilized faecal powder (20 min) in 10 ml of 90 per cent ethanol: distilled water (Wasser *et al.,* 2000). The supernatant was recovered following centrifugation (500g for 15 min). The pellet was resuspended in 5 ml 90 per cent ethanol, vortexed (1 min), and recentrifuged. Both ethanol supernatants were combined, dried completely, and redissolved in 1 ml methanol. Samples were diluted in assay buffer before RIA analysis.

Schwarzenberger *et al.* (2000) in their study on non-invasive monitoring of reproductive function in the Indian rhinoceros, used the elaborated method of faecal extraction (Brown *et al.,* 1994; Palme and Möstl, 1997; Goymann *et al.,* 1999; Möstl *et al.,* 1999) which yielded a cleaner and more concentrated extract by mixing 0.5 g of wet faeces with 0.5 ml of water and 4 ml of methanol and aliquots were analyzed by enzyme immunoassay. Similar method of extraction was followed by Merl *et al.*

(2000), Dehnhard *et al.* (2001) and Schatz and Palme (2001) in their studies on horses, roe deer and felines respectively.

Khan *et al.* (2002) while evaluating the storage of faecal samples of baboons for steroid analysis indicated that, storage of fecal samples is of critical concern because fecal bacteria metabolize fecal steroids within hours after deposit. Ethanol is often used as a preservative for fecal samples stored for several hours at room temperature. Subsequently, ethanol either alone or with sodium azide has been used as a preservative for short-term ambient temperature storage of faecal samples (Wasser *et al.,* 1997) or long-term storage for five months (Cavigelli, 1999) to 3.5 years (Curtis *et al.,* 2000).

Terio *et al.* (2002) suggested that storage of faecal samples at room temperature in ethanol is the best alternative to freezing for analysis of steroid hormone concentrations. In contrast, drying of samples in either a solar or conventional oven resulted in variations in the measured concentrations of steroid hormones. Similar methods were suggested by Wasser *et al.* (1998) and Lynch *et al.* (2003) on their studies on primates.

Möstl *et al.* (1999) had indicated that there was a significant increase in the concentration of measured cortisol metabolites in bovine, equine and porcine faeces after storage for one hour, four hours and 24 hours, respectively at room temperature. In frozen samples this effect was diminished after thawing samples at 40^0C.

Faecal samples from juvenile African elephants were collected within 30 min of defaecation and stored at -20^{0}C. Oven dried (100^{0}C) sample was powdered and mixed thoroughly and a 0.5 g sub sample was mixed with 10 ml of 80 per cent ethanol, shaken for 30 min and centrifuged at 1700 x g for 15 min. One milliliter of the supernatant was drawn off and stored at -20^{0}C until EIA analysis (Stead *et al.,* 2000). Similar methods of faecal extraction were used by Oates *et al.* (2002) and Stead-Richardson *et al.* (2002) in their study on short-beaked echidna and chuditch respectively.

Millspaugh *et al.* (2001) and Millspaugh *et al.* (2002) reported on stress responses in elk and white-tailed deer respectively, placed dried faeces (0.2 g) in a

test tube with 2 ml of 90 per cent methanol and vortexed faeces at high speed in a multi-tube vortexer for 30 minutes. Samples were then centrifuged at 2200 rpm for 20 min, and the supernatant was saved and stored at -20^{0}C until assayed.

Möstl and Palme (2002) advised that, the faecal samples for steroid analysis in stress monitoring studies should be frozen immediately to avoid changes after defaecation or bacterial enzymes inactivated by heating or drying.

2.8 FAECAL AND URINE CORTISOL CONCENTRATIONS

Stress measurement need to be sufficiently benign so as not to influence the endocrine response they are intended to measure. Measurement of plasma cortisol concentration provides a single point-in-time estimate. Furthermore, because cortisol is secreted in a pulsatile fashion in many species, accurate assessment of circulating activity can only be made by collecting multiple blood samples over time. Alternatively, measuring urinary and faecal steroid excretion has the advantage of providing an integrated index of cortisol production over the period preceding sampling (Brown *et al.,* 1995; Morrow *et al.,* 2000; Stead *et al.,* 2000).

Brown *et al.* (1995) studied the importance of urinary cortisol analysis for monitoring adrenal activity in elephants by ACTH challenge. One African (*Loxodonta africana*) and one Asian elephant (*Elephas maximus*) were given three injections of ACTH (1.25 mg) at two hour intervals and the serum and urinary cortisol levels were found out. Six hours after ACTH injection, urinary cortisol concentrations showed twenty fold and forty fold increase in African and Asian elephant, respectively.

Beerda *et al.* (1996) using a model of insulin-induced hypoglycemia, reported on stress-induced responses in urinary cortisol relative to cortisol responses in plasma in dogs. The Cortisol^{NE}/creatinine concentrations in undisturbed conditions were 11.9 ± 1.2^{12} and the levels after insulin treatment were found to be 28.2 ± 7.1^{6}. They concluded that urine collection is a valid non-invasive method to establish stress-induced cortisol responses in the dogs.

Palme *et al.* (1996) made a comparative study to gain more information about the excretion of steroid hormones and establish non-invasive steroid

monitoring procedures in farm animals by intravenous ^{14}C-steroid hormone infusion studies in sheep, ponies and pigs. Excretion of cortisol metabolites were 72 per cent, 59 per cent and 93 per cent in urine, and 28 per cent, 41 per cent and 7 per cent in faeces of sheep, ponies and pigs, respectively.

According to Stead *et al.* (2000) a number of glucocorticoid metabolites were detected in elephant faeces using HPLC analysis, and a group of them may be collectively described as 11,17-dioxoandrostanes(11,17-DOA). Similarly, an enzyme immunoassay was established for measuring trace amounts of cortisol or corticosterone immunoreactive metabolites in faecal samples of domestic ruminants (Palme and Möstl, 1997; Palme *et al., 1999*) and in faecal samples of other species, such as roe deer, horses, pigs, okapis and rhinos (Möstl and Palme, 1998).

Palme *et al.* (1999) in their study on measurement of faecal cortisol metabolites in ruminants tested the biological relevance of enzyme immunoassay for measuring glucocorticoids non-invasively by determining faecal cortisol metabolites. After ACTH administration maximal concentrations of faecal cortisol metabolites were reached about 10 hours after blood cortisol peaked, and peak concentrations in cattle and sheep ranged from 745 to 1954 and from 445 to 3622 nmol/kg faeces, respectively.

Palme *et al.* (1999) and Möstl *et al.* (1999) reported that, 11,17-DOA concentrations in faecal samples of ponies showed basal levels in the range of 2.3-35.3 nmol/kg faeces, in pigs between 6.9-19.1 nmol/kg faeces, in cattle faeces between 34-445 nmol/kg, and in sheep faeces between 93-1031 nmol/kg, respectively.

Terio *et al.* (1999) in a study on faecal cortisol metabolite analysis for non-invasive monitoring of adrenocortical function in the cheetah exposed the cheetahs to a variety of situations anticipated to increase cortisol secretion. Increased faecal corticoid metabolite excretion was observed 24-72 hr after exposure to these exogenous stressors.

Bahr *et al.* (2000) in their radiometabolism study compared the data on the time course, route, and characteristic of excreted [^{3}H] cortisol metabolites in three non-human primates: the common marmoset, the long-tailed macaque, and the

chimpanzee by injecting a low dose (40-100 μCi) ^{3}H-labelled cortisol intravenously and the excreta collected over a five-day period post-injection. In all the three species, peak radioactivity in faeces was detected between eight and 24 hours in the marmoset and after 22 and 26 hours in the macaque and chimpanzee, respectively.

Brown (2000) in a study on reproductive endocrine monitoring of elephants for assisting captive management, indicated how hormone monitoring can be used to better manage elephants and monitored steroid activity non-invasively through the measurement of excreted steroid metabolites in urine and faeces. Concentrations of urinary cortisol throughout the day in three Asian elephants averaged 15.2 ± 1.6 ng/mg Cr from 0800 to 1000 hours, 11.5 ± 4.4 ng/mg Cr from 1000 to 1200 hours, 10.4 ± 0.7 ng/mg Cr from 1200 to 1400 hours, and 6.5 ± 0.9 ng/mg Cr from 1400 to 1600 hours.

Merl *et al.* (2000) in their study measured the concentration of 11,17-dioxoandrostanes using enzyme immunoassay in faecal samples of horses experiencing painful episodes. Before castration, median concentrations of 10.5 nmol/kg faeces were measured and on days one and two after castrations, median values increased upto 26.2 and 50.0 nmol/kg faeces, respectively, and decreased thereafter. In animals with colic, all horses excreted more than 33 nmol/kg faeces for various periods.

Stead *et al.* (2000) injected four juvenile African elephants with 2.15 mg synthetic adrenocorticotrophic hormone and the concentrations of serum cortisol and faecal cortisol metabolites were determined using enzyme immunoassay. Serum cortisol increased between 4 fold and 7fold, reaching highest recorded values (526-652 nmol/L) after 2 hours. Basal values of faecal 11,17-DOA and corticosterone equivalents ranged from 21 to 168 nmol/kg and 33 to 133 nmol/kg, respectively. ACTH-induced peaks were between 572-1104 per cent (11,17-DOA) and 160-353 per cent (corticosterone) higher than basal values. These peak concentrations occurred 20-25.5 h after injection.

Teskey-Gerstl *et al.* (2000) measured the levels of faecal 11,17-DOA concentrations of hares following stress and found that levels in individual hares increased by upto fivefold on one of the following two days.

Bamberg *et al.* (2001) in their study on excretion of corticosteroid metabolites in urine and faeces of rats by ACTH challenge test found that, there was a marked diurnal variation in the amount of corticosteroid metabolites measured in the faecal samples.

Brown *et al.* (2001) in an adrenocorticotrophic hormone challenge in four black rhinoceros males demonstrated that the clearance rate of corticoid metabolites into faeces was ~24 hours. Faecal corticoid concentrations did not differ between males and females, but overall means were higher in the black (41.8 ± 3.1 ng/g) than in the white (31.2 ± 1.7 ng/g) rhinoceros, with this difference being more prominent in males.

Dehnhard *et al.* (2001) indicated the physiological relevance of faecal cortisol metabolites to adrenocortical activity in roe deer with an adrenocorticotrophic hormone challenge test and found that cortisol metabolite concentrations exceeded pre-treatment levels (31-78 ng/g) up to 13 fold (183-944 ng/g) within 8-23 h.

Pucher *et al.* (2001) in their study on three semi-wild ranging male Asian elephants, found that average cortisol levels were 72, 190 and 113 ng/g faeces. Cortisol levels could be tentatively related to the character of the bulls, the extraordinary calm bulls had the lowest, and the aggressive bull the highest values.

Schaltz and Palme (2001) in their radio-infusion experiment found out that, following injection of ACTH, detected an increase in faecal cortisol metabolites above baseline values using a corticosterone radioimmunoassay.

Millspaugh *et al.* (2002) used adrenocorticotrophic hormone challenges to determine faecal glucocorticoid metabolite concentrations for monitoring adrenal activity in white-tailed deer and found that there was a pronounced rise in glucocorticoid excretion, which decreased to pre-treatment concentrations at about 30 hours and remained relatively stable thereafter.

Möstl and Palme (2002) indicated that faecal concentrations of cortisol metabolites reflect the total amount excreted and enable large scale longitudinal studies, so that an animal acts as its own control, reducing the inter-animal variation.

The results by Millspaugh *et al.* (2002) suggested seasonal difference in lag times of glucocorticoid secretion in white-tailed deer. So, when trying to determine the effect of a particular stressor on an animal, it is important to know how long it takes to detect changes in stress hormones in faeces.

Wielebnowski *et al.* (2002) in their study on impact of social management on adrenal and behavioural activity in the cheetah found that average baseline concentrations of faecal corticoids ranged from 28.9 ± 1.74 to 59.2 ± 1.8 ng/g, whereas peak values ranged from 104.2 ± 2.6 to 289 ± 3.1 ng/g.

2.9 URINE CREATININE

Brown *et al.* (1995) in their study on urinary cortisol analysis for monitoring adrenal activity in elephants indexed urinary cortisol concentrations against creatinine concentrations to account for fluctuations in fluid intake.

Beerda *et al.* (1996) in their study on stress-induced responses in dogs provided strong support for the urinary C^{NE} / C (non-extracted urinary cortisol / creatinine) ratio as valid non-invasive indicator of acute stress in dogs.

Bahr *et al.* (2000) in their radiometabolism study on primates indexed urinary radioactivity against creatinine to adjust variations in water content and expressed as dpm/mg Cr. Creatinine measurement was performed by microtiter plate analysis.

Urine requires additional processing steps in the analysis of creatinine to account for variation in fluid intake, and hydrolysis of steroid conjugates to liberate assayable free steroids (Brown, 2000).

2.10 TEMPERATURE AND RESPIRATION

Asian elephant's normal body temperature is 95^0F to 98.6^0F, though several ranges have been reported, most notably differing in the minimum value for the range. Fluctuations in body temperature greater than 3.0^0F indicate the animals were experiencing a significant heat load. Actual temperatures greater than 100^0F indicate considerable fever or thermoregulatory difficulties (Benedict, 1936).

Asian elephants in their native habitat experiences temperatures ranging from below 32^0F to 104^0F (Sukumar, 1989).

Schmidt (1990) indicated that the respiration rate of the elephant varies with excitement. Calm elephants take an average of four to six breaths/minute, whereas excited elephants may take more than 15 breaths/minute. The average body temperature of normal Asian elephant ranges from 36 to 37°C.

Animals can tolerate a range of environmental temperatures over which their bodies can safely maintain a required body temperature and this range is dependant on species, prior exposure, and type of housing. Environmental temperatures outside this range can disrupt thermoregulation processes and cause the animal to suffer (Randall, 1993; Hahn, 1999)

Nair (1996) in a study on Asian elephants indicated that the initial respiratory rate and rectal temperature were 8.83±0.30 and 36.13±0.04^{0}C, respectively and it increased to 17.16±0.30 and 37.25±0.06^{0}C, respectively after three hours of continuous work and after one hour of rest, the values dropped significantly.

Parrot *et al.* (1997) in a study on the responses of heart rate to road transport in sheep showed that heart rate increased during loading and transport.

Das *et al.* (1999) in a study on buffaloes showed that there was an increase in pulmonary frequency and rectal temperature in relation to the environmental factors, where the ambient temperature and solar radiation was maximum.

Silanikove (2000) indicates that high ambient temperatures, high direct and indirect solar radiation, humidity and environmental stressing factors impose strain on animals and welfare can be measured by discrete measures such as changes in hormone level, body temperature, respiratory rates, heart rates and behaviour.

Andrade (2001) evaluated the repeated handling on heart rates and respiratory rates of Brahman cattle during restraint in a squeeze chute and found that respiratory rates (2.2 breaths/min) and heart rates (5 beats/min) showed a decline than normal while the animals were restrained.

Prasad (2001) in a study on draught efficiency of elephants in timber mills indicated a diurnal variation in rectal temperature between 96.1^{0}F to 97^{0}F and the respiratory rate varied between eight per minute to 11 per minute in working elephants.

Toscano *et al.* (2001) studied the effect of environmental conditions and body temperature of circus elephants transported during relatively high and low temperature conditions. Recorded body temperature for hot weather and cold weather trials averaged 97.2^0F and 97.3^0F, respectively and the body temperature ranged from 95^0F to 98.6^0F. Elevation in body temperature of two to three degrees is not considered a problem in elephants or most other mammals. The lack of larger increase in body temperature in the elephants clearly indicated that they could easily cope with daily environmental temperatures that approach 100^0F in the shade as well as travel related activity.

Materials and Methods

3. MATERIALS AND METHODS

3.1 EXPERIMENTAL MATERIALS

3.1.1 Location and Animals

Guruvayoor ($10^0$35' N, $76^0$0' E) is a temple town which is located in the coastal belt of Thrissur district of Kerala state in India. The elephant camp at Punnathur Kotta of the Guruvayoor Devaswom is world renowned for the largest numbers of captive elephants (63) herd which are kept in solitary confinement. The present study was conducted from July 2002 to July 2003, utilizing the male elephants over 15 years old of the camp.

3.1.2 Experimental Groups

The animals were dived into seven groups based on the type of stress as given below.

Group I - Animals in the stage of musth

Group II - Animals that are transported or traveled.

Group III - Animals under circumstances of any disease or injury.

Group IV - Animals that are extensively restrained and controlled.

Group V - Animals not under shelter during summer season.

Group VI - Animals not under shelter during monsoon.

Group VII - Animals not coming under any of the category mentioned above, considered as control.

3.2 BEHAVIOURAL DATA COLLECTION

A preliminary study was conducted to familiarize with the subjects and their behaviour for developing methods of measurement, and compiling a detailed ethogram. The behavioural observations were made from 0800 to 1600 hours during the period of observation to coincide with the daily routines of the camp. Behavioural observations were called "events" when the behavioural pattern is of short duration and "states" when the behavioural pattern is of relatively long

duration (Martin and Bateson, 1993). A total of thirty two hours of observation in each group with eight hours of observation on each animal was recorded. A total of 224 hours of behavioural observation were made for the present study.

3.2.1 Recording Method

3.2.1.1 Focal Sampling/Focal-Animal Sampling

Focal-Animal sampling is a sampling method in which, all occurrences of specified actions of an individual, or specified group of individuals, are recorded during each sample period, and, a record is made of the length of each sample 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 amount of time and recording all instances of its behaviour – usually for several different categories of behaviour (Martin and Bateson, 1993).

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

3.2.2 Ethogram

Ethogram, which is a catalogue of descriptions of the discrete, species typical behaviour patterns that form the basic behavioural repertoire of the species (Lehner, 1987) for the present study was developed from the diverse array of behavioural descriptions for elephants provided by various authors (Poole *et al.,* 1997; Langbauer Jr, 2000; Scott, 2002). The ethogram for the present study is given in Table 1.

A method developed by Scott (2002) to score the temporal gland secretion (TGS) and urine dribbling during musth was modified for use in the present study, since both these behaviours were present during most/or all of the time when the study was conducted on musth elephants. The score chart was given in Table 2.

3.2.3 Behavioural Data Collection Chart

The behavioural data collection sheet developed by Stead (2000) for measuring the behaviour of African elephants is modified for the present study including relevant and more important behavioural measures as per Table 1. A detailed data collection sheet is given in the Appendix 1 and 2.

Table 1. Ethogram

S.No	Behaviour	Description
1	*Feed/Drink*	*Ingestion of food and consumption of water.* Often this will involve the individual gathering food or water with its trunk and lifting it into its mouth
2	Dusting/Mudding	Individual lifts quantities of sand, dust, mud, or dirt and tosses onto own body with trunk. Includes water bathing, when individual douses own body with water
3	Spray water/saliva	Regurgitation of water/ sucking saliva from stomach and sprayed over body
4	Blowing body	Individual directs trunk towards underside or genitals and blows on to those areas. Does not any substrate such as dirt or sand in this case
5	Manipulate object or environment	Individual moves, pushes, tosses or picks up objects within its environment; such as grass, rocks, sticks, dirt or stand with foot, mouth or trunk, object is not eaten
6	Trunk up	Lifting trunk up above head towards a stimulus
7	Eyes wide	Whites of eye visible
8	Face check	Touching the mouth, face, or temporal gland with the trunk. It is self directed and a sign of apprehension

S.No	Behaviour	Description
9	Flehmen	The flattened surface of the tip of the trunk (the nostrils) is brought to the mouth and the trunk tip is placed into contact with the roof of the mouth. The elephant invariably has brought its trunk in contact, or in near contact, with something before flehming.
10	Digging	Individual is removing substrate with foot or tusk
11	Head high	Head held high so that tusks are horizontal to ground
12	Tail high	Tail held horizontally
13	Object throw at keeper	Individual throws faeces, rocks or other objects towards keeper
14	Object throw at public	Individual throws faeces, rocks or other objects towards public/ visitors
15	Aggression to trainer	Individual makes aggressive move or actions towards trainer
16	Penis jerking/Masturbation	Erect S-shaped penis repeatedly hitting ventrum
17	Rubbing/grooming	Individual rubs body against a substrate such as pillars, trees or a wall.
18	Chain pulling – trunk/tusk	Pulling the chain tied to the limbs with trunk/tusk
19	Chain pulling - foot	Pulling the chain tied to the limbs with foot
20	Ear flapping	Flapping both ears for communication or thermoregulation
21	Ear waving	Only right or left ear flaps back-and-forth while other ear remains still
22	Vocalization	Bawl, roar, chirp, growl and other
23	Low rumble	Low frequency quiet sound
24	Loud rumble	Low frequency loud sound

S.No	Behaviour	Description
25	Cry	Loud high pitched through mouth
26	Snort	Brief push of air through trunk
27	Squeak	Short high pitched sound through trunk
28	Soft trumpet	Medium pitched sound through trunk
29	Loud Trumpet	High pitched loud sound through trunk
30	Head shake	Vigorous shaking of head – ears flap against the body
31	Head weave	Head rocking from side to side repetitively
32	Head toss	A repeated figure-eight movement of the head and trunk
33	Body sway	Swaying the body from side to side repetitively
34	Trunk curl	Curling of the trunk tightly inwards
35	Trunk twitch	Twitching the trunk back and forth
36	Trunk bounce	Bouncing or dragging the distal portion of the trunk on the ground may or may not be accompanied by boom vocalization
37	Trunk twiddle	Twirling trunk around in circles, no object held
38	TGS	Temporal gland secretion - seen as a moist patch on side of face
39	Urine dribble	Continual dribbling of urine from the sheathed penis of a musth bull. Gives back leg a black, shiny appearance. Also has chemical communication function.
40	Work with trainer	Individual is actively engaged with trainer. May or may not be correctly following commands.
41	Self-directed behaviour	Individual touches, rubs or grooms own body. May use mouth, trunk, or appendages to contact any area of the body.

S.No	Behaviour	Description
42	Other solitary	Individual is engaging in a behaviour not covered in the ethogram descriptions above

Table 2. Ethogram – Score chart

Visible temporal gland secretion scale for male elephants

Score	Temporal Gland Secretion (TGS)
0	None visible
1	At eye level
2	Below eye level but not in line with mouth
3	In line with corner of the mouth
4	All the way to the chin

Visible urine dribbling scale for male elephants

Score	Urine dribbling
0	None visible (normal urination, legs dry)
1	Several wide streams of urine
2	Urine expelled with a force equal to that during normal urination
3	Occasional drops of urine, and/or legs wet from recent dribbling of urine
4	Steady series of drops with no pause between drops or urine discharged in a thin stream with no breaks

3.2.4 Combined Behaviour Variables

All the behavioural measures observed for this study were grouped into combined variables as shown below for the ease of statistical analysis.

1. Maintenance behaviour – feeding/drinking, dusting/mudding, spray water/saliva and blow body.

2. Exploratory/investigatory activities – manipulate object/environment, trunk up, eyes wide, face check and flehmen.
3. Aggressive/agonistic activities – digging, head high, tail high, object throw at keeper, object throw at public and aggression to trainer.
4. Self-directed behaviour – masturbation and grooming.
5. Comfort activities – chain pulling by trunk/tusk, chain pulling by feet, ear flap and ear wave.
6. Vocalizations – low rumble, loud rumble and cry/snort/squeak.
7. Trumpeting activities – soft trumpet and loud trumpet.
8. Stereotypic behaviour – head shake, head weave, head toss, body sway and trunk curl/twitch/bounce/twiddle.
9. Other behaviours – temporal gland secretion, urine dribbling, work with trainer and other solitary behaviours.

3.3 FAECAL SAMPLES

3.3.1 Collection and Storage

Faecal samples were collected randomly from four elephants in each group. Samples were collected within 30 minutes of defaecation. A single faecal bolus was hand mixed and from that a handful of faeces was placed into a plastic freezer bag with the addition of 90% ethyl alcohol as a preservative and stored at -20 ^{0}C till the preparation for extraction and RIA analysis (Stead *et al.,* 2000).

3.3.2 Extraction for Radioimmunoassay

Frozen faecal samples were dried in conventional oven (~50°C until dry; i.e., weight no longer changes). Each sample was powdered and mixed thoroughly. A 0.2 g sub-sample was mixed with 5 ml of 90 per cent 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 keep from boiling dry. The extract was brought up to pre-boil levels with 90 per cent ethanol and centrifuged at 1500 rotations per cent minute (rpm) for 20 minutes. The extracts were poured off into another storage vial. To the remaining faecal pellets, 90 per cent ethanol was added again and vortexed for 30

seconds and centrifuged at 1500 rpm for 15 minutes. The first and the second extract were combined. The extract was dried down and reconstituted in one ml of methanol, and vortexed for brief period. The methanol extracts were stored at -20 ^{0}C until RIA analysis (Brown *et al.*, 2002).

3.4 URINE SAMPLES

3.4.1 Collection and Storage

Urine samples were collected randomly from four elephants in each group as "free-catch" (i.e., mid-stream) using a clean plastic container. More than one sample collected from the same animal on the same day was pooled to form a single sample before storage. Temporary preservation of urine samples was done by adding 10 per cent ethanol and/or 0.1 per cent sodium azide. The samples were centrifuged at 2500 rpm for 15 minutes to remove the dirt and other cellular contaminants and 5 ml of the supernatant was stored at -20 ^{0}C until analysis (Brown *et al.*, 2002).

3.5 RADIOIMMUNOASSAY OF URINARY AND FAECAL CORTISOL

Urinary and faecal cortisol concentrations were determined using Clinical Assays™ GammaCoat™ [^{125}I] Cortisol radioimmunoassay kit (DiaSorin, Stillwater, Minnesota, U.S.A).

Principle

The unknown concentration of a hormone, H, 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 the presence of H. This competition reaction is easily calibrated by constructing a standard curve indicating how much H* binds to the antibody as a function of [H]

Procedure

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

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

b. 10 μL of samples (urine samples and extracted faecal samples) were added to each tube.

2. 1.0 mL of tracer-buffer reagent was added to each tube and mixed on a vortex mixer set at low speed.

3. The tubes were incubated for 45 minutes in a 37 ± 2 ^{0}C water bath.

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

5. The tubes were counted in a Gamma Counter (1480 WIZARD™ Automatic Gamma Counter) for one minute with windows suitably suited for iodine-125.

6. A standard curve was plotted with counts 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 CREATININE ESTIMATION

Urinary creatinine concentration was estimated by the method described by Brown *et al.*, (2002) using enzyme immunoassay technique.

Principle

Creatinine reacts with picric acid under alkaline conditions to form a yellow-coloured product that can be measured at 490 nm. The yellow-colour intensity will be directly proportional to creatinine concentration.

Reagents and standards required

1. Standard values used are 100, 50, 25, 12.5, and 6.25 μg/mL.

2. 0.1 g of anhydrous creatinine was added to 100 mL of 0.01N HCL (1 mg/mL stock)

3. The stock was diluted by adding 10 mL of the 1 mg/mL stock to 90 mL of 0.02N HCL making a 100 μg/mL concentration (top standard).

4. Top standard was serially diluted 2-fold using 200 μL stock plus 200 μL distilled water and mixed well.

5. Distilled water was used as 0 (blank).

6. Standards of 0.75N NaOH and 0.4N picric acid.

Samples

1. Urine samples were diluted at the rate of 1:50 in distilled water

Procedure

1. 50 μL per well of standard and sample in duplicate were added to the plate (Tarsons Elisa plate – Cat # 931196)

2. 50 μL per well of tap water were added to all wells using Eppendorf micropipette.

3. 50 μL per well of 0.75N NaOH were added to all wells using Eppendorf micropipette.

4. 50 μL per well of 0.4N picric acid using Eppendorf micropipette.

5. The plate was shaken briefly to mix and incubated at room temperature for 30 minutes.

6. The plate was read on MULTISKAN MS EIA plate reader at 490 nm.

7. The plate values were used to interpolate the unknown values from the standard curve.

3.7 OBSERVATION OF OTHER PHYSIOLOGICAL PARAMETERS

Physiological changes in terms of respiration rate and rectal temperature were recorded thrice in the morning, noon and evening on all the days whenever the behavioural observations were made.

3.7.1 Respiratory Rate

Respiratory rate was ascertained by holding lower portion of the trunk and placing the palm of the hand close to the nostrils of the elephants and counting by feeling the number of times the elephant expires air through the nostrils. To countercheck the count, the movement of the flank region was also noted and correlated.

3.7.2 Rectal Temperature

Rectal temperature of the elephants was taken by inserting full length of a clinical thermometer in the rectum and holding its bulb against the rectal mucous membrane for about one minute.

3.8 STATISTICAL ANALYSES

Urinary and faecal cortisol values were analyzed using "t" test to determine the significance of correlation, and ANOVA single factor test was used to compare the values within the group and between the groups. All the behavioural parameters were grouped into combined variables and a General Linear Model (GLM) was fitted to the data and Fishers test was used to test the differences between groups. Since the GLM used to process the data is not normally distributed and the test found no significant differences, Kruskal-Wallis test for non-parametric statistical analysis was used to find the statistical differences in behaviour within and between groups (Snedecor and Cochran, 1985; Siegel and Castellan, 1988).

Results

4. RESULTS

4.1 BEHAVIOURAL OBSERVATIONS

Behavioural observations were observed using focal animal sampling technique using a behaviour data collection chart. A total of thirty two hours of observation in each group with eight hours of observation on each animal was recorded. Behavioural observations of the individual animals recorded during each hour of observation were combined to show an average occurrence of events (number of events occurred in an hour) or average time spent (minutes per hour) performing each behavioural measure. The behaviours observed in this study were grouped into maintenance behaviour, exploratory behaviour, aggressive/agonistic behaviour, self-directed behaviour, comfort behaviour, vocalization, trumpeting, stereotypic behaviour, temporal gland secretion, urine dribbling, work with trainer and other solitary behaviours. All the behaviours were observed by focal animal sampling technique except the temporal gland secretion and urine dribbling, which was observed using score chart. Fisher's test was used to test for differences within groups. The Kruskal-Wallis test and Newman-Keul's test for non-parametric data analysis was used for those variables which found no significant difference from Fisher's test.

4.1.1 Maintenance Behaviours

Mean time (minutes per hour) spent performing maintenance activities such as feeding/drinking (Plate. 1) and mean number (number of activities per hour) of time performing dusting/mudding, spraying water/saliva and blow body activities were shown in Table 3 and Fig. 1.

4.1.1.1 Feeding/Drinking Activity

The subjects spent maximum time feeding in monsoon (group VI) with a mean time of 41.40 ± 4.28 minutes/hour, while the subjects in musth spent least time for feeding with a mean time of 16.28 ± 3.48 minutes/hour. There was significant differences ($P<0.05$) between groups as (Table 3).

A

B

C

D

E

F

A,B - Feeding C,D - Drinking E- Dusting F - Water splashing

Plate. 1. Maintenance behaviours

Table 3. Mean number of maintenance actions (n) and mean time spent performing maintenance activities (minutes) per hour with standard deviation

	Feeding/Drinking (min)	Dusting/mudding (n)	Spray water/saliva (n)	Blow body (n)
Group I	16.28 ± 3.48^{c}	1.25 ± 0.67^{c}	0.15 ± 0.09^{c}	0.25 ± 0.05^{cd}
Group II	26.35 ± 11.19^{abc}	3.01 ± 0.83^{b}	0.48 ± 0.34^{bc}	0.51 ± 0.23^{bc}
Group III	22.68 ± 6.88^{bc}	0.79 ± 0.31^{c}	1.01 ± 0.44^{bc}	0.67 ± 0.24^{b}
Group IV	30.41 ± 5.88^{abc}	0.98 ± 0.46^{c}	0.54 ± 0.27^{bc}	0.21 ± 0.14^{d}
Group V	35.26 ± 2.78^{ab}	4.25 ± 1.46^{a}	2.18 ± 0.72^{a}	1.08 ± 0.19^{a}
Group VI	41.40 ± 4.28^{a}	1.25 ± 0.31^{c}	1.20 ± 0.49^{b}	0.56 ± 0.08^{bc}
Group VII	31.58 ± 11.65^{abc}	1.94 ± 1.05^{bc}	0.94 ± 0.19^{bc}	0.67 ± 0.19^{b}

Means ± standard deviation in rows bearing different superscripts for each parameter differ significantly ($P<0.05$)

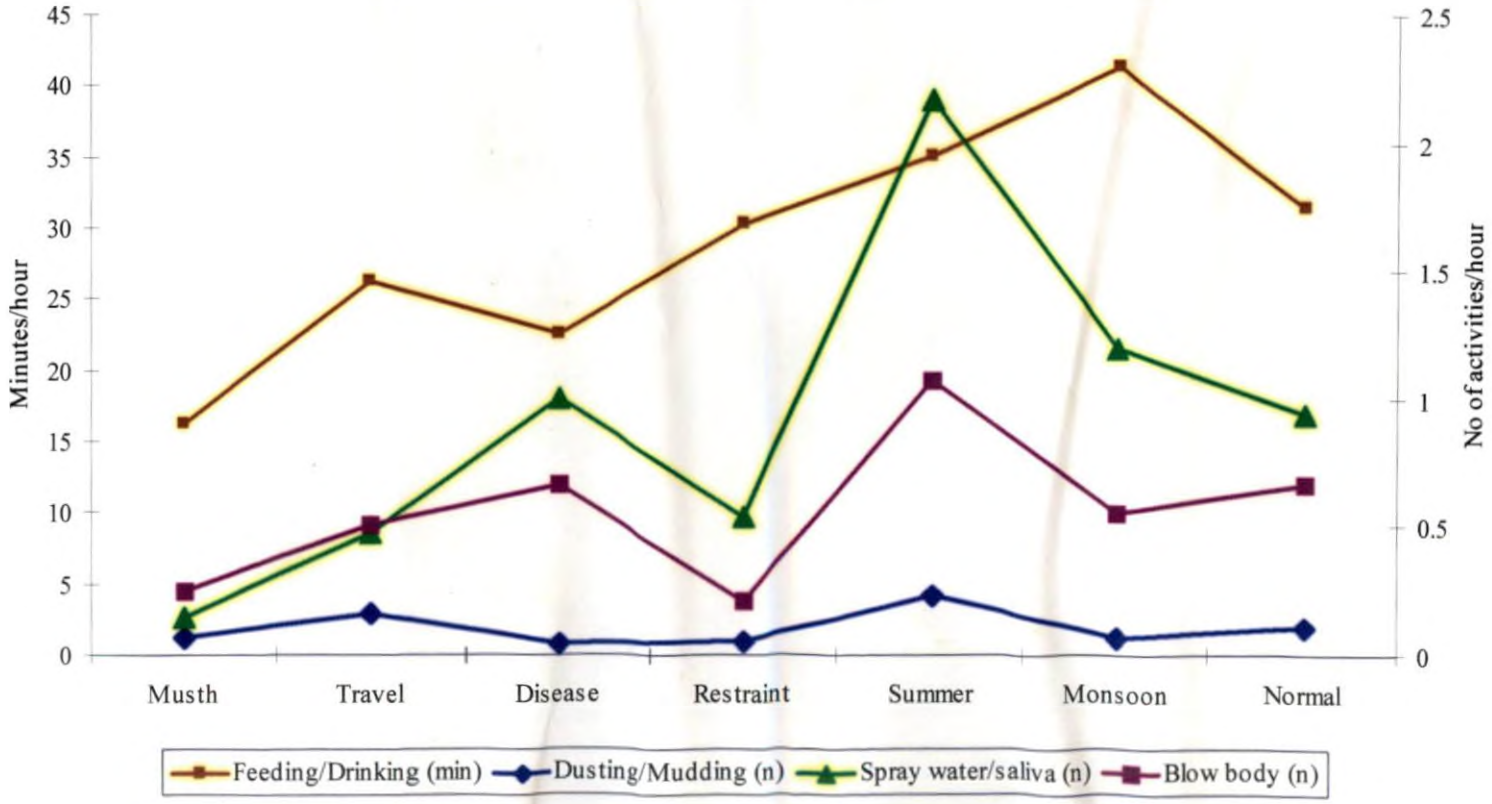

Fig. 1. Mean number of maintenance actions (n) and mean time spent performing maintenance activities (minutes) per hour

4.1.1.2 Dusting/Mudding Activity

Tossing mud/dust (Plate. 1) over the body was noticed to be highest during summer (group V) with a mean frequency of 4.25 ± 1.46 activities/hour, while the lowest frequency was noticed in the animals under disease or injury (group III). The significant differences between groups were shown in Table 3.

4.1.1.3 Spray Water/Saliva

Spraying water/saliva over the body (Plate. 2) was highest during summer and lowest during musth with mean frequency of 2.18 ± 0.72 and 0.15 ± 0.09 activities/hour, respectively. Significant differences between groups were shown in Table 3.

4.1.1.4 Blow Body

The highest mean frequency of this activity was performed by animals during summer (1.08 ± 0.19 activities/hour), while the lowest frequency was performed by animals under restraint in group IV (0.21 ± 0.14 activities/hour). Table 3 shows the statistical significance between other groups performing this activity.

4.1.2 Exploratory/Investigatory Behaviours

4.1.2.1 Manipulate Object/Environment

As shown in Table 4 and Fig. 2 mean time performing this activity (Plate. 2) was significantly higher ($P<0.05$) during musth with a mean time of 2.38 ± 0.43 minutes/hour, than animals in other group.

4.1.2.2 Trunk up Activity

Mean number of trunk up activities (Plate. 2) performed in group III, IV, VI and VII were 0.48 ± 0.15, 0.37 ± 0.11, 0.41 ± 0.16 and 0.41 ± 0.09 activities/hour, respectively. The significant difference from other groups is shown in Table 4.

4.1.2.3 Eyes Wide

The highest frequency of 0.23 ± 0.06 activities/hour in group VI (Table 4) differed significantly ($P<0.05$) from group I with the lowest frequency of 0.05 ± 0.03.

Table 4. Mean number of exploratory actions (n) and mean time spent performing exploratory activities (minutes) per hour with standard deviation

	Manipulate object/environment (min)	Trunk up (n)	Eyes wide (n)	Face check (n)	Flehmen (n)
Group I	2.38 ± 0.43^{a}	0.15 ± 0.14^{b}	0.05 ± 0.03^{b}	1.03 ± 0.16^{ab}	0.50 ± 0.18^{a}
Group II	1.05 ± 0.31^{b}	0.09 ± 0.07^{b}	0.09 ± 0.06^{ab}	0.98 ± 0.20^{abc}	0.09 ± 0.03^{c}
Group III	0.91 ± 0.55^{b}	0.48 ± 0.15^{a}	0.12 ± 0.07^{ab}	0.16 ± 0.15^{d}	0.16 ± 0.09^{c}
Group IV	1.57 ± 0.45^{b}	0.37 ± 0.11^{a}	0.10 ± 0.08^{ab}	1.09 ± 0.13^{ab}	0.38 ± 0.15^{ab}
Group V	1.14 ± 0.33^{b}	0.15 ± 0.13^{b}	0.18 ± 0.11^{ab}	1.28 ± 0.27^{a}	0.24 ± 0.05^{bc}
Group VI	0.85 ± 0.19^{b}	0.41 ± 0.16^{a}	0.23 ± 0.06^{a}	0.65 ± 0.20^{c}	0.28 ± 0.09^{bc}
Group VII	1.11 ± 0.39^{b}	0.41 ± 0.09^{a}	0.14 ± 0.04^{ab}	0.84 ± 0.18^{bc}	0.15 ± 0.05^{c}

Means ± standard deviation in rows bearing different superscripts for each parameter differ significantly ($P<0.05$)

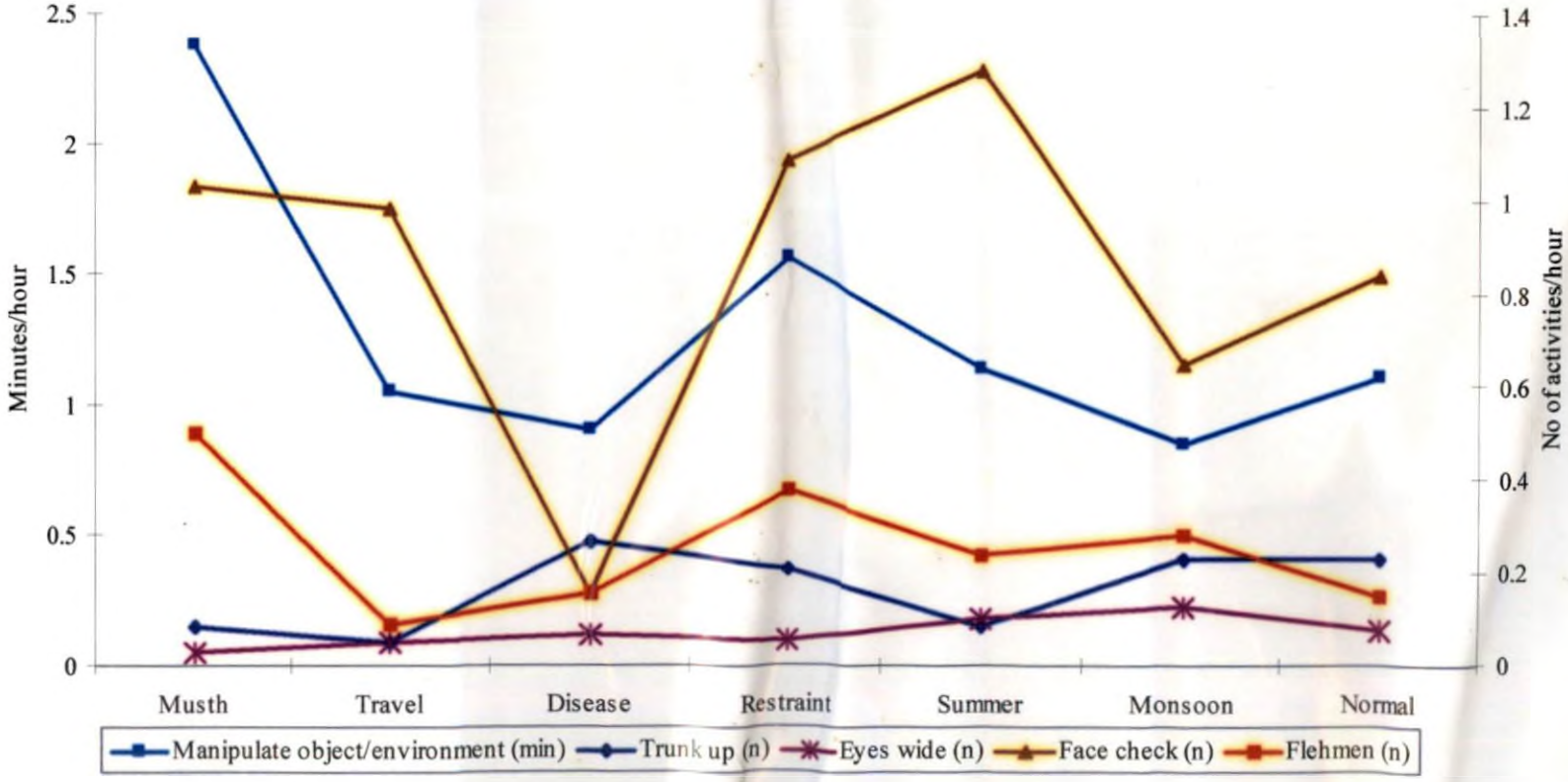

Fig. 2. Mean number of exploratory actions (n) and mean time spent performing exploratory activities (minutes) per hour

A

B

C

D

E

F

A,B - Manipulate object **C,D - Face check** **E - trunk raising** **F - Flehmen**

Plate. 2. Exploratory/Investigatory behaviours

4.1.2.4 Face Check

The significant difference ($P<0.05$) in face check activity (Plate. 2) performed by group III with groups I, IV, V, VI, VII were shown in Table 4.

4.1.2.5 Flehmen

The highest frequency of flehmen reflex (Plate. 2) was performed by elephants in musth (0.50 ± 0.18). The lower frequencies recorded in group II, III and IV were significantly higher ($P<0.05$) than those in groups V, VI and VII.

4.1.3 Aggressive/Agonistic Behaviours

4.1.3.1 Digging

The frequency of digging activity (Plate. 3) performed did not differ significantly ($P>0.05$) between groups (Table 5).

4.1.3.2 Head high

Musth elephants performed the highest mean frequency of head high (Plate. 3) activity (1.20 ± 0.31), which differed significantly ($P<0.05$) from other groups (Table 5).

4.1.3.3 Tail High and Aggression towards Trainer

Subjects in group I had shown to perform highest mean frequency of tail high activity (0.84 ± 0.31) and aggression towards trainer (2.08 ± 0.40), which significantly differed ($P<0.05$) from other groups (Table 5).

4.1.3.4 Object Throwing at Keeper and Public

Object throwing at keeper and public (Plate. 3) as shown in Table 5 was highest ($P<0.05$) in group I with mean frequency of 1.59 ± 0.43 and 0.56 ± 0.11 activities/hour, respectively and the significant difference between other groups were shown in the Table 5 and Fig. 3.

A

B

C

D

E

F

A,B - Digging **C,D - Head high** **E,f - Throwing objects**

Plate. 3. Aggressive/Agonistic behaviours

Table 5. Mean number of agonistic/aggressive actions (n) with standard deviation

	Digging (n)	Head high (n)	Tail high (n)	Object throw at keeper (n)	Object throw at public (n)	Aggression to trainer (n)
Group I	0.13 ± 0.07^{a}	1.20 ± 0.31^{a}	0.84 ± 0.31^{a}	1.59 ± 0.43^{a}	0.56 ± 0.11^{a}	2.08 ± 0.40^{a}
Group II	0.06 ± 0.07^{a}	0.92 ± 0.1^{b}	0.15 ± 0.13^{b}	0.21 ± 0.03^{c}	0.05 ± 0.03^{c}	1.02 ± 0.38^{b}
Group III	0.09 ± 0.06^{a}	0.54 ± 0.14^{c}	0.28 ± 0.09^{b}	0.14 ± 0.04^{c}	0.15 ± 0.03^{b}	0.56 ± 0.13^{b}
Group IV	0.09 ± 0.04^{a}	0.47 ± 0.19^{c}	0.18 ± 0.13^{b}	0.58 ± 0.23^{b}	0.09 ± 0.06^{bc}	0.93 ± 0.42^{b}
Group V	0.10 ± 0.05^{a}	0.61 ± 0.2^{c}	0.49 ± 0.21^{b}	0.10 ± 0.06^{c}	0.03 ± 0.03^{c}	0.89 ± 0.59^{b}
Group VI	0.05 ± 0.04^{a}	0.12 ± 0.0^{d}	0.51 ± 0.27^{b}	0.19 ± 0.13^{c}	0.17 ± 0.09^{b}	0.47 ± 0.23^{b}
Group VII	0.08 ± 0.06^{a}	0.04 ± 0.01^{d}	0.22 ± 0.08^{b}	0.64 ± 0.32^{b}	0.11 ± 0.05^{bc}	0.74 ± 0.48^{b}

Means ± standard deviation in rows bearing different superscripts for each parameter differ significantly ($P<0.05$)

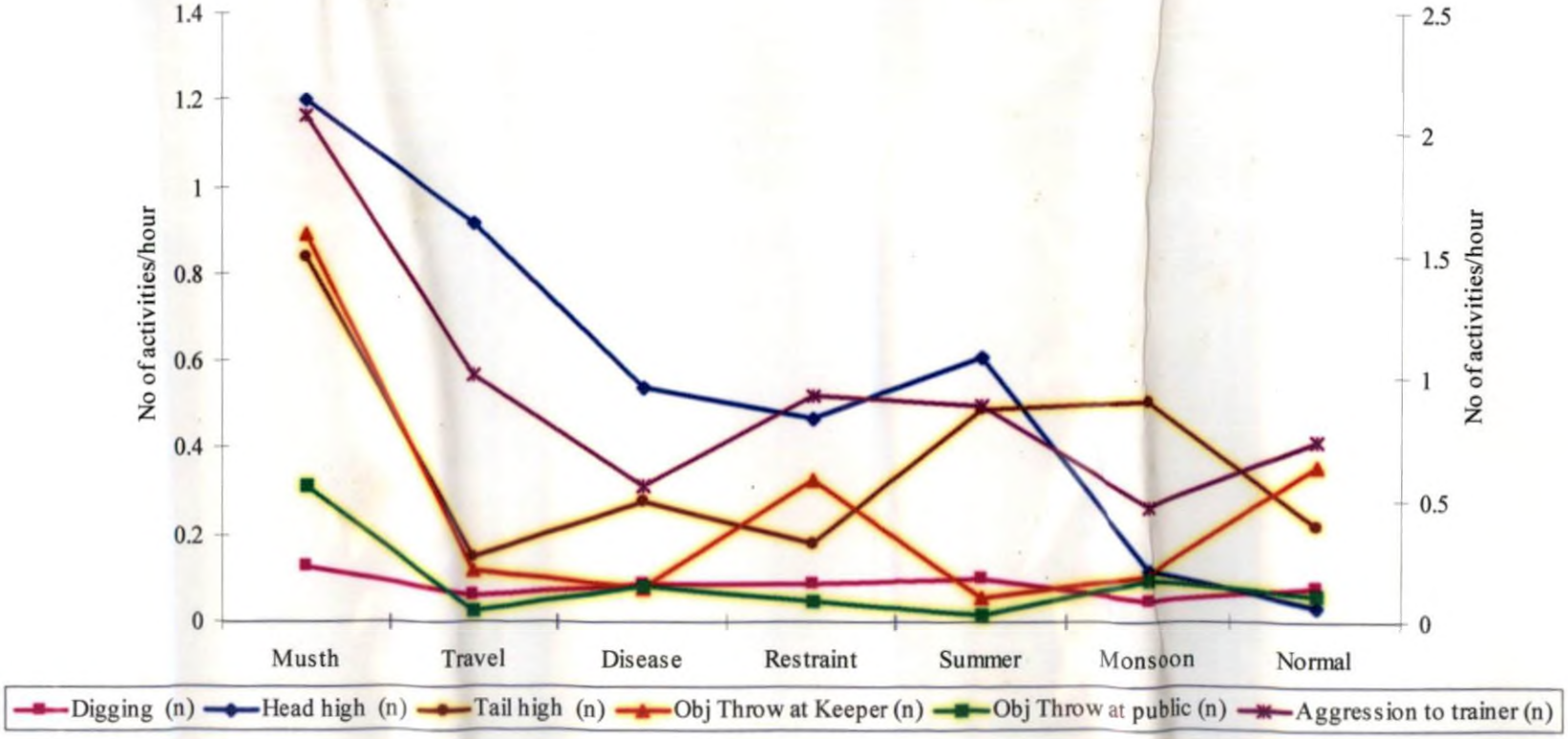

Fig. 3. Mean number of agonistic/aggressive actions (n)

4.1.4 Self-directed Behaviours

The mean time spent performing grooming activity and mean number of times performing masturbation by animals in different groups were show in Table 6 and Fig. 4.

4.1.4.1 Masturbation

The animals performing masturbation did not differ significantly between groups as shown in Table 6.

4.1.4.2 Grooming

Mean time spent performing grooming activities (Plate. 4) were significantly higher ($P<0.05$) in group I when compared to groups II, III and VII, respectively. Statistical differences between other groups are presented in Table 6.

4.1.5 Comfort Behaviours

4.1.5.1 Chain Pulling by Trunk/Tusk and Feet

As shown in Table 7, group II and IV did not differ significantly from group V and VII, while all other groups differ significantly ($P<0.05$) between them in the mean performance of chain pulling activity by trunk/tusk. The mean performances of chain pulling activity by feet (Plate. 4) did not differ significantly among groups.

4.1.5.2 Ear Flap and Ear Wave

Mean time performing ear flapping was highest in group I and II (Table 7) with a mean time of 18.12 ± 5.39 and 17.10 ± 9.29 minutes/hour, respectively, while group IV and V were having lowest mean time of 5.84 ± 1.34 and 6.54 ± 4.46 minutes/hour, respectively. Groups III, VI and VII shown significant difference ($P<0.05$) from other groups. The mean time performance of ear wave activity and the significant difference between groups were listed in Table 7 and Fig. 5.

4.1.6 Vocalizations

4.1.6.1 Low Rumble and Loud Rumble

Low rumbling was highest in group I and the lowest mean frequency observed in groups II, III and V. Statistically significant difference between other

Table 6. Mean number of self-directed actions (n) and mean time spent performing self-directed activities (minutes) per hour with standard deviation

	Masturbation (n)	Grooming (min)
Group I	0.015 ± 0.01^{a}	0.090 ± 0.07^{a}
Group II	0.006 ± 0.00^{a}	0.008 ± 0.00^{b}
Group III	0.002 ± 0.00^{a}	0.005 ± 0.00^{b}
Group IV	0.004 ± 0.00^{a}	0.035 ± 0.01^{ab}
Group V	0.003 ± 0.00^{a}	0.070 ± 0.03^{ab}
Group VI	0.010 ± 0.01^{a}	0.041 ± 0.01^{ab}
Group VII	0.006 ± 0.00^{a}	0.015 ± 0.00^{b}

Means ± standard deviation in rows bearing different superscripts for each parameter differ significantly (P<0.05)

Table 7. Mean number of comfort actions (n) and mean time spent performing comfort activities (minutes) per hour with standard deviation

	Chain pulling by trunk (n)	Chain pulling by feet (n)	Ear flap (min)	Ear wave (min)
Group I	0.085 ± 0.01^{a}	0.091 ± 0.11^{a}	18.12 ± 5.39^{a}	38.10 ± 2.18^{a}
Group II	0.021 ± 0.01^{cd}	0.014 ± 0.00^{a}	17.10 ± 9.29^{a}	25.82 ± 4.03^{bc}
Group III	0.010 ± 0.00^{d}	0.047 ± 0.00^{a}	11.05 ± 3.22^{ab}	14.20 ± 5.90^{d}
Group IV	0.028 ± 0.01^{cd}	0.019 ± 0.00^{a}	5.84 ± 1.34^{b}	31.08 ± 2.88^{ab}
Group V	0.009 ± 0.01^{d}	0.025 ± 0.03^{a}	6.54 ± 4.46^{b}	28.49 ± 4.09^{b}
Group VI	0.054 ± 0.03^{b}	0.034 ± 0.12^{a}	11.08 ± 2.52^{ab}	18.94 ± 2.44^{cd}
Group VII	0.035 ± 0.02^{c}	0.028 ± 0.02^{a}	10.81 ± 3.62^{ab}	29.66 ± 9.15^{ab}

Means ± standard deviation in rows bearing different superscripts for each parameter differ significantly (P<0.05)

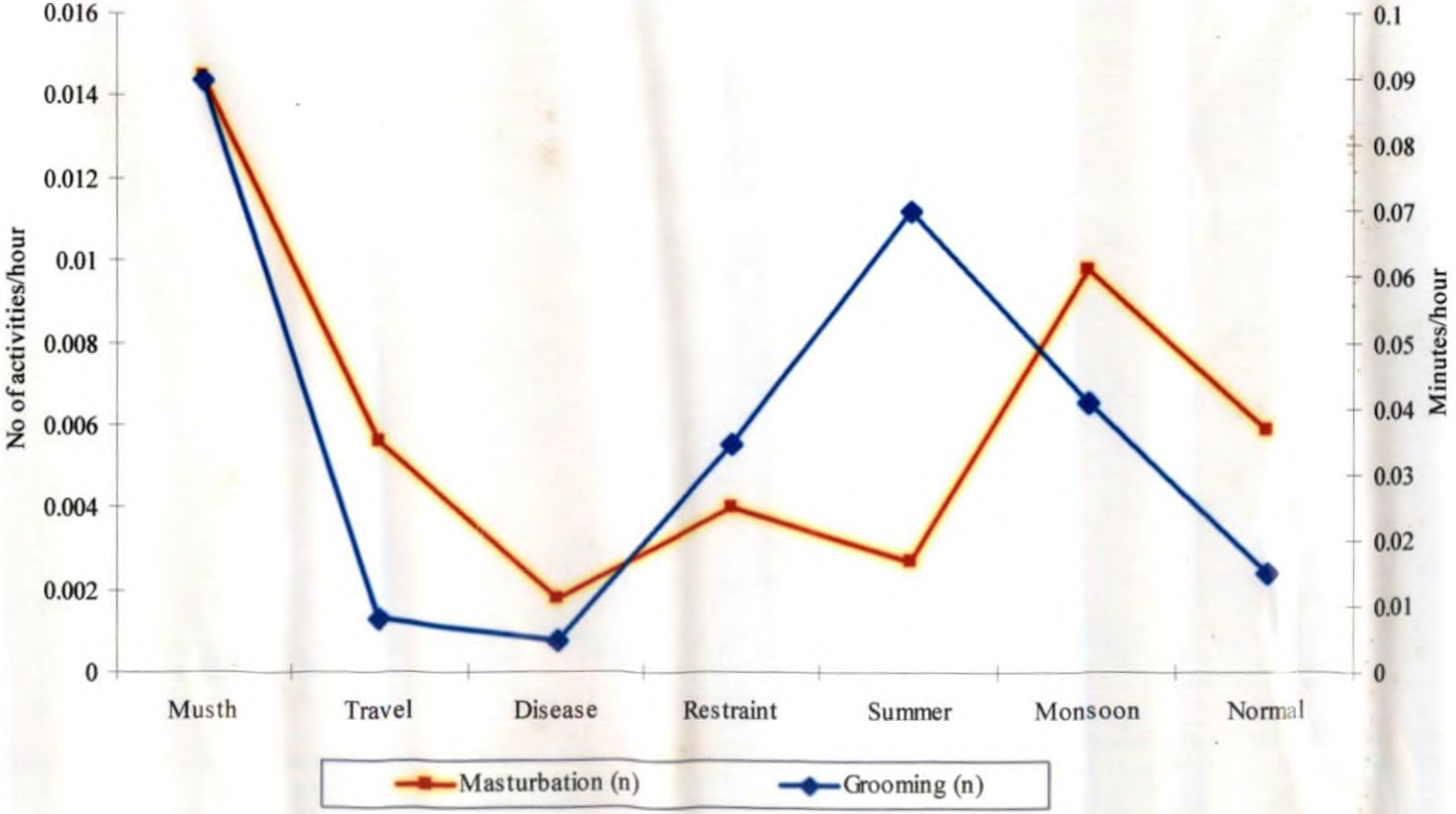

Fig. 4. Mean number of self-directed actions (n) and mean time spent performing self-directed activities (minutes) per hour

A

B

C

D

E

F

A,B - Grooming C,D - Chain pulling E,F - Trumpeting

Plate. 4. Trumpeting, Self-directed and Comfort behaviours

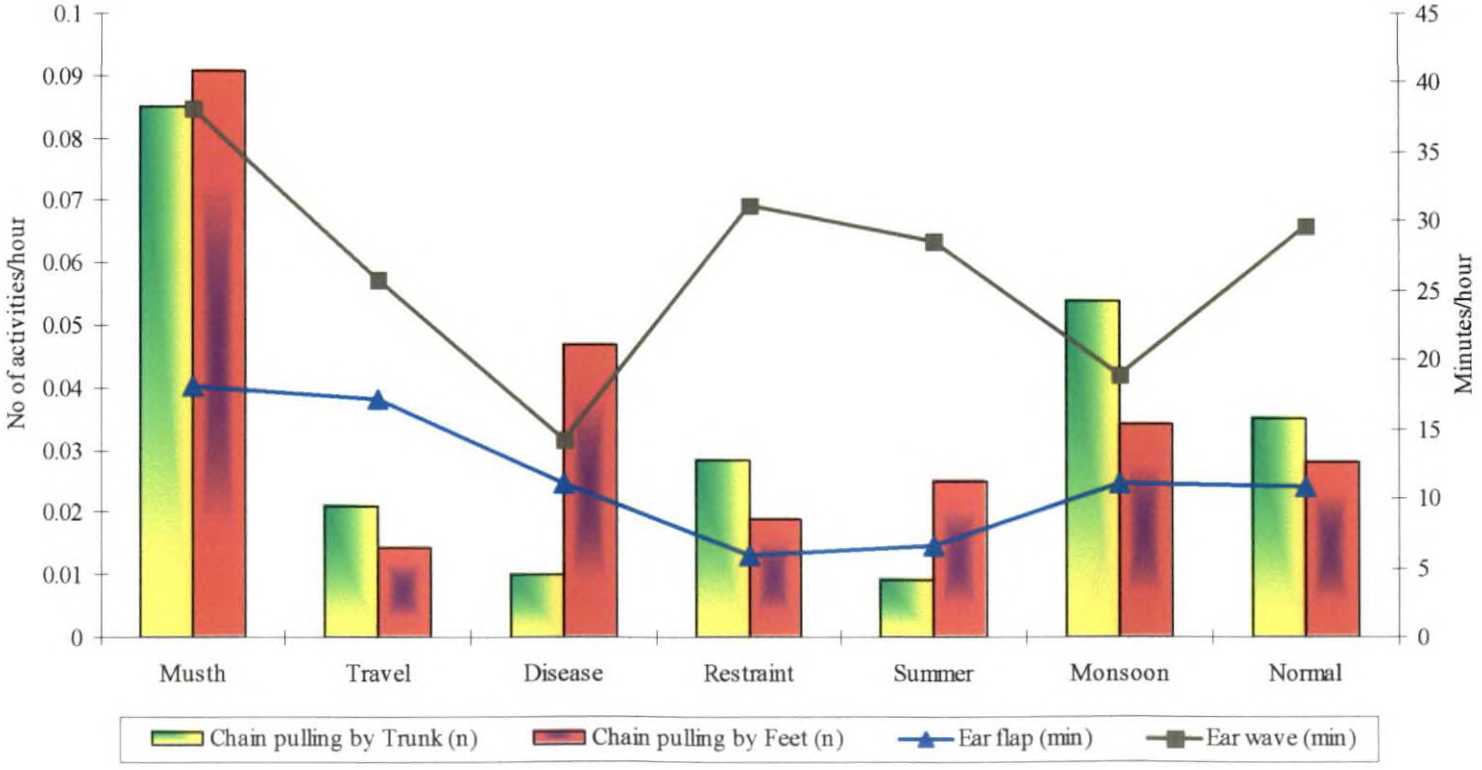

Fig. 5. Mean number of comfort actions (n) and mean time spent performing comfort activities (minutes) per hour

groups was listed in Table 8. Mean performance of loud rumbling differ significantly between groups as shown in Table 8.

4.1.6.2 Cry/Snort/Squeak

Mean performances of these vocalizations with the statistically significant difference between groups were presented in Table 8 and Fig. 6.

4.1.7 Trumpeting

Both low and loud trumpeting (Plate. 4) was found to be highest during musth with mean frequency of 1.09 ± 0.19 and 0.84 ± 0.14 activities/hour, respectively as shown in Table 9 and Fig. 7. The recordings of significant differences in trumpeting between groups were shown in Table 9.

4.1.8 Stereotypic Behaviours

4.1.8.1 Head Shake, Head Weave and Head Toss

Performance of stereotypic behaviour in all the groups were showing significant difference ($P<0.05$) with variations in the individual subject's performance. The mean frequency of performing these stereotypic behaviours were shown in Table 10 and Fig. 8 with their statistical significance between groups.

4.1.8.2 Trunk Curl/Twitch/Bounce/Twiddle

The performance of trunk related stereotypic behaviours were highest in the animals under musth with a mean time of 4.18 ± 1.87 minutes/hour, while the subjects in other groups do not differ significantly between them as shown in Table 10.

4.1.9 Temporal Gland Secretion and Urine Dribbling

The highest mean score showing the temporal gland secretion and urine dribbling (Plate. 5) were noticed during musth in the subjects with the respective scores being 4 ± 0.82 and 2.25 ± 0.96. The temporal gland secretion was also noticed in groups VI and VII, while urine dribbling was absent in groups III, VI and VII. The difference in the mean scores in other groups and their statistical difference were presented in Table 11 and Fig. 9.

Table 8. Mean number of vocalization (n) with standard deviation

	Low rumble (n)	Loud rumble (n)	Cry/snort/squeak (n)
Group I	0.94 ± 0.27^{a}	1.25 ± 0.41^{a}	0.09 ± 0.06^{ab}
Group II	0.05 ± 0.03^{d}	0.68 ± 0.24^{a}	0.04 ± 0.03^{bc}
Group III	0.09 ± 0.06^{d}	0.91 ± 0.41^{a}	0.11 ± 0.04^{a}
Group IV	0.58 ± 0.16^{b}	0.57 ± 0.12^{a}	0.02 ± 0.02^{c}
Group V	0.16 ± 0.05^{d}	0.67 ± 0.12^{a}	0.08 ± 0.04^{abc}
Group VI	0.41 ± 0.02^{bc}	1.01 ± 0.31^{a}	0.07 ± 0.03^{abc}
Group VII	0.31 ± 0.17^{cd}	0.99 ± 0.45^{a}	0.12 ± 0.08^{a}

Means ± standard deviation in rows bearing different superscripts for each parameter differ significantly ($P<0.05$)

Table 9. Mean number of trumpeting (n) performed with standard deviation

	Soft trumpet (n)	Loud trumpet (n)
Group I	1.09 ± 0.19^{a}	0.84 ± 0.14^{a}
Group II	0.75 ± 0.17^{b}	0.05 ± 0.02^{d}
Group III	0.05 ± 0.03^{d}	0.61 ± 0.07^{b}
Group IV	0.24 ± 0.13^{c}	0.18 ± 0.05^{cd}
Group V	0.09 ± 0.08^{cd}	0.07 ± 0.02^{d}
Group VI	0.18 ± 0.08^{cd}	0.48 ± 0.20^{b}
Group VII	0.17 ± 0.12^{cd}	0.25 ± 0.10^{c}

Means ± standard deviation in rows bearing different superscripts for each parameter differ significantly ($P<0.05$)

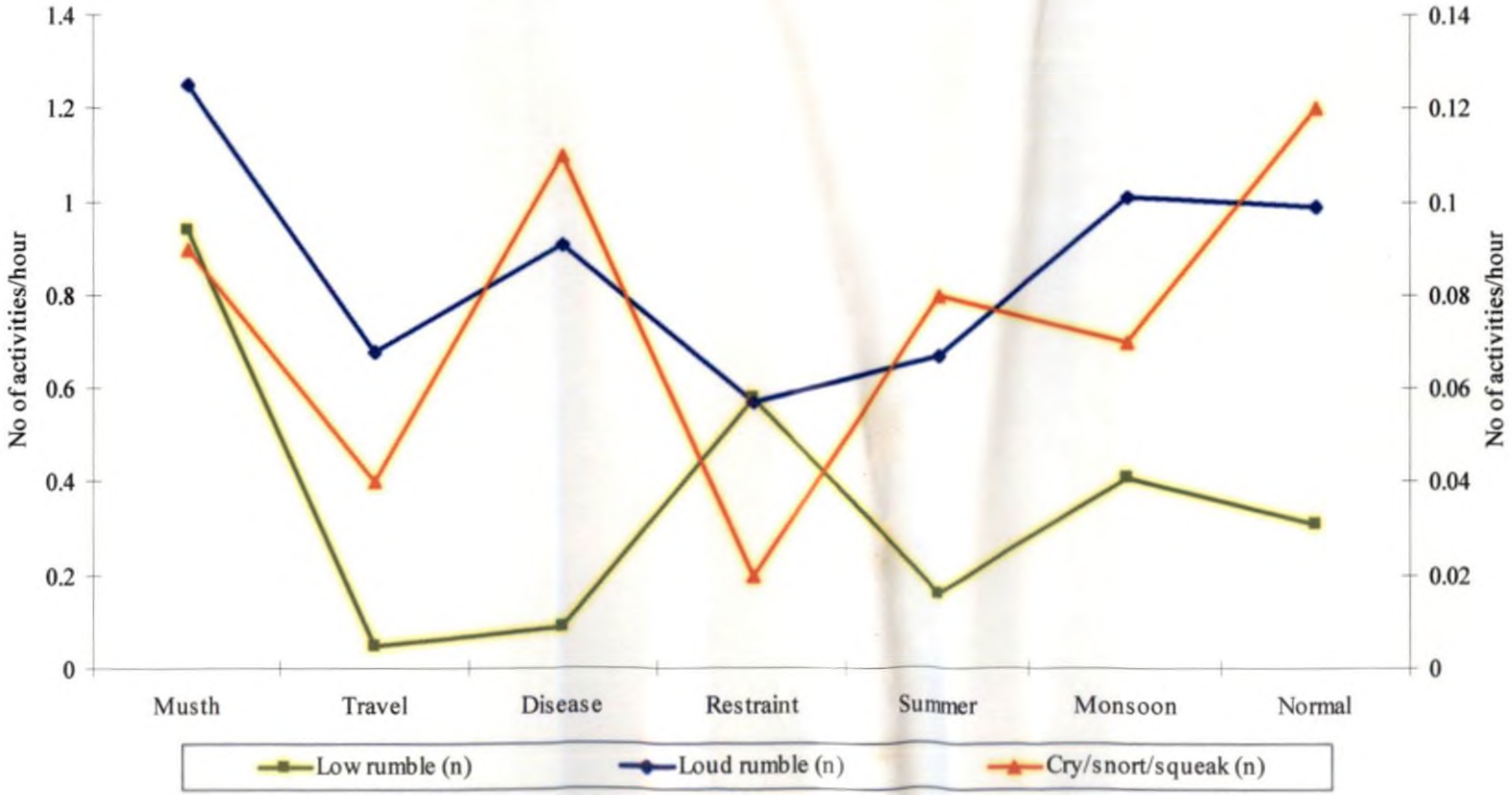

Fig. 6. Mean number of vocalizations (n) per hour

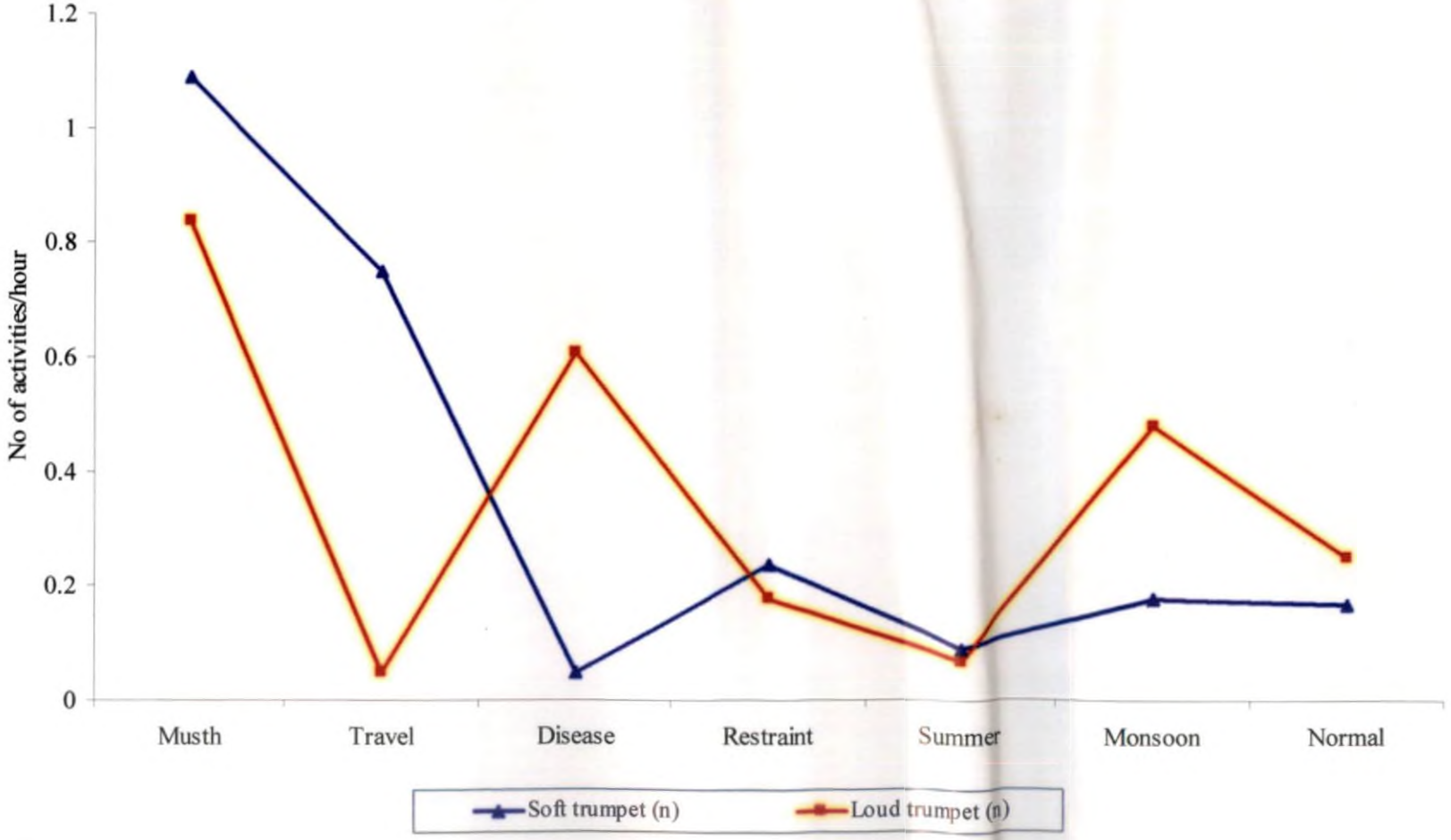

Fig. 7. Mean number of trumpeting (n) performed per hour

Table 10. Mean time spent performing stereotypic activities (minutes) per hour with standard deviation

	Head shake (min)	Head weave (min)	Head toss (min)	Trunk curl/twitch/bounce/twiddle (min)
Group I	5.47 ± 1.74^{a}	3.68 ± 1.20^{a}	1.08 ± 0.15^{ab}	4.18 ± 1.87^{a}
Group II	2.15 ± 0.84^{cd}	2.54 ± 1.07^{ab}	0.94 ± 0.37^{abc}	2.08 ± 0.63^{b}
Group III	3.08 ± 0.91^{bc}	1.08 ± 0.53^{c}	0.57 ± 0.09^{c}	$1.54 \pm .0.53^{b}$
Group IV	1.29 ± 0.31^{d}	2.17 ± 0.45^{bc}	0.67 ± 0.20^{bc}	0.82 ± 0.24^{b}
Group V	4.10 ± 0.93^{b}	0.98 ± 0.44^{c}	1.18 ± 0.22^{a}	1.92 ± 0.48^{b}
Group VI	1.14 ± 0.14^{d}	1.38 ± 0.27^{bc}	1.29 ± 0.16^{a}	1.09 ± 0.29^{b}
Group VII	2.08 ± 0.46^{cd}	2.55 ± 1.25^{ab}	0.30 ± 0.59^{a}	2.04 ± 1.11^{b}

Means ± standard deviation in rows bearing different superscripts for each parameter differ significantly ($P<0.05$)

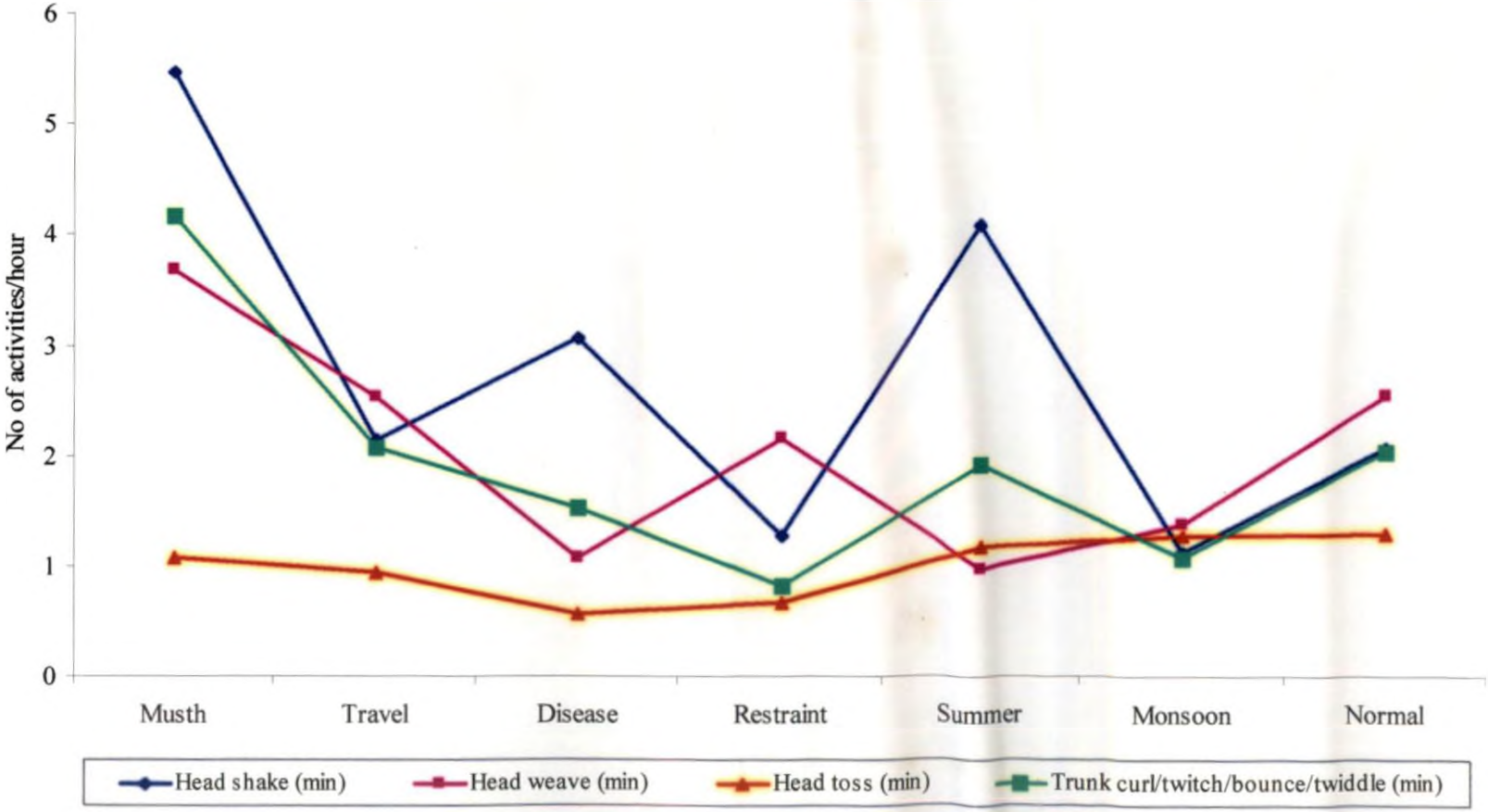

Fig. 8. Mean time spent performing stereotypic activities (minutes) per hour

A

B

C

D

E

F

A,B - Temporal Gland Secretion C,D - Urine dribbling E,F - Work with trainer

Plate. 5. Work with trainer, Temporal gland secretion and Urine dribbling behaviours

Table 11. Mean score showing temporal gland secretion (TGS) and urine dribbling and mean time spent performing work with trainer and other solitary behaviours (minutes) per hour with standard deviation

	Temporal gland secretion (score)	Urine dribbling (score)	Work with trainer (min)	Other solitary behaviour (min)
Group I	4 ± 0.82^{a}	2.25 ± 0.96^{a}	5.18 ± 0.74^{c}	5.22 ± 1.42^{a}
Group II	0.25 ± 0.50^{c}	0.25 ± 0.50^{b}	17.25 ± 7.30^{a}	1.18 ± 0.69^{c}
Group III	0.5 ± 0.58^{bc}	0	10.48 ± 1.67^{bc}	2.14 ± 0.80^{bc}
Group IV	1.5 ± 1.50^{b}	0.5 ± 0.58^{b}	6.98 ± 1.21^{c}	2.69 ± 1.18^{b}
Group V	1 ± 1.00^{bc}	0.5 ± 0.58^{b}	12.34 ± 5.72^{b}	1.08 ± 0.49^{c}
Group VI	0	0	14.55 ± 4.31^{b}	1.55 ± 0.47^{bc}
Group VII	0	0	15.44 ± 7.13^{b}	2.84 ± 1.52^{b}

Means ± standard deviation in rows bearing different superscripts for each parameter differ significantly ($P<0.05$)

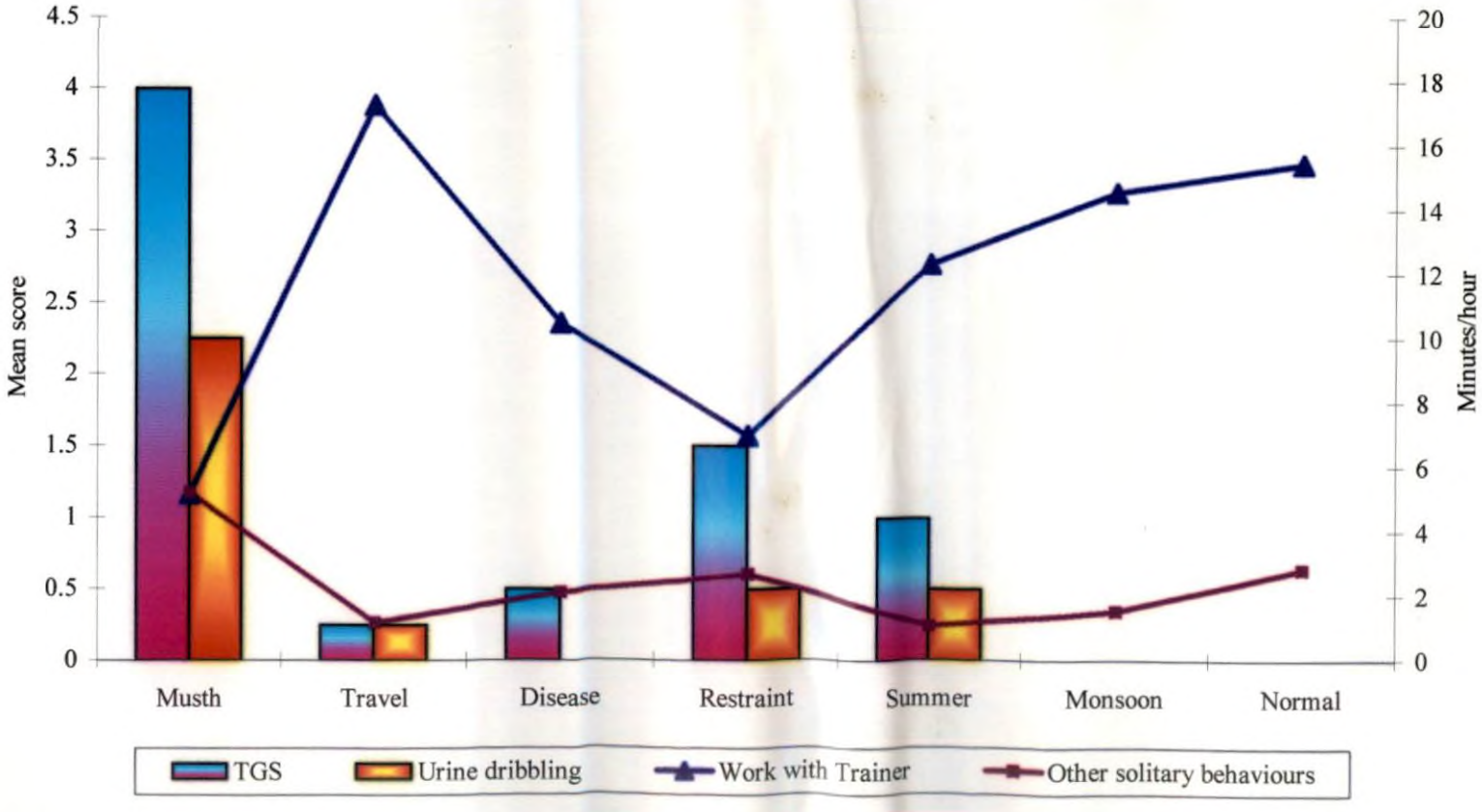

Fig. 9. Mean score showing temporal gland secretion (TGS) and urine dribbling and mean time spent performing work with trainer and other solitary behaviours (minutes) per hour

4.1.10 Work with Trainer

Mean time spent performing work with the trainer (Plate. 5) was highest in group II and lowest in Groups I and IV. Groups V, VI and VII differ significantly ($P<0.05$) from all other groups except group III (Table 11).

4.1.11 Other Solitary Behaviours

As presented in Table 11, the mean time spent performing other solitary behaviours were found to be highest in group I with a mean of 5.22 ± 1.42 minutes/hour and the lowest time in group II with a mean of 1.18 ± 0.69 minutes/hour. The significant difference ($P<0.05$) in the time spent performing other solitary behaviours were presented in the Table 11 and Fig. 9.

4.2 GLUCOCORTICOID CONCENTRATIONS

4.2.1 Urine Cortisol Concentrations

Variations in the cortisol concentrations of individual animals within each group were shown in Table 12. Mean urine cortisol level during musth was found to be 143.60 ± 30.07 ng/mg Cr which is significantly ($P<0.05$) higher than basal cortisol levels and cortisol concentration of other groups. Mean cortisol levels of 73.55 ± 11.95 ng/mg Cr and 69.13 ± 11.63 ng/mg Cr during summer and monsoon did not differ significantly with the mean basal level of cortisol 59.98 ± 8.45 ng/mg Cr. The mean cortisol values during travel, disease and restraint did not differ significantly from other groups as shown in Table 13. Mean cortisol concentration, range and median values for each group were shown in Table 13 and Fig. 10.

4.2.2 Faecal Cortisol Concentrations

Environmental stressors and other stressful events resulted in an increase of faecal cortisol metabolite (11,17-dioxoandrostanes) concentrations. A group of glucocorticoid metabolites collectively described as 11,17-dioxoandrostanes (11,17-DOAs) were detected in elephant faeces using radioimmunoassay. Individual differences in the basal and peak values of faecal cortisol metabolites were observed (Table 14). Basal values of faecal 11,17-dioxoandrostanes ranged from 58.29 to 583.11 nmol/kg (median: 168.91 nmol/kg). Highest mean concentration of faecal cortisol metabolites were found in group I (449.02 ± 86.31 nmol/kg). Mean faecal

Table 12. Urinary cortisol values (ng/mg Cr) under different stressful events

Urine Cortisol (ng/mg Cr) (n=20)						
Group I	Group II	Group III	Group IV	Group V	Group VI	Group VII
129.673	48.024	80.04	35.41	108.95	25.66	22.56
27.59	24.012	59.064	241.38	35.7	124.65	68.32
63.457	55.2	88.32	143.01	45.21	18.96	16.28
48.024	114.68	211.65	37.81	23.84	47.68	17.99
38.626	28.428	104.88	88.32	48.74	94.25	51.48
135.191	33.672	348.98	354.66	118.95	158.36	39.54
43.056	48.3	110.36	19.04	103.55	29.55	115.8
113.119	254.91	325.24	60.70	17.66	34.58	12.33
289.33	128.45	104.328	165.54	78.34	147.64	49.66
242.792	41.4	65.964	137.95	154.72	9.84	84.22
57.939	88.32	15.456	93.81	128.59	119.32	22.35
60.698	22.908	154.504	110.36	38.41	24.61	38.99
315.22	47.472	18.768	179.34	14.99	38.69	47.66
22.072	55.21	19.044	15.49	120.14	95.61	125.43
10.488	80.04	211.54	11.32	38.7	18.48	118.5
240.033	301.55	20.7	104.33	212.45	12.95	91.2
551.8	37.812	28.704	55.20	58.68	101.85	72.66
187.612	94.57	14.352	281.08	19.22	154.38	89.51
206.925	301.83	55.486	59.06	35.68	30.18	100.52
88.32	54.648	19.872	118.69	68.52	95.35	14.52

Table 13. Mean urine cortisol (ng/mg Cr) concentration with the standard error, range and median values under different stressful events

	Mean ± SE	Range	Median
Group I	143.60 ± 30.07^{a}	10.48 – 551.80	100.72
Group II	93.07 ± 19.78^{ab}	22.91 – 301.83	54.92
Group III	102.86 ± 22.44^{ab}	14.35 – 348.98	73.02
Group IV	115.62 ± 20.65^{ab}	11.32 – 354.66	99.67
Group V	73.55 ± 11.95^{b}	14.99 – 212.45	53.71
Group VI	69.13 ± 11.63^{b}	9.84 – 158.36	43.19
Group VII	59.98 ± 8.45^{b}	12.33 – 125.43	50.57

Means ± standard error in rows bearing different superscripts for each parameter differ significantly ($P<0.05$)

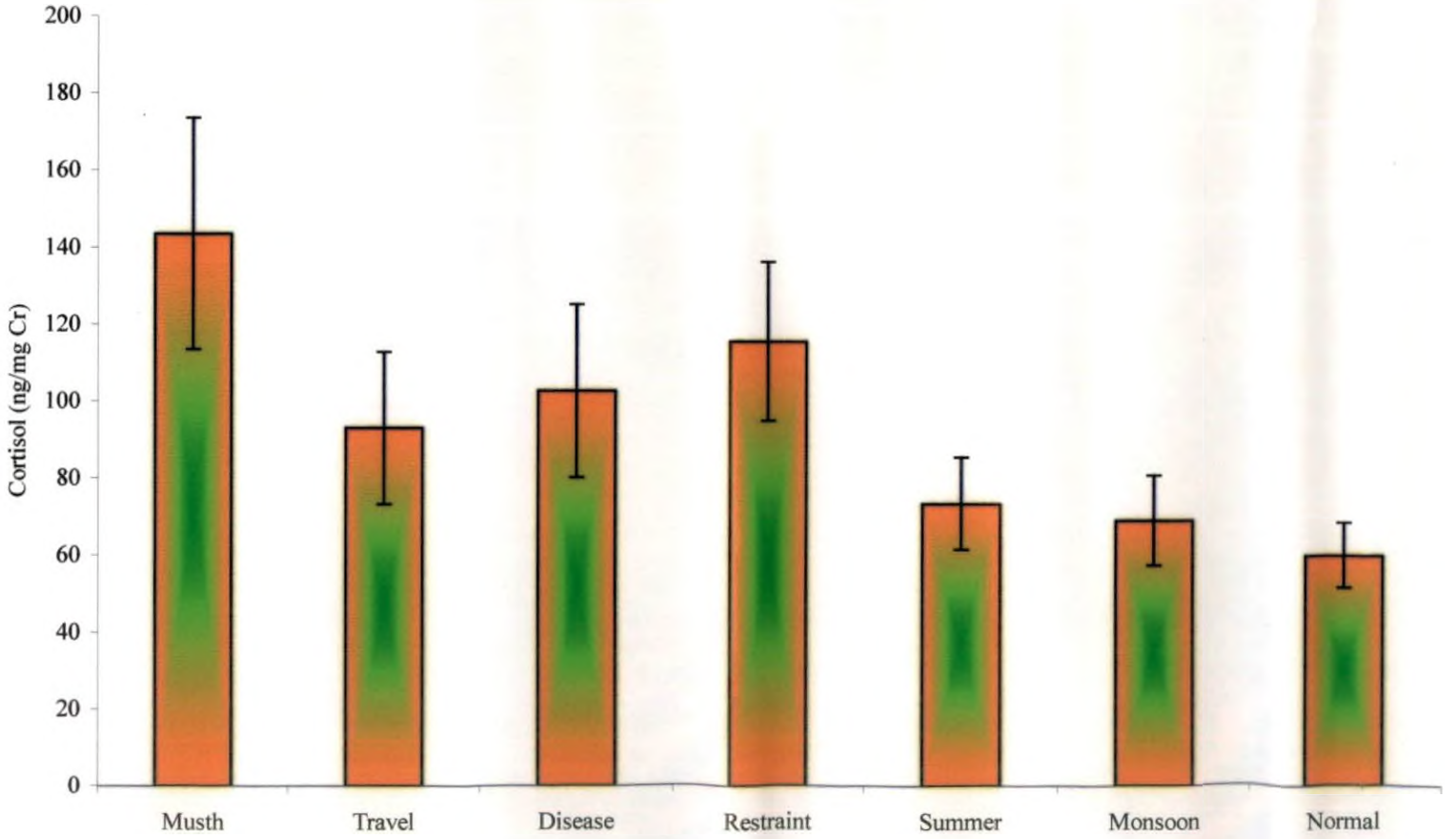

Fig. 10. Mean Urinary Cortisol (ng/mg Cr) values in different groups

Table 14. Faecal cortisol (11,17-DOA) concentration under different stressful events

Faecal Cortisol (nmol/kg) (n=10)						
Group I	Group II	Group III	Group IV	Group V	Group VI	Group VII
480.15	215.26	325.1	891.35	336.31	544.68	336.54
861.36	384.1	631.05	584.15	459.31	394.61	222.37
75.29	654.15	354.28	354.26	254.26	284.61	254.19
834.3	198.48	648.92	187.96	284.1	119.81	583.11
548.16	321.05	111.54	361.25	269.81	100.8	109.31
119.26	251.92	217.06	148.99	348.12	234.15	94.25
348.22	457.25	348.24	254.19	185.33	189.34	64.58
265.18	115.29	148.37	321.62	586.34	312.4	58.29
325.8	184.35	289.02	151.21	475.28	114.79	149.55
632.482	219.5	281.6	291.17	179.67	200.81	188.27

Table 15. Mean faecal cortisol (nmol/kg) concentration with the standard error, range and median values under different stressful events

	Mean ± SE	Range	Median
Group I	449.02 ± 86.31^{a}	75.29 – 861.36	414.19
Group II	300.14 ± 50.71^{ab}	115.29 – 654.15	235.71
Group III	335.52 ± 56.81^{ab}	111.54 – 648.92	307.06
Group VI	354.62 ± 72.07^{ab}	148.99 – 891.35	306.40
Group V	337.85 ± 41.94^{ab}	179.67 – 586.34	310.21
Group VI	249.60 ± 44.31^{b}	100.80 – 544.68	217.48
Group VII	206.05 ± 50.44^{b}	58.29 – 583.11	168.91

Means ± standard error in rows bearing different superscripts for each parameter differ significantly ($P<0.05$)

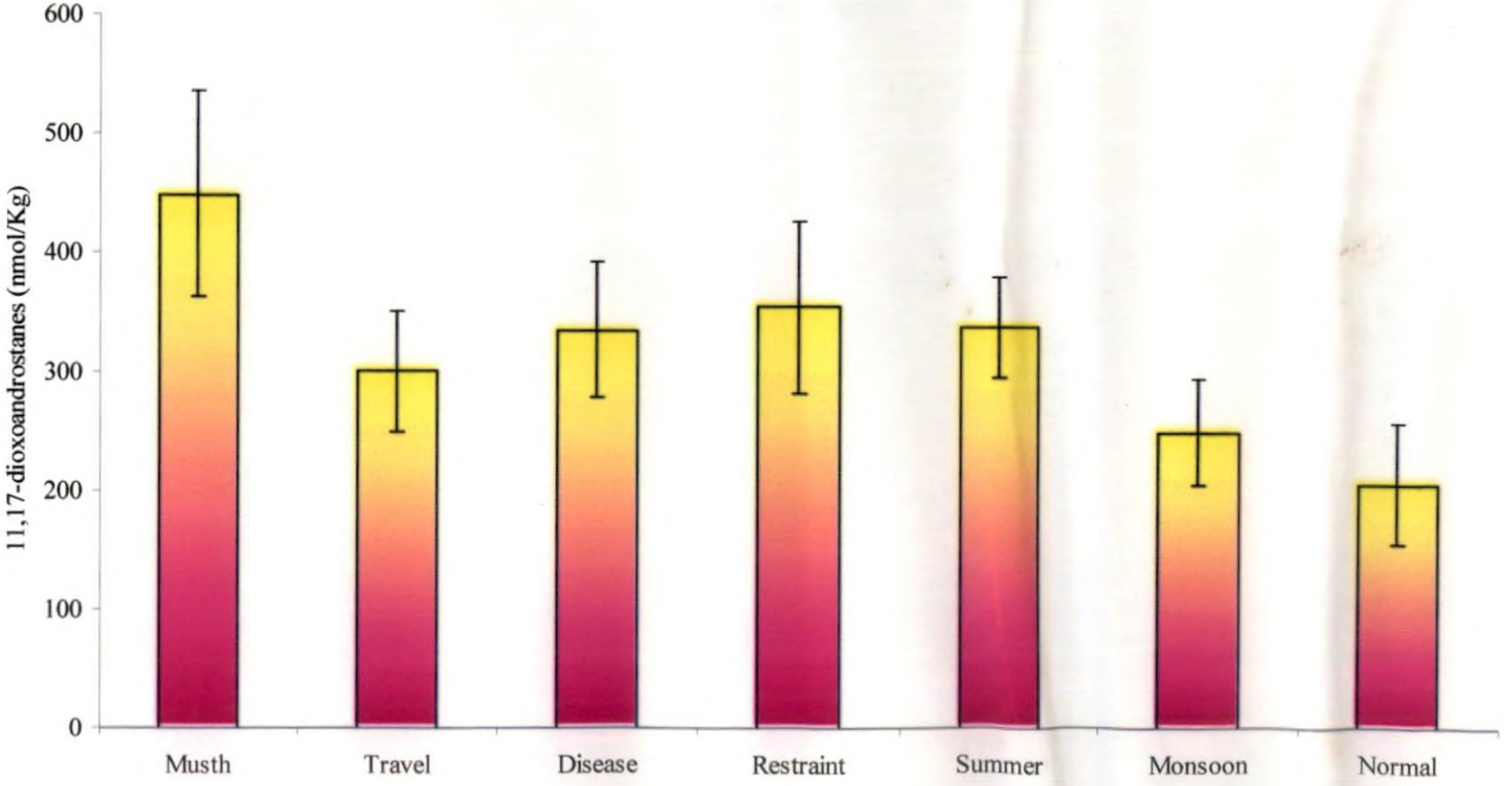

Fig. 11. Mean Faecal Cortisol (nmol/Kg) values in different groups

cortisol metabolite concentrations in group VI (249.60 ± 44.31 nmol/kg) did not differ significantly from the basal value, while other groups showed significant difference ($P<0.05$) from the basal concentration (Table 15 and Fig. 11).

4.2.3 Comparison of Urine and Faecal Cortisol Concentrations

The mean cortisol concentrations in urine samples and mean concentrations of cortisol metabolites (11,17-DOA) in faecal samples collected concurrently in all the stressful events to measure the advantages of non-invasive stress monitoring by urine and faecal cortisol levels showed a similar trend in the changes of cortisol levels in all the events with the highest cortisol concentrations in group I and the lowest cortisol concentrations in group VII as shown in Fig. 12.

4.3 URINE CREATININE CONCENTRATIONS

The individual values of urinary creatinine concentrations for indexing the cortisol values were shown in Table 16. During summer the animals showed the highest mean concentration of 0.211 ± 0.023 mg/ml with a median value of 0.190 mg/ml and a range of 0.081 to 0.480 mg/ml and found to be significantly different ($P<0.05$) from the other groups. The creatinine values of other groups did not show any significant difference from the basal value of 0.109 ± 0.012 mg/ml (Table 17 and Fig. 13). Comparison of mean urine creatinine concentrations against mean urine cortisol concentrations in each group of animals were shown in Fig. 14.

4.4 BEHAVIOUR AND CORTISOL

The correlation between mean performances of behaiovral events and the mean values of faecal cortisol metabolites and mean values of urine cortisol concentrations found out during the present study were shown from the Fig. 15 to Fig. 30.

4.5 OTHER PHYSIOLOGICAL OBSERVATIONS

4.5.1 Rectal Temperature

The mean rectal temperature showed a slight increase from the morning levels with the mean temperature in the noon, but returned back to the morning levels in the evening in all the groups (Table 18 and Fig. 31), but the values were within the normal range with no abnormal deviation except in few cases where such

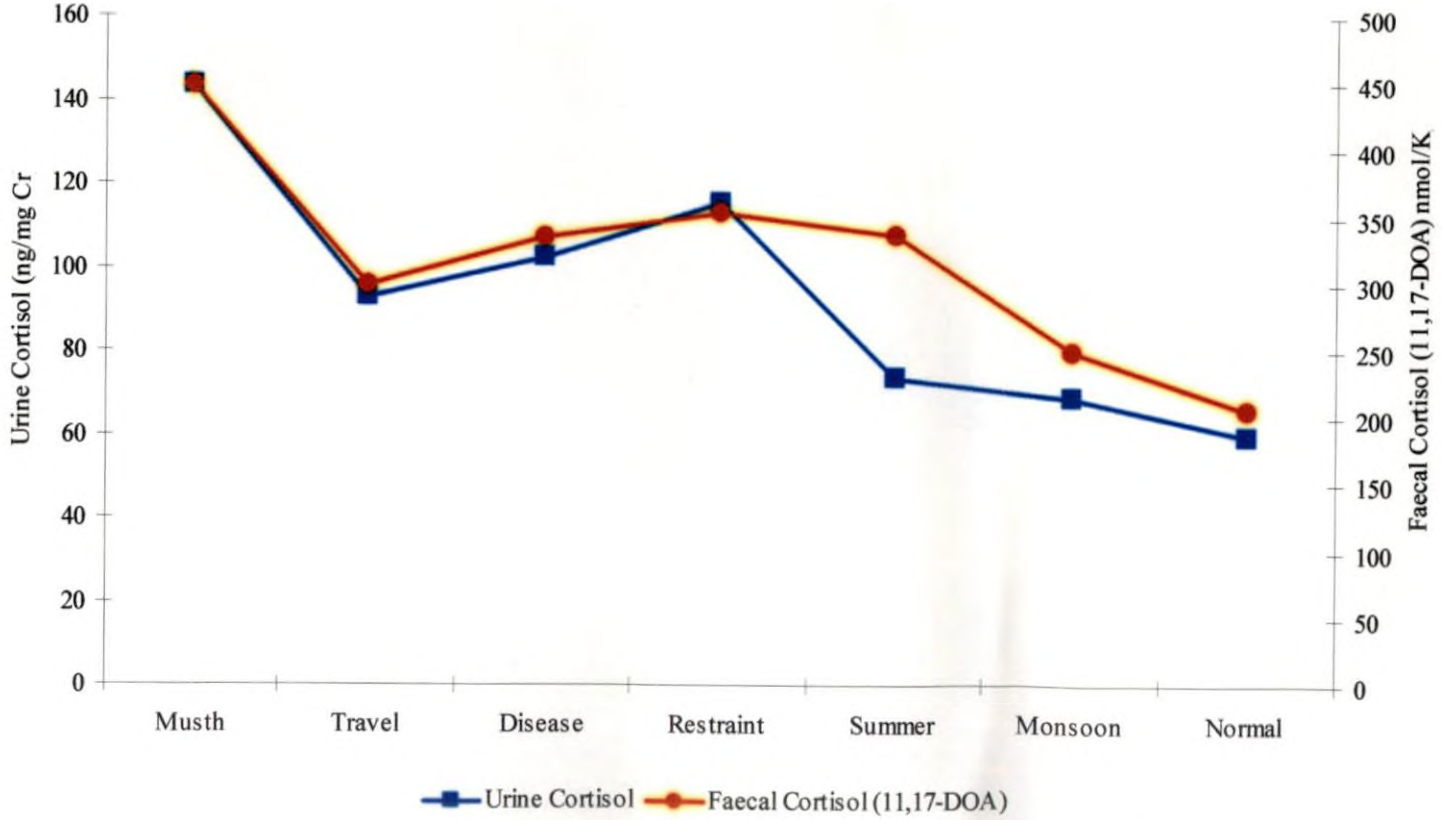

Fig. 12. Mean Urinary and Faecal Cortisol values in different groups

Table 16. Urine creatinine values (mg/ml) under different stressful events

Urine Creatinine (mg/ml) (n=20)						
Group I	Group II	Group III	Group IV	Group V	Group VI	Group VII
0.045	0.162	0.102	0.3	0.125	0.094	0.107
0.125	0.039	0.098	0.084	0.17	0.047	0.082
0.101	0.041	0.211	0.099	0.192	0.062	0.06
0.23	0.081	0.147	0.245	0.48	0.0847	0.105
0.095	0.056	0.084	0.025	0.348	0.18	0.12
0.054	0.0612	0.035	0.074	0.21	0.065	0.064
0.143	0.2	0.029	0.085	0.081	0.084	0.048
0.1	0.084	0.14	0.066	0.156	0.152	0.08
0.105	0.09	0.1	0.145	0.14	0.281	0.094
0.12	0.105	0.16	0.165	0.21	0.057	0.04
0.09	0.045	0.043	0.095	0.19	0.15	0.188
0.035	0.135	0.078	0.112	0.28	0.084	0.156
0.0514	0.118	0.214	0.087	0.081	0.17	0.069
0.211	0.202	0.068	0.1	0.098	0.038	0.144
0.061	0.13	0.055	0.21	0.19	0.17	0.16
0.055	0.148	0.175	0.071	0.17	0.166	0.084
0.068	0.19	0.012	0.025	0.28	0.057	0.072
0.084	0.098	0.012	0.035	0.17	0.094	0.254
0.094	0.114	0.01	0.04	0.36	0.105	0.18
0.152	0.115	0.1	0.064	0.28	0.07	0.064

Table 17. Mean urine creatinine (mg/ml) concentration with the standard error, range and median values under different stressful events

	Mean ± SE	Range	Median
Group I	0.101 ± 0.012^{b}	0.035 – 0.230	0.095
Group II	0.111 ± 0.011^{b}	0.039 – 0.202	0.110
Group III	0.094 ± 0.014^{b}	0.010 – 0.214	0.091
Group VI	0.106 ± 0.016^{b}	0.025 – 0.300	0.086
Group V	0.211 ± 0.023^{a}	0.081 – 0.480	0.190
Group VI	0.111 ± 0.014^{b}	0.038 – 0.281	0.090
Group VII	0.109 ± 0.012^{b}	0.040 – 0.254	0.089

Means ± standard error in rows bearing different superscripts for each parameter differ significantly ($P<0.05$)

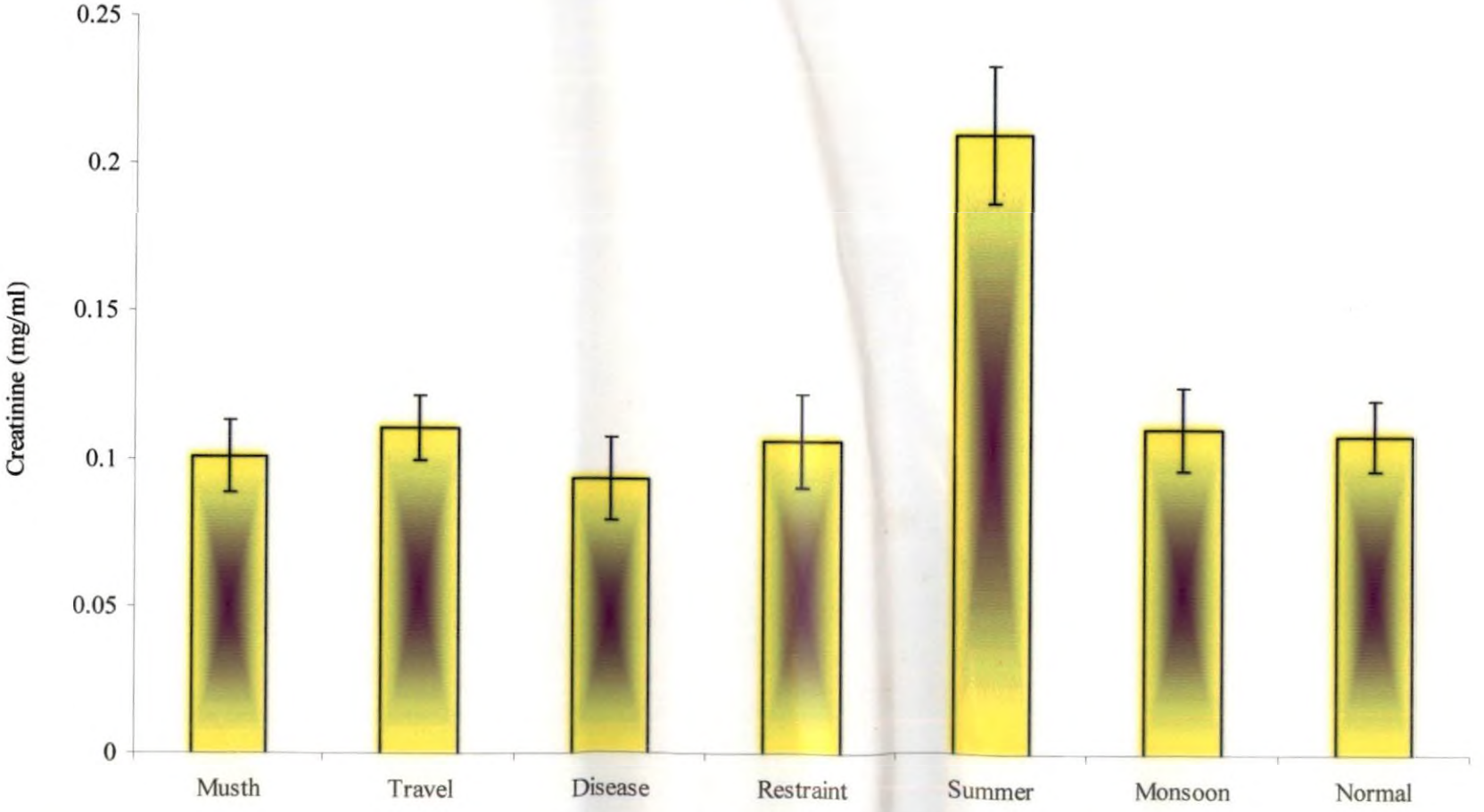

Fig. 13. Mean Urinary Creatinine (mg/ml) values in different groups

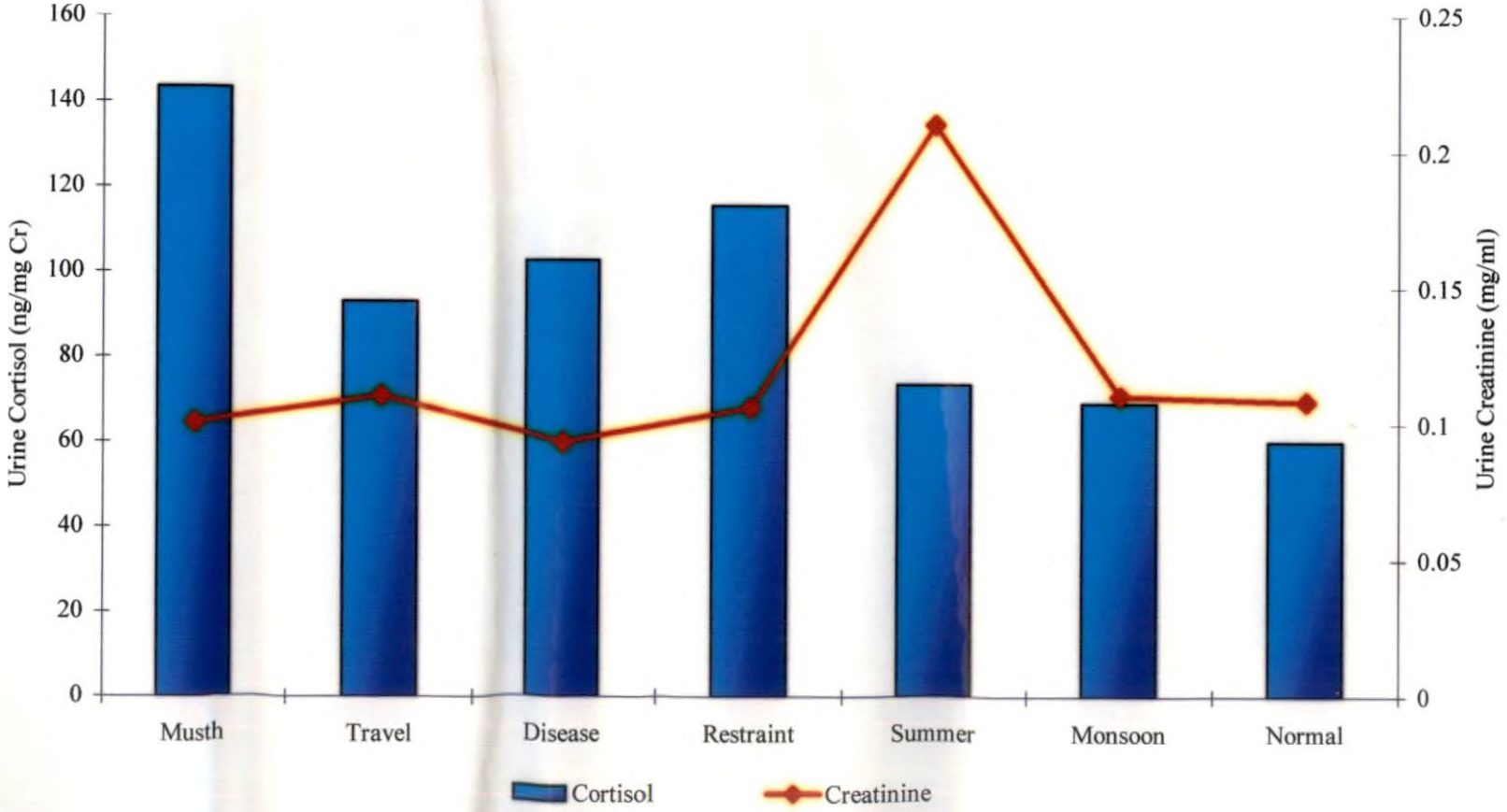

Fig. 14. Mean Urinary Cortisol and Urinary Creatine Concentrations in different groups

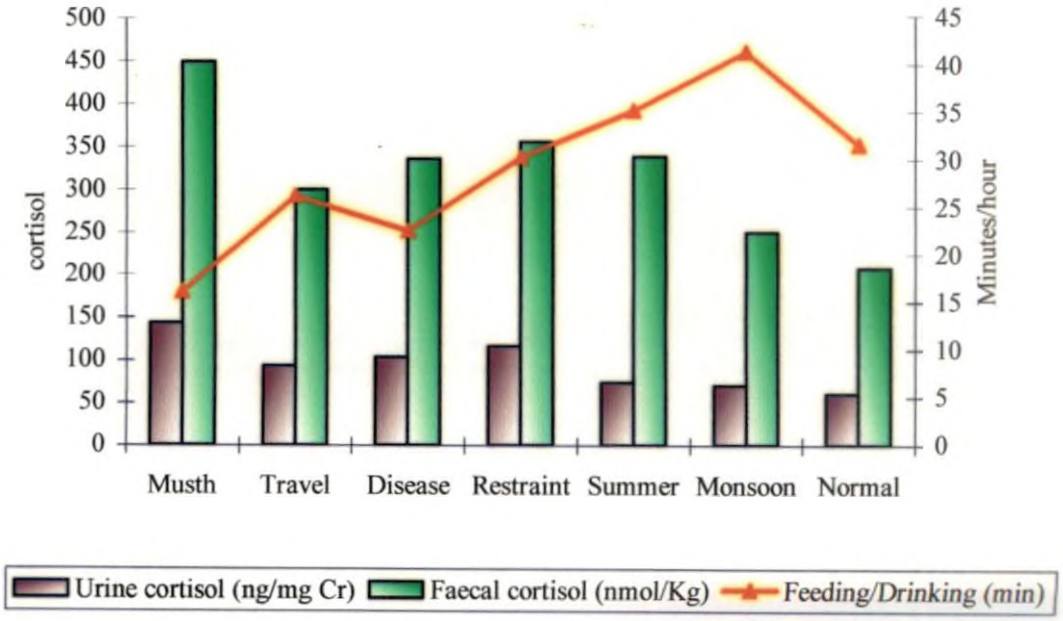

Fig. 15. Comparison of cortisol concentrations with maintenance behaviour

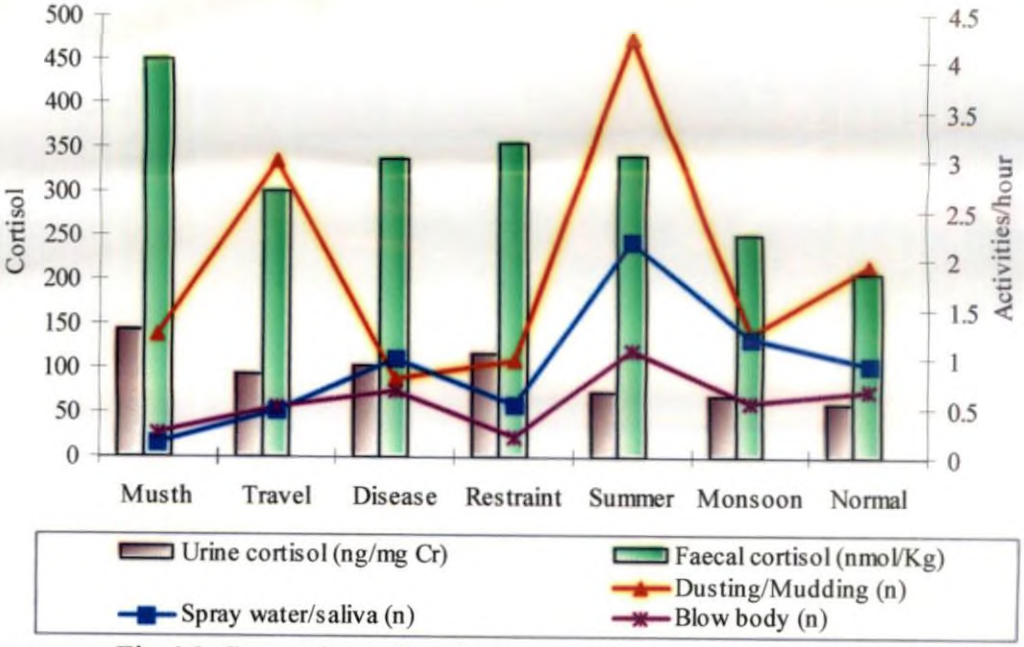

Fig. 16. Comparison of cortisol concentrations with maintenance behaviour

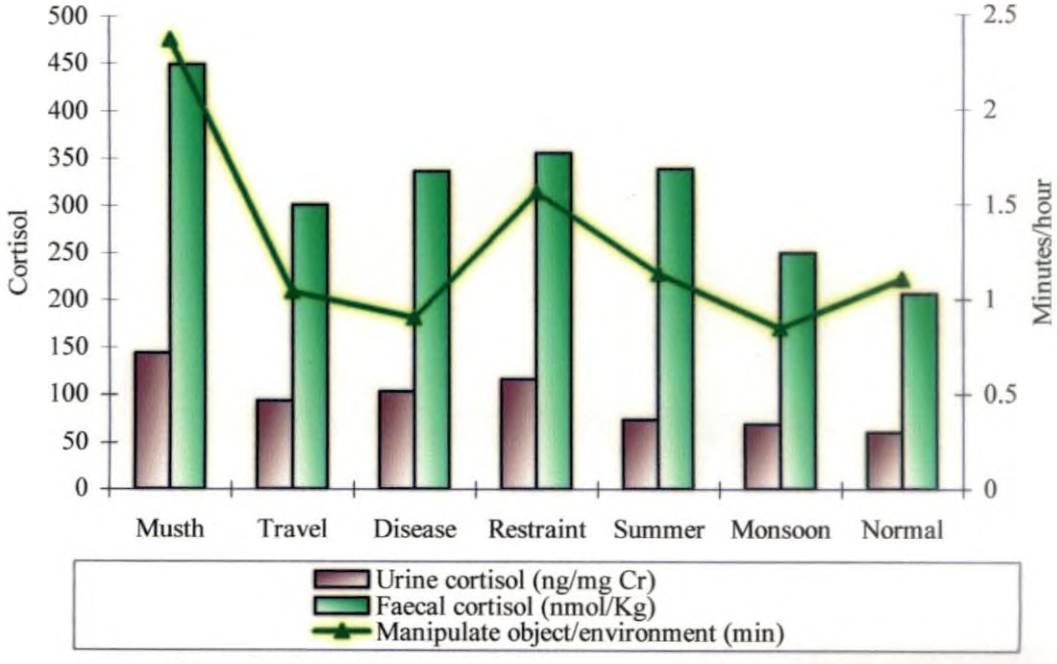

Fig. 17. Comparison of cortisol concentrations with exploratory behaviour

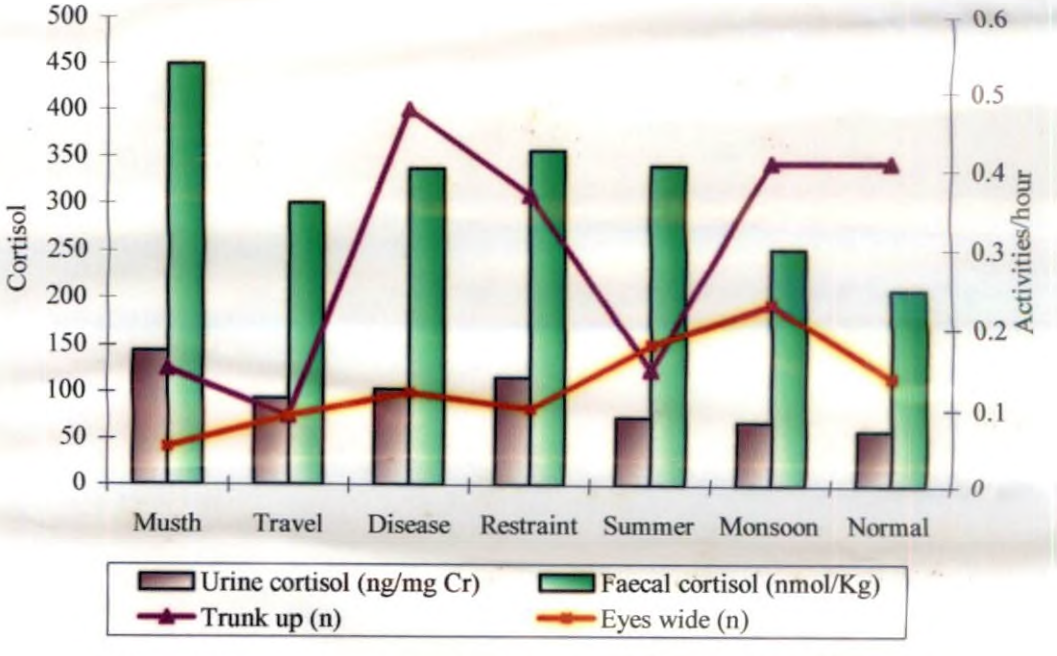

Fig. 18. Comparison of cortisol concentrations with exploratory behaviour

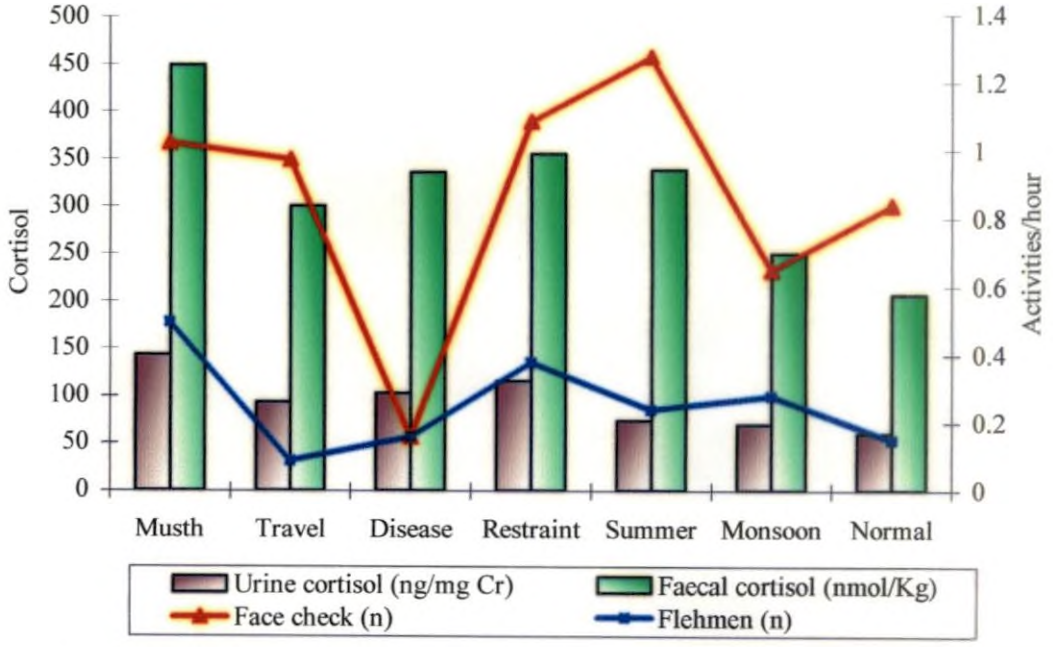

Fig. 19. Comparison of cortisol concentrations with investigatory behaviour

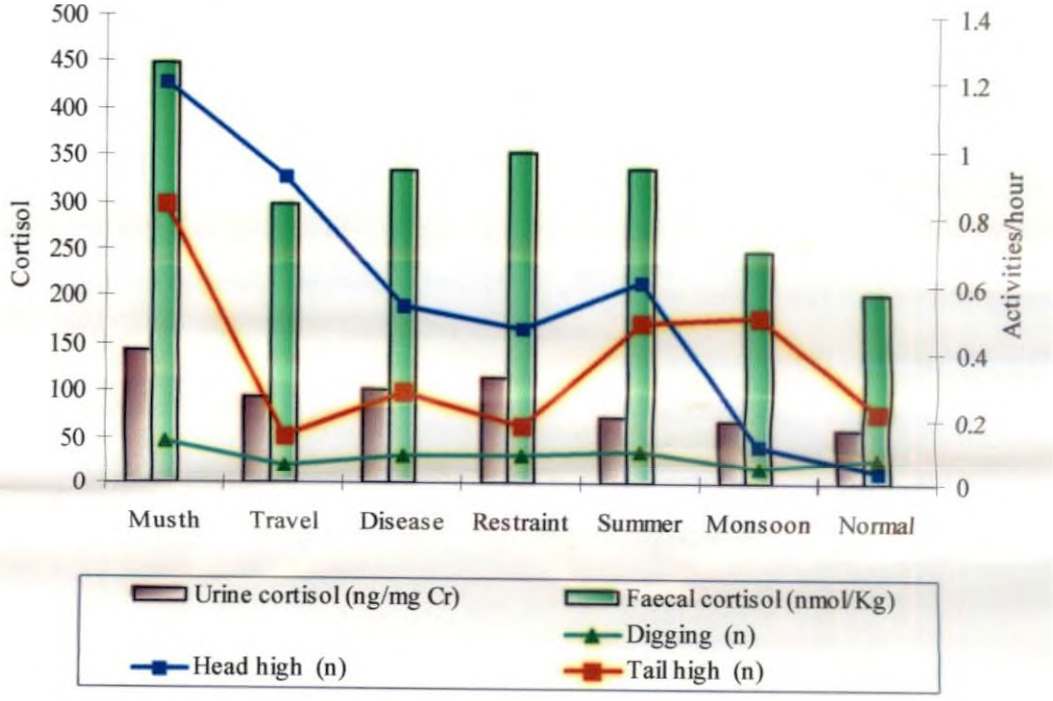

Fig. 20. Comparison of cortisol concentrations with agonistic behaviour

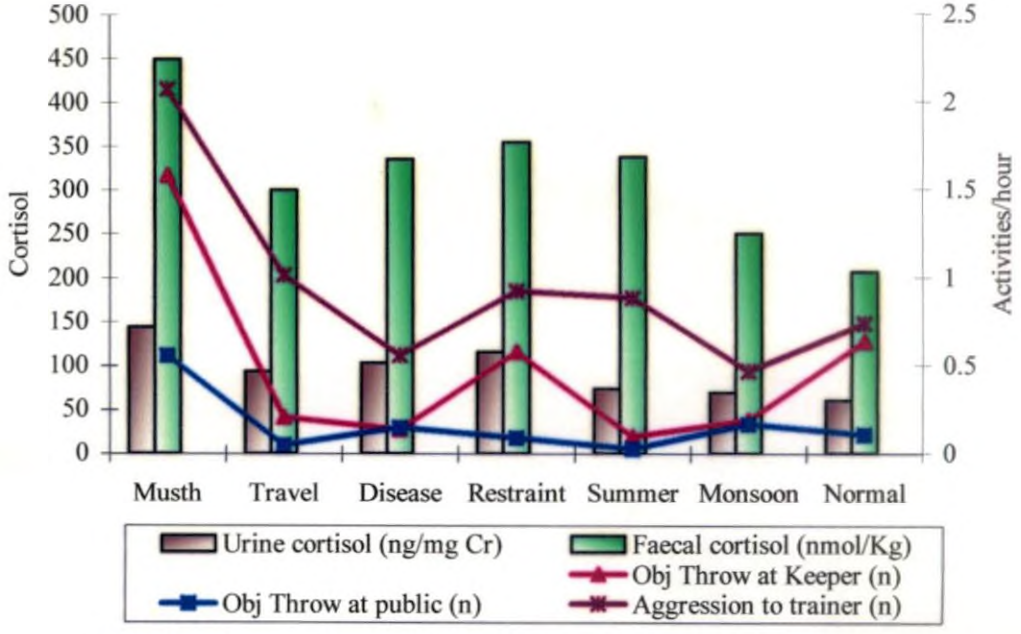

Fig. 21. Comparison of cortisol concentrations with aggressive behaviour

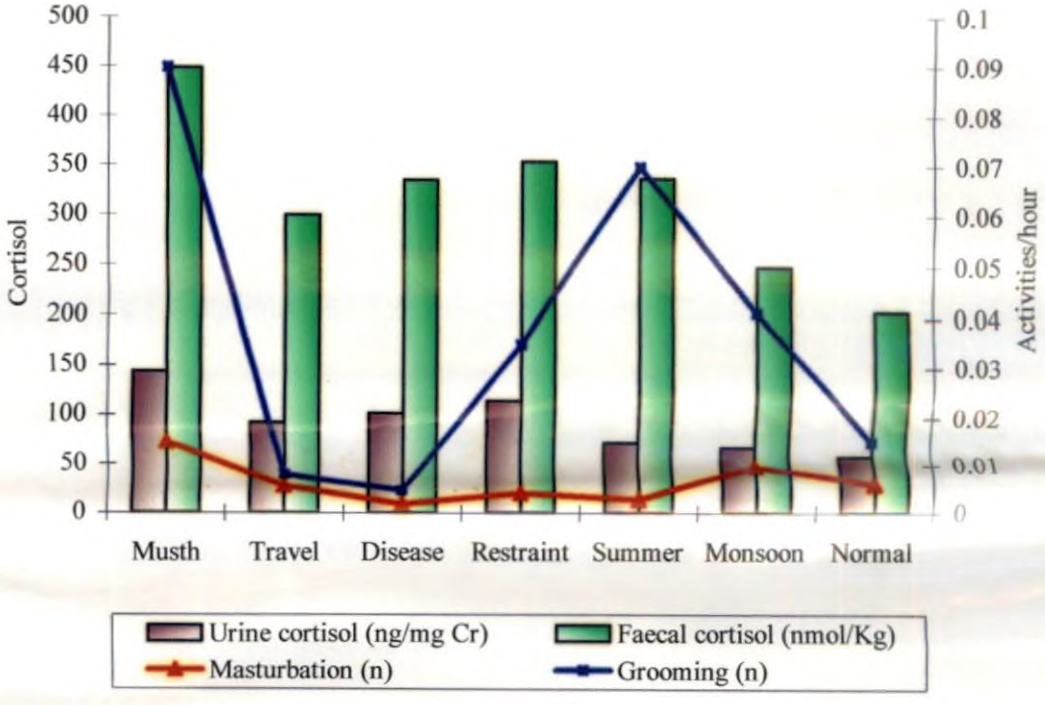

Fig. 22. Comparison of cortisol concentrations with self-directed behaviour

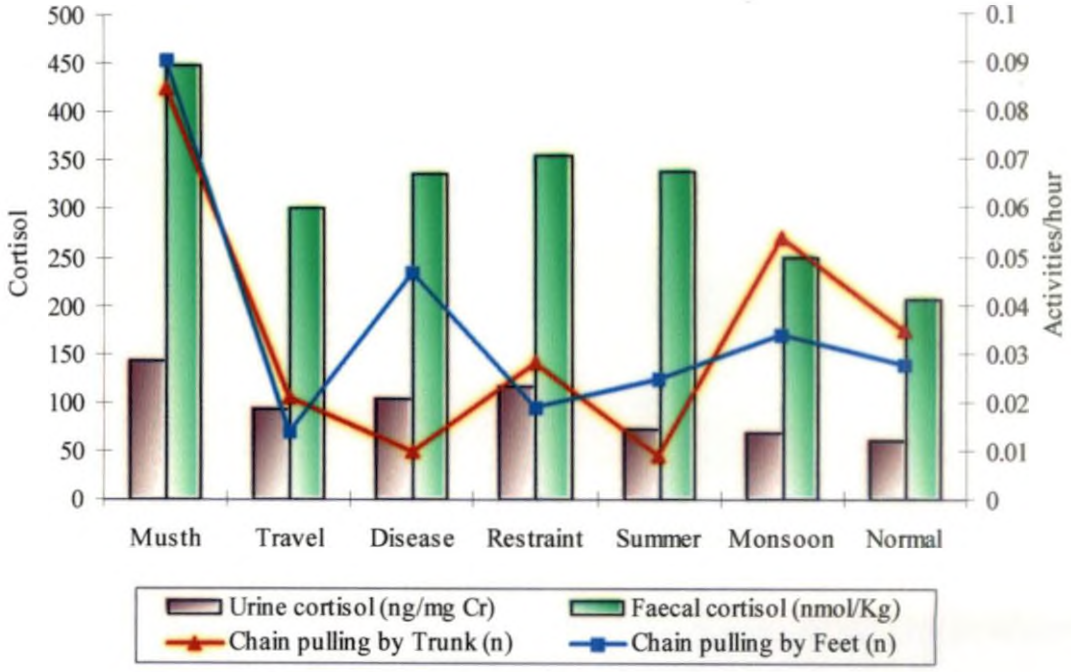

Fig. 23. Comparison of cortisol concentrations with comfort behaviour

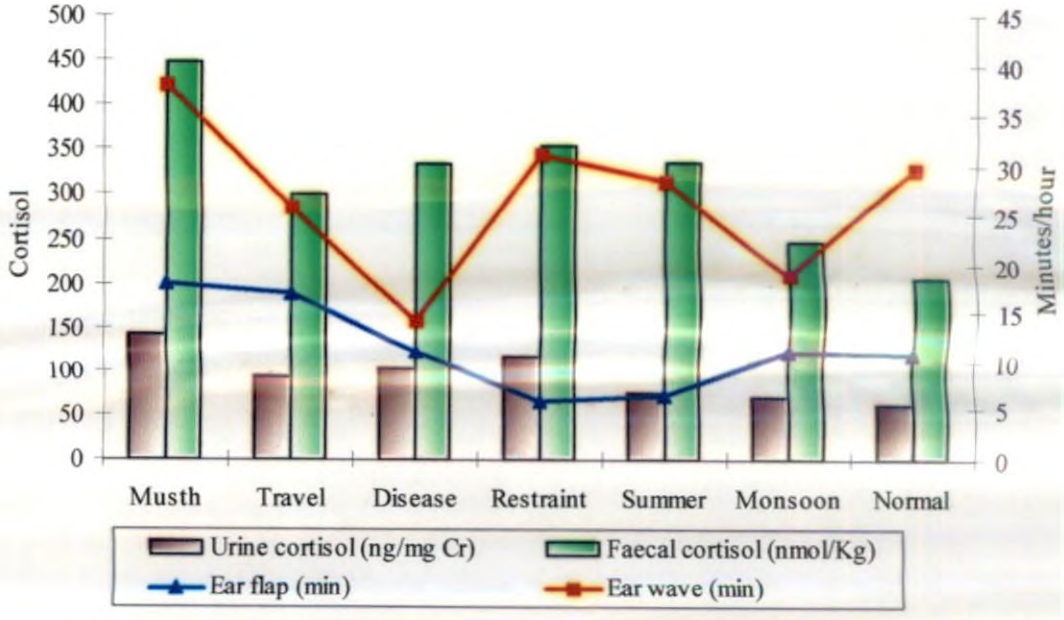

Fig. 24. Comparison of cortisol concentrations with comfort behaviour

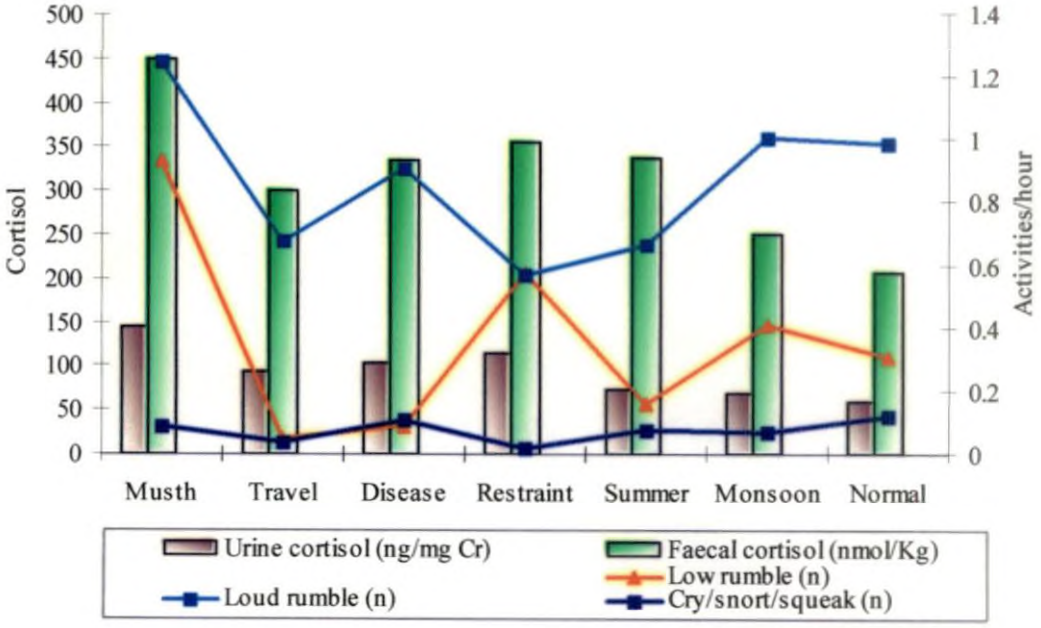

Fig. 25. Comparison of cortisol concentrations with vocalization behaviour

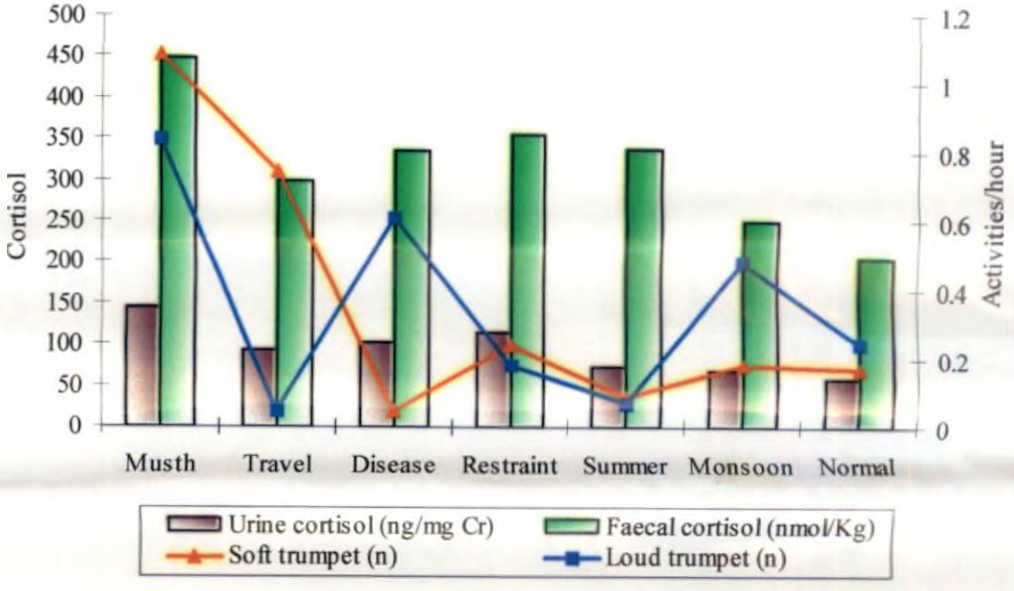

Fig. 26. Comparison of cortisol concentrations with trumpeting behaviour

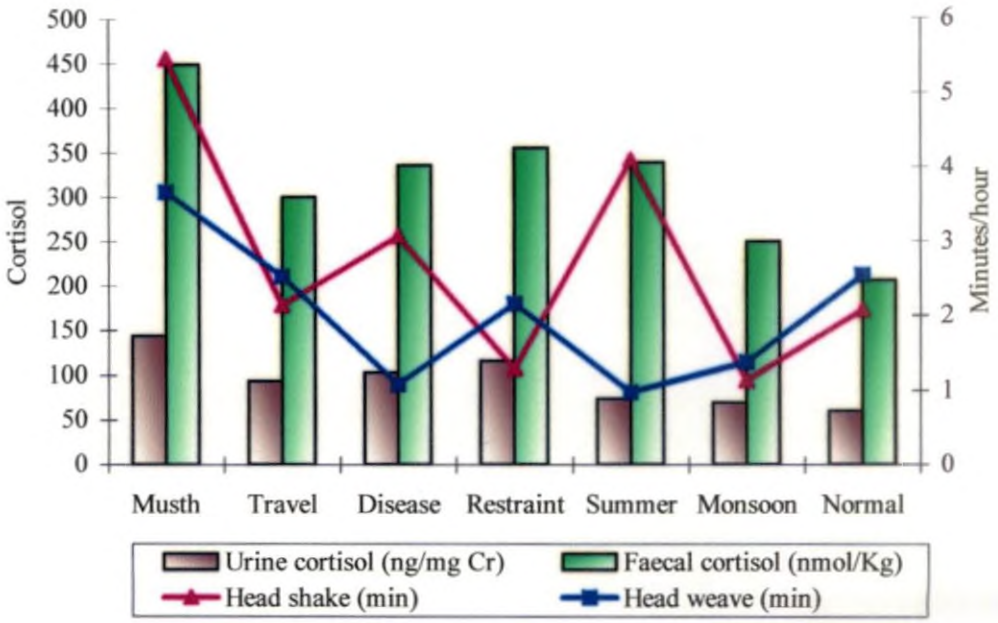

Fig. 27. Comparison of cortisol concentrations with stereotypic behaviour

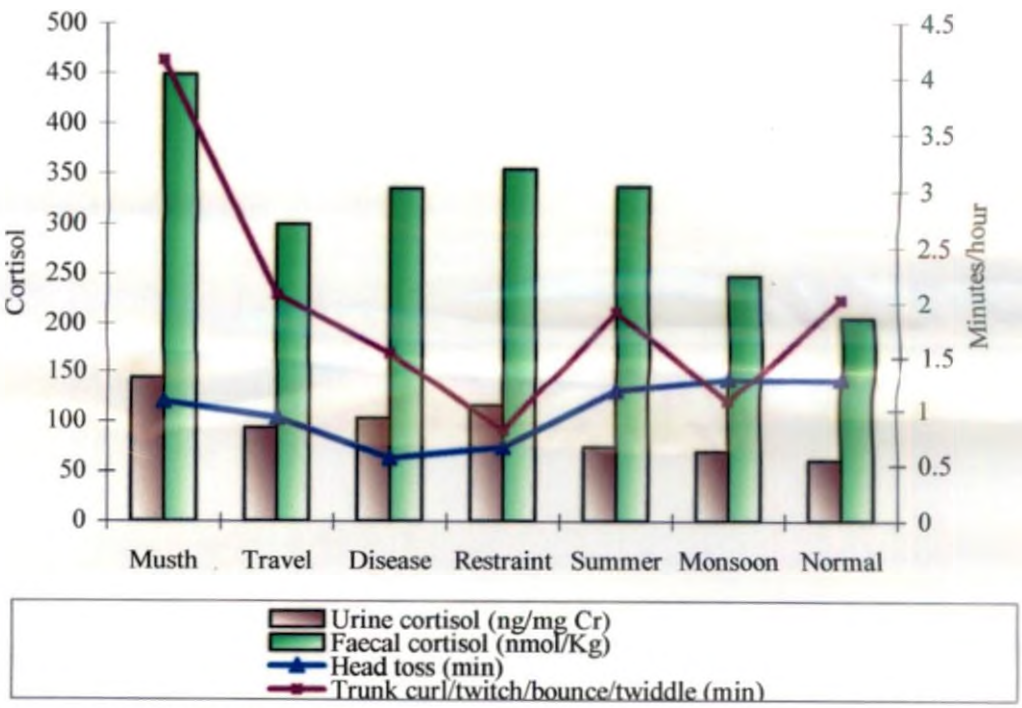

Fig. 28. Comparison of cortisol concentrations with stereotypic behaviour

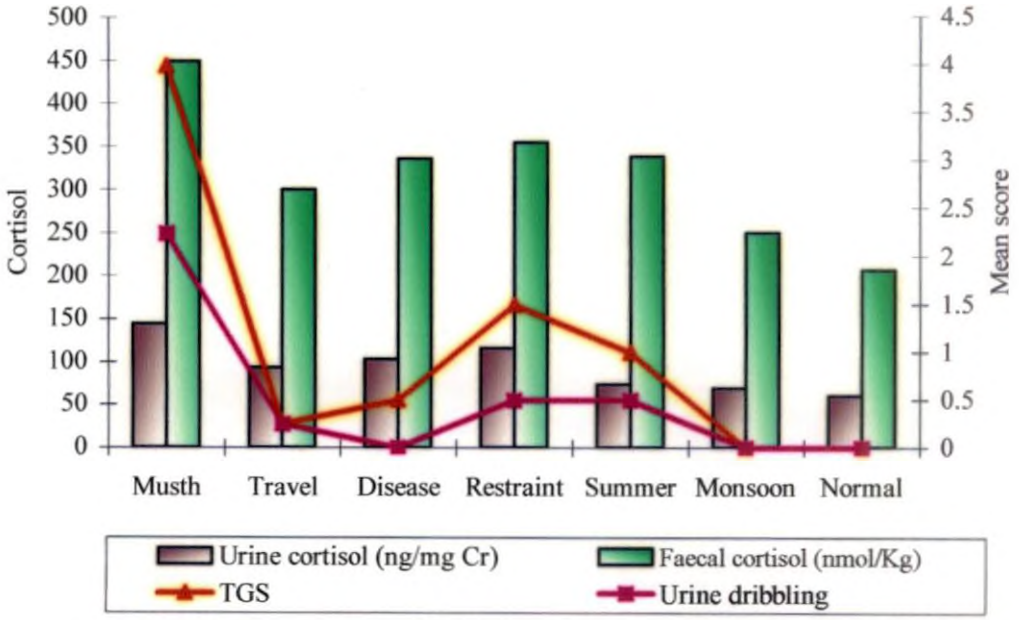

Fig. 29. Comparison of cortisol concentrations with temporal gland secretion and urine dribbling

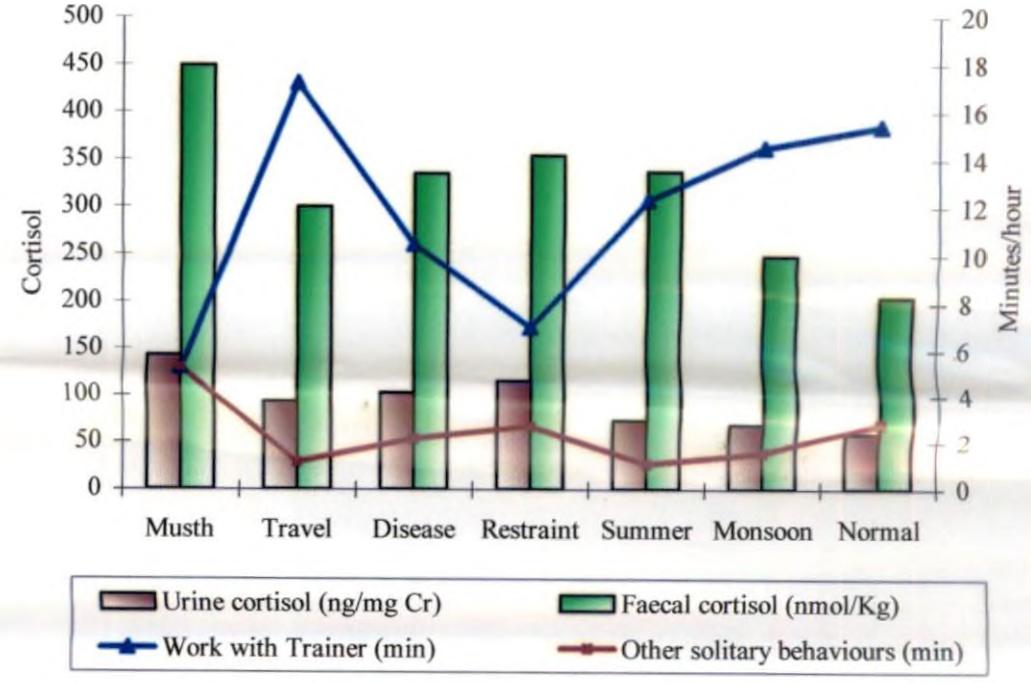

Fig. 30. Comparison of cortisol concentrations with other solitary behaviour

individual differences are not presented in the Table. Rectal temperature of the subjects in musth was not presented because of the difficulty in handling the animals.

4.5.2 Respiratory Rate

The mean respiratory rate was shown to be highest in the group IV with the morning, noon and evening readings showing 12.4 ± 0.8, 11.8 ± 0.58 and 11.6 ± 0.87 breaths/minute, respectively, while the lowest rates found in group VII with 07.2 ± 037, 09.4 ± 0.51 and 07.6 ± 0.51, respectively in the morning, noon and evening. The variations in the respiratory rates of other groups were listed in Table 19 and shown in Fig. 32. The respiratory rate of the subjects in musth was not presented because of the difficulty in collecting the values from the musth animals.

Table 18. Mean rectal temperature with standard errors of elephants in different groups

Mean rectal temperature (°C)			
	Morning	Noon	Evening
Group I	-	-	-
Group II	36.64 ± 0.05	36.62 ± 0.07	36.56 ± 0.06
Group III	36.76 ± 0.05	36.68 ± 0.04	36.62 ± 0.10
Group IV	36.66 ± 0.10	36.62 ± 0.05	36.68 ± 0.06
Group V	36.66 ± 0.05	36.74 ± 0.04	36.74 ± 0.04
Group VI	36.56 ± 0.06	36.60 ± 0.04	36.46 ± 0.02
Group VII	36.30 ± 0.07	36.48 ± 0.08	36.36 ± 0.13

Table 19. Mean respiratory rate with standard errors of elephants in different groups

Mean respiratory rate (breaths/minute)			
	Morning	Noon	Evening
Group I	-	-	-
Group II	11.0 ± 1.05	11.8 ± 0.80	10.4 ± 1.03
Group III	11.4 ± 1.03	11.6 ± 1.03	11.0 ± 0.95
Group IV	12.4 ± 0.81	11.8 ± 0.58	11.6 ± 0.87
Group V	10.4 ± 0.68	10.2 ± 0.58	10.4 ± 0.68
Group VI	08.6 ± 0.51	09.8 ± 0.37	09.6 ± 0.40
Group VII	07.2 ± 0.37	09.4 ± 0.51	07.6 ± 0.51

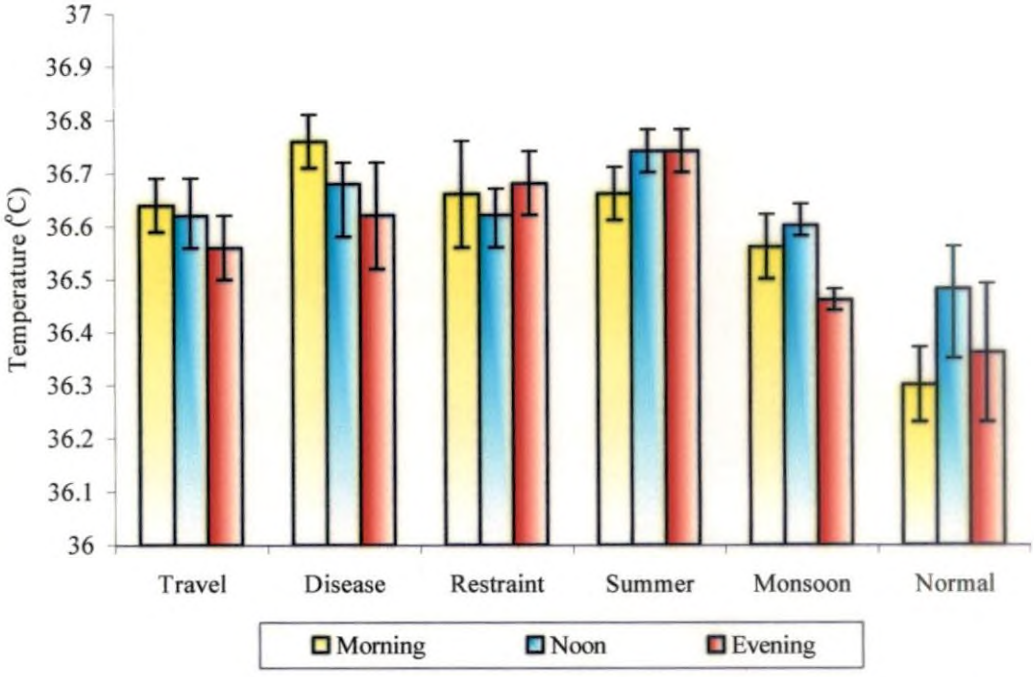

Fig. 31. Mean rectal temperature(°C) of elephants with standard errors under different groups

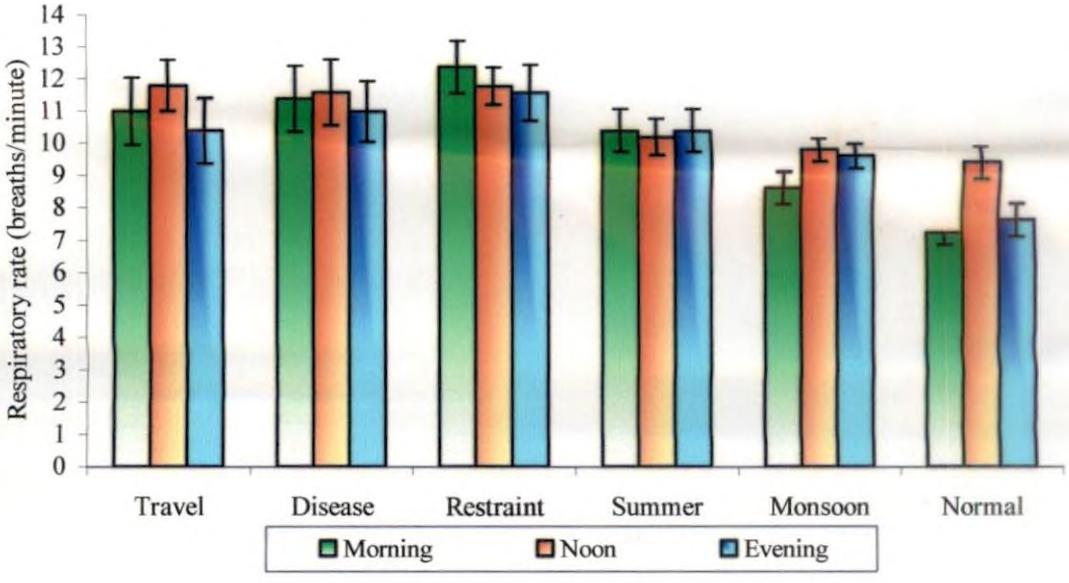

Fig. 32. Mean respiratory rate (breaths/minute) of elephants with standard errors under different groups

Discussion

5. DISCUSSION

The effect of different stressors on the changes in the behaviour and levels of the stress hormone cortisol in urine and faeces of captive male Asian elephants are discussed.

5.1 STRESS AND BEHAVIOURAL MEASURES

The present study showed a strong relation between stress and behaviour. These results are in accordance with Orchinik (1998), who observed that, modulation of behaviour is an integral component of stress response.

Behavioural observations in the present study were based on checklists used in a semi-natural environment. Observations of animal behaviour were done in a natural or semi-natural environment by notes, checklists of behaviour patterns, video recording, and/or some combination of these (Denberg and Banks, 1969; Stead, 2000).

Behavioural measures and sampling methods used for this work are akin to the methods used previously by Garaï (1997), Friend (1999), Schulte (2000), Stead (2000) and Scott (2002). Altmann (1974) and Martin and Bateson (1993) indicated that the individuals may differ enormously in their behaviour. These views were credible for the present study which showed variations in the behaviour between individuals.

Combining the behaviours into maintenance behaviour, exploratory/ investigatory behaviour, aggressive behaviour, self-directed behaviour, comfort behaviour, vocalizations, trumpeting, stereotypic behaviour and other solitary behaviours for observations during the present study is in agreement with Stead (2000).

Behavioural measures obtained from simple observations as an index of stress during the present study with measures including the intensity, duration and frequency of responses are in line with the views of Fraser and Broom (1990),

Broom and Johnson (1993) and Alados *et al.* (1996), who indicated that the complexity of behavioural patterns should be measured under situations of stress.

Several behaviours increased in frequency and duration in musth males and they tended to explore more of their yard area and spend more time investigating the area compared to when they were not in musth. These findings are in agreement with the findings of Scott (2002) who showed elevation in the performance of some behaviours during musth when compared to other stages, indicating the level of stress during musth.

5.1.1 Combined Behavioural Variables

5.1.1.1 Maintenance Behaviour

The elephants in normal group spent 52.6 per cent of the observed time feeding and drinking. These results were lower than the values of 79 per cent reported by Douglas-Hamilton and Douglas-Hamilton (1975) in wild elephants and 75 per cent by Stead (2000) in juvenile African elephants in captivity. Several authors (Wyatt and Eltringham, 1974; Guy, 1976) have also reported 66.7 per cent to 75 per cent of mean time spent in feeding. However these observations were carried out over periods of twenty four hours. But the results of 36 per cent and 57 per cent of mean feeding time during hot and wet weather respectively, and 46.5 per cent mean feeding by Stead (2000) by diurnal observations are more comparable to this study. Observations limited to diurnal periods and feeding at two predetermined times in a day, may be cited as the reasons for the lower values obtained during the present study. Time spent on feeding and drinking during musth, travel and disease respectively was found to be less compared to the mean value of control group, which indicated higher stress level in this group.

Increased frequency of dusting and mudding behaviours in group I, II and V during the present study might be attributed to the prolonged exposure to direct solar radiation. The skin of an elephant can be upto three centimeter thick and is highly sensitive to touch and temperature variation, and has been suggested that covering the skin with dust and mud provides protective layers, insulating it from the sun and reducing irritation by insect pests (Sikes, 1973). Stead (2000) suggested that the inability to dust is a factor that could contribute to stress, and that sand should be

made available to captive elephants. During summer and monsoon, elephants performed dusting and mudding activity at seven per cent and 2.1 per cent of the time respectively, and this exceeded the time observed by Guy (1976) in the hot and wet seasons (one and 4.3 per cent, respectively), while the results coincided with the 7.6 per cent observed by Stead (2000).

The elephants in musth showed decreased interest in food and spent the lowest time for feeding and drinking compared to the other groups, the findings were in congruence with the results of Scott (2002).

In the present study the elephants in normal group spent an average of 52.6 ± 19.4 per cent of the time on feeding, where as Friend (1999) stated that, elephants spent an average of 33 ± 1.2 per cent time for eating. The feeding time of elephants in disease condition were in line with the findings by Friend (1999), while the animals in other groups spent either more time or less time feeding depending on the level of stress.

5.1.1.2 Exploratory/Investigative Behaviour

During the present study, animals in disease and monsoon performed the exploratory behaviours with more frequency compared to other group. Stead (2000) opined that the frequency of investigative actions reflect the extent to which the animals have habituated to their environment and cannot be used as indicators of stress per se. Stress lead to a reduction in variability and complexity of exploratory behaviour (Alados *et al.*, 1996).

The frequency of performance of investigatory behaviour exhibited by the subjects in the present study supported the suggestion that elephants possess high levels of intelligence and memory for adaptive behaviours (Douglas-Hamilton and Douglas-Hamilton, 1975; Adams and Berg, 1980; Chevalier-Skolnikoff and Liska, 1993). Of all non-primate mammals, elephants have been cited to use tools with the highest frequency and diversity. These results suggest that exploratory behaviour is not an indicative of stress, but a repertoire of behaviours naturally exhibited by elephants (Adams and Berg, 1980; Chevalier-Skolnikoff and Liska, 1993; Wickler and Seibt, 1997).

The frequencies of manipulating objects by elephants in present study, which showed a maximum of 2.38 ± 0.43 minutes/hour during musth, are not directly comparable to the results of Chevalier-Skolnikoff and Liska (1993), who reported an average of 22.8 activities of tool use by elephants in captivity. The contradiction is due to the difference in the methods used for data collection in both studies.

The highest performance of flehmen response (0.5 ± 0.18 acts per hour) during musth in the present study is attributed to the pheromaonal stimuli from TGS and musth urine of the same elephant or neighboring elephants in musth, which are in line with the views of McKay (1973), Eisenberg *et al.,* (1971) and Saseendran (1994), who indicated that an elephant's trunk acts as a major tactile organ and carrier of olfactory information.

5.1.1.3 Aggressive/Agonistic Behaviour

The results of the present study indicated that the animals in musth exhibited more number of aggressive actions compared to the animals in other group is a direct indication of the intensity of stress experienced by the animals in musth and can be used to measure stress. The findings are in agreement with Jainudeen *et al.* (1972), Cooper *et al.* (1990), Saseendran (1994) and Scott (2002), who stated that musth in the male Asian elephant is characterized by elevated serum testosterone levels and increased aggression. Garaï (1997) cited that aggression, fear, insecurity, unfamiliar surroundings, loss of family could induce a stress response from an animal. Wait and Buchanan-Smith (2001) attributed that reduction in feeding time and changes in feeding schedule during musth lead to the rise in the rates of self-directed and aggressive behaviours and Stead (2000) indicated that head high behaviour, tail high behaviour and aggression can be used as an indicator of stress.

The throwing of objects by elephants towards humans with higher frequency in stressful situations compared to the normal group are contradictory to the reports by various authors (Wyatt and Eltringham, 1974; Douglas-Hamilton and Douglas-Hamilton, 1975; Stead, 2000) who could not observe any object throwing by captive elephants.

5.1.1.4 Self-directed Behaviour

Stead (2000) indicated that the absence of grooming facilities and hot weather in a captive environment contributed to the development of stress. Present results (0.090 ± 0.07 minutes per hour) contradicts the previous study, which showed more grooming time (0.94 minutes per hour) compared to the present study, the reason of which can be attributed to the inadequate availability of grooming tools for the subjects in the present study.

The very low frequency of masturbation noted during musth and in other events during the present study contradicts the results obtained by Saseendran (1994), who observed increased frequency of the masturbation activity in captive Asian elephants during the musth followed by pre-musth stages and no activity during non-musth stage.

5.1.1.5 Comfort Behaviour

Increased frequency of ear flapping and ear waving during musth in the present study may be attributed to a mode of thermoregulation (Benedict and Lee, 1938), and a mode of auditory communication to other elephants and aid in the dispersion of the chemical signals emitted from the temporal gland (Scott, 2002).

The frequency and time spent in performance of comfort behaviours during the present study correlated with the stressful situations and the results are in coherence with Gruber *et al.* (2000) who observed increase in comfort behaviours in chained elephants than in penned elephants.

5.1.1.6 Vocalizations

High frequencies of low and loud rumbles noticed in animals under musth during the present study are in accordance with Poole (1987).

In the present study low rumbles were more in group I, but there were no significant differences in the loud rumbles between all other groups. Poole *et al.* (1988) observed low rumbles by elephants in all types of situations and found to be most frequent type of vocalization used to convey a specific massage.

Vocalizations during musth and restraint indicated the intensity of stressful situations and these results are in similarity with Lay *et al.* (1992) and Grandin

(1997), who observed vocalization as a behavioural indicator of stress in cattle, and were correlated with increased heart rate and cortisol production.

5.1.1.7 Trumpeting

Increased trumpeting during musth, followed by restraint and disease indicated a higher level of emotional turmoil, such as aggression, frustration, fright etc. supports the assumptions by Garaï (1997), who stated that stressed animals vocalized more than less stressed animals.

5.1.1.8 Stereotypic Behaviour

The time spent in performance of stereotypic behaviours was highest during musth and restraint, while showing no significant difference between other groups of elephants under study. Scott (2002) stated that the stereotypic behaviours are indicator of stress and characteristic of individual animals. Performance of stereotypic behaviours may be attributed to boredom, inability to perform the species typical behaviours, sub-optimal environmental conditions and sub-optimal levels of psychological welfare (Mason, 1991; Marriner and Drickamer, 1994; Wiedenmayer and Tanner, 1995). Since stereotypies emerge from a situation where variation in the environment was absent, environmental enrichment could promote natural behaviours and prevent animal from acquiring stereotypies or performing stereotypies already developed (Broom, 1991; Gonyou; 1994; Schmidt, 1995; Fischbacher and Schmid, 1999). McBride and Cuddleford (2001) and Möstl and Palme (2002) also reported increased glucocorticoid production and stereotypies during stress. Increased stereotypic behaviours in circus elephants kept in solitary confinement in picket lines compared to paddocks was observed by Friend and Parker (1999).

The present study recorded highest incidence of weaving compared to other stereotypic behaviours are in line with the findings by Rushen *et al.* (1993) and Friend (1999), who indicated weaving as the single most common stereotypic behaviour observed in elephants, followed by head bobbing and trunk tossing. The increased frequency observed prior to feeding or watering suggests the effects of arousal and motivation.

Increased performance of stereotypies and other abnormal behaviours during the present study may be attributed to inadequacies in social organization and continuous restraint in captivity preventing the animals from performing the genetically acquired behaviour patterns. Similar views were conferred by Garaï (1997), who indicated that, a restriction in behavioural complexity and possibly even deprivation of certain emotional requisites, could lead to long lasting psychological deficiencies leading to abnormal behaviours.

5.1.1.9 Other Behaviours

The elephants in musth during the present study showed maximum score for both the temporal gland secretion and urine dribbling, with negligible score in other groups, are in accordance with the results obtained by Jainudeen *et al.* (1972), Cooper *et al.* (1990) and Saseendran (1994), who indicated that, secretions from the paired temporal scent glands on either side of the face and dribbling of urine from the penile prepuce are the characteristic signs of musth in Asian elephants. The reason for these behaviours may be attributed as a means of chemical communication and could be used as an honest signal to conspecifics, especially as only well-nourished males are capable of experiencing musth. Stead (2000) concluded that temporal gland secretion can be used in conjunction with a description of the context within which it takes place as an indicator of stress.

The temporal glands secreted under conditions of stress, fear and excitement was considered as the only recorded visible sign of extreme stress in elephants under musth (Buss *et al., 1976*; Adams *et al., 1978*). Schulte (2000) has also conferred to the similar findings that animals in musth had continuous urine dribbling and an increased secretion of thick fluid from the temporal gland.

There were no citations to support the mean time spent performing work with the trainer and other solitary behaviours.

5.2 STRESS, CORTISOL AND ANIMAL WELFARE

Elevated faecal and urine cortisol secretion after stress observed in the present study was in accordance with the findings of Moberg (1985, 1990) and Wielebnowski (2002).

The application of the faecal glucocorticoid measurement to assess welfare during the present study showed good correlation between behavioural observations, environmental stressors and faecal glucocorticoid metabolite concentrations, which is akin to the findings by Stead *et al.* (2000). Similar views were conferred by Palme *et al.* (2000), Grandin (1997) in cattle and Molony and Kent (1997) in lambs, who stated that, a combination of both physiological and behavioural measures for the assessment of stress and discomfort should be taken into account.

Increased cortisol concentrations after stress during the present study stresses the importance of environmental enrichment for the animals in view with the results of Markowitz *et al.* (1978), Wilson (1982), Tripp (1985) and Carlstead *et al.* (1993) who suggested environmental enrichment for better welfare and it reduced cortisol concentrations in the animals under stress.

Use of faecal and urine cortisol metabolites to assess stress, is in accordance with Brown *et al.* (1995), Wasser *et al.* (1996) and Stead *et al.* (2000) who found that the concentrations of faecal and urine cortisol metabolites for non-invasive monitoring of adrenocortical activity in elephants could help optimize the capture, transport, husbandry of elephants and be useful in investigating stress in free-ranging situations.

5.3 URINE AND FAECAL GLUCOCORTICOID MEASURES

Measurement of glucocorticoids, such as cortisol, using non-invasive methods in this study as a potential physiological indicator of stress, is in agreement with the views of Moberg (1985), Graham and Brown (1996) and Monfort *et al.* (1998), who indicated that, analysis of excreted corticoid metabolites in the urine and faeces is often found to be a better indicator of stress and offered advantages of safety and ease of sample collection, and the data generated were comparable to the circulating hormonal profiles.

Wide variation in faecal and urine cortisol responses of the individual subjects within a group observed during the present study may be due to the reasons suggested by Kent *et al.* (1993) and Dinnis *et al.* (1997), who found that variation in cortisol responses to treatment is common and this variability rises our capacity to

detect small, between-group differences and diminishes the numbers of animals required in each group.

Collection of urine by midstream catch, additional processing steps in the analysis of creatinine to account for variation in fluid intake and methods used for collection and processing of faecal samples during the present study are in accordance with the findings of Brown and Lehnhardt (1995) and Brown (2000), who used similar techniques to measure endocrine status in elephants. Brown (2000) also indicated that, even though faecal samples are easier to collect without disturbing the subject, analyses are hampered by a more laborious and expensive sample preparation process and the lack of a suitable index (such as creatinine) to standardize results, but the best results for faeces were obtained when samples obtained were dried and faecal powder is separated from fibrous material.

An elevated faecal and urine glucocorticoid level in elephants has been observed after the stressful events during the present study, which is in agreement with the findings of Dathe *et al.* (1992), Brown *et al.* (1995) and Morrow *et al.* (2000) after ACTH challenge test.

The mean basal urine cortisol value of 59.98 ± 8.45 ng/mg Cr obtained during the present study is in accordance with the concentrations of urinary cortisol values obtained by Brown (2000) in three Asian elephants, 15.2 ± 1.6 ng/mg Cr, 51.5 ± 4.4 ng/mg Cr and 46.5 ± 0.9 ng/mg Cr and the values varied considerably, throughout the day.

5.3.1 Musth and Cortisol

The results of the present study which showed highest mean faecal cortisol metabolites of 449.02 ± 86.31 nmol/kg during musth compared to the basal values is in consonance with the results of Schwarzenberger *et al.* (2000) in Asian and African elephants, who observed an elevation in the level of faecal cortisol metabolites during musth in males of both species indicating that musth is the most stressful event.

Increased faecal and urinary corticosteroid excretions during aggressive interactions while the animal was restrained or in musth are in congruence with the findings of Wallner *et al.* (1999), who demonstrated that aggressive interactions

produced prolonged physiological changes in individuals, which can be monitored in the excreted steroids in Barbary macaques.

A good correlation of increased frequency of stereotypic and aggressive behaviour with concurrent increase in excreted cortisol concentrations during musth is in accordance with the findings by Schulte (1999) in elephants, which showed that regular hormonal assessment and correlating it with behavioural measures are important to understand the phenomenon of musth in male elephants.

5.3.2 Travel/Transport and Cortisol

The large increase in mean value of 11,17-DOA after travel (300.14 ± 50.71 nmol/kg) from the basal value (206.05 ± 50.54 nmol/kg) in the present study showed that, travel is a stressful event and the results are in similarity with the observations on ponies by Möstl *et al.,* (1999) and in cattle by Palme *et al.* (2000) after transport

Elevated faecal cortisol metabolites after transport observed in the present study may be due to the stress arising from transport. Similar results were obtained by Tarrant (1990), Palme *et al.* (2000), Schwarzenberger *et al.* (2000), Denhard *et al.* (2001) and Möstl *et al.* (2002) who indicated an increase in the concentrations of the faecal cortisol metabolites which occured about 12 hours after transport and was mostly due to confinement.

5.3.3 Disease and Cortisol

Increase in the mean faecal cortisol metabolites during disease conditions (335.52 ± 56.81 nmol/kg) from the basal value (206.05 ± 50.54 nmol/kg) during the present study are in accordance with Nelson and Demas (1996) who observed increased faecal cortisol metabolites in elephants which suffered parasitic load and it may also provide useful indices of immunosuppression in elephants under stress.

Increased mean urinary cortisol value of 102.86 ± 30.07 ng/mg Cr from the basal value of 59.98 ± 8.45 ng/mg Cr in diseased conditions observed during the present study. The finding is in accordance with Miller *et al.* (1991b), who reported elevated urinary cortisol values in bighorn sheep during an outbreak of pasteurellosis.

The findings of the present study indicated that, the disease level is of considerable importance in welfare assessment of animals, which should be correlated with adrenal activity and behavioural measures. Similar views were conferred from the results obtained by Broom (1991).

5.3.4 Restraint/Handling and Cortisol

Restraint and handling of elephants during the present study significantly elevated the faecal cortisol levels (354.62 ± 72.07 nmol/kg) from the mean basal levels (206.05 ± 50.44 nmol/kg), suggesting that handling affected the levels of cortisol. Stead *et al.* (2000) also observed that cortisol levels in faeces began to rise before ACTH was injected. These findings are in accordance with Fulkerson and Jamison (1982), Sire *et al.* (1995) and Schatz and Palme (2001), who reported that physical restraint can produce an increase of faecal cortisol levels within 15 minutes of handling.

Increased urinary cortisol concentrations observed during handling and restraint (115.62 ± 20.65 ng/mg Cr) from the basal values (59.98 ± 8.45 ng/mg Cr) of the subjects in the present study, are similar with the results of increased urine cortisol levels observed in macaques exposed to restraint and tethering (Crockett *et al.,* 1993), captive non-domestic felids translocated to new habitats (Carlstead *et al.,* 1992) and domestic sheep subjected to confinement (Berman *et al.,* 1980).

Increase in cortisol concentrations during restraint/handling in the present study is in congruence with the findings by Möstl and Palme (2002) and Cook *et al.* (2000).

Morrow *et al.* (2000) observed decrease in urine cortisol four to seven days after an imposed stressor suggested a compensatory decrease in cortisol production as the animal adapted to the new situation and same reasons may be attributed to the decreased cortisol values in some subjects due to compensatory adaptation. Similar results were obtained by Ladewig and Smidt (1980) in response to tethering and restraint in cattle and horses (Merl *et al* 2000).

The present study which accounted for measuring both physiological and behavioural measures to assess stress are in accordance with the results obtained by Grandin (1997), who indicated that restraint and handling are very strong

psychological stressors in cattle, and to accurately assess the animals reaction, a combination of behavioural and physiological measurements will provide the best overall welfare of the animal and found that the cortisol levels were elevated in restrained cattle.

5.3.5 Season and Cortisol

The cortisol metabolite concentrations in faeces during the present study were significantly correlated with socioecological pressure such as season, with high concentrations in the dry season compared to the monsoon season and basal values. These findings are in agreement with the findings of Foley *et al.* (2001) in African elephants, Cavigelli (1999) in ring-tailed lemurs and Kotrschal *et al.* (2000) in geese. They also indicated that higher variability of faecal metabolite values within and between individuals necessitates considerable sample size and attributed the reasons for individual variations to be the retention time of gut contents.

Seasonal differences in the faecal glucocortcoid concentrations noted in the present study are analogous with the results obtained by Millspaugh *et al.* (2001) in elk deer, who indicate that the data from their study suggested seasonal variability in faecal glucocortcoid excretion.

5.4 OTHER PHYSIOLOGICAL MEASURES

5.4.1 Rectal Temperature

Animals in group V which was directly exposed to the solar radiation has the highest mean rectal temperature, which is similar to the findings by Nair (1996) who observed increased rectal temperature in elephants under direct solar radiation after three hours of continuous work. The increase in rectal temperature with in the normal range of elephant's body temperature of 36^0C to 37^0C during the present study confers with views of Benedict (1936) who indicated that the temperature within this range do not affect the animal and this cannot be considered as a significant sign of stress. The results were also in line with Toscano *et al.* (2001) who observed that the temperatures during transport, summer and monsoon season were within the range that the healthy elephants have the physiological and behavioural ability with which to cope. They also indicated that elevation in body temperature of two to three degrees is not considered a problem in elephants.

5.4.2 Respiratory Rate

The respiratory rate was found to be highest in group IV followed by group V indicating that restraint and summer are the most stressful events. Elephants in group II showed increased respiratory rate compared to group VII, which is supported by Nair (1996), who indicated that elephants showed a gradual rise in respiration rate as the duration of work increased, however there were no citations to support the results from the other groups.

Grandin (1997) showed an increase respiratory rate in cattle during restraint and transport and Broom (1991) showed increased respiratory rate during diseased conditions and they correlated positively with behavioural and physiological measures. Their findings contradict the results of the present study, which do not show any significant correlation between respiratory rates and the physiological and behavioural measures.

Summary

6. SUMMARY

The care and management of elephants in captivity has become an intricate combination of art and science and, the importance of furthering scientific research for the benefits of elephants is obvious. The present study using non-invasive methods to assess stress in captive elephants was undertaken to evolve management plans to optimize conditions for behaviour and health, thus ensuring the well being of elephants maintained in captivity.

The present study was conducted in captive male Asian elephants (*Elephas maximus*) at the elephant camp in Punnathur Kotta under the Guruvayoor Devaswom Temple during the period from July 2002 to July 2003. The objective of the study was to measure the behavioural responses in captive male Asian elephants to specified stressors. The animals were divided into seven groups, group I (musth), group II (travel), group III (disease), group IV (restraint), group V (summer), group VI (rainy season) and group VII (normal). A total of thirty two hours of behavioural data were collected from four animals in each group, with eight hours of observation on each animal and the data were converted into one hour average performance. Twenty samples of urine and ten samples of faeces were collected from each group to determine the concentration of cortisol metabolites and urine creatinine concentrations. The creatinine concentrations of urine were measured to counteract the difference in the fluid intake by individual animals. The urine and faecal samples were collected and stored at - 20°C until it was processed and analyzed for measurement of cortisol metabolites by radioimmunoassay technique and urine creatinine concentrations by enzyme immunoassay technique using standard procedures. Other physiological measures like rectal temperature and respiratory rates were also recorded from each group.

The behavioural variables measured to indicate the levels of stress were combined to form grouped variables for the ease of statistical analyses and recorded by using focal-animal sampling technique. The behavioural data were converted to activities performed per hour and the mean value analyzed statistically. The

behavioural variables were expressed as activities performed per hour (frequency) or mean time spent performing per hour (event) depending on the type of the variables.

Mean performance of maintenance behaviour and their statistical significance ($P<0.05$) were analyzed to measure the activities indicative of stress. It was found that mean time spent feeding was highest in group VI with 41.40 ± 4.28 minutes/hour and lowest in group I with 16.28 ± 3.48 minutes/hour, indicating that the animals feed less during more stressful situations. Other maintenance behaviours like dusting/mudding, spraying water/saliva and blow body activities, although showed variations in their performances were not statistically significant variables indicative of stress.

The performances of agonistic/aggressive actions like digging have not shown any significant between them while the tail high activity was more in group I with a mean performance of 0.84 ± 0.31 activities per hour, but the other groups do not differ significantly between them. Other activities like head high, object throwing and aggression towards trainer were significantly higher in group I compared to the normal group, while the performances in other groups showed non-significant relation between groups.

Performance self-directed behaviours like masturbation do not differ significantly between groups, while grooming was found to be highest in group I while showing non-significant difference between groups. These behaviours cannot be accounted as behaviours indicative of stress.

Mean time performing the comfort behaviour, chain pulling by feet does not show any significant difference between groups, indicative of the discomfort to the animals due to prolonged tethering irrespective of the stressors. Other comfort behaviours like chain pulling by trunk/tusk, ear flap and ear wave was performed with highest frequency in group I with the mean values of 0.085 ± 0.01, 18.12 ± 5.39 and 38.10 ± 2.18 activities per hour respectively, while other groups showed non-significant ($P<0.05$) difference between them and can be considered as the measures of stress.

Mean number of low rumbles and loud rumbles performed were highest in group I with 0.94 ± 0.27, 1.25 ± 0.41activities per hour, respectively. Low rumbles

between other groups differ significantly, while loud rumble showed non-significant difference between them. Other vocalization like cry/snort/squeak was insignificant to be used as a measure of stress. Trumpeting behaviour occurred more frequently in group I when compared to other groups, while there was significant difference ($P<0.05$) between groups. Trumpeting always was accompanied by aggressive behaviour. Vocalizations cannot be used as a reliable indicator of stress because of their infrequent performances.

Head related stereotypic behaviours like head tossing, head weaving and head shaking was performed frequently in group I with a mean time of 1.08 ± 0.15, 3.68 ± 1.20 and 5.47 ± 1.74 minutes per hour, respectively and the other groups differed non-significantly between them. Trunk related stereotypies were highest during musth at 4.18 ± 1.87 minutes per hour, while the other groups do not differ significantly between them. Stereotypic behaviours are significant indicators of stress since stereotypies are performed due to boredom, inability in performing the species typical behaviours, sub-optimal environmental conditions and suboptimal levels of psychological welfare.

The score obtained for temporal gland secretion and urine dribbling was highest for group I with a mean score of 4 ± 0.82 and 2.25 ± 0.96, while other groups was significantly lower that group I. Temporal gland secretion and urine dribbling can be used in conjunction with a description of the context within which it takes place as an indicator of stress. Performance of work with trainer and other solitary behaviours are insignificant to measure as an indicator of stress.

On analyzing the urine cortisol concentrations it was found that the highest mean cortisol concentration of 143.60 ± 30.07 ng/mg Cr in group I followed by group IV, III, II, V, VI and VII with mean cortisol values of 115.62 ± 20.65 ng/mg Cr, 102.86 ± 22.44 ng/mg Cr, 93.07 ± 19.78 ng/mg Cr, 73.55 ± 11.95 ng/mg Cr, 69.13 ± 11.63 ng/mg Cr and 59.98 ± 8.45 ng/mg Cr, respectively indicating the level of stress in each group compared with the group VII considered as normal.

Mean urinary creatinine concentrations of the subjects in each group showed the mean values of 0.101 ± 0.012 mg/ml, 0.111 ± 0.011mg/ml, 0.094 ± 0.014 mg/ml, 0.106 ± 0.016 mg/ml, 0.211 ± 0.023 mg/ml, 0.111 ± 0.014 mg/ml and 0.109 ± 0.012

mg/ml in group I, II, III, IV, V, VI and VII, respectively. Urine creatinine values did not show any significant difference except group V, indicative of the individual variations in the fluid intake by animals irrespective of the stressful events.

Since cortisol is as such absent in the faeces of elephants, a group of cortisol metabolites called 11,17-dioxoandrostanes (11,17-DOA) were measured in the elephants to document the level of stress in each group. Mean faecal cortisol metabolite concentrations were found to be highest in group I with a mean vale of 449.02 ± 86.31nmol/Kg followed by 354.62 ± 72.07 nmol/kg in group IV, 337.85 ± 41.94 nmol/kg in group V, 335.52 ± 56.81 nmol/kg in group III, 300.14 ± 50.7 nmol/kg in group II, 249.60 ± 44.31 nmol/kg in group VI and 206.05 ± 50.44 nmol/kg in group VII, respectively differing significantly between groups ($P<0.05$) indicating the intensity of stress each group when compared with the values in groupVII.

On comparing the mean urine cortisol values and mean faecal cortisol metabolite values in all the groups, it was found that the mean values in all the stressful events and in normal group followed a similar trend indicating the reliability of the techniques for non -invasive monitoring of stress in elephants using the urine and faecal cortisol values.

The mean rectal temperature and respiratory rate do not show any significant variation from the normal group irrespective of the intensity of stressors, which shows non-significant difference between them. These values were within the range and indicate that the healthy elephants have the physiological and behavioural ability with which to cope with varying conditions of stress.

It could be summarized that behavioural measures, urine cortisol values and faecal cortisol metabolite values can be used as a reliable feed-back free non-invasive method to monitor stress and welfare in captive elephants. The results suggest that integrating the endocrine assessment, such as cortisol levels in the urine and faeces, with other evaluations based on behaviour could potentially provide a more meaningful measure of stress in captive living male Asian elephants.

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* Originals not consulted

APPENDIX Ia

DATA COLLECTION SHEET

Date: Time: Location: Weather: Name:

Time	fd	dm	ws/db	moe	t,f,e	fl	agt	tt,tp	dht	mas	gr	cf,ct	ef/w	voc	tru	strh	strt	tgs	ud	wt	os
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APPENDIX Ib

BEHAVIOUR ABBREVIATIONS FOR DATA COLLECTION SHEET

fd	-	Feed/Drink
dm	-	Dusting/Mudding
ws/db	-	Spray water/saliva and blowing body
moe	-	Manipulate object or environment
t, f, e	-	Trunk up, face check and eyes wide
fl	-	Flehmen
agt	-	Aggression to trainer
tt. tp	-	Object throwing at public and object throwing at keeper
dht	-	Digging, head high and tail high
mas	-	Masturbation/Penis jerking
gr	-	Grooming
cf.ct	-	Chain pulling by trunk/tusk and chain pulling by foot
ef/w	-	Ear flapping and ear waving
voc	-	Vocalization, low rumble, loud rumble, cry, snort and squeak
tru	-	Trumpeting, loud trumpet and soft trumpet
strh	-	Head toss, head sway and body sway
strt	-	Trunk curl, trunk twitch, trunk bounce and trunk twiddle
tgs	-	Temporal gland secretion
ud	-	Urine dribbling
wt	-	Work with trainer
os	-	Other solitary behaviours

BEHAVIOURAL RESPONSES IN CAPTIVE MALE ASIAN ELEPHANTS (*Elephas maximus*) TO SPECIFIED STRESSORS

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ABSTRACT

Assessing the welfare status of captive male Asian elephants is a growing area of concern, as ethical questions are raised about the psychological well-being of animals maintained in captivity. The study was undertaken as a synthesis of approach to measure stress through physiological and behavioural studies to quantify the welfare and ensure the well-being of elephants in captivity. Captive male elephants maintained at the "elephant camp" in Punnathur Kotta under the Guruvayoor Devaswom Board were utilized for the present study. The animals were divided into seven groups: group I (musth), group II (travel), group III (disease), group IV (restraint), group V (summer), group VI (monsoon) and group VII (normal). All the behavioural and physiological data were collected from the individual animals in the above mentioned groups.

A total of forty two behaviours grouped into combined variables, were measured by focal-animal sampling technique using a behaviour check sheet to identify the behaviours indicative of stress. A total of thirty two hours of behavioural data were collected from four animals in each group, with eight hours of observation on each animal. The data were then analyzed using non-parametric tests to determine the significance of stress related behaviours. Most of the behaviours were performed with higher significance in group I followed by other groups in conjunction with a description of the context within which it takes place as an indicator of stress. The results indicate that musth is the single most stressful event. The prominent stress related behaviours identified during the present study were stereotypes, temporal gland secretion and aggression. The frequency or intensity of performance of other behaviours was in line with the severity of the stressful event. A good correlation between the cortisol values and behaviour scores indicates that both physiological and behavioural measures can be used in conjunction as potential non-invasive methods to assess stress. Other physiological measures like rectal temperature and respiratory rate could not be used as a measure of stress, since the healthy subjects have the physiological and behavioural ability to cope with varying conditions of stress to maintain the normal homeostasis.

Urine and faecal samples collected from animals in each group were stored at -20°C until it was processed and analyzed for measurement of cortisol metabolites by radioimmunoassay technique and urine creatinine concentrations by enzyme immunoassay technique using standard procedures. Urine creatinine concentrations were analyzed to counteract the variations in fluid intake by individual animals. Mean urine cortisol concentrations were found to be highest during musth (143.60 ± 30.07 ng/mg Cr) followed by restraint (115.62 ± 20.65 ng/mg Cr), disease (102.86 ± 22.44 ng/mg Cr), travel (93.07 ± 19.78 ng/mg Cr), summer (73.55 ± 11.95 ng/mg Cr) and monsoon (69.13 ± 11.63 ng/mg Cr) when compared to the mean values in normal group (59.98 ± 8.45 ng/mg Cr), indicative of the level of stress in each group. Similarly mean faecal glucocorticoid metabolite concentrations (11,17-dioxoandrostanes) were highest during musth (449.02 ± 86.31 nmol/kg) followed by restraint (354.62 ± 72.07 nmol/kg), summer (337.85 ± 41.94 nmol/kg), disease (335.52 ± 56.81 nmol/kg), travel (300.14 ± 50.71 nmol/kg) and monsoon (249.60 ± 44.31 nmol/kg). Both the urine cortisol concentrations and faecal cortisol metabolite concentrations followed a similar trend in the values indicating that, both the methods can be used as a measure for reliable indicator of stress. The results suggest that, musth is the most stressful event and monsoon season the least stressful event.

The results of the present study suggest that, integrating the endocrine assessment, such as cortisol levels in the urine and faeces, with other evaluations based on behaviour could potentially provide a more meaningful measure of stress in captive living male Asian elephants and can help resolve the managemental problems. The study underlines the feasibility of measuring faecal and urine cortisol metabolites combined with behavioural measure as a non-invasive approach, to answer questions such as animal welfare and stress in captive elephants.