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Ph.D. Thesis:

Circadian rhythmicity of captive eland in temperate zone – the influence of environmental stimuli to behaviour and heart rate.

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Declaration

I do hereby declare that I have written Ph.D. Thesis alone under supervision of Assoc. Prof. MVDr. Alica Kočišová, CSc. and Radim Kotrba, Ph.D. I used only literature sources listed in the cited literature. I agree with display of this Ph.D. Thesis for the public in the library at the Czech University of Life Sciences in Prague, Czech Republic.

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1. INTRODUCTION

The physical environment of life is characterised by several major periodicities that derive from the motions of the earth and the moon relative to sun. From its origin some billions of years ago, life has had to cope with pronounced daily and annual cycles of light and temperature (Pittendrigh, 1981). The rhythmic behaviour of organisms in their natural habitats is surely one of the most obvious features of any ecosystem. Therefore, biological cycles of different durations can be found in most of living organisms with the most evident among them are the daily rhythms. Daily variations in the behaviour of organisms must have been among the earliest of biological observations of primitive man (Menaker, 1969). Many organisms have evolved biological clocks to time their metabolic processes rather than simply responding to the daily light-dark cycle transitions. Circadian (from "circa" about; "dies" – a day) clocks are among these biological clocks, and they time biological events that occur per 24 hours (Sharma, 2003). As a part of evolutionary adaptation of a species, behavioural rhythms play a major role in the ecological relations of a species (Aschoff, 1958). It is assumed that the adaptive value of the circadian clocks in periodic environment brings the organisms a fitness advantage. Each organism process number of physiological and behavioural rhythms which are synchronized to each other and consequently they are confronted with stimulus from the external environment.

The generally accepted view is that practically all animals have an oscillating system, own circadian periodicity which under the influence of environmental stimuli (so called "zeitgeber") may be coupled (so called "entrained") to an external stimuli (so called "forcing oscillation") (Nielsen, 1984). Many sub-functions of the organism are included in most complex functions, such as activity and feeding, which are thus modulated by distinct biological time patterns (for instance, reproduction or digestion). Aschoff (1958) presumed that the circadian rhythm is the most influential factor and all the other rhythms are more or less related to it. Biological rhythms also provide information on physical parameters, such as the nutritional state, social status or stress. Therefore, they could be the tools to describe the

situation of individuals and groups of animals (Tester and Figala, 1990). For instance, the level of heart rate can be a good indicator of physiological state of an animal, e.g. the level of metabolism, motion or stress.

As Pépin et al. (2006) has stated, an important area of research on circadian rhythms particularly in ruminants is the effect of the light-dark cycle on the rest-activity rhythm, associated with both environmental stimuli (e.g. sunrise/sunset, temperature, available vegetation, activity of predators) and internal physiological processes (e.g. feeding, digestion, reproductive state, growth). Knowledge of these values for a species is a precondition for identification of unusual anomalies of diagnostic importance (Berger et al., 1999). The flexibility in activity rhythms in animals based on housing or breeding system under commercial farming is highly relevant. Livestock maintained under ranch extensive conditions may be expected to exhibit activity rhythms quite different from rhythms of livestock maintained in individual cages or pens with changed environmental and social conditions. These differences may be significant in relation to aspects of management such as light regime, time of feeding, forage supplements, exercise and reproduction (Tester and Figala, 1990).

Several studies, describing diurnal patterns and time budget allocations in different animal species, were made to better recognize the influence of external and internal impulses to the certain species. As previously mentioned knowledge of these factors and their impact on animals give us a potential to production. economic utilization and enhance to prevent health. physiological, physical or behavioural disorders of animals under captive breeding. Diurnal patterns of the feeding behaviour were observed in layer hens (Gallus g. domesticus) (Gunnarsson et al., 2008) in which constant illumination caused growth disorders. Taweel et al. (2006) had studied the diurnal rhythm of dairy cattle (Bos taurus) feeding behaviour at different stocking systems which concluded that dairy cows reduced their daylight feeding time when maximum daily ambient temperature exceeded 25°C. DeVries and von Keyserlingk (2005) proved that time and frequency (DeVries et al., 2005) of feed delivery affects the feeding and lying patterns of dairy cows, i.e. the feeding time increases under a certain timing of feed delivery

by 12.5%. Diurnal fluctuations in activity were measured also in free-ranging ruminants like elk (*Cervus canadensis*) in which feeding declined from summer to winter while resting increased. Majority of daily feeding periods coincided with sunrise and sunset (Green and Bear, 1990). Owen-Smith (1998) found out that high ambient temperature had less influence on foraging time than on overall activity level in kudu (*Tragelaphus strepticeros*). Kudus compensated hot conditions by foraging for longer time during the afternoon when conditions became cooler. Lewis (1977) noted that eland (*Taurotragus oryx*) penned overnight and therefore forced to forage for most of the day, showed greatly reduced activity when the temperature reached about 32° C.

Game ranching (Bothma, 2002) and game farming (Bothma and van Rooyen, 2005) is widespread in South Africa, but exotic game ranching was practised in North America as an alternative to cattle ranching due to its better adaptation to hot and dry climate (Lambrecht, 1983). According to FAO (Scherf, 2000), eland is probably the most suitable African species for experimental breeding and domestication in tropical and subtropical Africa, but presently established at zoos and research centres. Eland seems to be also perspective species for domestication and meat production not just for Africa, but also for temperate climate of Europe (Kotrba and Ščevlíková, 2002).

First evidence about attempts to breed various species of antelopes is known from the ancient Egypt (Spinage, 1986). Later on, in the 19th century, the exploration of eland breeding occurred in region of South Africa, British Islands, former Rhodesia, East Africa and forms southern Russia. In 1859 eland's joint has been subjected to the quality food experiment and it was classified as a new and superior kind of meat available at that time in Europe (Spinage, 1986). Recently again, the interest in the eland breeding with the view of potential domestication has risen (Treus and Lobanov, 1971; Kotrba and Ščevlíková, 2002). Eland has been subject of many studies investigating the possibility of breeding and domestication, for example, Lightfoot and Posselt (1977), Lewis (1977, 1978), Treus (1983).

One of the biggest herds of elands bred in Europe is located at Czech University of Life Sciences farm at Lány in the Czech Republic. It is possible to expect some differences in behaviour between wild eland in Africa and tame captive eland on the farm in the Czech Republic. There are still many unanswered questions concerning the suitable or optimal breeding/farming technology of eland in the temperate climate of the Czech Republic. Therefore, this study would like to contribute to the limited knowledge about circadian behavioural activity of captive eland and concurrently bring new information for the direct application into the management of herd at the farm at Lány.

2. <u>LITERATURE REVIEW</u>

2.1. Biological rhythms

Already our ancestors were confronted with the rhythmic and cyclic nature of animal behaviour since early in recorded history (Rusak and Zucker, 1975). The main features which are possible to observe in the nature are related to seasonal rhythms (changes of photoperiod) in areas distant from equator. Temporal information is provided to the animal through the length and direction of change of the photoperiod (Moore and Marler, 1988). As Rusak and Zucker (1975) explained, for example, homeothermic species in temperate and arctic climates must adapt to seasonal changes in temperature, predator pressure, and the availability of food and protective cover. In doing so they undergo hibernation, estivation, migration, seasonal and opportunistic breeding, and delayed uterine implantation. Social reorganization and changes in fur quality and coloration are also among the adaptations observed in mammals on a seasonal basis (Rusak and Zucker, 1975). Physiological and behavioural responses must be initiated well in advance of the seasonal conditions they are designed to meet. Hibernating mammals store food or body fat long before snow cover appears and before cold makes feeding impossible and hibernation becomes essential for survival. Birds show precise timing of premigratory fattening and development of functional reproductive organs in advance of their arrival at distant breeding ground (Farner, 1970). The reproductive cycles of several mammals and birds are known to persist as circannual rhythms in laboratory environments that lack seasonal cues (Pittendrigh, 1981).

The seasonal influence on behaviour and physiology of animals also play key role in the management of domestic animals in extensive farming, concerning the reproduction. Certain breeders living in arid regions reproduce only after favourable events such as rainfall (Hansson, 1972).

Other crucial features influencing the behaviour of animals are the stimuli which occur during approximately 24 hours. All free-living animals divide their lives into periods of rest and of activity, and these periods are tied to the changes of ambient factors repeating through the diel (Nielsen, 1984). This daily cycle of biological activity based on a 24-hour period is called circadian rhythms. These rhythms include the biochemical, physiological and behavioural processes of living entities (plants, animals, fungi etc.) (Sharma, 2003). We can assume that the circadian timing system which generates daily rhythms of behaviour permits organisms to anticipate predictable events in their environment, and thus increases the probability of locating essential resources (e.g. food and mates), avoids predators, and ultimately increases the likelihood of survival (Holmes and Mistlberger, 2000; Sharma, 2003). Since the time itself is a finite resource animals must manage it and divide it to maximize reproductive success (Altmann, 1974a; Dunbar, 1992b), and time management strategies depend not only on an animal's habitat and life stage, but also on its social environment (Pollard and Blumstein, 2008). Finally, according to Tester and Figala (1990), daily biological rhythms of the organism express adaptation to the annual change of the photoperiod.

2.2. Circadian rhythms

2.2.1. General consideration

We can find already the fossil evidence that corals and nautiloids have demonstrated daily rhythms as long ago as 400 million years (Wells, 1963; Kahn and Pompea, 1978). These organisms exhibit daily growth layers (Neville, 1967) which Sharma (2003) explains by the Earth rotation around the sun. However it doesn't reveal anything about an endogenous circadian clock at that time (Sharma, 2003).

Day-night cycles caused by the Earth's rotation around its axis have influenced living organisms to a great extent. Most organisms have evolved to coordinate their activities with it. Some have chosen day or night, while others escaped daily temporal constraints altogether by living in constant conditions such as deep in the oceans or in natural caves (Pittendrigh, 1960). It is believed that an organism possessing circadian clocks gains fitness advantage in two ways: (i) by synchronizing its behavioral and physiological processes to cyclic environmental factors (extrinsic adaptive value); (ii) by coordinating its internal metabolic processes (intrinsic adaptive value) (Sharma, 2003).

Light – dark cycle is acknowledged to be dominant environmental factor for mammalian rhythms (Rusak and Zucker, 1975; Sharma, 2003; Nielsen, 1984, Hastings et al., 1991). Other environmental factors can be weather, food supply and disturbance (Tester and Figala, 1990). The changes observed in animal activity rhythms after human disruption of their environments (Kavanau, 1969) also suggest an important role in modifying mammalian rhythms.

Among biological factors, mentioned by Rusak and Zucker (1975) and Tester and Figala (1990), are social environment, reproduction (courtship), maternal care, nutritional status and food storage and fat reserves. Social factors may synchronize self-sustaining rhythms of individuals (Aschoff et al., 1971). The likely efficiency of social stimuli is indicated by the observation that nondominant animals modify their feeding and activity rhythms to avoid conflict with dominant animals (Rusak and Zucker, 1975). The cycle of maternal behaviour has been proposed as a social stimulus that may contribute to the development of rhythmicity in the young (Ader and Grota, 1970). Perhaps the most extensively documented social influences are connected with reproductive rhythms. In some species synchronization of estrus can be effected by the presence of males (Bronson, 1971).

Other stimuli include electrical fields and cyclic pressure changes (Hayden and Lindberg, 1969). Temperature cycles, although effective as stimuli for poikilotherms, are ineffective for mammals. Mammals, in common with other animals, show excellent temperature compensation in preserving periodicity within narrow limits (Rawson, 1960). Pittendrigh (1954, 1974) and Sweeney and Hastings (1960) also noted that the period of an endogenous rhythm shows the stability over a wide range of temperatures.

Circadian clocks have a genetic basis and recently several genes involved in clock mechanisms have been identified, characterized and their roles explained. The molecular mechanisms regulating circadian rhythms, based on transcription-translation, have been described in a variety of organisms such as cyanobacteria, Neurospora and Drosophila (*Drosophilla melanogaster*) (Sharma, 2003). The information has been obtained also for mammals (Reppert and Weaver, 2001). In addition, these genes also influence several vital metabolic cycles and therefore they are believed to play an essential role in adaptive mechanisms (Dvornyk et al., 2003). Cyanobacteria were the first prokaryotes reported to possess circadian clocks controlled by a cluster of three genes, KaiA, KaiB, and KaiC, even when growing with doubling times that are considerably faster than 24h (Johnson et al., 1996; Kondo et al., 1993). From this fact it can be clearly inferred that the rhythm is produced by an endogenous mechanism which can be called self-sustained oscillation or circadian clock or biological clock (Nielsen, 1984).

The way that biological events are tied to the natural changes of the diel is explained by a coupling of the internal clock (the self-sustained oscillation) together with the external 24-hour rhythm (the forcing oscillator). The specific part of external rhythm, which acts as an effector (e.g. the change of illumination), is called the zeitgeber (time-cue, or synchronizer or entraining agent). The whole coupling process is called entraining (Nielsen, 1984; Pittendrigh, 1993; Czeisler and Wright, 1999). It is assumed that multicellular organisms contain many oscillators, each of which may have different characteristics and periods (Rusak and Zucker, 1975). A single rhythmic function (e.g. locomotor activity) may reflect the operation of two or more independent oscillators that normally are strongly coupled but may in fact show different natural frequencies and different sensitivities to light. One oscillator can only be phase-advanced by light and the other only phasedelayed. Together these oscillators can account for effects that up to now have been interpreted in terms of a single oscillator with differentially sensitive phases (Pittendrigh, 1974).

The extrinsic adaptive value of the circadian clocks can be explained on the fact that since a number of cellular functions are affected by light, it is conjectured that organisms might have restricted some of their metabolic processes to night to avoid adverse effects of light (Pittendrigh, 1993). Several organisms are also known to shift various UV-sensitive cellular processes such as DNA replication to night to avoid exposure to deleterious UV radiations (Nikaido and Johnson, 2000). Another example of adaptation to the environmental conditions is the initiation of eclosion activity of insects several hours prior to dawn which coincides with the optimal humidity level (Pittendrigh, 1960). Timing is also crucial in relation to other organisms: predators hunt when prey are most active and related species that feed on similar diets can avoid certain types of competition by foraging at different times of day (Owings and Lockard, 1971). Concerning intrinsic adaptive value, the temporal coordination of internal metabolic events is assumed to be an important benefit resulted from circadian clocks. Incompatible processes, which may require different physiochemical conditions for their successful functioning, appear to be separated efficiently in the time and space with the aid of circadian clocks (Moore-Ede et al., 1982). Therefore, the circadian clocks, as they are known now, are perceived to be an evolutionary adaptation primarily to daily cycles of the environment (Aschoff, 1964; Enright, 1971; Hoffman, 1976). Several cyclic factors like light/darkness, temperature, and humidity may have initially played key role as selection pressure for circadian clocks, while later on the rhythmic activities of prey, predators, opening of flowers etc. may have fine-tuned them (Sharma, 2003).

Arhythmicity was found only in animals living in an environment with constant illumination (or darkness). All other animals have a daily rhythm of the activity which may be diurnal, nocturnal or crepuscular; in the last case it may have two periods of activity each diel, at dusk and at dawn (Nielsen, 1984). Question is whether the adaptive values are also significant for animals living in constant conditions like deep seas and in caves. Some authors like Park et al. (1941) and Nielsen (1984) mentioned studies which did not reveal any presence of endogenous rhythm in the observed organisms at all. Secondly, there have been found organisms which exhibit highly variable rhythms with only occasional circadian periodicity or rhythms with non-circadian periodicities (Blume et al., 1962; Jegla and Poulson, 1968). And thirdly, there were observed organisms living in constant conditions which clearly showed circadian rhythms (Trajano and Menno-Barrreto, 1996; Koilraj et al. 2000; Mead and Gilhodes, 1974). Nielsen (1984) states that the overt periodicity - the free-running rhythm - should express the true periodicity of the oscillations in the environment with constant ambient factors. And this should be in contrast to the rhythm influenced (entrained) by an external stimulus which ordinarily is the change of the illumination. These relations have been summarized by Aschoff (1960). His Circadian Rule suggest that light lengthens or shortens the period of an endogenous oscillation (Aschoff, 1964), thus if the light-active animal is kept at a permanent illumination, than the rhythm continues with a period longer than normal and at a permanent darkness is shorter than normal. For instance,

Aschoff (1951) tested the hypothesis on mice with the following result: in permanent light the rhythm prolonged from 24 hours to 25.8 hours and in permanent darkness it shortens to 23.2 hours. In night-active animals the relationship between the illumination and the parameters is the reverse.

Considering the presumption that the light-dark cycle change is the dominant stimulus of entrainment in mammals then the pathway should be normally, but not exclusively, through ocular receptors (Rusak and Zucker, 1975; Nielsen, 1984). However, we can't perceive the circadian clocks as centrally located in the organism with exclusive role of processing the information from external environment. The response of the organism is based on joint neural, chemical and hormonal control (Rusak and Zucker, 1975; Pittendrigh, 1981; Nielsen, 1984; Sharma 2003).

2.2.2. Circadian rhythms in technical terms

Since the issue of circadian rhythms is quite complex, it is necessary to explain the main terms used. The first who used the term "circadian" was Halberg (1959). He stated the definition: "As the activity periods in a constant environment are repeated with intervals different from 24 hours, they are called circadian." A very wide variety of biological rhythms have been shown to be "circadian" and the self-sustained oscillation is by many biologists considered to be universal, ubiquitous part of living matter (Pittendrigh, 1960). Pittendrigh (1993) and Czeisler and Wright (1999) describe circadian rhythm as an overt expression of an internal timing mechanism that measures daily time. Menaker (1969) defines the issue clearly and intelligibly: Organisms are not passive responders. They have internal, accurate time-measuring systems or "clocks". The environment acts on the organism to keep the clock set to "correct" time.

Daily rhythms behave in much the same way as do some physical oscillators. In fact, a major conceptual tool of daily rhythms has been the specific analogy between the biological data and the behaviour of oscillating physical systems; this led to adoption of a specific terminology (Menaker, 1969; Rusak and Zucker, 1975).

The overview of the terminology:

• *Rhythm* – A part of a recurring oscillation.

- Cycle The repeating unit of the rhythm.
- Period (τ) The duration of a single cycle; i.e. the time between successive recurrences of a specified phase of a cycle designated as τ for the biological rhythm and T for the zeitgeber.
- Phase The instantaneous state of an oscillation. Any point within a cycle.
- Phase shift A displacement of an oscillation along the time axis, designated $\Delta \phi$ and $\Delta \Phi$ for biological rhythms and zeitgeber, respectively. A shortening of the period is a phase advance while lengthening is a phase delay. Phase shifts can be produced by the application of an external force at a particular point or phase in the cycle for a given time.
- *Phase angle* The value on the abscissa corresponding to a particular phase of a cycle, this is given as some fraction of a total cycle (hours, degrees, radians) and is represented by φ for a biological rhythm and Φ for a zeitgeber.
- Phase angle difference The difference between corresponding phase angles of two coupled oscillators.
- Phase relationship (Ψ) The relationship between a phase of the rhythm and the light-dark cycle.
- Frequency The reciprocal of the period; i.e. if $\tau = 0.5$ hr then the frequency is 2 cycles per hr.
- Zeitgeber A periodic environmental factor that entrains a biological rhythm, the light – dark cycle (LD) is the paramount example of a zeitgeber.
- Free-running rhythm, Endogenous rhythm A self-sustaining rhythm not entrained to any zeitgeber and therefore displaying its spontaneous period, usually under constant conditions. A certain natural period exhibited by the oscillator.
- Entrainment The coupling of an endogenous rhythm to a zeitgeber such that both display the same period (synchronization). The external force synchronizes or entraines the oscillator. The oscillator, instead of oscillating with its own free-running period, will oscillate with the period of the "driving" or entrained force.

 Circadian rhythm – An endogenous rhythm with a period of approximately 24 hr. The range of deviation permitted for classification as a circadian rhythm is undefined, but limits between 21 and 27 hr are usually suggested.

(Menaker, 1969; Rusak and Zucker, 1975; Pittendrigh, 1981; Daan, 2000; Sharma, 2003).

Each period of a circadian rhythm (τ) consists of an active part (α) and a rest part (ρ). These parameters together with the total activity during the period (level of excitement) are used in the Aschoff's Circadian Rule. Furthermore, the active time of the period is a larger part of the whole period, and there is more activity during the period in bright permanent light than in dim light or in darkness. Or expressed in the "circadian" idiom: Spontaneous frequency (1/ τ), the proportion α : ρ , and the level of excitement are all positively correlated with the light intensity (Nielsen, 1984).

Furthermore, the circadian clocks with τ close to 24h are usually more stable than those with τ far removed from 24h (Pittendrigh and Daan, 1976; Sharma and Chandrashekaran, 1999). Therefore, it appears that natural selection has produced circadian clocks in order to achieve optimally stable Ψ in the face of fluctuating internal and/or external environment, and hence the non-24h nature of circadian clocks may be a well-planned strategy of natural selection rather than an error (Sharma, 2003).

2.2.3. Development of the view

The first one who studied the problem was Réaumur in 1734. He described how the moths if kept in dark boxes became active at the same time as the free-living ones. During the day they remained immobile, but at the fall of night and sometimes already close to sunset, they moved their wings and tried to fly as much as their narrow enclosures permitted. More important breakthrough made Ingeborg Beling in this field who observed the behaviour of honey bees (*Apis mellifera*). In 1929 she published a study which described the problem of the 24-hour rhythm in behaviour of animals. She found that bees indeed have a time-sense which permits them to be trained to return for food at any time of the diel. Furthermore, she found that this ability was independent of ambient factors, and finally, that the time-sense can only be working with 24 hour intervals (reviewed by Nielsen, 1984).

Hemmingsen and Krarup (1937) and later Johnson (1939) found that the duration of the periods of activity in rats and mice, respectively, varied with the intensity of illumination.

Prior to 1950, there was very little interest among biologist in daily rhythms either in the field or in the laboratory (Menaker, 1969). In general, animal behaviourists within the American psychological tradition have not given adequate weight to the role of biological clocks in their questions. They have struggled with behavioural periodicity in their theories of motivation. The issue was why a given animal is active at one time and quiet at another; why it eats, copulates, sleeps or fights at one time of day and not at another (Rusak and Zucker, 1975).

The structure of the circadian concept was essentially built up during the 1950's, the most important work since 1960 has been from a more physiological viewpoint. It has concentrated mostly on two problems: 1) to locate the site of the controlling mechanism and 2) the mechanism by which the endogenous rhythm is adjusted to the time-cue. Much attention was given to the problem of whether the stimulus for the activity is exogenous, completely controlled by ambient factors, or endogenous with continuation of the rhythm expressed even if all ambient factors are kept constant (Nielsen, 1984).

The entrainment of circadian systems has been a close issue to Colin Pittendrigh and Jürgen Aschoff who have been concerned and challenged by the problem of entrainment throughout their career. Pittendrigh attributed special value to the transitions of light to darkness and vice versa that would generate instantanenous nonparametric phase shifts, while Aschoff's starting point was the parametric dependence of circadian period on constant light (Daan, 2000). Later on, Frank A. Brown and his co-workers (1970) have found that many rhythms in a constant environment are not circadian but remain strictly 24-hour rhythms.

The first serious effort to locate the site of the endogenous mechanism was made in 1954 by Janet Harker. She found that in cockroach (*Periplaneta americana*) four neurosecretory cells in the suboesophageal ganglion were

necessary to maintain a circadian rhythm; furthermore, by transplanting of these cells she found that the rhythm was restored.

Lately the main focus has been turned to the internal processes of organisms and their relation to circadian rhythms as neurosecretion (Cymborowsky, 1970), acethylcholinesterase (Cymborowsky et al., 1970), RNA synthesis (Cymborowsky and Dutkowski, 1970) and spontaneous electric activity of the central nervous system (Vijayalakshimi et. al, 1977). Others have focused on hormones from the epiphysis region of higher vertebrates. It has been possible to show that activity of the pineal gland in birds (Zimmerman and Menaker, 1979), and suprachiasmic nuclei in mammals are associated with rhythmic behaviour (Rusak and Zucker, 1975, 1977; Menaker and Brinkley, 1981).

2.3. Time budget and behaviour

Foraging and other activities generally occur in bouts, interrupted by periods of inactivity, and these vary in relative duration over the daily cycle (Owen-Smith, 1998). In connection with the annual changes in weather regimes that cause seasonal fluctuations in availability and quality of forage (Hobbs et al., 1981; Baker and Hobbs, 1982), the length and distribution of active and inactive bouts may vary through the diel.

Practically, we could divide animal's time budget into four general behavioural categories: subsistence (foraging/feeding), locomotion (moving/ travel), rest (inactivity) and 'other' (including active social behaviours). Because rest is the least energetically intensive activity, and the least conspicuous, it serves as default behaviour (Iwamoto and Dunbar, 1983; Altmann and Muruthi, 1988; Dunbar and Dunbar, 1988; Dunbar, 1992b; Korstjens et al., 2006). Any active-time demands imposed by increased sociality (e.g. travel time, foraging time and/or active social time) would reduce the time available for resting, leading to the negative correlation observed. A physiological or ecological need for rest, resulting from digestion constraints, heat dissipation/conservation, bodily repair or other factors, may render a species unable to meet other categorical time demands imposed by sociality (Dunbar, 1988; Janson, 1998; Williamson and Dunbar, 1999; Korstjens et al., 2006).

Digestion constraints are one physiological cause for needed rest (Dasilva, 1992; Korstjens and Dunbar, 2007) especially in animals which need extended time for feed processing like ruminants have and are in a particular concern for species with leafy herbivorous diets. Another physiological need for rest is thermoregulation. In hot climates or those with little cover, certain species may be forced to spend the warmest hours of their day hiding in the shade or otherwise resting to avoid overheating (Dunbar, 1988, 1992b; Stelzner, 1988, Lewis 1978). In cooler climates or during cold seasons, the converse may be true: resting may be vital for conserving heat (e.g. 'sunning' behaviour, Morland, 1993). For any habitat in which animals frequently experience temperatures outside their thermoneutral zone, resting time may be essential (Pollard and Blumstein, 2008). In addition to influencing behaviour, body size also has implications for how animals respond to changing environmental conditions (du Toit and Yetman, 2005).

A time-allocation perspective may also prove useful for wildlife conservation and management. If resting time constrains social group size in species living in social groups, anything that disrupts resting time (e.g. anthropogenic disturbance) may have longer term ramifications on social stability (Pollard and Blumstein, 2008). Analysing the time structure of longterm and continuously measured activity and feeding could be a useful method to follow the general living conditions, especially the nutritional situation and to detect stressful conditions (Berger et al., 1999).

Studies on different animal species were done to observe animal's circadian rhythm either to describe animal's behaviour under natural environmental conditions or to identify the response of the organism to the change of external environmental conditions. Usually, in farm animals, the studies are focused on improving living conditions, productivity, welfare and management of the species. There is a lack of information about time budget and circadian rhythm based on proximal conditions of eland. The published results are just descriptive (e.g. Lewis, 1977, 1978). Most of the studies of the eland are focused on feeding ecology, reproductive and social behaviour.

McKeegan and Deeming (1997) examined the effect of day time, gender and group size on behaviour of adult ostriches (*Struthio camelus*). Results showed that the time budget of adult breeding ostriches is greatly affected by gender. In males, although mating and associated displays take up only a small amount of time, territorial pacing induced by breeding condition can account for up to a third of the time budget. Similarly, in females, while time spent actually mating is small, the extra costs of egg production during the reproductive cycle mean that long periods of time must be spent foraging to meet this demand. Being a member of a larger group affected the time budget through a reduced need for vigilance but also increased competition for food.

The effect of natural and artificial light, respectively, on feeding and perching of layer hens were studied by Gunnarsson et al. (2008). They worked on an assumption that light rhythm is important in controlling many of the behavioural patterns on chicken. Also they draw on findings of Möller et al. (1999) that permanent light reduces the welfare of the birds and that an appropriate resting period is essential for normal development. Results showed that light rhythm affects the diurnal feeding behaviour of chicken.

Diurnal patterns and time courses of activity and feed availability were investigated in three generations of farmed American mink (*Mustela vison*) subjected to three different feedings routines; normal farm feeding (close to average ad libitum), ad libitum, and restricted feeding. The results indicate that mink fed restrictively synchronize their daily activity rhythm to the expected time of feeding. In addition, a high feeding motivation appears to be a precondition for circadian anticipatory activity in mink whereas anticipatory activity of mink fed ad libitum or close to ad libitum may be provoked by stimuli from the actual feeding procedure (Hansen and Møller, 2008).

Time allocation may in fact be an important mechanism through which predation risk, resource needs and other factors influence the evolution of group size. Pollard and Blumstein (2008) conducted a comparative study using 50 species of diurnal primates as a model taxon for cohesive groupliving animals. They expect time allocation to be an important cause and consequence of differences in sociality among species. Their results suggest that a broad-scale evolutionary relationship exists between time allocation and social group size.

Berger et al. (1999) conducted investigations on four horses from a group of 12 Przewalski mares. The results showed the level of activity on daytime to be higher than that at night. Only on hot summer days, activity at night was higher than during daylight hours. There clearly was noted a resting phase before sunrise, almost during the whole year. The subsequent activity peak correlated with dawn. The general pattern of activity and feeding over the year was closely related to sunrise and sunset. The level of activity was lowest in winter; whereas feeding was lowest in summer.

2.4. Studies conducted on ruminants

As mentioned previously the knowledge of the time budget and the circadian rhythm of the particular species of the ruminant can reveal more about the requirements, adaptability and management of the species. Time which ruminants spent on foraging/feeding varies according to species and the feeding type. According to Piccione et al. (2007), the anatomy and function of the rumen suggest that the ruminants have evolved an ingenious system to deal with low-quality fibrous feeds. The system allows for rapid ingestion of feed and for a more leisurely reprocessing of the fermenting mass.

Common basic principles are visible when comparing the strategies of feeding of most free-ranging herbivores. They respond to decreasing availability of suitable food either by taking in smaller quantities and selecting only easily digestible plants or parts of plants like concentrate selectors; e.g., giraffe (*Giraffa camelopardalis*) and roe deer (*Capreolus capreolus*), or by consuming more food of lower quality like grass and roughage eaters; e.g., cattle and sheep (Hofmann, 1989). A third one varies between the two first, by changing its physiology and behaviour and by that being adapted to food conditions (intermediate type; e.g., eland *Taurotragus oryx* and red deer *Cervus elaphus*) (Berger et al., 1999).

A basic premise is that if all species in a mammalian herbivore assemblage use the same quality food and ingest it at roughly the same rate, then daily time spent feeding and walking in search of food is expected to increase with body mass due to increased metabolic requirements (Peters, 1983; Calder, 1984; Schmidt-Nielsen, 1984; Hudson, 1985).

Many physiological and ecological attributes of animals scale significantly with body mass, and since these attributes influence behaviour, it is to be expected that behavioural patterns should also scale in some ways with animal size (Peters, 1983). Larger herbivores can, however, ingest food at faster rates, enabling them to tolerate a wider range of diet qualities than smaller herbivores (Bell, 1971; Geist, 1974; Jarman, 1974). As a result, larger herbivores may be able to reduce their foraging time, despite having greater absolute intake requirements, by increasing their intake rate and reducing their food quality. Owen-Smith (1988, 1992) found a positive relationship between daylight foraging time and body mass for African grazing and browsing ruminants.

Du Toit and Yetman (2005) compared the diurnal activity budgets of four sympatric species of African browsing ruminant that differ widely in body size. Diurnal time spent feeding and moving in a savanna ruminant guild was strongly positively correlated with body mass. This is clearly different to the pattern found for temperate species (Belovsky and Slade, 1986; Mysterud, 1998; Pérez-Barbería and Gordon, 1999), so there must be environmental factors that override physiological and morphological factors in determining how ruminant activity budgets scale with body mass. The negative relationship between body size and activity suggests that larger ruminants in the temperate region indeed include a lower-quality diet leading to longer rumination time and therefore less time spent active (Mysterud, 1998). Mysterud (1998) suggested as well that in tropical ruminants the larger species might spend comparatively more time feeding during the day. Furthermore, among African ruminants the larger browsers seem to spend more time foraging than similar-sized grazers (Owen-Smith, 1992). One likely reason is that the ratio of feeding: ruminating frequency differs between grazers (lower) and browsers (higher) due to differences in the fermentation rates (Owen-Smith, 1982) of grass (slower) and browse (faster). This implies that, in savanna environments, the foraging time budgets of the larger species are more susceptible to thermoregulatory constraints than those of the smaller ones. Ruminants may be able to at least partially compensate for lower quality diets by increasing rumination time (Hofmann, 1989).

Indeed, both small and large ungulates have been found to significantly reduce their overall daytime activity during period of high ambient temperature (Jarman and Jarman, 1973; Leuthold and Leuthold, 1978; Belovsky and Slade, 1986; Klein and Fairall, 1986; Owen-Smith, 1998).

The impact of timed restricted feeding on the behavioural circadian organization of sheep was investigated by Piccione et al. (2007). Total activities of sheep subjected both to 12-h light: 12-h dark (12:12 LD) cycle and to periodic food-availability during the dark phase were recorded. During the restricted feeding treatment, most activity occurred in the dark. Activity in the dark was confined to the food restriction time and a food anticipatory activity was clearly present. Restricted feeding-entrained activity demonstrated the existence of food entrainable and food anticipatory activities in sheep, a diurnal mammalian species. Restricted night-time feeding in sheep substantially shifts the main bout of activity from diurnal to nocturnal. As a consequence, diurnal activity was reduced (Piccione et al., 2007). The effects of the daytime on the ingestive behaviour of groups of five sheep grazing monocultures of ryegrass (Lolium perenne) or white clover (Trifolium repens) were measured by Orr et al. (1997). There was an interaction between time of day and herbage species with intake rate increasing over the day for grass and clover. These changes in feeding behaviour were associated with diurnal changes in the chemical composition of the two herbages (Orr et al., 1997). Odyuo et al. (1991) found that feeding time and dry matter intake were significantly higher in the lactating group of buffaloes (Bubalus arnee) than in the non-lactating group of buffaloes, both reared under loose housing system. Generally, buffaloes spent significantly more time eating during the day time (06:01 a.m.-06:00 p.m.) and on rumination during the night time (06:01 p.m.-06:00 a.m.). Diurnal patterns of feeding activity were displayed with four peaks, three during day and one at night. Rumination activity started increasing from evening and reached the peak period during the night. DeVries et al. (2003) described the presence of dairy cows at the feed alley which was highest during the daytime and early evening (06:00 a.m. to 06:00 p.m.) and the lowest during late evening and early morning hours. Later DeVries and his team (2005) focused on feeding behaviour of lactating dairy cows concluding that group housed dairy cows increased their daily feeding time with increased frequency of feed delivery. To determine the influence of lamb presence on the dairy ewes' circadian

system were monitored 16 different endocrine parameters, besides heart rate (Morgante et al., 2008). The results indicate that lamb presence influences ewes' metabolism. Most variables lost the rhythmicity that they show in not pregnant and not lactating ewes. Lamb presence also influenced urea circadian rhythm; the time of urea peak was shifted when ewes were housed with lambs. Continuous heart rate monitoring did not show heart rate modifications due to animal responses to sampling procedures (Morgante et al., 2008).

Taweel et al. (2006) had studied the diurnal rhythm of dairy cows feeding behaviour at different stocking systems with the aim to quantify the effect of daily ambient temperature on this diurnal rhythm. The results showed that dairy cows reduced their daylight feeding time when maximum daily ambient temperature exceeded 25 °C. Bite rate and chewing rate were not influenced by management or ambient temperature. Under both stocking systems, bite rate was maximal and chewing rate minimal during the evening grazing bout. This indicates that dairy cows were trying to maximize their intake at evening time before darkness falls, by changing their jaw movements into more bites and fewer chews, regardless of the stocking system they were exposed to. When maximum daily temperature exceeds 25°C, the animals start responding by decreasing their daylight feeding time (Taweel et al., 2006). In contrast to this finding, Owen-Smith (1998) concluded that the effect of ambient temperature on daily activity and foraging time of the greater kudu (Tragelaphus strepticeros) was weak. Even over the midday period, when temperatures were highest, activity level remained relatively little influenced by temperature conditions. However, thermal stress can limit the time that large herbivores can devote to foraging activity (Owen-Smith, 1998) the energetic requirements of ruminants belonging to browsers/concentrate selectors seems to override the effect of ambient temperature.

The influence of different management systems on dairy cow's grooming, exploration and abnormal behaviour was observed by Krohn (1994). The frequencies of all type of exploratory behaviours (sniffing and licking the equipment or the ground) were 2-3 times higher in the tie-stall than in loose housing systems. Daily exercise in a yard supported normal social behaviour, self-grooming and investigative behaviour and decreased bar-bitting. Whistance et al. (2007) focused on defecation behaviour of dairy cows under different housing systems – straw yards or cubicle systems. The results indicated that in the majority of instances the cattle did avoid bodily contact with freshly deposited faeces through avoidance behaviour. The increased level of non-avoidance of faeces which occurred in cattle housed in cubicles indicated that housing design might have had a negative impact on the expression of eliminate-avoidance behaviour. Aland et al. (2002) noted significantly more defecations and urinations of dairy cattle during the milking and feeding periods than during the resting periods.

The lactating period and warm weather increased the water intake by beef cattle grazing hill-country in New Zealand (Bagshaw et al., 2008). The influence of the season was not significant. However, the seasonal cycles had significant impact on feeding and resting of free-ranging rocky mountain elk (Green and Bear, 1990). Feeding behaviour declined by 10% from summer to winter while resting increased by 10%. Diurnal feeding decreased 70% when animals migrated from summer to winter ranges. Major daily feeding periods coincided with sunrise and sunset. Animals alternately fed and rested throughout the day on summer range. While on winter range, animals rested for extended periods during daylight, but fed and rested during the night (Green and Bear, 1990). Similar patterns were found in moose (Alces alces) (Cederlund, 1988). In midwinter, the active periods tended to be synchronized in a polyphasic pattern with 5-6 cycles/day. But in winter, the activity cycles were reduced to 2-3/day. The animals were most active around sunrise and sunset throughout the winter, mainly during the sunset when the moose were active more than 50% of the time. This would be not dependent only on ambient temperature, but also on diet quality, like fiber content and availability of nutrients. Pépin et al. (2006) observed 24-hours activity budgets of red deer hinds and concluded that hinds spent more time active in winter (13 h) or in spring (12.5 h) than in summer (10 h). The proportion of time spent active differed between seasons, during the period 1-3 h after sunrise, the time spent active was the highest in winter and the lowest in summer. In summer, the proportion of time spent active was the lowest during both the 1-3 h before and the 1-3 h after sunset periods. Longer active periods after sunset in winter may reflect adjustments made by hinds to obtain enough

food when it became scarce and when weather conditions remained without snow. However, whatever the season, the hinds attune their activity similarly to the 2 h surrounding sunrise and sunset (Pépin et al., 2006). Such a bimodal crepuscular pattern with prominent peaks around sunrise and sunset has been found for most northern free-ranging ungulates, i.e. chamois (*Rupicapra rupicapra*) (Couturier, 1938), roe deer (Bubenik, 1960; Turner, 1979; Cederlund, 1989), white-tailed deer (*Odocoileus virginianus*) (Beier and Mc Cullough, 1990), red deer (Bubenik and Bubenikova, 1967; Georgii and Schröder, 1978, Georgii, 1981), Spanish ibex (*Capra pyrenaica*) (Alados, 1986), moose (Cederlund, 1989; Cederlund et al.,1989) and rocky mountain elk (Green and Bear, 1990).

Colman et al. (2001) tested whether sunlight and insect harassment were important proximate factors (zeigebers) controlling feeding and lying patterns of wild reindeer (*Rangifer tarandus tarandus*). With no insect harassment, feeding and lying each exhibited a polycyclic rhythm with, on average, 4 bouts at equal intervals during 24 hours period. When insect were absent, the feeding activity was slightly longer than lying during the day, with the insect present, both feeding and lying occurred mainly at night. Reindeer did not attune their activity to the sunrise and sunset, demonstrating that daylight is not most likely a powerful zeitgeber for reindeer during summer (Colman et al., 2001). Collins and Smith (1989) focused on twenty four hour behaviour patterns of free-ranging reindeer in winter. Almost 50% of the total activity animals dedicated to feeding and rested more during night than day.

During daytime, variations in several activities were observed in the Pyranean chamois (*Rupicapra pyrenaica*), e.g. grazing, resting and other behaviour watching, moving, etc (Ciofolo and Boissier, 1992). The grazing was more prominent in the morning and afternoon, diminishing rapidly in the middle of the day, at which time the resting phase increased. Other activities usually remained at a constantly low level. Although the fluctuations were observed from one season to another, the time of their occurrence, and their magnitude, varied with the season, age, and sex of the chamois (Ciofolo and Boissier, 1992). The diurnal fluctuations observed in the chamois appear to depend on 2 main factors related to the animal itself and those related to the dynamic environment around it. Factors related to the animal itself depend on its age and sex. The rhythm of activity in young animals progressively resembles that of the adults. The rhythm of the male adult is altered markedly during the rutting period. As regards environmental factors, the chamois adjusts its activities to the season. Grazing and resting are closely related to the temperature variations during the day. In addition, certain activities increase depending on the season (Ciofolo and Boissier, 1992).

Several authors have suggested that grazing is the main daytime activity of the chamois and the Pyranean chamois (around 50%), followed by resting (20-28%) and change of location (13-18%) (Pépin et al., 1991). Other investigators (Hutter and Glauser, 1974) also noticed that the chamois was foraging for food during both day and night, particularly during winter. During daytime, a marked periodicity in the activities of the animals was observed. In spring and winter, feeding took place mostly from 11 a.m. to 2 p.m. and from 3 p.m. to 5 p.m., when the thaw was maximal. During summer, the animals grazed early in the morning and late in the evening with a rest in the middle of the day. Crispi (1977) suggested a better synchronization of the activities when the weather conditions were bad.

Different nutrient requirements stemming from differences in body size and the need for growth and reproduction suggest that the different age-sex classes may benefit from different allocation of their time budget. Côté et al. (1997) noted that milk dependent muskoxen calves (*Ovibos moschatus*) tended to spend more time lying and standing and less time feeding than other agesex classes.

2.5. Heart rate – endogenous biological rhythm

Endogenous biological rhythms are considered to be a manifestation of autonomic organism's activity. One of these rhythms is also heart rate. The stimulus that can induce a reaction of an organism resulting in change of heart rate, can be for example feeding (Baldock et al., 1988; Mesteig et al., 2000), exercise (Nilssen et al., 1984), social interaction (Marchant et al., 1995; Pollard et al., 1993), handling (Price et al., 1993; Pollard and Littlejohn, 1995; Carragher et al., 1996; Marchant et al., 1997) or transport (Baldock and Sibly, 1990). Primarily, stress causes sudden changes in behaviour, and heart rate.

Monitoring of heart rate or heart rate variability allow to determine accurately and reliably the influence of handling on animals. Therefore, heart rate is commonly used as one of the approaches to the welfare of farmed animals. In cattle, heart rate was recorded during different tagging procedures (Lay et al., 1992a). Effects of handling processes as closure, transport and blood collection (Stephens and Toner, 1975), sorting and loading to the vehicle (Stermer et al., 1981), treatment during parturition (Hemsworth et al., 1989) and during milking (Hemsworth et al., 1989; Royle et al., 1992) were observed. Variability in heart rate may be a sign of health state; therefore, it requires more attention in farmed animals (Goldberg, 1991).

2.6. Eland (*Taurotragus oryx*) – the description of the species

Eland (Taurotragus oryx; Pallas, 1766) is the one of the largest antelopes in the world (Hofmann, 1973; Underwood, 1979). Three subspecies are distinguished according morphological traits T. oryx oryx (southern T. oryx livingstonii (central distribution) and T. oryx distribution). patersoniatus (north-eastern distribution). This not fully follows the classification based on mitochondrial DNA. The main difference for subspecies determination is the number and size of white stripes on body (Estes, 1993) and measures of cranial areas. There are significant differences between sexes, especially in body size and weight. Eland males are 135 - 178cm high, females 125 - 150 cm. Males weight varies between 400 - 947 kg with average 690 kg, females 390 - 595 kg with average 450 kg. Physical maturity is reached in 5 - 7 years in males and in 3 - 4 years in females. Sexual maturity is reached in 15 - 24 month, gestation period last 271 - 279 days and lactation period last 130 - 210 days (Kingdon, 1997).

2.6.1. Geographical range

Eland formerly occurred throughout the savanna woodlands of eastern and southern Africa, extending into high-altitude grasslands and the arid savannas and scrublands of the Kalahari and Karoo in southern Africa. It has been eliminated from more than half of its former range by the expansion of human populations, and their numbers have decreased dramatically since the 1970s as a result of civil wars and their aftermath in countries such as Uganda, Rwanda, Angola and Mozambique. They are now extinct in Burundi. However, eland have been reintroduced to a number of game ranches and private rangeland in southern Africa (particularly Republic of South Africa), and this has done much to bolster numbers. In addition, animals have been introduced widely outside of their natural range; for example, although their natural range in Namibia is restricted to the north-eastern parts, they now occur widely on game ranches in the southern and central parts (East, 1999).

2.6.2. Behaviour - activity patterns of eland

Eland is very adaptable to different habitats (Kerr et al., 1970). In general, eland during dry season depends on evergreen woody plants and during rainy season, especially in the beginning, stays on grasslands, which also provide sufficient quality brows (Fabricius and Mentis, 1990; Watson and Owen-Smith, 2000).

The main difference in daily activity patterns of eland in the dry and rainy season is in distribution of time spent on diurnal feeding activities. In winter, there are periods of grazing alternating with rumination, while browsing tends to occur in the early morning and evening (Underwood, 1975). In summer, on the other hand, there is a distinct tendency for animals to ruminate during the afternoon, while browsing has a three to four hourly cycle throughout the day, usually at the expense of grazing. Much more time eland spent on feeding activities during the dry season than in the rainy season (Underwood, 1975). Kerr et al. (1970) noted that eland usually grazed only in the beginning of rainy season, and were browsing in the late rainy season and during dry season. During whole year eland diet consists of up to 94 % from browsing and only 6 % from grazing. Although Wallington et al. (2007) did not noticed grazing during late wet season and in dry season, Nge'The and Box (1976) published that eland is grazing during whole year, and their diet is composed from grazing from up to 70 %. Such different results point to the intake would be largely dictated by availability of high quality food which can be mainly determined by region and climatic conditions.

Treus (1983) observed that animals spent 30-50 % of the day time on feeding and drinking. Adult males which did not take part on reproduction spent the least time on feeding; the young orphans fed the longest time. Lewis (1977) studied the day-time activity of night-enclosed eland, oryx (*Oryx gazella*), buffalo (*Syncerus caffer*) and cattle in a hot, semi-arid area in Kenya which showed that, on average, eland spent 70% of the day feeding, oryx 60% and buffalo and cattle 52%. There were also within species differences, depending on whether or not the individual was pregnant, lactating or sucking. Lactating eland fed for an average of 0.4 hour (5%) longer than adult males.

Littlejohn (1968) observed that eland utilizes at least 29 species of grasses and 57 shrubs, trees and herbs, which point out that eland is more browser than grazer. This statement confirms many other authors (e.g. Kerr et al., 1970; Watson and Owen-Smith, 2002). The most important factor influencing the selection of woody species by eland is total fibre content in shoots (Watson and Owen-Smith, 2002). Although preferred species have high leaf:stem ratio and low content of fibre, such as leafy forbs and broad-leafed shrubs, Watson and Owen-Smith (2000) observed that major part of eland diet form species with low leaf:stem ratio. This was probably caused due to ability of eland to sufficiently select a diet of low total fibre. Eland can achieve this by consuming a young green shoots of woody species and eating large portions of preferred species with high leaf:stem ratio.

Eland feed the longest time in the morning from 5 a.m. to 9 a.m.; the shortest feeding time occurs in the afternoon from 14 p.m. to 16 p.m. There were noted 2-3 feeding bouts during the night. The longest resting bout occurs before dawn (Treus, 1983). Although nocturnal rumination occurred during all night, there were peaks in this activity before and after midnight in eland (Lewis, 1977).

Besides feeding, the second most time-consuming activity for eland, buffalo and cattle is walking. The eland spend very little time resting (Lewis, 1977). Browsing herd of captive elands can walk 6 – 8 km per day (Treus, 1983).

Milked elands drink 1-2 times/day as well as free-ranging animals do. Elands ruminate in standing position from 7 minutes to 2h35 per day; the youngest ones at the age of 23 days ruminate solely in lying position for 1h52. Adult males ruminate in standing position 2h35 per day. Stud males spend ruminating the longest time 6h11 per day. Animals of milked herd had two activity peaks per day – from 5 a.m. to 10 a.m. and from 15 p.m. to 20 p.m., animals of free-ranging herd had 3 activity peaks per day – from 5 a.m. to 9 a.m., from 14 p.m. to 16 p.m. and from 17 p.m. to 21 p.m. (Treus, 1983). The distribution of activity throughout the day of all species was similar and the mobile activities of feeding and walking tended to be concentrated in the early morning and late evening.

Animals defecate and urinate after resting bout, in total, they urinate 5-7 times/day and defecate 3-4 times/day. Usually eland rest 6-11 hours (25-40% of the day), they spend in lying position 19-32% of the time, sleeping 13-22% of the time and ruminating in lying position 27% of the time (Treus, 1983). All non-feeding activities show an increase during the rainy season, the most prominent being "inactive". This presumably reflects either increased inactivity because of daytime heat, i.e. it may be an artefact of daylight-only observations, or else the lack of need for other activities: e.g. food is more nutritious, therefore there is less feeding (Lewis, 1977). The annual cycle of activities, particularly feeding activities, is highly correlated with cycles in rainfall, vegetation, temperature and other aspects of the ecology of the eland (Underwood, 1975).

Lewis (1977) in the study of four species - eland, oryx, buffalo and cattle, observed that all species spent significantly less time feeding during periods of high ambient temperature and values for solar radiation. The eland spent significantly more time resting when ambient temperature and thermal reradiation values were high. The time spent ruminating by eland increased when ambient temperature, solar radiation and thermal re-radiation values were high. Underwood (1975) found that this pattern correlate with the season. Although the day in summer was longer, most activities showed fewer peaks than in winter. This was most obvious in active feeding, in ruminating and in walking. This was probably an adaptation to the afternoon heat, since the main resting activity had its peak from 14.00 - 17.00 h. Total resting activities showed the afternoon peak. Feeding began to increase in the late

afternoon and presumably peaked again after dark. The indications are that eland do more active feeding during the night in summer than in winter. Lewis (1977) is also convinced that nocturnal feeding is important to wild eland. Underwood (1975) noted four main phases of activity during daylight during winter. The earliest was a sequence of intensive feeding, then ruminating followed by walking. At noon and in the early afternoon, the groups fed and ruminated in approximately the same spot for two hours or more, then began a walking/browsing bout. There were distinct peaks, suggesting that an internal clock partly governed the activities.

Important strategy how to avoid the impact of solar radiation and high temperature is to hide in shade. Lewis (1978) noted that animals sought shade in the middle of the day. Eland are basically browsing animals (Hofmann, 1973) and are therefore more likely to have been feeding on the trees and shrubs which were casting shade. Consequently they were more likely to be in the shade while feeding. The distribution of time spent in the shade during the day indicates that there were peaks for eland between 13h00 and 14h00. The time spent in the shade by eland was significantly greater on days of high ambient temperature, solar radiation and thermal re-radiation; the time spent in the shade during days of high wind speed (Lewis, 1978).

Lewis (1978) has also focused on the level of synchronization during observed activities in eland. Tinbergen (1953), Scott (1962) and Sinclair (1970) describe the term synchronization as animals in a flock or herd doing the same thing at the same time. Feeding was noted as the best synchronized activity; walking, resting and shade behaviour showed very poor synchronization.

2.6.3. <u>Captive breeding potential of the species</u>

Farming of eland has been known not only from African continent but also from Europe. Well-known is the Askania-Nova ZOO in Ukraine which imported four eland males and females in 1892 from Africa. Until 1968, in total, 408 animals were bred here (Treus and Kravchenko, 1968). Breeding of eland in Africa has number of advantages in comparison with cattle; eland better transform low-quality forage into quality meat and have more effective water management as an adaptation to the dry environment (Posselt, 1963). African tribes have had not probably the reason or needs to domesticate eland, because of its high density in wild together with low density of human population (Kotrba et al., 2004).

Since the colonialism era, eland breeding on ranches have occurred mainly in South and East Africa. Ranches usually offer the possibility of big game hunting; they run photo safari, ecotourism, provide accommodation in hotels and produce high quality game meat (Kotrba et al., 2004). Eland had been transported also to American continent as a promising species for domestication (Treus, 1983; Lambrecht, 1983).

In spite of the successful domestication, in low rainfall area the eland was found to have no advantages over cattle; not being able to search for succulent and dew-laden food at night, it required both a richer diet and as much water as cattle. Eland do much better in higher rainfall areas, and is the species which has been chosen from the outset for farming trials. This has been said to be misguided because it only superficially resembles a cow. Its physiology and nutritional requirements are quite different, as well as are an agile, far-ranging species, able to leap a 2 m fence with ease. Nevertheless, it has many characteristic in its favour; it is easily tamed (but it is always potentially dangerous because if it's sharp horns) and it produces both a high quality meat and milk (Spinage, 1986).

The milk is exceptional in having approximately twice the protein and fat of cow's milk, and in being much richer in calcium and phosphorus, but the sugar content is similar (Spinage, 1986). Eland can produce 7.5 kg nutritious, creamy milk per day (Hall, 1975). Eland's milk is easier to preserve, it stays fresh much longer than cow's milk. It is kept for 2 hours on fresh air and then being stored at 37°C it can be kept for up to 8 month (Uspenskii and Saglanskii, 1952).

In Russia it is valued for medical purposes and is alleged to be effective in the treatment of gastric ulcers and injuries (Spinage, 1986). In Askania Nova ZOO, eland were milked and the milk was used for treatment of tuberculosis, skin eczema and gastric ulcers (Treus and Kravchenko, 1968). Given the right circumstances the eland was more productive than cattle in Africa, thus an eland bull may weigh 565 kg at three and half years, compared with cattle under the same feeding regime reaching less than 500kg (Spinage, 1986).

Several reasons are advanced as to why wild ungulates would be better for meat production than conventional stock under similar conditions in marginal areas; one of these reasons being that they make better use of habitat. Cattle select only a limited number of grass species, in contrast to the much wider range usually chosen by antelopes, which take in addition forbs and may browse on bushes and trees. More important perhaps is their resistance to many diseases of stock, especially trypanosomiasis. Another of the reasons advanced in favour of antelopes is that they are more productive, that is, they produce a given quality of meat more quickly. This is because they both breed and grow more quickly on their preferred diet; but the mixed feeders and browsers generally require a richer diet than do cattle. Like deer therefore, the meat of African antelopes is low in fat, so that the marbling effect found in beef does not occur. The low-fat meat of antelopes could therefore well be the meat of the future. Finally, in their favour, antelopes have less effect on the habitat than does the same density of cattle, not creating trampling and erosion, due to the fact that they spread out more while feeding and follow narrow paths to watering points (Spinage, 1986).

As well as advantages for domestication of eland there are disadvantages too, for instance, high costs of feed and fences. Eland require higher amount of feed to be kept in healthy physical condition comparable with free-living eland. In tropical and subtropical zones eland feed also at night to avoid heat stress which is risky mainly in light of higher activity of predators. Therefore, high fence is inevitable (Hillmann, 1979).

The situation is slightly different in temperate zone where costs of the eland breeding are equal to or little bit smaller than the cattle breeding; with the exception of the cost of fences which is rather demanding (Kotrba et al., 2004).

Natural behaviour of free-living animals is during domestication largely modified by man interference. If we want animals to successfully cope with new environment, we have to adapt breeding technology to its requirements and create favourable conditions for its basic physiological functions and potential abilities (Kotrba et al., 2004).

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3. <u>OBJECTIVE OF THE THESIS AND HYPOTHESIS</u>

The aim of this work is to evaluate circadian rhytmicity related to feeding and resting behaviour in farmed eland in an environment where the temperature was constant, circadian light rhytmicity was equally distributed, the quantity and quality of feed remain the same thorough the year, feed and water availability was ad libitum and social group was stable.

- H1. Elands will spend more time by feeding behaviour in low ambient environment (winter) due to a higher energetic demand and increased metabolic rate.
- H2. The time spent by active feeding (behaviour related to ingestion) will be longer at night than in the course of the day when the time spent by non active digestion should predominate. If the natural antipredatory behaviour occurs, elands would sleep and rest/digest longer during the day than night, because predators of elands are more active during the night in areas of their natural occurrence.
- H3. Juvenile elands after weaning will spend by ingestion of feed more time and have higher frequency of ingestion/digestion bouts than adults due to larger feed intake.
- H4. Juvenile elands after weaning will spend more time lying and sleeping than adult animals.
- H5. The males of eland will have different rhytmicity of feeding behaviour (longer ingestion/digestion bouts and lower frequency of bouts) due to its large body size and lower energetic needs proportional to its body size.
- H6. The elands as a ruminant will spend the same time (regular distribution) for the behaviour related to ingestion and digestion of feed in the case of equal distribution of light and temperature regime. Elands as a ruminant will have regular requirements for feeding time and its distribution during 24 h based on processes in rumen and consecutive digestion physiology, but only in the case of equal distribution of environmental conditions (temperature, light regime).
- H7. The behaviour related to feeding will be based on the influence of the environmental factors called "zeitgeber effect" or it is influence of innate internal timing (internal "clocks"). We suppose elands to be active with ingestion activities (feeding, moving) an hour before and after dawn and dusk rather than with digestion activities (recumbent, standing and ruminating) as a consequence of the internal timing.
- H8. Elands will be more active (moving without feeding) 1-3 hours before the feeding time as a consequence of a regular time of feed delivery (effect of "entrainment") or start to feed after the delivery of fresh feed (effect of "forcing oscillation").
- H9. Male will have lower frequency of heart rate because of its larger body size; and with increasing body size heath rate should decrease.
- H10. Heart rate will change during change of physical activity.

4. METHOD

4.1. Location

• Farm Březová

This study has been started in 2005 on farm Březová which is located 8 km south-east from Kutná Hora (49° 08' N, 15° 04' E). Farm Březová, as the first placement of elands bred under supervision of Institute of Tropics and Subtropics (ITS), Czech University of Life Sciences Prague (CULS Prague).

Average altitude of this locality is 370 m above sea levels. Average annual temperature was 8.4°C and average annual precipitation was 595 mm in 2005 (ČHMÚ, Praha, Ruzyně, 2012).

Elands (*Taurotragus oryx*) were group-housed in straw bedded pens in a barn measuring 9.5 x 23 m. Barn was originally built for cattle and lately adapted for elands. Concrete floor was covered by straw bedding and cleaned once a week. Breeding technology on a deep litter was chosen to maintain a sufficient temperature in a relatively spacious barn (Kotrba et al, 2004). Animals had free access to paddock with an area of 2.2 ha from April to November. Paddock was enclosed by a high fence dimension 2.5 - 2.8 m.

The animals were housed permanently inside the barn during our observation to maintain the environmental conditions constant (ambient temperature) and avoid the disturbance by external noise or strangers etc. and to provide the same type of feed.

The elands were fed twice a day with concentrated feed and fodder beets, meadow hay and water were available ad libitum.

• Farm Lány

Because of the transportation of the herd to the new location, the study has continued in the newly built barn on the farm Lány (50° 07' N, 13° 57' E) which is located 43 km north-west from capital city Prague. The farm Lány is located in the same region as the original farm Březová.

Average altitude of this locality is 421 m A.S.L.. Average annual temperature was 9.4°C and average annual precipitation was 527 mm in 2008

and average annual temperature was 9.1°C and average annual precipitation was 596 mm in 2009.

Elands were group- housed in the pen barn measuring 16 x 16 m with an access to pasture with an area of 2.5 ha. The pasture is divided to 2 parts to control breeding.

Feeding was delivered regularly twice a day and animals were fed by feed mixture and water ad libitum. Basic feed mixture was based on corn silage (60%), lucerne haylage (30%), meadow hay (7%), and barley straw (3%). This mixture contained 16.6 % of crude protein and 16.2 % of crude fibre. Animals were kept on a deep litter closed in pen barn during whole trial in relatively stable conditions, i.e. in relatively stable social composition of the herd and in relatively stable external environment thanks to the proper thermal insulation of the barn.



Figure 1: Pen barn on farm Březová (Kotrba, 2005)



Figure 2: Pen barn on farm Lány (Al Hakimová, 2007)

4.2. Monitored animals

The herd of elands nowadays reached fifty two animals. Original breeding herd consisted of 5 animals born in ZOO Dvůr Králové nad Labem starting 2002 Their ancestors has been imported to the Czech Republic from East Africa between 1969- 1971 (Vágner, 1973).

We observed during every sampling day together ten elands that were born in captivity and their complete genealogies were available. The group consisted of different sex and age classes to correspond with the natural social conditions. We have chosen for the study two adult males, four adult females and four juveniles. Animals could be individually distinguished by natural (horns, size) and man-made (coloured numbered ear tags) features.

We have determined three age classes:

- Adult male older than 3 years, female older than 2 years (with or without a calf);
- Juvenile young animal after weaning (7-10 months until 3 years in males and 2 in females);
- Calf animal from the birth till weaning.

Name of observed animal	Date of birth	Sex
Name of observed animat	(YYYY-MM-DD)	
Ninja	1994-02-10	Male
Niagara	1997-02-06	Female
Libres	1997-02-12	Female
Prima	1997-05-11	Female
Lydie	1997-05-16	Female
Staple	2003-08-17	Female
Dakarka	2003-10-16	Female
Krul	2003-12-17	Male
Lojza	2003-12-20	Male
Lia	2004-05-22	Female
Niombu	2004-07-15	Male
Limpopo	2004-11-13	Male
Lorie	2007-02-16	Female
Nikola	2007-03-21	Female
Varna	2007-03-22	Female
Dulu	2007-04-28	Female
Lysinka	2007-07-03	Female
Sydney	2007-12-19	Female
Viktorka	2008-02-02	Female
Drak	2008-02-08	Male
Lejdy	2008-02-24	Female
Vavřín	2008-02-25	Male
Lutu	2008-03-05	Male
Cavalia	2008-04-20	Female
Singi	2008-10-21	Male
Dajan	2009-04-26	Male

Tab. 1: Identification data of observed animals

4.3. Observing method

As mentioned previously, the management of the time budget is crucial to utilization of available resources. The circadian rhythms play an important role in timing the "right" activity to proceed at the right time to properly use the available resources. To recognize the circadian rhythms of eland we determined several activity patterns that we observed by 24-hours scan sub group sampling (Altmann, 1974) inside the barn. We determined following activities:

Active behaviour	Passive behaviour	External influence
Moving	Standing	Feeding by keeper
Feeding	Standing and ruminating	Handling
Walking and ruminating	Recumbent	
Drinking	Recumbent and ruminating	
Excretion (urinating, defecating)	Sleeping	
Comfortable behaviour (self care)		
Grooming		
Agonistic behaviour		
Suckling		
Trying to suck		
Play behaviour		

Tab. 2: Determined activities

Two observers alternated every two to six hours. Activity of each animal was recorded manually in special tables by recording the onset and termination of each activity. The minimal sampling period was one minute. Defecation, urination, agonistic behaviour and flehmen was recorded when occurs. We dimmed the lighting in the pen barn during night to simulate natural night "light" emitted by Moon. We recorded the temperature in the stable as well. The time of sunrise and sunset was obtained from specialized astronomical observatory Kleť (České Budějovice).

Research started in March 2005 and continued during the years 2008 and 2009. In total, the data were taken during 264 hours. The dates of 24 h observation are following:

4th - 5th March 2005

11th - 12th March 2005

18th - 19th March 2005

25th - 26th March 2005

21st - 22nd March 2008

28th - 29th March 2008

- 4th 5th April 2008
- 1st 2nd August 2009

21st - 22nd August 2009

28th - 29th August 2009

16th - 17th October 2009

4.4. Heart rate monitoring

In the first phase of the experiment, along with determined activities, we also recorded heart rate of one adult male and one adult female. Heart rate should reflect the physiological state of an animal (metabolic rate, exercise, stress etc.) and therefore it should be different during different activities. A stressful situation usually causes sudden changes in behaviour and heart rate. The ability to recognize when an animal is under stress is a part of responsible breeding and management processes used to improve the welfare of farmed animals (Pollard et al., 1993).

Changes in heart rate may be an important parameter to study the responses of animals to external stimulus and to study the quality or quantity of the stressor. These changes in heart rate may indicate individual response to stress and susceptibility to stress (Hopster and Blokhuis, 1994).

This experiment was performed on farm Březová in March during the winter housing of animals and period of equinox.

The study group consisted of two captive born, tame individual elands, 11 years old male (named Ninja, live weight 660 kg) and 8 years old female (named Prima, live weight 350 kg). Total number of animals in the herd was eight; one adult male, four adult females and three calves (age from six month - one year). Male Ninja was penned separately and female Prima was in a group of other animals. They had no physical contact, only visual.

The male was dominant to all animals and studied female was the second highest ranking among females. These two animals were deliberately chosen for the experiment because they reacted relatively calmly to the handling and they allowed the physical contact with humans. The study animals were trained to access the restrain box every 14 days during one year and we also made about 20 attempts to accustom them to the belt with HR recorder.

To monitor the levels of HR in eland we observed determined behaviour by 24-hours focal sub group sampling (Altmann, 1974) in the pen barn. We determined the behaviours that could affect HR and the type of behaviour were following: standing and ruminating; recumbent and ruminating; standing; standing and feeding; movement; sleeping; excitement during feeding by keeper; stressful events and handling in restrain box. Feeding by keeper took place twice a day. The movement was associated with the walking or movement of an animal thorough the pen barn. Sleep was defined as the position at which the animal was lying with its eyes closed and its head was twisted backward on the abdominal part (Kiley-Worthington, 1978). Situations, when animals were disturbed, startled or during handling, were considered as potential stressful situations.

Two observers alternated every six hours. Behaviour of each animal was recorded by observation and written down in special tables by noticing onset and termination of each type of behaviour. The animals were closed inside the pen barn during our observations to maintain the environmental conditions constant (ambient temperature) and avoid the disturbance by external noise or strangers etc.. The temperature in the pen barn was recorded as well, since it is a factor that could affect the HR.

We used least invasive method to monitor the HR. The most common type of sensor used in previous studies for monitoring was Polar ® Sport Tester. It has been successfully used in humans (Karvonen et al., 1984; Seaward et al., 1990), horses (*Equus caballus*, Sloet van Oldruitenborgh-Oosterbaan, et al. 1988), cattle (Hopster and Blokhuis, 1994; Pollard and Littlejohn, 1995; Mattielli et al., 1997; Brosh et al., 1998; Mohr, 2002), pigs (Marchant et al., 1995, Marchant et al., 1997), reindeer (*Rangifer tarandus*, Mesteig et al., 2000), deer (Pollard et al., 1993, Price et al., 1993; Carragher et al., 1996) and alpaca (*Vicugna pacos*, Pollard and Littlejohn, 1995).

Earlier studies of precision and accuracy of Polar ® Sport Tester (PST) showed that it provides sufficient and valid data on HR in young children and even adults during various exercises (Treiber et al., 1989; Seaward et al., 1990). Evans and Rose (1986) and later by van Sloet Oldruitenborgh-Oosterbaan et al. (1988) found that PST provides valid and reliable records of HR in horses. Hopster and Blokhuis (1994) dealt with the detailed testing of commercial monitor Polar ® Sport Tester in dairy cows and concluded that PST is a suitable instrument for monitoring of HR and HR variability in dairy cows. Mesteig et al. (2000) used this system in reindeer and HR values recorded by PST achieved with minimal deviation values measured by ECG.

We used the device Polar S610 for our experiment. It consisted of two steel electrodes size of 17.5 cm2 which were connected to a signal carrier. Data recorder about a size of digital watch was attached 20 cm far from the signal carrier and all components were attached to elastic belt. One electrode was placed in the ventral part of the humerus, the second electrode was placed 15 cm above the first one in the shoulder part. The recorder was attached in the dorsal part of the withers (Fig. 3). Scan of heart rate was carried out every 15 seconds; for that reason, the record could be stored in the recorder for 24 hours.

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Figure 3: Female Prima with attached heart rate recorder Polar S610 (Mojžíšová, 2005).

4.5. Statistical data analyses

Data were analyzed using the SAS System V 9.2 (SAS Inst. Inc., Cary, NC). The associations between the percentage of time spent by specific behaviour and the fixed effects were tested using a General Linear Mixed Model (GLMM). The percentage of time spent by specific behaviour was included as a dependent variable and evaluated independently for 18 behaviour patterns described above. The independent fixed effects in the full model consisted of the class variable 'Day/Night' phase. Another class variable was 'sex'- male or female and 'age'- adult, juvenile and calf. The last class variables were the 'season'- Spring, Summer, Autumn and Winter and 'year' of collection. To account for the repeated measures on the same animal the individual animal was included as a random factor, using PROC MIXED. The significance of each fixed factor in the GLMM was assessed using an F-test. The least-squares-means (LSMEANs) were used to find differences between the classes tested by a t-test. For multiple comparisons we used the Tukey-Kramer adjustment.

To account for differences during one hour before and after dawn and dusk and three hours before morning feeding we compared proportion of time spent by ingesting behaviour with digestive behaviour by t- test.

Data of HR were analysed using SAS system 9.1. The differences of behaviour on HR in male and female was evaluated using one-way Analysis of Variance for repeated measures, where the HR was dependent variable and type of behaviour was independent variable.

5. <u>RESULTS</u>

5.1. Evaluation of activity budgets

5.1.1. <u>Seasonal activity budgets (Hypothesis 1)</u>

The proportion of time the elands spent by feeding, resting and moving were different between seasons. There was a significant **increase in feeding** $(F_{(3,87.9)} = 8.87; p < 0.0001)$ and a **decrease in moving** $(F_{(3,190)} = 25.78; p < 0.0001)$ from summer to winter. The proportion of time elands spent by **feeding and resting increased** during the same period and was the longest in winter in comparison to summer (Fig. 4). The hypothesis was supported.

There was significant increase in feeding time from spring to winter. Eland spent by **feeding 17%** of the time in spring, **16%** in summer, **21%** in autumn and **27%** in winter. Eland were active mostly in spring/summer season; their activity "**moving**" decreased in autumn and remains very low during winter. Elands were moving **14%** of the time in spring, **19%** in summer, **10%** in autumn and **5%** in winter.

An increase in activity had a fluctuating tendency. Eland were resting in "recumbent and ruminating" position ($F_{(3,85.2)} = 15.65$; p< 0.0001) 28% of the time in spring, 23% in summer, 26% in autumn and 42% in winter.

Eland spent the shortest time "standing and ruminating" ($F_{(3,75.4)} = 24.33$; p< 0.0001) in winter, it did 6% of the time. Hence further, the time eland spent "standing and ruminating", has increased. It did 12% of the time in spring, 21% in summer and 25% in autumn.

Activity "standing" ($F_{(3,190)} = 25.72$; p< 0.0001) significantly decreased from spring to winter. Eland were standing up to 16% of the time in spring, 7% of the time during summer, 5% in autumn and 10% in winter.

The percentage of time spent for other than above mentioned behavioural variables during different seasons did not differ.



Time spent by activity according to season

Figure 4: Seasonal activity budgets.

5.1.2. Daily activity budgets (Hypothesis 2)

Eland fed ($F_{(1,164)} = 33.42$; p< 0.0001) higher proportion of time during the daylight hours, accounting for 23% of total daily time. Nocturnal feeding took 17% of the total daily time (Fig. 5). The hypothesis was not supported.



Time spent by feeding according age group and day light

Figure 5: Daily activity budgets – feeding.

The 24-hour pattern of eland activity was characterized by alternating bouts of feeding and resting. Eland **rested shortly during day** and spent the **night mostly in "recumbent and ruminating**" position.

Eland were motionless standing ($F_{(1,190)} = 7.69$; p = 0.0061) during the day a higher proportion of time than during the night, i.e. they were "standing" 11% of the time during the day and 8% during night (Fig. 6).



Time spent by standing according age group and day light

Figure 6: Daily activity budgets – standing.

Correspondingly, eland were resting more at night that during a day, they spent "recumbent and ruminating" ($F_{(1,167)} = 99.24$; p< 0.0001) 23% of the time during day and 37% of the time during night (Fig. 7).

Time spent by recumbent and ruminating according age group



Figure 7: Daily activity budgets – recumbent and ruminating.

Eland spent slightly more time "standing and ruminating" ($F_{(1,170)} = 12.63$; p = 0.0005) during night, about 18% of the time; compared to a day when eland spent 14% of the time by this activity (Fig. 8).



Time spent by standing and ruminating according age group and day light

Figure 8: Daily activity budgets – standing and ruminating.

Eland spent by sleeping higher proportion of time during the night than during day. They were "sleeping" ($F_{(1,163)} = 15$; p = 0.0002) **1.5%** of the time during the day and **3.7%** during night (Fig. 9).



Time spent by sleeping according age group and day light

Figure 9: Daily activity budgets - sleeping

Eland movements were associated with feeding and with movements to and from feeding area. Eland spent by walking higher proportion time during day than during night. They were "moving" ($F_{(1,172)} = 4.02$; p = 0.0466) 11% of the time during the day and 12% during night (Fig. 10).



Time spent by moving according age group and day light

Figure 10: Daily activity budgets – moving.

The percentage of time spent for other than above mentioned behavioural variables during day or night did not differ.

5.1.3. Influence of age on activity budgets (Hypotheses 3 and 4)

We found significant differences in the proportion of feeding time ("feeding") between the age classes in interaction with time of the day.

Calves spent by "feeding" ($F_{(2,168)} = 3.16$; p= 0.0448) 16% of the time during daylight hours and 12% during night, juveniles 28% and adults 27% during daylight and 16% and 22% during night, respectively (Fig. 5). There were differences in feeding, but we cannot support Hypothesis 3.

Moreover, there is a significant difference between the amount of time that calves and adults devote to rest and in what position are resting. The results show that **calves** most often **rest in recumbent position** in contrast with **adults** that more often **rest in standing position**.

Calves spent the highest proportion of time in "recumbent and ruminating" ($F_{(2,19.9)} = 9$; p = 0.0016) position, i.e. 38% of the time in

comparison with juveniles **29%** and adults that rested **23%** of the time in this position (Fig. 7). The first part of Hypothesis 4 was supported.

Correspondingly, calves spent lower proportion of time to "standing and ruminating" ($F_{(2,22.1)} = 7.06$; p = 0.003) than juveniles and adults. Calves were "standing and ruminating" **11%** of the time; compared to juveniles **17%** and adults that spent up to **20%** of the time in this position (Fig. 8).

Eland calves spent by sleeping higher proportion of time than older animals. They were "**sleeping**" ($F_{(2,163)} = 7.38$; p = 0.0063) **5%** of the whole time during in comparison to **2%** in juveniles and adults **1%** (Fig. 9). The second part of Hypothesis 4 was supported.

Eland calves spent by playful behaviour ($F_{(2,190)} = 11.25$; p < 0.0001) higher proportion of time than juveniles and adults. They were "playing" 2% of the whole time during in comparison to 0.2% in juveniles and 0.3% in adults.

5.1.4. Influence of sex on activity budgets (Hypothesis 5)

Any difference of observed behaviour were affected by sex of animal, thus hypothesis 5 was rejected. Males and females spent equally the same percentage of time by the same activities.

5.1.5. <u>Circadian rhytmicity and activity budgets during equal light</u> <u>distribution (Hypothesis 6)</u>

There were not differences in proportion of time spend by feeding, walking and standing and ruminating during a day or night but there were differences in activity "standing" ($F_{(1,112)} = 10.39$; p = 0.0017), activity "recumbent and ruminating" ($F_{(1,100)} = 123.84$; p< 0.0001), activity "sleeping" ($F_{(1,112)} = 11.88$; p = 0.0008).

Eland were motionless standing during the day a higher proportion of time than during the night, i.e. they were "standing" **15%** of the time during the day and **11%** during night.

Correspondingly, eland were resting more at night than during a day, i.e. they spent "recumbent and ruminating" **30%** of the time during day and **49%** of the time during night.

Eland spent by sleeping higher proportion of time during the night than during day. They were "sleeping" **0.8%** of the time during the day and **4%** during night.

The Hypothesis 4 was accepted only for ingestion activities, i.e. feeding, moving (walking) and digestion during standing.

5.1.6. Feeding behaviour based on environmental or internal timing (Hypothesis 7)

The prediction based on influence of the environmental factors called "Zeitgeber effect" or on influence of innate internal timing (internal "clocks") is that elands should be active with ingestion activities (feeding, moving) an hour before and after dawn and dusk rather than by digestion (recumbent, standing and ruminating) as a consequence of the internal timing as the same pattern observed in African nature.

We found that activities connected to digestion and ingestion are similarly (without significant difference, p > 0.05) distributed around dawn and dusk. Thus, the Hypothesis 7 was rejected.

5.1.7. Feeding behaviour based on environmental or internal timing (Hypothesis 8)

The prediction was that elands should be more active (moving without feeding) 3 hours before the feeding time as a consequence of a regular time of feed delivery (effect of "entrainment") or start to feed just after the delivery of fresh feed (effect of "forcing oscillation").

We found that proportion of time animals spent for activities connected to digestion was **61%** and to ingestion only **20%** one to three hours before regular feed delivery ($t_{(1)}$ = 6.45, p=0.0003). This was changed to feeding after fresh feed was delivered.

Thus, the effect of "entrainment" was not confirmed and hypothesis 8 was not supported.

Elands started to feed after the delivery of fresh feed (effect of "forcing oscillation"). Feeding behaviour has started in all animals when fresh feed was delivered and did not show any variability.

5.2. Heart rate monitoring

5.2.1. The difference in heart rate during activity in eland male and <u>female</u>

Data from one adult male (Ninja) and one female (Prima) were evaluated. The analysed data were taken during 7 days (66 hours together) for male and 6 days (49 hours) for female.

Generally, male had always lower mean levels of heart rate (HR) during different behaviour in comparison to the female. Hypothesis 9 was supported (Fig. 11). The average value of HR in female was **67.58 bpm** (Tab. 3) and the average value of HR in male was **48.97 bpm** (Tab. 4).

Hypothesis 10 pronouncing the assumption that HR changes when changing activities, was fully confirmed. The method of analysis of variance confirmed that the HR is different in different activities in both male $(F_{(7,3933)} = 393.46, p < 0.0001)$ and also in female $(F_{(11,2951)} = 259.95, p < 0.0001)$ (Fig. 11).

Low level of HR for both animals was measured during activity "recumbent". The HR in eland at rest ranged of values from 40.99 to 62.15 bpm ($F_{(1,2780)} = 10947$; p< 0.0001) (Fig. 11).

However, we could monitor that female had significantly the lowest level of HR during activity "**sleeping**" ($F_{(11,2951)} = 259.95$; p< 0.0001) (Fig. 4). Activity "sleeping" was observed in female only and the HR was recorded at 57.32 bpm (\pm 3.84 bpm). We were not able to record this activity in male from technical reasons.

The average value of HR during "standing and ruminating" ($F_{(1,256)} = 312.86$; p< 0.0001) was lower in both animals than the general average value of HR - in female 64.33 bpm (± 5.60 bpm) and in male 48.05 bpm (± 8.98 bpm). The average value of HR in female reached during "standing" ($F_{(1,905)} = 595.25$; p< 0.0001) 69.86 bpm (± 7.32 bpm), HR was slightly lower in male 52.03 bpm (± 11.33 bpm) (Fig. 11).

HR increased in both animals during "**movement**" ($F_{(1,616)} = 313.84$; p< 0.0001). The average value of HR during "movement" was higher than the general average value of HR – in female 74.82 bpm (\pm 7.24 bpm) and in male 57.48 bpm (\pm 12.53 bpm) (Fig. 11).

Significant increases in HR were recorded during the "excitement during feeding" ($F_{(1,471)} = 193.79$; p< 0.0001) and "handling" of animals ($F_{(1,25)} = 41.10$; p< 0.0001) (Fig. 11).

The average value of HR in female reached during "excitement during feeding" 71.94 bpm (\pm 7.58 bpm), HR was slightly lower in male 60.14 bpm (\pm 10.44 bpm).

Stress caused the largest increase of HR (Fig. 11). Highest measured HR was recorded during the handling of animals like stay in the restrain box, weighing, driving of animals and during littering. Handling and stressful environment produced in both animals following values: in male 67.06 bpm, which represents a difference of 26.07 bpm compared to state of rest (40.99 bpm); in female, it was recorded 102.41 bpm, which is a difference of 40.26 bpm from the average value during rest (62.15 bpm).

Stressful events ($F_{(1,36)} = 26,88$; p< 0,0001), like sudden noise outside the pen barn or unexpected entry of a stranger to the stable, caused increase of HR as well. During these situations HR was in female 79.12 bpm (± 10.32 bpm) and in male 62.01 bpm (± 7.96 bpm) (Fig. 11).



Figure 11: Differences between averages HR measured during different type of behaviour in male and female. Asterisk indicate significance (p<0.0001).

6. <u>DISCUSSION</u>

The everlasting alternation between light and darkness and the concomitant oscillation in environmental temperature provides systematic variation in the environment to many organisms on Earth. Those organisms have adjusted their physiology to those environmental changes to profit optimally from the daily changes (Hut and Beersma, 2011).

The allocation of each organism's time is related firstly to intrinsic factors which include - in case of an animal - digestive systems, sex, age, and physiological condition. Secondly, to environmental factors like forage quantity/ quality, light distribution, predation risk, influence of intraspecific social factors or interspecific factors, human activity, and climatic conditions.

We based our investigation on arrangement of relatively stable environmental conditions in the pen barn, i.e. excluding predation risk, minimizing human disturbance and providing ad libitum feed and water. Generally, we can conclude that the time eland spent feeding, resting and moving were different between day and night, season and age.

Seasonal Activity Budgets – We assumed that elands would spend more time by feeding behaviour in low ambient environment (winter) and this was supported, as seasonal time budget reflected changes in elands' activity. The time eland spent by feeding increased from spring to winter. Eland fed 17% of the time during spring and 16% during summer, 21% of the time during autumn and 27 % of the time during winter. The sex and age did not prove as a significant factor in elands' seasonal activity budgets. Therefore, we assume that weather conditions and thermoregulation appears most important factor for amount of feed intake, since predation was not directly addressed, and forage quantity/ quality remained unchanged during the year.

The time budget for feeding reflected both feeding conditions and the annual pattern of body condition. We could conclude that thanks to *ad libitum* presence of feed, eland could cover their higher energy needs for thermoregulation from daily dry matter intake and therefore, their energy reserves were not depleted. We could visually observe that every individual remained in good body condition, i.e. the visibility of pelvis and ribs remained standard (not thin and not very fat).

On the contrary to our observation in eland, other ruminants, like Przewalski horses, have evolved cycles of voluntary food intake as an adaptation to the seasonal change in food availability and quality. The Przewalski horses voluntarily decreased the dry matter intake during winter while the diet was highly fibrous, and accelerated feeding while the food was rich in crude protein in order to maximize the rate of energy uptake from forage (Kuntz et al., 2006). A reduction of food intake during winter and a shift towards energy reserves as the major metabolic fuel is advantageous if the energetic and time costs of foraging and digestion outweigh the possible energy uptake (Kuntz et al., 2006).

If we look at the seasonal activity patterns of other herbivores we have to bear in mind that seasonal adjustments of herbivores feeding behaviour varies among the species and habitat used. The free ranging animals can use greater variety of habitats since it may increase activity time because different habitats provide suitable thermal conditions for activity at different times of day. This principle has different display in different climatic zones. According to our results, elands' behaviour was most similar to that observed in species living in temperate zone rather than species from tropical climate.

Species in tropical climate zone mostly exhibit a decrease in activity as average daily temperature increases as could observe Belovsky and Slade (1986), Lewis (1977) and Underwood (1975). Lewis (1977) observed eland, oryx, buffalo and cattle in Kenya, all species spent significantly less time feeding during periods of high values of ambient temperature and solar radiation during a day. Underwood (1975) noted that in eland in South Africa this pattern correlate with the season. Much more time eland spent on feeding activities during the dry season than in the rainy season. This may be also based on lower night temperature especially during dry winter season.

Similar experience to our investigation made Arnold et al. (2004) in red deer, where animals spent higher proportion of time for feeding. It has increased during autumn and was even higher during winter than during summer and declined sharply with the onset of plant growth in April. Berger et al. (1999) found that feeding of Przewalski horse accounted for 62% of total activity in spring and 40% in summer, 55% of total activity was feeding during autumn and during winter. In summer, horses increasingly shifted their search for food to the hours of night to avoid disturbance by flying insects and high temperatures.

Females and juveniles of spanish ibex shown the tendency to feed more in winter-spring than during rut and summer as well (Alados, 1986).

Contrary to that Green and Bear (1990) have observed in free-ranging elk where feeding time decreased from 55% during summer to 45% during winter.

Locomotor activity varied correspondingly throughout the year. Eland have decreased active movement from spring to winter. Eland were moving (walking) 14% of the time during spring and 19% during summer, 10% during autumn and 5% of time during winter. This result supports our assumption that reduction in winter activity conserves energy and minimizes the negative energy balance. Moen's (1976) observation of white-tailed deer in the winter led to this assumption as well. The level of activity was lowest in winter in a mare group of Przewalski horse (Berger et al., 1999). Total locomotor activity of red deer increased during spring, remained high during summer (66%), and declined continuously after the end of rut to the low winter level (51%) (Arnold et al., 2004).

Most studies on temperate zone cervid's and bovid's activity patterns reported reductions in winter activity and increases during spring and summer (Craighead et al., 1973; Cederlund, 1981; Georgii, 1981; Georgii and Schröder, 1983; Jingfors, 1982; Roby and Thing, 1985).

Daily Activity Budgets – The eland circadian activity was characterized by alternating bouts of feeding, resting and moving. We expected that elands would feed longer at night than during a day and this may indicate a persistence of natural antipredatory behaviour of eland in captivity. Nevertheless, our results disprove this hypothesis because eland spent feeding 23% of time during a day and 17% of time during night. We may suggest that night enclosure of eland reduced their susceptibility to predation, thus eland could increase activity during the day without the likelihood of being captured. Also time spent by sleeping was the longest during night.

Lewis's (1977) observation of eland has concluded in similar knowledge – night enclosed eland spent 70% of the day time by feeding. But in this study is missing explanation about night access to the feed.

In contrast, Alados (1986) could observe the spanish ibex feeding before dawn on moon-lit nights in their home range. Green and Bear (1990) have measured that nocturnal feeding of elk reached up to 44% of total daily feeding time during winter and nocturnal feeding exceeded diurnal feeding during 7 months of year. Colman et al. (2001) found significant difference between feeding of wild reindeer during no-insect days (50% for daytime and 44% for night time) and insect days (18% for daytime and 45% for night time) in summer. Even though, we could not avoid the presence of insect in the pen barn, the number of insect and its harassment could not have significant impact on eland feeding behaviour.

Relatively short part of the daytime and night time (23% and 17%, respectively) dedicated to feeding activities could be explained by ad libitum access to feed and water and stable feed mixture during whole observation.

Influence of sex and age on activity budgets – No significant difference was found between the length of ingestion bouts of adult males and the bouts of other herd members.

We presumed that males' body size would predetermine males for different feeding behaviour. The importance of body size in activity patterns was expected because of metabolic, thermoregulatory and predation considerations. Peters (1983) and Calder (1984) are hypothesizing that large animals must spend more time feeding to satisfy their larger energy needs or large animals cannot be as active as small animals, because their energy costs for locomotion are greater.

We did not prove that adult males would have significantly longer ingestion bouts and lower frequency of bouts due to its large body size. Nevertheless, there is a certain trend indicating that adult males feed longer than adult females, juveniles and calves. Similarly, we proposed that juveniles after weaning would feed longer than adults and have longer digestion bouts than adults. We assumed that juveniles' more intensive catabolic metabolism would require more intensive feeding than the adults' one and that calves could compensate their energy needs by breast-feeding. We found statistically significant difference in proportion of time spent by feeding between the adults, juveniles and calves. Our data showed that juveniles fed the longest time during a day, i.e. 28% of the time and 16% of the time during night; in comparison with adults that fed 27% of the time during daylight and 22% during night. However, the hypothesis cannot be supported even the differences are only 1 % of total time. The difference between the age classes is not great and the explanation may lie with the fact that the data could be influenced by presence of pregnant and lactating adult females in the herd. Lewis (1977) found that the longest feeding time was in pregnant and lactating females.

According to Belovsky and Slade (1986), activity time significantly increases with body size due to increased tolerance to extreme thermal regimes with increasing body size. As well, Mysterud's (1998) exploration of data on African ruminants showed that total time spent by feeding increased isometrically with increasing body weight. Opposite conclusion made Pérez-Barbería and Gordon (1999) who found a significant negative relationship between activity time and body mass. This conclusion would be in correspondence with the observations of Treus (1983) who noted that the adult males of farmed eland which did not take part on reproduction spent the least time on feeding; the young orphans fed the longest time.

According to the type of stabling used for our study and based on our personal observations we may also suppose that juveniles could have fewer opportunities to reach the fodder due to hierarchy order. The question is whether juveniles use special feeding strategy under such conditions. We may assume juveniles to increase their feed intake in order to compress the feeding time, since the fodder quality is high and there is no necessity to search for optimum quality feed. Our assumption might be supported by the length of digestion bouts that significantly differ among age classes. Calves were recumbent and ruminating the longest time 38%, juveniles rested in this

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position 29% of the time and adults the shortest time 23% of the day and night time. Similarly, we could observe the positive relationship between the body size, age class and the length of "sleeping" activity. Calves were sleeping the longest time (5%) by comparison with juveniles (2%) and the adults (1%). Hypothesis stating that juveniles will be sleeping more than adults was supported.

Activity budgets during equal light distribution – The behaviour of eland could be described as a short-term, cyclic rhythm of alternating periods of activity and rest during 24 hours of equal light distribution.

We noted that eland rested more during night than day. Thus, when observation of behaviour was done in some previous studies only during daytime it may be significantly biased.

This study of activity patterns revealed that the proportion of time elands spent by feeding, walking and "standing and ruminating" did not differ during day and night, it shows that these activities were equally distributed during day and night with equal light distribution.

However, we recorded significant differences in the way of elands' rest. They prefer to rest in a standing position during a day, i.e. 15% of the daytime, rather than during night, i.e. 11% of the night time. On the other hand, eland rest in recumbent position more often at night (49%) than during a day (30%), and sleeping occurs more often during night (4%) than during a day (0.8%).

Feeding behaviour based on environmental or internal timing – Many social ruminants tend to exhibit synchronized cycles of behaviour with daily peaks of activity at dawn and dusk. Whether circadian rhytmicity with two peaks occurs in free-ranging elands were not described yet. Nevertheless, many authors have reported this phenomenon in various free-ranging animals like Green-finches (*Chloris chloris*) (Aschoff, 1966), Spanish ibex (Alados, 1986), moose (Cederlund et al., 1988), rocky mountain elk (Green and Bear, 1990) and cattle (Ray and Roubicek, 1971; Hafez and Boissou, 1975; Ruckenbusch and Bueno, 1978). No increase in proportion of time based on circadian rhytmicity was recorded for feeding over 24-h periods in elands. We did not find any prominent active peak/peaks around sunrise and/or sunset for captive eland in this study, the hypothesis was not supported. We found that activities connected to digestion and ingestion, were similarly distributed around dawn and dusk. Similar conclusion made Colman et al. (2001) in wild reindeer where no effect of sunrise and sunset on activity patterns was found. On the other hand, Collins and Smith (1989) reported that free-ranging reindeer attuned their 24 hour feeding rhythm to sunrise and sunset. Colman et al. (2001) explain this discrepancy by the effect of the insect's harassment, which activity are affected by light and climate, on feeding patterns of reindeer and this may have been confused with a crepuscular feeding pattern of ruminants in the earlier studies.

We do not suggest that the insect harassment would play significant role in our study; we rather inclined to explanation that the phase shift between daylight and night may not act as a primary triggering factor (Zeitgeber).

We may consider that the influence of the light-dark transition may be overridden by more powerful Zeitgeber, which could be the delivery of fresh feed to feed alley. This assumption is based on the finding that elands performed resting activities like digestion (standing and ruminating, recumbent and ruminating) 1-3 hours before the feeding time rather than proactive behaviour like ingestion (feeding, moving towards feed alley). This was changed to feeding after fresh feed was delivered (effect of "forcing oscillation") when all the elands started to feed. Our hypothesis that elands will be more active (moving without feeding) 1-3 hours before the feeding time as a consequence of a regular time of feed delivery was not supported. We aimed to find whether elands would anticipate the feeding process 1-3 hours before the feeding time since the feeding occurs every day at the same time during whole year. We supposed elands to move more during the selected time like walking without feeding which could be explained like nervousness and expectations (effect of "entrainment" to regular factor).

Nevertheless, elands dedicated 61% of the time to activities connected to digestion and only 20% to activities connected to ingestion. The delivery of

fresh feed has been previously described as a strong factor influencing feeding patterns in dairy cows (Tanida et al., 1984; Haley et al., 2000; DeVries et al., 2003a; Wagner-Stroch and Palmer, 2003). DeVries and von Keyserlingk (2005) found that not only the fresh feed delivery but the time of feed delivery as well has a great impact on the amount of feed consumed and on the overall daily feeding pattern of group-housed dairy cows. The amount of feed consumed also increased with increasing the frequency of feed delivery in dairy cows and the animals adapted their feeding behaviour to the new distribution of the feeding time over the course of the day (deVries et al., 2005).

The difference in heart rate during activity in eland male and female – Heart rate (HR) monitoring in eland brought about the physiology of these animals interesting knowledge.

The particularly interesting is the difference between basal levels of average HR in male (48.97 bpm) and in female (67.58 bpm), where the difference of these values makes up 18.61 bpm. It was also possible to find differences in HR during all scored type of behaviour between male and female.

Higher HR in male (60.14 bpm) during feeding is probably due to nervousness during the feed delivery; in female, in addition to nervousness, the HR may be also increased by the frequency of aggressive interactions with other animals (71.94 bpm). As already described by Marchant et al. (1997), feeding environment, which allows contact between animals is characterized by increased HR and increased cardiac variability, probably due to the short-term responses to agonistic behaviour.

Our findings may be similar to those recorded by Price et al. (1993), who have registered an increase in HR in deer during the isolation by 27 bpm; Carragher et al. (1996) monitored even higher values during the process of capture and closure of deer to fixation box when HR increased to 111 bpm, i.e. by 55.7 bpm. Baldock and Sibly (1990) reached the same conclusion in sheep (difference of 20 bpm during isolation). Unlike Marchant et al. (1997), we did not expect that the social isolation of male would be a stressful environment and therefore the reason for increased HR in male compared to female HR, which was housed in a group. This assumption is based on the social needs of male who often seeks solitude during common grazing. On the contrary, female had consistently higher levels of basal HR. The question remains, why was this level consistently higher, we assume that these values affects different weight and age of the animals.

It is clear that values measured during recumbency and rumination are closest to the values monitored in deer (Mesteig et al., 2000; Nilssen et al., 1984, Carragher et al. (1997). On the contrary, average HR in cows registered during stress free situations reached 105.1 bpm (with a minimum of 50 bpm and a maximum of 125.0 bpm) (Mohr et al., 2002; Hopster and Blokhuis, 1994; Brosh et al., 2004). Probably, this fact is affected by the high production of cattle that influence HR in these animals.

HR could be affected by different weight and age of the animals, different amount of time devoted to individual activities, the housing technique and interactions among the animals during the trial. The effect of age is evident between the monitored male and female. Male was longer recumbent and ruminating (Treus, 1983), it was longer standing and also it performed movement longer, unlike other adult animals. Another factor is the influence of feed intake, which showed in their studies Mesteig et al. (2000). This factor cannot be assessed in this work, and it may be a topic of further research.

The results could serve as the new knowledge about the physiology of eland, in which the monitoring of HR in relation to the behaviour has not been done. Considering the prospects of eland breeding, results could serve as a basis for assessing welfare in future breeding (Treus and Lobanov, 1971; Kotrba and Šcevlíková, 2002).

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7. <u>CONCLUSION</u>

While introducing new management to breeding, we should be aware that the new breeding conditions should respect the natural needs of animals. By creating the environment that meets the biology of animals we succeed to produce animals with outstanding health and physical condition, under perfect well-being and resulting in successful reproduction.

This study of activity patterns was concluded on a 24 hour observation basis and revealed that there are distinct seasonal and circadian phases of eland's activity and rest. In general, feeding dominated eland's activity followed by rest and moving. The pattern of alternating bouts of feeding and resting is due to separate time required to feeding and rumination.

Our observations noted nocturnal activity in eland. Feeding and resting bouts occurred in the course of the night; nevertheless, nocturnal feeding time did not predominate over the proportion of diurnal feeding time. The sex and age did not prove as significant factor influencing eland's feeding behaviour, the herd performed highly synchronous behaviour.

Elands did not attune their activity to the set points of sunrise and sunset, demonstrating that the phase shift between daylight and night may not act as a primary triggering factor (Zeitgeber). The regular feeding time did not prove as well to be important factor in eland's feeding behaviour. We may suggest that the most powerful Zeitgeber could be the delivery of fresh feed to feed alley.

Significant seasonal changes in eland's feeding and moving behaviour were documented. Feeding increased from summer to winter while moving decreased. Thermal constraints were compensated by increased feeding time during winter not by prolonged rumination. The decline in locomotor activity closely corresponded with the increased feeding during winter. This strategy elands most likely used to conserve energy and to minimize the negative energy balance. Seasonal activity was most likely related to ambient temperature, as it is generally important factor influencing animals' behaviour, since thermoregulation could have limiting impact on activity because certain season presents heat gains or losses to the animal.

Differences in HR were found in all scored type of behaviour between male and female; the basal levels of average HR were recorded in male (48.97 bpm) and female (67.58 bpm).

The greatest influence on level of HR (the highest values) had handling, stressful events and movements. The increase in HR (for female up to 102.41 bpm) was response to these stimuli. Increased HR was associated also with feeding and the delivery of fresh feed by the keeper.

The lowest HR value for both animals was recorded during sleep and recumbency, followed by the time spent resting (standing and ruminating).

Due to the assumption that HR serves as an important indicator of adaptability; this aspect requires more attention for farmed animals.

Common eland as native ungulate from eastern and southern Africa had previously demonstrated the great potential of this species to adapt rapidly to farming conditions, human contact or the contact with other species at the common grazing.

We presumed to suggest several recommendations based on the results of this study. In the case of commercial eland breeding we recommend to increase the amount of energy in feed for overcoming unfavourable climatic conditions during winter without declines of weight gains. According to our results the time devoted to feeding had risen by 11% during winter in comparison to summer.

Since the delivery of feed was shown to have the greatest impact in terms of stimulating elands to feed; the increased frequency of feed delivery could be considered. Frequent delivery of the feed improves access to the feed for all herd members. Firstly, during the peak feeding periods when fresh feed is provided, subordinate individuals may not be displaced as frequently when fed more often.

Secondly, it may reduce the amount of feed sorting. The natural tendency for elands to continually sort the feed and push it away while eating, results in much of the feed being tossed forward where it is no longer within reach. This was monitored a particular problem since feed was delivered to a feed alley. Thus, we recommend to push the feed closer to the elands in between feedings as a means of ensuring that elands have continuous access to the feed. The effect of the feed push-ups on feeding behaviour patterns of elands may be studied.

Eland's circadian rhythmic behaviour proved that nocturnal feeding comprise a not negligible part of the whole circadian feeding time. Therefore, this behaviour could be utilized in the case of commercial fattening of young bulls.
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9. ATTACHEMENT

R-Square	Coeff Var	Root MSE	Mean HR value
0.493056	8.364883	5.653755	67.58918

9.1. Tab. 3: Average value of HR in female Prima.

9.2. Tab. 4: Average value of HR in male Ninja.

R-Square	Coeff Var	Root MSE	Mean HR value
0.412295	17.64709	8.642566	48.97445

9.3. Tab. 5: Time of sunrise and sunset during observation days in Czech Republic (Kočer, Michal. Observatory and planetarium České Budějovice and Kleť observatory. Kleť astronomical almanac, 2001-2010 [online].

Date	Time of sunrise	Time of sunset
	(HH:mm; a.m.)	(HH:mm; p.m.)
4th March 2005	6:41	5:50
5th March 2005	6:39	5:52
11th March 2005	6:26	6:02
12th March 2005	6:24	6:04
18th March 2005	6:11	6:13
19th March 2005	6:09	6:15
25th March 2005	5:56	6:24
26th March 2005	5:53	6:26
21st March 2008	6:04	6:19
22nd March 2008	6:02	6:20
28th March 2008	5:48	6:30
29th March 2008	5:46	6:31

4th April 2008	5:33	6:41
5th April 2008	5:31	6:42
1st August 2009	4:32	7:46
2nd August 2009	4:34	7:45
21st August 2009	5:02	7:10
22nd August 2009	5:03	7:08
28th August 2009	5:12	6:56
29th August 2009	5:14	6:54
16th October 2009	6:27	17:10
17th October 2009	6:29	17:08